

OPIS
Listă 10 publicații relevante
Candidat pentru obținerea atestatului de abilitare în Domeniul Medicină:
CATANA ANDREEA

1. **Cătană A**, Pop M, Hincu BD, Pop IV, Petrișor FM, Porojan MD, Popp RA. The XRCC1 Arg194Trp polymorphism is significantly associated with lung adenocarcinoma: a case-control study in an Eastern European Caucasian group. *Onco Targets Ther.* 2015 Nov 27;8:3533-8. doi: 10.2147/OTT.S92361. PMID: 26664136; PMCID: PMC4669918.
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2. Porojan, M. D., **Cătană, A.**, Popp, R. A., Dumitrascu, D. L., & Bala, C. (2015). The role of NOS2A –954G/C and vascular endothelial growth factor +936C/T polymorphisms in type 2 diabetes mellitus and diabetic nonproliferative retinopathy risk management. *Therapeutics and Clinical Risk Management*, 11, 1743–1748. <https://doi.org/10.2147/TCRM.S93172>, [WOS:000366470300001](https://www.webofscience.com/wos/woscc/full-record/WOS:000366470300001), **FI 2,3; Q2**
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3. **Andreea Catana**, Eniko Kutasi, Florica Ana Chiș, Cristian Popița, Sanda Mariela Militaru. Kartagener Syndrome Associated with a Family History of Dextrocardia – First Patient to be reported in Romania. *Archives of Clinical and Biomedical Research* 6 (2022): 740-743, **FI 3.1; Q2**
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The *XRCCI* Arg194Trp polymorphism is significantly associated with lung adenocarcinoma: a case-control study in an Eastern European Caucasian group

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Abstract: DNA repair plays an important role in maintaining the integrity of the genome by repairing DNA damage induced by carcinogens. Certain genetic polymorphisms that occur in DNA-repair genes may affect the ability to repair DNA defects, and may represent a risk factor in carcinogenesis. The gene *XRCCI* is involved in DNA repair. The purpose of our study was to investigate the association between *XRCCI* Arg194Trp and Arg399Gln polymorphisms and the risk of lung cancer in a Romanian population. We recruited 222 healthy controls and 102 patients with lung cancer. Genotypes were determined by multiplex polymerase chain-reaction restriction fragment-length polymorphism. Statistical analysis (odds ratio, recessive model) revealed an increased risk for lung cancer for the homozygous 194Trp genotype ($\chi^2=0.186$, odds ratio 10.667, 95% confidence interval 1.309–86.933; $P=0.007$). Also, we found an association between the 194Trp allele and women with lung adenocarcinoma. In conclusion, the results of the study place the *XRCCI* Arg194Trp polymorphism among independent risk factors for developing lung cancer.

Keywords: lung cancer, *XRCCI* Arg194Trp, *XRCCI* Arg399Gln

Introduction

Lung cancer, the most common type of cancer, is nowadays the main cause of cancer deaths for men and women worldwide.¹ Over the last few years, the incidence of lung cancer has increased steadily.² The risk of lung cancer has been associated with different kinds of environmental and genetic factors. Exposure to environmental carcinogens and cigarette smoking are considered major etiologic factors for lung cancer.¹

It is well known that DNA repair plays an important role in ensuring the stability of the genome by repairing DNA damage induced by exogenous and endogenous carcinogens. It is accepted that polymorphisms that occur in DNA-repair genes may affect the ability to repair DNA defects, and may represent a risk factor in different malignancies, because of the change of base-excision repair (BER) functions.³ *XRCCI* is a crucial gene involved in DNA repair, specifically in the BER pathway. BER, nucleotide-excision repair, and double-strand-break repair are the main DNA-repair pathways described. Previous reports have indicated that certain genetic polymorphisms, particularly the *XRCCI*-gene variants, were associated with a high risk of malignancy, such as primary lung cancer,^{3,4} hepatocellular carcinoma,⁵ cervical cancer,⁶ childhood acute lymphoblastic leukemia,⁷ and gastric cancer.⁸

A genome-wide association study of 1,154 ever-smoking non-small-cell lung cancer patients, with genotyping of 317,498 tagging single-nucleotide polymorphisms and 1,137 ever-smoking controls in a Texas population of self-reported European descent,

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identified new DNA-repair polymorphisms associated with lung cancer.⁹ A newer, more comprehensive analysis of genome-wide association-study data of the DNA repair-capacity phenotype and genotypes with a much large sample size has confirmed the important role of genetic variation of DNA-repair genes and their role in lung neoplasia.¹⁰ The most common polymorphisms of the *XRCC1* gene are Arg194Trp (A194T, rs1799782), Arg280His (A280G, rs25489), and Arg399Gln (A399G, rs25487). DNA-repair capacity may be affected by these polymorphisms, and thus it may modulate cancer susceptibility.

Over the last few years, case-control studies have been performed to study the association between the *XRCC1* Arg194Trp and Arg399Gln polymorphisms and the risk of lung cancer.^{1,11} The purpose of this study was to investigate whether the *XRCC1* Arg194Trp and Arg399Gln polymorphisms were involved in susceptibility to lung cancer in a Romanian population.

Materials and methods

Patients and controls

The current study was approved by the ethics committee of the conducting institution (Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania). The study was conducted according to the Helsinki Declaration. Written informed consent was obtained from all subjects included in the present study.

A group of 324 individuals were included in the study (Caucasian subjects of Romanian and Hungarian ethnicity). The case group comprised 102 cases diagnosed with lung cancer. Lung cancer was confirmed by imaging (computed tomography scan), and histopathological examination and tumor-subtype classification were done according to World Health Organization criteria.^{12–18} The controls included 222 healthy volunteers with no history of any malignancy. All study participants were or had been active smokers, with an average tobacco consumption between 15 and 40 cigarettes a day for at least 10 years.

Genotypic analysis of *XRCC1* gene

Genomic DNA was extracted from 300 μ L venous blood samples using a Wizard[®] genomic DNA purification kit (Promega Corporation, Fitchburg, WI, USA) and ZymoBead[™] genomic DNA kit (Zymo Research Corporation, Irvine, CA, USA). Arg194Trp and Arg399Gln polymorphisms of the *XRCC1* gene were genotyped using the multiplex polymerase chain-reaction restriction fragment-length polymorphism technique. A total of 100 ng of genomic DNA was amplified

in a total volume of 25 μ L reaction mixture containing reaction buffer of 1.5 nM MgCl₂, 10 pmol of each primer, and 200 μ M of each deoxyribose nucleoside triphosphate, and 0.5 unit of Taq polymerase. Thermocycling conditions were carried out as follows: 94°C for 5 minutes and then 30 cycles of 94°C for 30 seconds, 62°C for 1 minute, 72°C for 45 seconds, and a final polymerization step at 72°C for 5 minutes (Mastercycler[®] Gradient; Eppendorf AG, Hamburg, Germany).

Genotyping analysis was interpreted according to the following criteria: the common allele corresponding with Arg at codon 194, resulting in a 293 bp fragment, and the variant allele corresponding to Trp in a fragment of 313 bp. For codon 399 of the *XRCC1* gene, the presence of 375 and 240 bp fragments, respectively, characterized the common allele corresponding to Gln, while the variant allele corresponding to Arg was defined by the presence of the undigested 615 bp fragment. Genotypic analysis of the *XRCC1* gene was adapted after a protocol performed by Abdel-Rahman et al in 2000.¹⁹

Statistical analysis

The distribution of genotype and allele frequency of each *XRCC1* polymorphism between different groups was compared by Fisher's exact test, followed by comparative analysis according to dominant and recessive models. For estimation of the relative risk and strength of association, we calculated odds ratios (ORs) at a 95% confidence interval (CI). We considered statistically significant a *P*-value \leq 0.05. Statistical analysis was carried out using SPSS 18.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

The common tumor type identified for the lung cancer group was squamous cell carcinoma (69.6%), with a frequency of 12.7% in women and 87.3% in men, followed by adenocarcinoma (18.6%), at 26.3% in women and 73.7% in men. Small-cell squamous carcinoma was found in 11.7% of patients. The average age at diagnosis was 62.2 years for squamous carcinoma (CI 60.4–63.9 years) and 60.3 years (CI 53.8–66.9 years) for small-cell lung cancer. The lowest average age of onset of clinical symptoms was recorded in patients with adenocarcinoma: 58 years (CI 53.9–64.7 years). Genotype distribution and frequency of alleles of the Arg194Trp and Arg399Gln polymorphisms of *XRCC1* in patients with lung cancer and controls are presented in Table 1.

Comparative analysis (Fisher's exact test) of dominant and recessive models for variant carriers of lung cancer risk is

Table 1 Genotype distribution and frequency of alleles of Arg194Trp and Arg399Gln polymorphisms of *XRCC1* in patients with lung cancer and controls

Polymorphisms	Variant	Lung cancer, n (%)	Controls, n (%)	OR (95% CI)	P-value
<i>XRCC1</i> Arg194Trp	Arg/Arg	89 (87.3)	197 (88.7)	0.983 (0.900–1.073)	0.706
	Arg/Trp	3 (2.9)	22 (10.0)	0.296 (0.090–0.969)	0.044
	Trp/Trp	10 (9.8)	3 (1.3)	7.254 (2.040–25.805)	0.002*
	Arg/Trp+Trp/Trp	13 (12.7)	25 (11.3)	0.622 (0.335–1.1521)	0.131
	Arg allele frequency	181 (88.7)	416 (93.7)	0.956 (0.907–1.008)	0.097
	Trp allele frequency	23 (11.3)	28 (6.3)	0.982 (0.605–1.595)	0.943
<i>XRCC1</i> Arg399Gln	Arg/Arg	43 (42.2)	112 (50.5)	0.835 (0.643–1.086)	0.179
	Arg/Gln	43 (42.2)	86 (38.7)	0.990 (0.749–1.307)	0.944
	Gln/Gln	16 (15.6)	24 (10.8)	1.451 (0.806–2.611)	0.214
	Arg/Gln+Gln/Gln	59 (57.8)	110 (49.5)	1.176 (0.941–1.443)	0.153
	Arg allele frequency	129 (63.3)	310 (69.8)	0.905 (0.802–1.022)	0.109
	Gln allele frequency	75 (36.7)	134 (30.2)	1.218 (1.040–1.426)	0.014*

Note: * $P \leq 0.05$.

Abbreviations: OR, odds ratio; CI, confidence interval.

presented in Table 2. Comparative analysis to assess the risk for lung cancer in the study group compared with the control group for variant allele *XRCC1* 194Trp carriers (Fisher test analysis, ORs, dominant model), identified a statistical significance risk for the study group ($\chi^2=0.135$, OR 2.675, CI 0.963–7.432; $P=0.052$). Also, statistical comparative analysis (odds ratios, Fisher test for recessive model) revealed an increased risk for lung cancer for the homozygous 194Trp genotype ($\chi^2=0.186$, OR 10.667, CI 1.309–86.933; $P=0.007$). In this context, we can say that the results of the study place the *XRCC1* Arg194Trp polymorphisms among independent risk factors for developing lung cancer.

Comparative analysis to assess lung cancer risk in the study group compared with the control group for the variant Arg399Gln *XRCC1* gene carriers (Fisher test analysis, ORs for dominant model) did not identify an increased risk for lung cancer ($\chi^2=0.036$, OR 1.160, CI 0.660–2.037; $P=0.606$). Statistical analysis according to Fisher's exact test and ORs for the recessive model did not reveal any significant differences among patients and controls ($\chi^2=0.044$, OR 1.276, CI 0.600–2.716; $P=0.526$).

Comparative analysis of the Arg194Trp and Arg399Gln polymorphisms of the *XRCC1* gene in patients with lung

Table 2 Comparative analysis of Arg194Trp and Arg399Gln polymorphisms of *XRCC1* in patients with lung cancer (Fisher's exact test)

Genotype	Model	χ^2	P-value	OR	CI
<i>XRCC1</i> Arg194Trp	Dominant	0.135	0.052	2.675	0.963–7.432
	Recessive	0.186	0.007*	10.667	1.309–86.933
<i>XRCC1</i> Arg399G	Dominant	0.036	0.606	1.160	0.660–2.037
	Recessive	0.044	0.526	1.276	0.600–2.716

Note: * $P \leq 0.05$.

Abbreviations: OR, odds ratio; CI, confidence interval.

cancer according to sex and histopathological type revealed an increased frequency of the 194Trp allele in women with adenocarcinoma compared to males with adenocarcinoma ($P=0.0003$). There were no other statistically significant associations between the 194Trp and 399Gln alleles and different histopathological types of lung cancer (Table 3).

Discussion

To our knowledge, this is the first study to perform a comparative analysis of the Arg194Trp and Arg399Gln polymorphisms of the *XRCC1* gene in patients with lung cancer in Eastern Europe. Overall, our study provides evidence that the Arg194Trp polymorphism is associated with a high risk of lung cancer.

When we performed subgroup analyses, we observed that the *XRCC1* Arg194Trp polymorphism was significantly associated with the risk of lung cancer. According to the literature,

Table 3 Comparative analysis of Arg194Trp and Arg399Gln polymorphisms of *XRCC1* gene in patients with lung cancer according to sex and histopathological type (Fisher's exact test)

Histopathological type	Allele	Males	Females	P-value
Squamous cell carcinoma	194Trp	12	4	0.108
	194Arg	50	5	
	399Gln	14	3	
	399Arg	38	6	
Adenocarcinoma	194Trp	1	6	0.699
	194Arg	12	0	
	399Gln	3	3	
	399Trp	10	3	
Small-cell carcinoma	194Trp	3	2	0.320
	194Arg	4	3	
	399Gln	1	1	
	399Trp	6	4	

adenocarcinoma is most commonly found in women and young smokers, whereas in men and older smokers, first place is represented by small-cell lung cancer.^{20,21} David-Beabes and London observed that the 194Trp allele was associated with a significantly decreased risk of lung cancer among African-Americans.¹¹

Statistical analysis revealed a frequency of 5.5% for the variant 194Trp allele of the *XRCCI* gene, below those reported for the European and North American populations (11%–15%) and a frequency of 65% for the variant 399Arg allele codon for the *XRCCI* gene, above the statistical limits reported in the literature (40%–65%).²² The results of the present research place our country among populations with the lowest frequency for the Arg194Trp variant allele of the *XRCCI* gene.^{11,23}

Our study showed that there was no association between the *XRCCI* Arg399Gln polymorphism and the risk of lung cancer. Park et al found an association between the *XRCCI* 399Gln allele and increased risk of squamous cell carcinoma of the lung in male Korean patients (OR 1.66, 95% CI 0.99–2.79).³ Divine et al observed that the *XRCCI* 399Gln/Gln genotype was associated with a high risk of adenocarcinoma of the lung (OR 2.45, 95% CI 1.1–5.8).¹² An association between the *XRCCI* Arg399Gln polymorphism and risk for lung cancer in Asians has been found by Kiyohara et al; in contrast, no increase in this risk has been observed in Caucasians, highlighting that there is a particular distribution of allele variants in the European population.¹⁵

No relationship between lung cancer and the *XRCCI* Arg399Gln polymorphism was observed by Hu et al or Hung et al.^{13,14} In a meta-analysis, Wang et al reported no association between the *XRCCI* Arg399Trp polymorphism and lung cancer risk in the studied population.¹ In the same meta-analysis, when studies were stratified by control source, they reported that the variant *XRCCI* 399Gln/Gln and Arg/Gln or Gln/Gln genotypes had a protective effect for lung cancer (OR 0.73, 95% CI 0.58–0.92, and OR 0.86, 95% CI 0.77–0.97, respectively). Another meta-analysis conducted by Kiyohara et al found no association between *XRCCI* polymorphisms and an increased risk of lung cancer among Caucasians and Asians.¹⁵ No association was observed between an increased risk for the development of lung cancer in Turkish patients and the *XRCCI* Arg399Gln polymorphism.¹⁷

Another meta-analysis from eight eligible studies in Chinese populations reported that variant genotypes of *XRCCI* Arg399Gln might alter interindividual susceptibility to lung cancer.¹⁶ On the basis of the same meta-analysis,

Zheng et al did not observe an association between the 194Trp and 280His alleles and lung cancer risk.¹⁶

For the *XRCCI* Arg399Gln polymorphism, David-Beabes and London¹¹ observed some evidence of a reduced risk for the homozygous variant genotype among heavier smokers (African-Americans, OR 0.3, 95% CI 0.0–2.9; Caucasians, OR 0.4, 95% CI 0.2–1.0).

In a recent meta-analysis of 44 case-control studies, the variant homozygous Trp/Trp genotype of codon 194 showed an increased risk for developing lung cancer (OR 1.19, 95% CI 1.01–1.39), especially in Asians (OR 1.21, 95% CI 1.02–1.43).²⁴ Similar observations were reported by Jiang et al (OR 1.22, 95% CI 1.04–1.44).²⁵ In contrast, another meta-analysis found a decreased lung cancer risk among subjects with a variant heterozygous genotype (Arg/Trp) for the *XRCCI* Arg194Trp polymorphism (OR 0.88, 95% CI 0.79–0.97).¹ Dai et al did not find a similar association in the total population, but did in a population-based control. They also found the presence of the heterozygous genotype Arg/Trp was associated with a reduced risk of non-small-cell lung cancer (OR 0.69).²⁴

On the basis of a meta-analysis using 22 studies including 7,515 cases and 9,560 controls, Jiang et al failed to show an association between the *XRCCI* Arg194Trp polymorphism and the risk of squamous cell carcinoma, small cell lung cancer, adenocarcinoma, or other histologic types of lung cancer.²⁵

Currently, there are no specialized data about the distribution of these genetic variants in the Romanian population, but the results of the current study highlight some particularities of the studied population compared with existing data in the literature. An interesting finding is that the frequency of the Trp variant allele of the 194 codon of the *XRCCI* gene is lower common compared to the American, European, and Central Asian populations (~35%), and approaching values reported in the northern US and northern African populations (6%–11%).^{11,13,26–28}

Studies have shown different susceptibilities for smokers to develop lung cancer according to sex, with the assumption of differences in ability to repair DNA lesions, with a deficiency of these mechanisms in women.^{29,30} The results of our analysis revealed no statistically significant general difference between the sexes in the *XRCCI* 194Trp and 399Arg variants. However, we cannot confirm this was because cancer susceptibility does not involve just particular molecular features of DNA-repair pathways but other immunological and hormonal variables.

One of the major findings in the present study is that the variant 194Trp allele of the *XRCCI* gene was more

common in women with adenocarcinoma compared to men with adenocarcinoma, which could mean that this genetic variant could be an important inborn risk factor in the etiology of women's lung adenocarcinoma. Our results are in agreement with other studies in which this DNA-repair genetic variant was associated with lung adenocarcinoma in women, although most of these studies focused on nonsmoking women.^{31,32}

One of the limits of the present study is the small number of subjects and the fact that they were not followed thereafter to assess the association with different demographic characteristics in relation to other individual or pathological factors (disease stage and treatment). Because the study included Caucasian subjects with Balkan, Slavonic, Romanian, and Asian ancestry³³ as part of the Eastern European population, genetic characteristics may have varied due to demographic particularities; therefore, it is imperative to conduct large-scale studies to determine the allele frequency and appropriate connections of these genetic variants in lung cancer in Eastern European patients.

Conclusion

We can say that our statistical results place our study group among the populations with the lowest frequency for the Arg194Trp variant allele of the *XRCC1* gene. The findings of the study place the *XRCC1* Arg194Trp polymorphism among independent risk factors for developing lung cancer, particularly lung adenocarcinoma in women.

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Disclosure

The authors report no conflicts of interest in this work.

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The role of NOS2A –954G/C and vascular endothelial growth factor +936C/T polymorphisms in type 2 diabetes mellitus and diabetic nonproliferative retinopathy risk management

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Abstract: Type 2 diabetes mellitus (T2DM) remains one of the major health problems in Europe. Retinopathy is one of the major causes of morbidity in T2DM, strongly influencing the evolution and prognosis of these patients. In the last 2 decades, several studies have been conducted to identify the possible genetic susceptibility factors involved in the pathogenesis of the disease. However, there is little data related to the involvement of vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS) gene polymorphisms in the T2DM Caucasian population. The objective of this study was to identify a possible connection between NOS2A –954G/C (rs2297518) and VEGF +936C/T (rs3025039) polymorphisms and the risk of developing T2DM and nonproliferative diabetic retinopathy in a Caucasian population group. We investigated 200 patients diagnosed with T2DM and 208 controls. Genotypes were determined by multiplex polymerase chain reaction-restriction fragment length polymorphism. Statistical and comparative analyses (Fisher's exact test) for dominant and recessive models of NOS2A –954G/C and VEGF +936C/T polymorphisms revealed an increased risk of T2DM ($\chi^2=8.14$, $\phi=0.141$, $P=0.004$, odds ratio [OR] =2.795, 95% confidence interval [CI] =1.347–5.801; $\chi^2=18.814$, $\phi=0.215$, $P=0.001$, OR =2.59, 95% CI =1.675–4.006, respectively). Also, comparative analysis for the recessive model (using Pearson's chi-square test [χ^2] and the phi coefficient [ϕ]) reveals that the variant CC genotype of NOS2A gene is more frequently associated with T2DM without retinopathy ($\chi^2=3.835$, $\phi=-0.138$, $P=0.05$, OR =0.447, 95% CI =0.197–1.015). In conclusion, the results of the study place VEGF +936C/T polymorphisms among the genetic risk factor for T2DM, whereas NOS2A –954G/C polymorphisms act like a protective individual factor for nonproliferative retinopathy.

Keywords: type 2 diabetes mellitus, T2DM, retinopathy, +936C/T variant of VEGF gene, –954G/C of NOS2A gene

Introduction

Type 2 diabetes mellitus (DM) is a major public health problem, being one of the most common metabolic disorders.^{1,2} Even if the hyperglycemic status has a hereditary component with dominant expression in the phenotype, diabetes is considered as a multifactorial disease in which there is an imbalance associated with hereditary and environmental factors.^{3,4} Over the last 2 decades, genome-wide association studies, linkage analysis, candidate gene approach, and combined analysis of these candidate loci led to the identification of several molecular markers associated with the pathogenesis of T2DM and its complications.^{5–7}

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Diabetic retinopathy (DR) is one of the major complications in diabetic adults and considered as the major cause of new-onset blindness in these patients. This microvascular complication occurs rapidly in some patients, whereas it occurs in the late stages of diabetic evolution or does not develop at all in other patients.^{8–10}

Vascular endothelial growth factor (VEGF) is a potent angiogenic and vascular permeability factor; therefore, this gene and its polymorphic variants seem to play an important role in DR characterized by impaired vascular permeability, tissue ischemia, and neovascularization.^{11–13} The human *VEGF* gene is located on chromosome 6 (6p21.3) and highly polymorphic, with at least 30 single-nucleotide polymorphisms described in the literature.^{14–16} The particular +936C/T in the 3'-untranslated region of *VEGF* gene is one of the most common gene variants related to lower levels of plasma lipopolysaccharide-stimulated VEGF protein production in peripheral blood mononuclear cells in healthy individuals.^{17,18}

Human gene encoding for nitric oxide synthase (*NOS*) is located on chromosome 17 (17q11.2-12)¹⁹ and consists of a highly reactive intercellular signaling molecule, with anti-thrombogenic and antiplatelet regulatory activities, produced in the Muller cells and the retinal pigment epithelium²⁰ and highly expressed in retinal vessels, where it plays a major role in the regulation of vascular tone and macrophage remodeling.²¹ Although there is a sufficient evidence that endothelial NOS isoform is linked to diabetes and complications, such as retinopathy, inducible nitric oxide synthase (iNOS) remains unknown in terms of its association with DR. A pentanucleotide (CCTTT)*n* polymorphism was already proved to be associated with DR, although it is still questionable whether –954G/C polymorphism in the promoter region that affects the expression level of iNOS is also linked to DR.¹⁹

The objective of this study was to evaluate the possible association of +936C/T variant of *VEGF* gene and –954G/C of *NOS2A* gene in relationship with type 2 diabetes and nonproliferative retinopathy in a Caucasian of origin Eastern European population group.

Materials and methods

Patients and controls

The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of the “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania. Written informed consent was obtained from all subjects included in this study.

A group of 408 individuals, all Caucasians, were included in this prospective case-control study; among those,

200 patients with T2DM were included in the study group without a medical history of high blood pressure or dyslipidemia (independent risk factor for retinopathy). For both groups, fasting blood glucose, total serum cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, triglyceride level, weight, body mass index, abdominal circumference, and systolic and diastolic blood pressure were determined. For the study group, glycosylated hemoglobin (HbA_{1c}) levels were determined in addition. Also, ophthalmological assessment with binocular indirect ophthalmoscopy and a standard fundus retinography (Visucam Lite; Carl Zeiss Meditec AG, Jena, Germany) was carried out in all diabetic subjects. We mention that in this study, only diabetic patients without retinopathy or with incipient, early-stage nonproliferative retinopathy were included. The controls consisted of 208 healthy, nondiabetic volunteers, with negative chronic ophthalmological illnesses.

Genotypic analyses of NOS2A –954G/C (rs2297518) and VEGF +936C/T (rs3025039)

DNA samples were obtained from 400 µL peripheral blood, using Wizard Genomic DNA Purification Commercial Kit (Promega Corporation, Fitchburg, WI, USA). Genotyping was based on polymerase chain reaction (PCR)-restriction fragment length polymorphism technique. For specific DNA amplification, a total amount of 100 ng of genomic purified DNA was amplified in a volume of 25 µL reaction mixture containing 12.5 µL of PCR mastermix, a premixed, ready-to-use solution containing Taq DNA polymerase, deoxynucleotide triphosphates, MgCl₂, and reaction buffers (Promega Corporation); 7.5 µL free nuclease water; 1 µL of bovine serum albumine; 1 µL of each primer; and 1 µL of water suspended DNA. The amplification products were submitted to enzyme digestion (Fermentas; Thermo Fisher Scientific, Waltham, MA, USA) and analyzed by electrophoresis agarose gel (MetaPhor®; FMC BioProducts, Rockland, ME, USA), allowing detection by ethidium bromide staining of the corresponding genotypes. Specific thermocycling conditions and resulting DNA fragments are presented in Table 1. The genotypic analyses of NOS2A –954G/C (rs2297518) and VEGF +936C/T (rs3025039) were done in accordance with the studies of Kun et al²² and Guan X et al.²³

Statistical analysis

Statistical analysis was carried out using SPSS 18.0 for Windows software (SPSS Inc., Chicago, IL, USA). Demographic and clinical data were compared using the Pearson's chi-square test (χ^2) and the phi coefficient. The distribution

Table 1 Specific thermocycling conditions and resulting DNA fragments

Gene variant	Primer sequence	Thermocycling conditions	Restriction enzyme	Fragments
NOS2A and -954G/C	F3'-CATATGTATGGGAATACTGTATTTTCAG-5' R5'-TCTGAACTAGTCACTTGAGG-3'	94°C/30 s, 61°C/45 s, 72°C/75 s, and 72°C/10 min	BsaI	GG (437 and 136 bp) GC (573, 437, and 136 bp) CC (573 bp)
VEGF and +936C/T	F3'-AAGGAAGAGGAGACTCTGCGCAGAGC-5' R5'-TAAATGTATGTATGTGGGTGGGTGTGTCTACAGG-3'	94°C/30 s, 64°C/1 min, 72°C/75 s, and 72°C/5 min	NlaIII	CC (208 bp) CT (208, 122, and 86 bp) TT (122 and 86 bp)

Abbreviations: NOS, nitric oxide synthase; F, forward; R, reverse; s, second; min, minute; VEGF, vascular endothelial growth factor.

of genotype and allele frequency of each *NOS2A* -954G/C (rs2297518) and *VEGF* +936C/T (rs3025039) polymorphisms between different groups was compared by the Fisher's exact test, followed by comparative analysis according to dominant and recessive models. For estimation of the relative risk and strength of association, we calculate odds ratio (OR) at 95% confidence interval (CI). A *P*-value <0.05 is considered to be statistically significant.

Results

Demographic characteristic and clinical data of the diabetic subjects and controls are presented in Table 2. The average disease duration was 7.2±5.4 years. Statistical analysis did not reveal any differences between subjects and controls as sex distribution ($\chi^2=1.370$, $\phi=0.058$, $P=0.242$), body mass index ($P=0.219$), weight ($P=0.943$), abdominal circumference ($P=0.731$), systolic blood pressure ($P=0.312$), and diastolic blood pressure ($P=0.341$). Although average

mean age in control group is lower compared with the study group ($P=0.001$), this has no influence on the genetic polymorphisms, because they are present in individuals, without being age related.

None of the subjects from the control group or the study group had high cholesterol or triglycerides level.

Genotype distribution and frequency of alleles of *NOS2A* -954G/C and *VEGF* +936C/T polymorphisms in patients with diabetes and controls are presented in Table 3.

Comparative analysis (Fisher's exact test) for dominant and recessive models of *NOS2A* -954G/C and *VEGF* +936C/T polymorphisms was performed.

Comparative analysis to assess the diabetes risk in the study group with that of the control group for -954G/C of *NOS2A* gene variant carriers (Fisher's exact test analysis - ORs for dominant model) does identify a statistically increased risk value ($\chi^2=8.14$, $\phi=0.141$, $P=0.004$, OR =2.795, 95% CI =1.347-5.801). The statistical analysis

Table 2 Demographic characteristics and clinical data of the diabetic and control subjects

	Diabetic subjects (n=200)	Control subjects (n=208)	OR	95% CI	P-value
Age	63.84±12.59	54.78±15	1.231	0.843-1.781	0.001
Fasting blood sugar	122.9±41.64	88.4±11	1.132	0.723-1.825	0.376
Weight (kg)	81.4±17.3	81.7±13.9	1.241	0.872-1.721	0.943
Body mass index (kg/m ²)	27.8±6.1	25.1±5.1	1.132	0.763-1.739	0.219
Abdominal circumference (cm)	90.4±11.8	87.9±10.3	1.192	0.812-1.769	0.731
Systolic blood pressure (mmHg)	124.51±15.35	120.71±17.25	1.251	0.629-1.871	0.312
Diastolic blood pressure (mmHg)	72.03±10.07	69.73±9.17	1.272	0.682-1.897	0.341
Disease's duration (years)	7.2±5.4	N/A	N/A	N/A	N/A
Cholesterol (mg/dL)	196.64±46.45	188.73±36.74	1.328	0.742-1.802	0.493
HDL (mg/dL)	46.14±10.07	49.04±11.71	1.287	0.620-1.891	0.529
LDL (mg/dL)	98.88±9.77	91.22±10.23	1.321	0.686-1.832	0.319
Triglyceride (mg/dL)	159.48±48.30	154.03±83.75	1.378	0.789-1.875	0.683
HbA _{1c}	6.95±0.8	N/A	N/A	N/A	N/A
Males, n (%)	101 (50.5)	93 (44.71)	1.262	0.855-1.862	0.275
Retinopathy, n (%)	123 (61.5)	N/A	N/A	N/A	N/A

Notes: Data shown as mean ± SD unless otherwise specified.

Abbreviations: OR, odds ratio; CI, confidence interval; SD, standard deviation; N/A, not applicable; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA_{1c}, glycosylated hemoglobin.

Table 3 Genotype distribution and frequency of alleles in diabetic and control subjects

Polymorphisms	Variant	Diabetic subjects, n (%)	Control subjects, n (%)	OR (95% CI)	P-value
NOS	G/G	92 (46)	149 (71.63)	0.337 (0.223–0.508)	,0.001
	G/C	81 (40.5)	48 (23.08)	2.268 (1.477–3.483)	,0.001
	C/C	27 (13.5)	11 (5.29)	2.795 (1.346–5.801)	0.005
	G/C + C/C	108 (54)	59 (28.37)	2.964 (1.967–4.467)	,0.001
	G allele frequency	265 (66.25)	346 (83.17)	0.397 (0.285–0.552)	,0.001
	C allele frequency	135 (33.75)	70 (16.83)	2.518 (1.810–3.502)	,0.001
VEGF	C/C	118 (59)	164 (78.85)	0.386 (0.249–0.597)	,0.001
	C/T	60 (30)	38 (18.27)	1.917 (1.205–3.048)	0.007
	T/T	22 (11)	6 (2.88)	4.161 (1.650–10.492)	0.001
	C/T + T/T	82 (41)	44 (21.15)	2.590 (1.674–4.005)	,0.001
	C allele frequency	296 (74)	366 (87.98)	0.388 (0.268–0.563)	,0.001
	T allele frequency	104 (26)	50 (12.02)	2.571 (1.775–3.725)	,0.001

Abbreviations: OR, odds ratio; CI, confidence interval; NOS, nitric oxide synthase; VEGF, vascular endothelial growth factor.

according to Fisher’s exact test – ORs for the recessive model – also reveals significant differences among patients and controls ($\chi^2=27.712$, $\phi=0.261$, $P=0.001$, OR =2.965, 95% CI =1.967–4.468).

Comparative analysis to evaluate the diabetes risk in the study group with that of the control group for +936C/T polymorphism of *VEGF* gene carriers (Fisher’s exact test analysis – ORs for dominant model) does identify a statistically increased risk value ($\chi^2=18.814$, $\phi=0.215$, $P=0.001$, OR =2.59, 95% CI =1.675–4.006); the same statistically significant differences were highlighted by using Fisher’s exact test – ORs for the recessive model ($\chi^2=10.506$, $\phi=0.160$, $P=0.001$, OR =4.161, 95% CI =1.650–10.493).

DR was also assessed by using the same Fisher’s exact test – ORs. Comparative analysis for the dominant model to analyze DR in relation to the variant genotype for –954G/C of *NOS2A* gene did not reveal any statistically significant differences ($\chi^2=1.661$, $\phi=-0.091$, $P=0.197$, OR 0.685, 95% CI =0.385–1.219), whereas comparative analysis for the recessive model highlighted that the variant CC genotype is more frequently associated with diabetes without retinopathy ($\chi^2=3.835$, $\phi=-0.138$, $P=0.05$, OR =0.447, 95% CI =0.197–1.015).

Comparative analysis to analyze the diabetic non-proliferative retinopathy in relation to the variant genotype +936C/T of *VEGF* gene did not reveal any statistically significant differences for neither the dominant nor the recessive models ($\chi^2=0.215$, $\phi=0.033$, $P=0.643$ OR =1.147, 95% CI =0.642–2.052 and $\chi^2=1.316$, $\phi=0.081$, $P=0.251$, OR =1.769, 95% CI =0.661–4.739, respectively).

Nonproliferative DR assessed in relationship with HbA_{1c} and variant genotypes of both investigated polymorphisms also found no statistical differences ($\chi^2=5.750$, $\phi=0.182$,

$P=0.016$, OR =2.171, 95% CI =1.146–4.113 for –954G/C of *NOS2A* variant and $\chi^2=5.288$, $\phi=0.143$, $P=0.021$, OR =3, 95% CI =1.158–7.772 for +936C/T of *VEGF* variant).

Discussion

Over the past 2 decades, the number of people diagnosed with T2DM has more than doubled globally, making it one of the major public health challenges to all developed countries. DR is the most common vascular complication of T2DM characterized by hemostatic abnormalities, increased vascular permeability, and tissue ischemia followed by neoangiogenesis, and it occurs in ~75% of patients within 15 years of evolution.²⁴ As this complication is one of the major causes of blindness in general population, the etiopathogenesis of this complication has been extensively studied in recent years.

However, only some of the diabetics develop this retinopathy, and this proves that there are a series of individual genetic factors involved in the long-term outcome of microvascular diabetic complications. Therefore, it is important to identify the genetic susceptibility factors for DR, which could help us to clarify the pathogenesis, evolution, and adopt adequate treatment.²⁵

The risk factors for DR include disease’s length (period of time), fasting glucose, HbA_{1c}, and in addition high blood pressure, lipid serum levels, and genetic factors. The genetic risk factors remain a new, innovative field, requiring clear more scientific data that are involved in developing DR. We decided screening the subjects without high blood pressure and dyslipidemia and with a satisfactory glycemic control of the diabetes, especially for showing the role of genetic factors in DR.

Although *NOS2A* –954G/C and *VEGF* +936C/T polymorphisms and their relevance with diabetes and DR were studied

in several different studies^{4,5} in the last years, there are few data regarding the distribution and importance of these two genetic variants in Caucasian population with diabetes.

Genetic variants of VEGF with dysregulated expression are implicated in many chronic proliferative disorders, with markedly elevated protein levels in the vitreous and aqueous fluids in the eyes of patients with diabetes.²⁴ As VEGF may play an important role in the pathogenesis of DR, several polymorphisms have been studied in the *VEGF* gene. A meta-analysis found significant associations between +936C/T (rs3025039) and DR susceptibility in Asian populations;¹⁶ in accordance with other research group, a meta-analysis found that +936C/T polymorphism of *VEGF* gene was related to DR in Korean populations;²⁶ however, another study on Asian population demonstrated no significant association of this polymorphism with diabetes and associated retinopathy.²⁷ Our results come in agreement with these studies, placing +936C/T variant of *VEGF* gene among the genetic risk factors involved in the pathogenesis of type 2 diabetes and associated retinopathy.

Even if there are valid data regarding the distribution of this variant in the general population, it is still not clear whether there is a conclusive evidence for an association of this polymorphism with diabetes and retinopathy in East European Caucasian population.

It is well known that functional hyperemia in diabetic patients is severely decreased. The loss of this vascular response could starve the retina for oxygen and glucose, putting neurons at risk, and therefore contributing to retinal pathology.²⁸ Neuronal or glial retinal dysfunction observed in early stages of DR is associated with altered neurovascular signaling, leading to the loss of functional hyperemia. Because functional hyperemia is reduced and iNOS expression is increased, genetic polymorphisms of NOS2A gene are the suitable candidate molecular markers for the study of DR. Also, chronic inflammation has been proposed to be involved in the pathogenesis of obesity-related insulin resistance and type 2 diabetes,²⁹ and also a new study has shown that iNOS plays an important role in the pathogenesis of insulin resistance in vivo.³⁰ Another proof that iNOS may be involved in DR is that restoring functional hyperemia by inhibiting iNOS may slow the progression of the disease.²⁸

To our knowledge, there are no valid data regarding the assessment and relationship of -954G/C NOS gene polymorphism and diabetes in Caucasian population. -954G/C NOS was reported as a genetic risk variant in the inflammatory bowel disease in Spanish population or gastric cancer in the

Chinese population.¹⁹ The results of our study show that variant CC genotype of -954G/C NOS gene is associated with T2DM but is less commonly found in subjects with DR. Most of the late studies focused on the study of the pentanucleotide (CCTTT)*n* repeat polymorphism in *iNOS* gene, result suggesting that this particular genetic variant seems to act like a protective factor against retinopathy in patients with T2DM.^{19,30} Although our study is among the first case-control study focused on the association of NOS2A -954G/C and VEGF +936C/T genetic polymorphisms with the risk of T2DM and DR in the eastern European population, our study still has some limitations due to the relatively small sample size and lack of information regarding the evolution of retinopathy in these diabetic patients.

Conclusion

In this study, +936C/T polymorphism of *VEGF* gene is highly associated with DR; therefore, this genetic variant is confirmed to be an independent genetic risk factor for non-proliferative retinopathy. As for -954G/C polymorphism of *NOS* gene, there is evidence that the variant genotype acts like a protective factor against retinopathy in patients with type 2 diabetes.

Studied genetic polymorphisms may play an important role in genetic predisposition to DR. This finding suggests the need for interventional approaches to identify asymptomatic patients at risk of developing retinopathy, which can benefit from an aggressive prevention of DR.

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Disclosure

The authors report no conflicts of interest in this work.

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Case Report

Kartagener Syndrome Associated with a Family History of Dextrocardia – First Patient to be reported in Romania

Andreea Cătană^{1,2,4*}, Enikő Kutasi¹, Florica Ana Chiș^{1,3}, Cristian Popița^{1,4}, Sanda Mariela Militaru^{1,2}

Abstract

Kartagener syndrome is a monogenic inherited disorder in the heterogeneous group of primary diseases of the cilia, with pulmonary and ENT (ear/nose/throat) involvement, situs inversus, and infertility. The condition is characterized by the clinical triad consisting of bronchiectasis, situs inversus, and chronic sinusitis. Most cases result from biallelic mutations in *DNAH5* and *DNAI1* genes, which encode the outer dynein arm, responsible for microtubule movement in cells. We are reporting a 14-year-old male patient who underwent genetic testing at 14, despite recurring sinus and ear infections from a very young age. A recently performed CT scan revealed dextrocardia, and further imagistic investigations showing situs inversus and genetic testing defined a compound heterozygous genotype for the *DNAI1* gene as Kartagener syndrome was confirmed. Heterozygous pathogenic variants were identified in both parents and the patient's brother. The case report provides details regarding the patient's diagnosis, the medical particularities of the case due to genotype-phenotype associations, and the psycho-emotional impact related to the diagnosis of a rare genetic disease. Potentially relevant family history is also discussed. This is the first patient with Kartagener syndrome to be reported and published in Romania.

Keywords: Kartagener Syndrome; Situs Inversus; ENT Infections; DNAI1 Gene

Introduction

Kartagener syndrome (MIM#244400) is a rare genetic disorder with autosomal recessive inheritance, characterized by primary ciliary dyskinesia. The clinical triad which defines the condition consists of situs inversus, chronic sinusitis, and bronchiectasis [1]. Due to the impaired motility of the cilia, patients are predisposed to developing recurrent ENT (ear/nose/throat) infection, chest infection, and infertility caused by immotile spermatozoa in males and ciliary dyskinesia in the fallopian tubes in female patients [2]. Kartagener syndrome is most commonly the consequence of defects in *DNAH5* and *DNAI1* genes, which encode the heavy, intermediate chain of dynein, a protein responsible for microtubule movement in cells. Although the mechanism is based mainly on axonemal impairment, the phenotypic presentation of the syndrome is highly heterogeneous.

Case presentation

We are reporting a 14-year-old male patient who presented in the Pneumology Department with recurring sinus and ear infections associated with coughing and excessive nasopharyngeal secretions. We mention that the

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patient comes from pregnancy with no complications; he was born at full term and did not present fetal distress or postnatal pathological clinical manifestations. In the first year of their life, he showed recurrent ear and nose infections, treated with non-steroidal anti-inflammatory drugs and antibiotics. Multiple allergies were investigated, and immunology, pneumology, and ENT investigations were performed, but no accurate diagnosis was established. During childhood, symptomatic treatment was alternatively administered depending on the severity of the recurrences, more severe respiratory episodes often necessitating withdrawal from school activities. During the past year, the patient's symptoms have worsened as he experienced decreasing tolerance to effort and persistent coughing throughout the day, affecting essential daily and social activities and altering his quality of life. Due to the epidemiological context and being diagnosed with Covid infection, the patient underwent a CT scan, and two bronchiectasis lesions were discovered along with dextrocardia. Interestingly, the mother mentioned that the maternal grandfather also had dextrocardia, but he was asymptomatic until he was 80. No other family members presented with dextrocardia or ENT-related symptoms, except for the mother, who had a mild form of chronic rhinosinusitis. The pneumologist suspected Kartagener syndrome, and the patient was addressed for genetic counseling. Given the suggestive clinical and imaging findings, after informed consent was obtained from both the patient and the parents, a comprehensive molecular panel was performed using an NGS combined with MLPA techniques which detect structural anomalies and deletions/duplications in 174 candidate genes for primary ciliopathies. The result revealed two pathogenic mutations in the *DNAH11* gene, c.1612G>A (p.Ala538Thr) and c.180G>A (silent) variant. Genetic testing was recommended for both parents and the patient's five-year-old brother, who were diagnosed as carriers of a mutant gene variant. The mother is a carrier for the c.1612G>A (p.Ala538Thr) variant and the father for the silent but disruptive, c.180G>A variant of the *DNAH11* gene, so we concluded that the patient inherited the mutations on different alleles of the gene and therefore he is a *DNAH11* compound heterozygous, which genetically defines Kartagener syndrome. The patient's brother carries the same pathogenic variant as the father, who is an asymptomatic carrier of the disease. Furthermore, a complete MR imaging was performed to evaluate other topographic anomalies in Kartagener syndrome, and situs inversus was described.

The patient and his family received genetic counseling and the necessary information to understand the disease and its implications. The patient was referred to a multidisciplinary approach with experience in the clinical management of primary ciliary dyskinesia. A primary care pneumologist, a respiratory rehabilitation nurse, and a general practitioner will monitor the evolution of the patient, together with a fertility specialist, if infertility will represent an issue for the patient in the future.



Image 1: Situs Inversus observed on MRI.

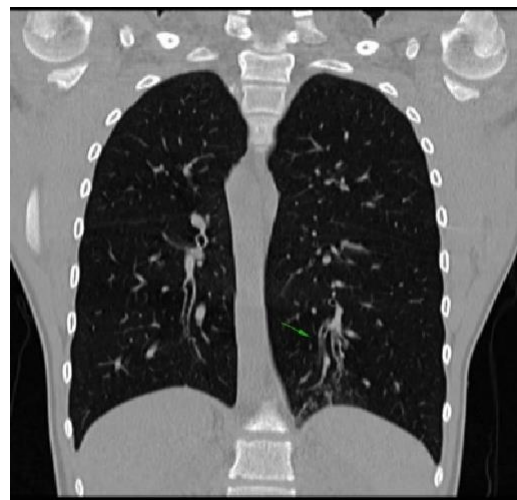


Image 2: Left lower lobe bronchiectases on CT scan (coronal reconstruction).

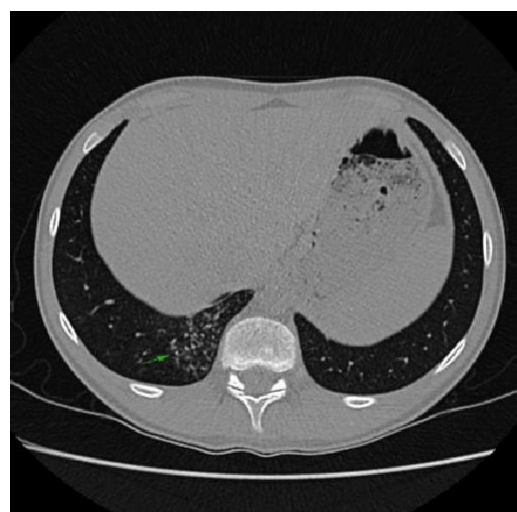


Image 3: Left lower lobe bronchiectases and mucocele on CT scan (axial plane).

Discussion

Before the NGS technology development, the diagnosis of Kartagener syndrome was solely clinical and based on the typical triad represented by recurring sinusitis, bronchiectasis, and situs inversus [3]. As for most rare genetic disorders, primary ciliary dyskinesia is associated with various lung pathologies like cystic fibrosis or asthma [4] or is used as an exclusion criterion [5], especially in countries where molecular testing is still a luxury tool [6]. Most affected individuals present symptoms from early childhood, a period when ENT infectious recurrences are common due to social interaction. Recurring sinus and ear infections can lead to complications such as hearing impairment, deviated septum, nasal polyps, or severe allergies [7]. All these manifestations have been previously reported as common signs in ciliary disorders [1,3]. Among our patient's most disturbing symptoms are chronic refractory rhinosinusitis, bronchitis episodes, productive coughing, and thick, mucoid rhinorrhea, which became more severe in the past year and severely affected his quality of life. Although there were early signs of the disease, genetic testing was recommended only after dextrocardia was documented in a CT scan to investigate potential pulmonary involvement due to Covid-19 infection. Interestingly, the patient's family history involves dextrocardia in the maternal grandfather, which did not cause any clinical manifestations. The grandfather did not undergo genetic testing for mutations that might be associated with primary ciliary dyskinesia. Recurring sinusitis was also present in the patient's mother, who carries a pathogenic variant of the *DNAI1* gene. While studies do not demonstrate a clear association between Kartagener syndrome and positive family history of recurring sinus infections or dextrocardia, it could represent an aspect worth evaluating. Although ciliopathies are monogenic, Mendelian disorders [8], the phenotype can be influenced by many non-Mendelian factors like locus heterogeneity, copy number variants oligogenic, multiple allelism, transposon-mediated mutagenesis, epigenetics, and of course, environmental factors [9]. An essential aspect of rare genetic diseases is the genotype-phenotype association, which can provide valuable information regarding the clinical features, evolution, and possible complications of various pathologies. An even more critical element in primary ciliary dyskinesia is its being characterized by significant clinical heterogeneity. Our patient presents two different pathogenic variants in both alleles of the *DNAI1* gene. Together with the *DNAH5* gene, *DNAI1* represents the most affected gene in Kartagener syndrome [3]. A recent review on PCD revealed that patients with *DNAH11* mutations had significantly better respiratory function with higher FEV1 values at diagnosis than patients with mutations in any of the other genes [10]. In our patient, upper airway manifestations prevail the clinical phenotype, such as recurrent rhinosinusitis and otitis, both present from early childhood. Currently, the respiratory function seems to be preserved; the functional respiratory examination

is within the normal range for his age. To our knowledge, there are no published cases of Kartagener syndrome with c.1612G>A (p.Ala538Thr) / c.180G>A *DNAI1* compound heterozygosity. The c.1612G>A variant is classified as pathogenic and reported as homozygous in Kartagener. The c.180G>A variant was considered until recently a variant of uncertain significance due to its silent change of amino acid structure. Still, algorithms developed to predict the effect of nucleotide changes on RNA splicing suggested that this variant may alter RNA splicing [11]. The compound heterozygous genotype may explain the phenotype dominated by ENT manifestations with a variant with moderate penetrance. Kartagener syndrome is invariably associated with infertility due to a lack of mobility in spermatozoa or obstructive azoospermia [12]. Studies show that patients with this diagnosis can achieve viable pregnancies with assisted conception consisting of intracytoplasmic sperm injection. Sperm motility can be enhanced with the help of pentoxifylline, or the process can involve random selection of sperm and fertilization using assisted oocyte activation [13,14]. Being able to assure biological fatherhood is an important issue. Although confirming the diagnosis helps improve patient management, pediatric genetic testing raises ethical concerns. Genetic counseling and family planning are essential in avoiding further pregnancies with the same condition as the affected family member [15]. Predictive testing can identify the genetic findings that imply a predisposition towards developing certain diseases and allow necessary prophylactic measures. Nevertheless, we cannot oversee the emotional impact a diagnosis of a rare illness means for both the pediatric patient and his family [16]. It is always possible that genetic testing reveals some secondary findings than the initial suspicion, so it is essential that the parents and the child, especially in the teenage years, understand all the possible results and outcomes of performing diagnostic genetic testing [17]. As for most rare genetic diseases, early diagnosis of primary ciliopathies like Kartagener syndrome is essential for preserving pulmonary function, assuring a better quality of life, and providing the necessary emotional support.

Conclusion

Kartagener syndrome is primary ciliary dyskinesia with autosomal recessive inheritance. The clinical trial is characterized by bronchiectasis, chronic sinusitis, and situs inversus. Infertility also represents an issue for Kartagener syndrome patients, but assisted conception can help achieve viable pregnancies. The patient we are reporting is a 14-year-old male who presented recurring ENT and pulmonary infections from the first year of his life. Nevertheless, pulmonary function is still preserved. When undergoing a CT scan searching for potential lung damage caused by Covid-19 infection, dextrocardia was observed, along with bronchiectasis lesions in the lower left lobe. Further imagistic investigations show situs inversus. Genetic testing, performed

using a combination of the NGS and MLPA techniques, revealed two pathogenic mutations in the *DNAH11* gene: c.1612G>A (p.Ala538Thr) and c.180G>A (silent) variants. Both the patient's parents and brother are heterozygous carriers of a pathogenic variant. The patient's family history shows that the mother suffers from chronic sinusitis. The maternal grandfather is known to have dextrocardia, but he did not undergo genetic testing. As potential complications can occur due to recurrent clinical manifestation, a multidisciplinary approach is required to ensure our patient's a better quality of life. Rare genetic disorders and their implications have a significant emotional impact on pediatric patients and their families, so psychological counseling must be included in case management.

Declarations

Ethics approval and Consent to Participate

Not applicable.

Patient Consent for Publication

The patient gave consent to publish the case details and associated images.

Acknowledgments

Not applicable

Competing Interests

The authors declare that they have no competing interests.

Availability of Data and Materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' Contributions

AC performed genetic consults and counseling; EC has seen and confirmed the raw data's authenticity; MSM interpreted the genetic test results. AFC and CP had clinical and imaging evaluations. All the authors have read and approved the final manuscript.

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Article

Hereditary Breast Cancer in Romania – Molecular Particularities and Genetic Counseling Challenges in an Eastern European Country

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Abstract: In Romania, breast cancer (BC) is the most common malignancy in women. However, there is limited data on the prevalence of predisposing germline mutations in the population in the era of precision medicine, where molecular testing has become an indispensable tool in cancer diagnosis, prognosis, and therapeutics. Therefore, we conducted a retrospective study to determine the prevalence, mutational spectrum, and histopathological prediction factors for hereditary breast cancer (HBC) in Romania. A cohort of 411 women diagnosed with BC selected upon NCCN v.1.2020 guidelines underwent an 84-gene NGS-based panel testing for breast cancer risk assessment during 2018–2022 in the Department of Oncogenetics of the Oncological Institute of Cluj-Napoca, Romania. A total of 135 (33%) patients presented pathogenic mutations in 19 genes. The prevalence of genetic variants was determined, and demographic and clinicopathological characteristics were analyzed. We observed differences among *BRCA* and non-*BRCA* carriers regarding family history of cancer, age of onset, and histopathological subtypes. Triple-negative (TN) tumors were more often *BRCA1* positive, unlike *BRCA2* positive tumors, which were more often the Luminal B subtype. The most frequent non-*BRCA* mutations were found in *CHEK2*, *ATM*, and *PALB2*, and several recurrent variants were identified for each gene. Unlike other European countries, germline testing for HBC is still limited due to the high costs and is not covered by the National Health System (NSH), thus leading to significant discrepancies related to the screening and prophylaxis of cancer.

Keywords: hereditary breast cancer; Romania; *BRCA*; non-*BRCA*



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1. Introduction

In Romania, 12,000 new BC cases are diagnosed annually, accounting for the second cause of cancer-related deaths after lung cancer [1]. Out of all diagnosed cases, 10% of BC are hereditary (HBC), the consequence of inherited or de novo predisposing mutations that define a group with increased malignancy risk compared to the general population. For the 11 million women in Romania, we do not have epidemiological data on the frequency of predisposing mutations in the general population. In addition, there are no national screening programs or reimbursed genetic testing. Nevertheless, more than 55% of cases are diagnosed in advanced clinical stages, with an overall survival rate below other European countries, defining a significant health concern [2]. *BRCA1* and *BRCA2* germline mutations

are responsible for about 60% of HBCs with an overall 60–80% lifetime risk; the other 40% are associated with other predisposing variants in moderate-to-high penetrance genes such as *PALB2*, *PTEN*, *TP53*, *CDH1*, *CHEK2*, *ATM*, and the MMR group [3,4]. Although *BRCA*-related HBCs are prevalent, current testing guidelines recommend panel testing to ensure extensive mutation spectrum coverage [5]. Finding the predisposing pathogenic variants is essential for identifying high-risk women in order for them to be further followed in intensive screening programs that allow an early diagnosis or even avoid the onset of the malignancy and provide proper genetic counseling for family members [6,7]. In addition, the current medical practice supports targeted molecular therapy with PARP inhibitors among women with advanced breast neoplasia and triple-negative histology who have *BRCA* germline mutations and precision treatment [8]. We assume that the incidence of HBC in Romania is the same as that reported in the Caucasian population; however, it is already well-known that there are differences regarding the distribution of pathogenic variants and associations with the malignant phenotype [9,10]. There is very little data related to the distribution and peculiarities of germline variants in women from Romania, as published data only refer to 500 patients [11–13].

2. Materials and Methods

The study was conducted in the Department of Oncogenetics of the Oncology Institute of Cluj-Napoca, Romania, between 2018 and 2022. It included patients with at least one NCCN v.2020 molecular testing criteria for HBC susceptibility genes (BC diagnosed before age 50, bilateral BC metachronous or synchronous BC, TNBC before age 60). Based on the initial genetic consult, signing the consent form, and individual financial possibilities, eligible patients were tested using extensive molecular panels in two certified NGS/MLPA laboratories (Invitae and Blueprint) on a panel of 84 high-, moderate-, and low-penetrance genes (*AIP*, *ALK*, *APC*, *ATM*, *AXIN2*, *BAP1*, *BARD1*, *BLM*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CASR*, *CDC73*, *CDH1*, *CDK4*, *CDKN1B*, *CDKN1C*, *CDKN2A*, *CEBPA*, *CHEK2*, *CTNNA1*, *DICER1*, *DIS3L2*, *EGFR*, *EPCAM*, *FH*, *FLCN*, *GATA2*, *GPC3*, *GREM1*, *HOXB13*, *HRAS*, *KIT*, *MAX*, *MEN1*, *MET*, *MITE*, *MLH1*, *MSH2*, *MSH3*MSH6*, *MUTYH*, *NBN*, *NF1*, *NF2*, *NTHL1*, *PALB2*, *PDGFRA*, *PHOX2B*, *PMS2*, *POLD1*, *POLE*, *POT1*, *PRKAR1A*, *PTCH1*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *RB1*, *RECQL4*, *RET*, *RUNX1*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *SMAD4*, *SMARCA4*, *SMARCB1*, *SMARCE1*, *STK11*, *SUFU*, *TERC*, *TERT*, *TMEM127*, *TP53*, *TSC1*, *TSC2*, *VHL*, *WRN*, *WT1*).

Genomic DNA obtained from peripheral blood samples was enriched for targeted regions using a hybridization-based protocol and sequenced using Illumina technology. All targeted regions were sequenced with $\geq 50\times$ depth and 20 bp of flanking intronic sequence. Reads were aligned to a reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. Promoters, untranslated regions, and other non-coding regions were not interrogated.

Only patients with pathogenic and potentially pathogenic mutations were included in the study, although 76 patients presented variants of uncertain significance (VUS) but were not included in the positive group statistical analysis. Data were organized using Microsoft Excel, part of the Microsoft Office 2019 suite (Microsoft Corp., Redmond, WA, USA). Data were then analyzed using R 4.2.2 (R Foundation) [14], RStudio (Posit Software, PBC, Boston, MA, USA) [15]. In addition, the following libraries were loaded in the workspace: *stringr* [16], *readxl* [17], and *GenVisR* [18]. To identify statistically significant differences in quantitative variables between groups, we used the Wilcoxon rank-sum (Mann–Whitney U) test. To identify statistically significant differences in frequencies of qualitative variables between groups, we used either the χ^2 test, or where its assumptions were violated, the Fisher test. Quantitative variables were expressed as mean (standard deviation), and qualitative variables were expressed as percentages.

3. Results

Of 624 women diagnosed with BC, 560 met at least one NCCN v.2020 molecular testing criteria, and 411 women underwent testing.

Among all 411 patients, 135 (32.8%) carried a pathogenic or likely pathogenic heterozygous germline mutation in 19 genes, including high-penetrant breast cancer genes like *BRCA1* (43–31.9%), *BRCA2* (19–14.1%), *PALB2* (12–8.9%), *TP53* (3–2.2%), and *CDH1* (2–1.5%), and moderate to low-penetrant genes *CHEK2* (25–18.5%), *ATM* (12–8.9%), MMR group *PMS2* (2–1.5%), *MSH3* (1–0.7%), *MSH6* (1–0.7%), *MLH1* (1–0.7%), *BARD1* (3–2.2%), *NF1* (3–2.2%), *MUTYH* (5–3.7%), *EGFR* (1–0.7%), *SDHB* (1–0.7%), *RAD50* (3–2.2%), *NBN* (2–1.5%), and *XRCC2* (2–1.5%). We identified 142 defects in all. Of these, 77 were different pathogenic variants, of which the most common defects were frameshift (46–32.4% of all detected defects) and missense (40–28.2%) variants, followed by nonsense (27–19%), deletion/insertion (21–14.8%), and intronic variants (8–5.6%). Seven patients in the group had two pathogenic mutations (Figures 1 and 2).

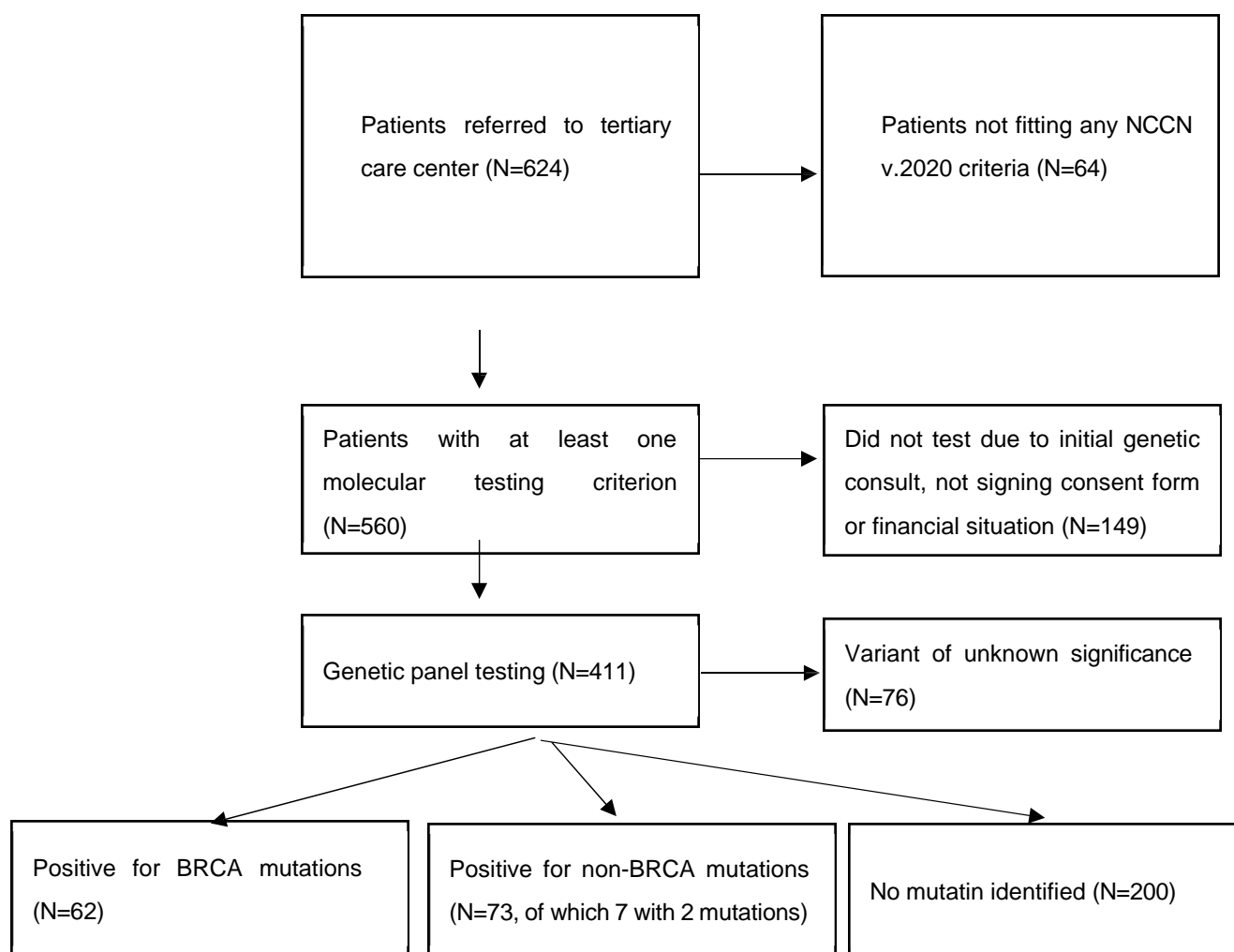


Figure 1. General characteristics of the cohort.

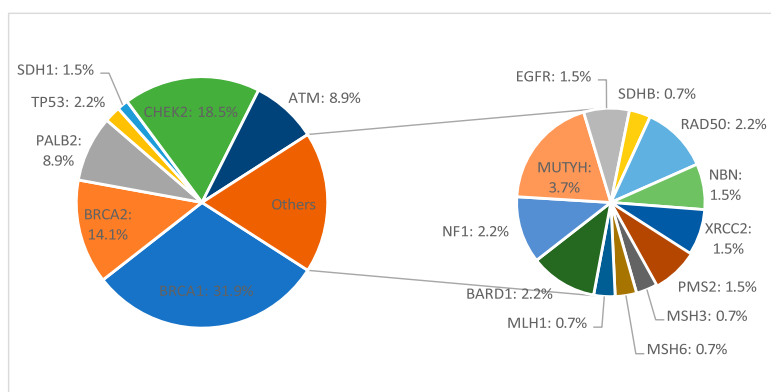


Figure 2. Mutation frequency in germline-positive patients with hereditary breast cancer.

Three recurrent variants (reported in more than three unrelated patients) c.3607C>T (p.Arg1203Ter), c.181T>G (p.Cys61Gly), and c.5266dupC (p.Gln1756Profs) accounted for (26) 60% of all reported variants were identified in the *BRCA1* gene. The c.172_175delTTGT (p.Gln60Argfs) frameshift variant was also recurrent in the *PALB2* gene, reported in (4) 33% of carriers, and c.1564_1565delGA(p.Glu522Ilefs) frameshift variant was found in (5) 41% of *ATM* carriers. Another recurring variant in the *CHEK2* gene, c.470T>C (p.Ile157Thr), was reported, accounting for a majority of (18) 72% of the pathogenic variants for this gene and 13% of all non-*BRCA* pathogenic variants in this cohort (Table 1).

Table 1. Pathogenic variants reported in breast cancer patients.

Gene	Mutation	Number of Patients
<i>BRCA1</i>	c.3607 C>T (p.Arg1203Ter)	12
	c.181T>G (p.Cys61Gly)	8
	c.5266dupC (p.Gln1756Profs)	6
	c.68_69delAG (p.GluValfs)	3
	c.1687C>T (p.Glu563Ter)	2
	c.843_846del (p.Ser282fs)	1
	c.212+1G>T	1
	c.4327C>T (p.Arg1443Ter)	1
	c.5030_5033delCTAA (p.Thr1677Ilefs)	1
	c.1018C>T (p.Arg340Ter)	1
	c.4065_4068del (p.Asn1355fs)	1
	c.3700_3704del (p.Val1234fs)	1
	c.737del (p.Asp245_Leu246isTer)	1
	c.5251C>T (p.Arg1751Ter)	1
	c.213-12A>G	1
	c.1636_1654del (p.Met546fs)	1
	c.211A>G (p.Arg71Gly)	1
<i>BRCA2</i>	c.9371A>T (p.Asn3124Ile)	3
	c.2808_2811del (p.Ala938Profs)	2
	c.5796_5797del (p.His1932fs)	2
	c.7878G>C (p.Trp2626Cys)	2
	c.2944A>C (p.Ile982Leu)	1
	c.7230delT (p.Phe2410Leufs)	1
	c.9253delA (p.Thr3085Glnfs)	1
	c.3545_3546delT (p.Phe1182Terfs)	1
	c.793+1G>A	1
	c.5576_5579del (p.Ile1859fs)	1
	c.3680_3681del (p.Leu1227fs)	1
	c.8680C>T (p.Gln2894Ter)	1
	c.729_732del (p.Asn243fs)	1
	c.5946del (p.Ser1982fs)	1

Table 1. Cont.

Gene	Mutation	Number of Patients
PALB2	c.172_175delTTGT (p.Gln60Agfs)	4
	c.2257C>T (p.Arg753Ter)	3
	c.93dupA (p.Leu32Thrfs)	1
	c.757_758del (p.Leu253fs)	1
	c.1037_1041del (p.Lys346fs)	1
	c.93dup (p.Leu32fs)	1
	c.1002C>A (p.Tyr334Ter)	1
TP53	C.586C>T (P.Arg196ter)	1
	c.1025G>C (p.Arg342Pro)	1
	c.916C>T (p.Arg306Ter)	1
SDH1	c.1531C>T (p.Gln511Ter)	2
ATM	c.1564_1565delGA (p.Glu522Ilefs)	5
	c.935dup (p.Leu312Phef*s6)	1
	c.6095G>A (p.Arg2032Lys)	1
	c.8585-2A>C	1
	c.5980A>T (p.Lys1994Ter)	1
	c.4768C>T (p.Leu1590Phe)	1
	c.5644C>T (p.Arg1882Ter)	1
	c.5932G>T (p.Glu1978Ter)	1
CHEK2	c.470T>C (p.Ile157Thr)	18
	c.902delT, p.(Leu301Trpfs*3)	3
	c.917G>C (p.Gly306Ala)	1
	c.1312G>T (p.Asp438Tyr)	1
	c.444+1G>A	1
	c.1100del (p.Thr367fs)	1
BARD1	c.1690C>T (p.Gln564Ter)	1
	c.632T>A (p.Leu211Ter)	1
	c.176_177del (p.Glu59fs)	1
RAD50	c.326_329del (p.Thr109fs)	3
PMS2	c.1239dup (p.Asp414fs)	1
	c.1076dupT(p.Leu359Phefs)	1
MSH3	c.2436-1G>A	1
MSH6	ex.1-6del	1
MLH1	c.544A>G (p.Arg182Gly)	1
MUTYH	c.650G>A (p.Arg217His)	3
	c.1187G>A (p.Gly396Asp)	1
	c.536A>G (p.Tyr179Cys)	1
NF1	ex.5 CNV	1
	ex.7del	1
	c.2410-1G>A	1
NBN	c.657_661del (p.Lys219fs)	2
SDHB	c.423+1G>A	1
XRCC2	c.190C>T (p.Arg64Ter)	2
EGFR	c.2061+2T>C	2

The mean age at the primary cancer diagnosis was 41.387 ± 8.084 for *BRCA* carriers; the mean age at primary cancer diagnosis was significantly higher both for non-*BRCA* mutation carriers (at 44.466 ± 7.02 , Wilcoxon rank sum $p = 0.007$) and for patients with no mutation (at 45.62 ± 7.367 , Wilcoxon rank sum $p < 0.001$).

The mean age at diagnosis for patients with a positive family history of cancer was 42.798 ± 7.054 versus 45.674 ± 7.607 for patients with no family history of cancer (Wilcoxon

rank sum $p = 0.002$). Among patients with no mutation, 24.5% had positive familial history. In contrast, among patients with any mutation, 49.2% had a positive familial history (54.2% among those with BRCA mutations and 45.2% among those with other types of mutation). There were statistically significant differences between patients with no mutations and both those with BRCA mutations ($\text{Chi}^2 p < 0.001$) and those with non-BRCA mutations ($\text{Chi}^2 p < 0.001$). At the same time, there were no differences among BRCA and non-BRCA carriers ($\text{Chi}^2 p = 0.302$). We did not have information about the family history of 3 patients with BRCA mutations.

The most common tumor histology in the cohort was invasive ductal carcinoma, found in 312 of all tested patients (75.9%), followed by invasive lobular carcinoma, 53 (12.9%), in-situ carcinoma, 30 (7.3%), and other rare histologies, 16 (3.9%). As for the germline-positive patients, the most common tumor histology was invasive ductal carcinoma 102 (75.5%), followed by invasive lobular carcinoma 13 (9.6%) and other rare histologies 11 (8.1%). In situ, non-invasive histologies were identified in 9 (6.7%) of diagnosed cases. There were statistically significant differences in the prevalence of tumoral histology types across the mutation types (Fisher exact test $p = 0.018$). Rare histologies had a lower prevalence in patients with no mutation (1%) than in patients with mutations, while lobular histology was much rarer among patients with BRCA mutations than the others. An interesting finding was that the indication for genetic testing for 42% of patients without familial history of cancer was the TN histology revealing a BRCA1 mutation. A total of 38% of Luminal A cancer patients with an onset <45 years of age and no familial history of cancer revealed BRCA2 or non-BRCA germline variants (Table 2).

Table 2. Histopathological and molecular characteristics for the cohort of 411 patients. † = 3 patients with BRCA mutations lacked information regarding family history of breast cancer and were excluded from analysis.

Variable	BRCA Mutation (N = 62)	Non-BRCA Mutation (N = 73)	TN (N = 76)	No Mutation (N = 200)
Diagnosis age	41.387 ± 8.084	44.466 ± 7.02	44.7	45.62 ± 7.367
Positive family history of cancer	32 (54.2%) †	33 (45.2%)	32 (42.1%)	49 (24.5%)
Histology				
Ductal (312–75.9%)	50 (80.6%)	52 (71.2%)	48 (63.1%)	162 (81%)
In situ 30 (30–7.3%)	4 (6.5%)	5 (6.8%)	11 (14.4%)	10 (5%)
Lobular (53–12.9%)	4 (6.5%)	9 (12.3%)	14 (18.4%)	26 (13%)
Others (16–3.9%)	4 (6.5%)	7 (9.6%)	3 (4.1%)	2 (1%)
Molecular subtype				
Luminal A (72–17.5%)	10 (16.1%)	15 (20.5%)	28 (36.8%)	19 (9.5%)
Luminal B (285–69.3%)	28 (45.2%)	49 (67.1%)	42 (55.2%)	166 (83%)
TN (54–13.1%)	24 (38.7%)	9 (12.3%)	6 (7.9%)	15 (7.5%)

The most common molecular subtype for germline-positive patients was Luminal B (77–57%), followed by triple negative (33–24.4%) and Luminal A (25–18%) subtypes. Luminal B histology was predominant in patients with no mutation compared to mutation carriers ($\text{Chi}^2 p < 0.001$, OR = 3.68 CI 95% 2.23–6.08). TN histology was predominant

among BRCA mutations, compared to the other groups ($\text{Chi}^2 p < 0.001$, OR = 6.71 CI 95% 3.57–12.65). The distributions of the molecular types in breast cancer patients with the BRCA and non-BRCA mutations differed significantly depending on the gene involved. The Luminal A subtype was prevalent in tumors positive for moderate-to-low penetrance mutations. The *BRCA1*-associated cancers were significantly more often TN than tumors harboring other mutations ($\text{Chi}^2 p < 0.001$, OR = 9.69 CI 95% 4.83–19.45). Luminal B subtypes, particularly Luminal B HER2-positive subtypes, were reported more frequently in *BRCA2* tumors but with no statistical significance compared to the other *BRCA1* and non-*BRCA* tumors ($p = 0.158$). The Luminal A subtype was more frequently associated with CHEK2-positive tumors than other mutations ($p = 0.013$).

The mean Ki67 index value was 45.194 ± 23.717 for *BRCA* carriers, significantly higher than for non-*BRCA* mutation carriers (35.808 ± 20.427 , Wilcoxon rank sum $p = 0.016$) but not compared to the mean value of Ki67 index in patients with no mutations (42.175 ± 19.681 , Wilcoxon rank sum $p = 0.366$) (Figure 3).

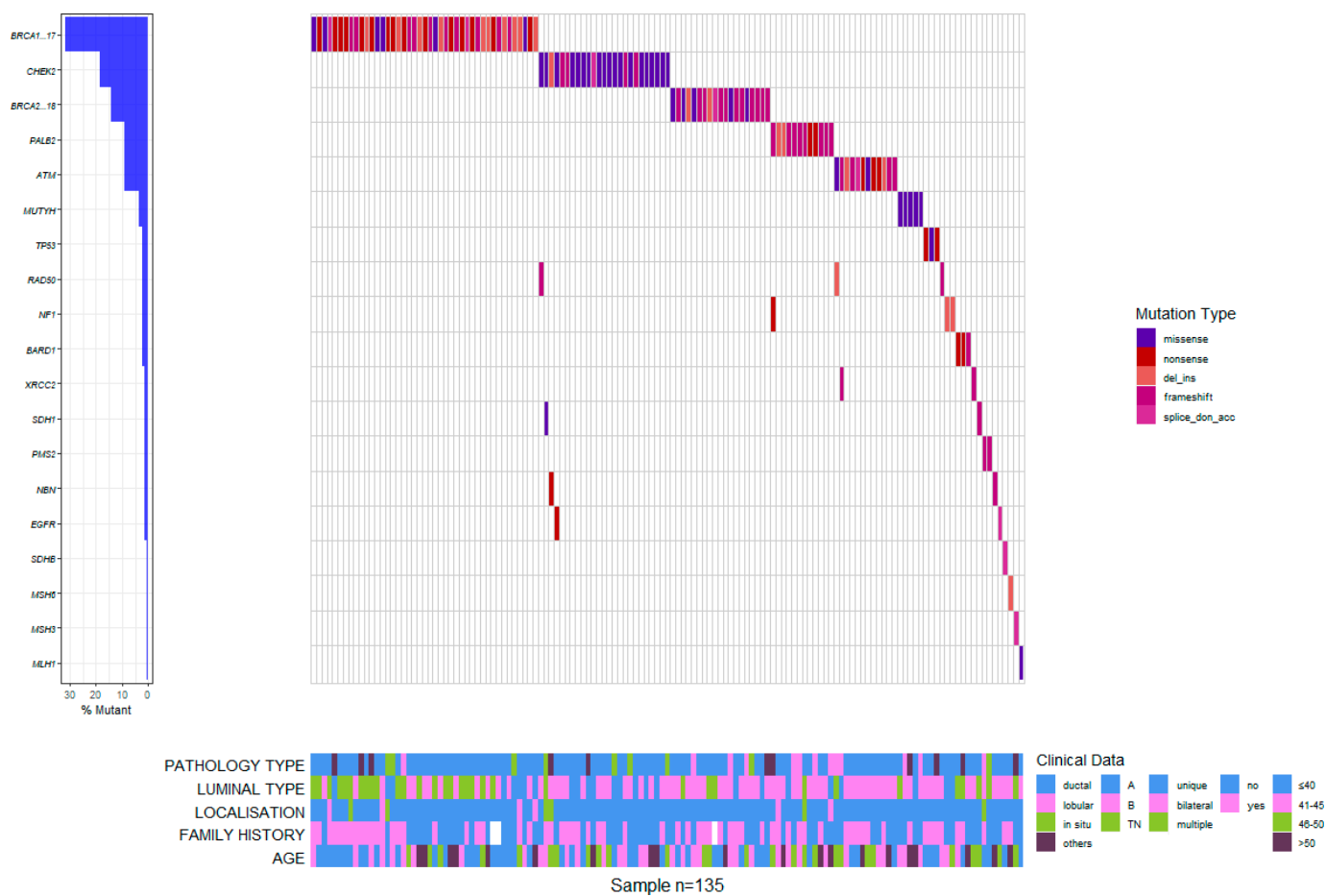


Figure 3. Waterfall plot of mutation profiles and clinical data for the Romanian cohort.

4. Discussion

4.1. Mutation Prevalence

In countries such as Romania, Bulgaria, Ukraine, Malta, Albania, Serbia, Bosnia, and Herzegovina, Macedonia, and Montenegro, surveys on *BRCA1* and *BRCA2* mutations have yet to be conducted, and no data are available. From Croatia, no conclusive data about the founder *BRCA1/2* mutation pattern is available, since only some individual mutations and benign variants were reported in one study [19].

It is complicated to estimate the prevalence of pathogenic mutations in the general population, considering that until now, there have only been two studies published about

the Romanian population. One, published in 2022, studying 250 women with breast cancer and 240 with ovarian cancer who underwent germline molecular testing for the detection of pathogenic *BRCA1* and *BRCA2* mutations, revealed that the most common variants identified were 5266delC, followed by 4218delG and c.68_69delAG for *BRCA1*, and c.9371A>T and c.1528G>T for *BRCA2* [11]. The other study conducted on 130 breast cancer patients tested by multigene panel analysis (*BRCA1*, *BRCA2*, *TP53*, *STK11*, *CDH1*, *PTEN*, *PALB2*, *CHEK2*, *ATM*), highlighted *BRCA1* c.3607C>T as the most common variant in the group, prevalent in triple-negative invasive carcinomas [12].

As expected, the most common mutations in our group were found in *BRCA1* and *BRCA2* genes, exceeding half of the reported mutations in this study. Frameshift and missense mutations leading to a complete or partial loss of tumor suppressor effect in *BRCA* genes were the most common defects. The c.3607C>T, c.181T>G missense variants, and c.5266dupC and c.68_69delAG(p.GluValfs) frameshift variant were recurrent in *BRCA1* carriers. The c.181T>G is one of the most common causes of breast and ovarian cancer in patients with Eastern European and Polish heritage and in Sicily [20–22], and therefore expected to be a common variant in our study. An older study in a Nord-Eastern region of Romania revealed that the *BRCA1* 5382insC mutation was not observed in any of the 120 breast and 50 ovarian cancer patients, contradictory when compared to reported data for the Romanian and Eastern European populations [23].

Interestingly, only one *BRCA2* mutation was found in 3 patients, the c.9371A>T (p.Asn3124Ile) missense variant, a founder defect in the eastern European population.

Only three other studies in the same demographics account for 320 patients with pathogenic mutations related to breast cancer. One study including 107 patients diagnosed with breast or ovarian cancer revealed that the c.5266dupC Ashkenazi founder mutation was the most common *BRCA1* pathogenic variant reported in 36.67% of cases, closely followed by c.3607C>T in 30% of cases. Only one *BRCA2* variant, c.9371A>T, was recurrent in this study [11]. Another smaller study, including 44 breast cancer patients revealed the same pattern in the *BRCA1* mutational prevalence; *BRCA1* c.5266dupC and c.3607C>T, *BRCA2* c.9371A>T were also prevalent in this group [13]. The previous study, including 56 patients, revealed a higher prevalence for *BRCA1* c.3607C>T variant than c.5266dupC and a recurrent *BRCA2* mutation, c.8755-1G>A [12].

Mutations in the *PALB2* gene, a high-penetrant gene associated with a 40–60% lifetime risk of BC, were reported as the third most common high-risk gene in this study. One particular variant, c.172_175delTTGT (p.Gln60Argfs), was recurrent in *PALB2* carriers and was reported in other studies to be associated with hereditary breast and pancreatic cancer [24,25]. There are no available data on *PALB2* mutation prevalence in the Romanian population. Only one study included East European and, thus, Romanian cancer-diagnosed patients, but with no detailed description of *PALB2* mutation [26]. With a frequency ranging from 0.5 to 1.0%, the truncating *PALB2* variant c.172_175delTTGT has recently been discussed in Central and Eastern Europe [27], Poland, Belarus, Germany, and Russia [28–30]. Considering the aforementioned, c.172_175delTTGT could be considered a *PALB2* founder mutation for the Romanian population. Another mutation, the c.2257C>T (p.Arg753Ter), also seems to be recurrent for the *PALB2* gene and has previously been reported in Poland and the Eastern-European population [31].

Germline testing for breast cancer should also include the *PALB2* gene, considering the frequency of pathogenic defects in the general population and because *PALB2* heterozygotes should be considered for the same therapeutic regimens and clinical trials as those for *BRCA1* and *BRCA2* carriers.

ATM is a moderately penetrant gene associated with a 20–40% risk for breast cancer. The recurrent Slavic founder mutation c.1564_1565delGA (p.Glu522Ilefs) frameshift variant was found in 41% of *ATM* carriers and previously reported in the Romanian population [14].

Consistent with currently available data, pathogenic variants in *CHEK2* were the most frequently identified after pathogenic variants in *BRCA1* or *BRCA2* genes. Debates on the impact of *CHEK2* pathogenic mutations on breast cancer risk are ongoing, with

emerging data to classify pathogenic mutations of *CHEK2* in moderate to low-penetrant variants [32]. The c.470T>C (p.Ile157Thr) variant was recurrent in our cohort, with more than half of *CHEK2* mutated patients carrying this mutation. Compared to other *CHEK2* pathogenic variants, c.470T>C has an attenuated association with BC, was not associated with non-breast cancers [33,34], and is probably modulated by other genetic factors or non-genetic risk factors to increase BC risk. Along with hormone-related risk factors [35], it has been shown that a family history of BC correlates with higher risks for women with *CHEK2* pathogenic variants [36–38]. Surprisingly, the common c.1100del *CHEK2* variant was reported only in one patient. On the other hand, two out of three patients carrying the c.902delT *CHEK2* moderate-risk variant had other germline pathogenic mutations in the *BRCA1* gene.

One unexpected finding in our study is related to the *MUTYH* gene. The association between *MUTYH* mutations and HBC risk is controversial, as there is a higher level of evidence that carriers homozygous for *MUTYH* pathogenic variants have an increased risk of BC [39]. However, a higher frequency of heterozygous *MUTYH* mutations in families with breast and colorectal cancer has also been reported compared to the general population [40,41]. We identified the c.650G>A recurrent heterozygous mutation in three patients in our cohort. Two patients had invasive lobular carcinoma and intestinal polyps, and the third had a parent diagnosed with colon cancer. Among other variants, the c.650G>A mutation is reported in ClinVar to be associated with invasive Luminal B breast carcinoma [42].

4.2. Molecular Subtypes Associations

Invasive carcinoma of the breast is considered a heterogeneous group of malignant epithelial tumors. Current data describes a wide range of morphological phenotypes and specific histopathological and molecular subtypes among sporadic and hereditary types but with significant differences between genotypes of germline mutations-associated tumors [43,44]. Most *BRCA1*-associated breast cancers are invasive ductal carcinomas of non-special type and fall into the “basal-like” intrinsic molecular subtype [45]. These triple-negative (TN) tumors lack estrogen, progesterone receptors, and human epidermal growth factor receptor 2 expressions. *BRCA2*-associated breast cancers are more likely to be found in the “luminal type” and share common characteristics with sporadic tumors [46]. Luminal A tumors have $Ki67 \leq 14\%$ and lack HER2 protein overexpression. Luminal B tumors have higher $Ki67$ values and are HER2-positive [47]. In our study, basal histology was a common finding in *BRCA1* patients, and luminal types were more commonly identified in *BRCA2* and non-*BRCA* carriers. Ductal in situ carcinoma (DISC) was a rare histological finding in our cohort as most diagnosed cases were symptomatic and therefore T2/T3, N/M > 1 at diagnosis. Less than 5% of cases were diagnosed as DISC, all part of annual screening due to a positive family history of breast cancer.

$Ki-67$ proliferative marker is considered an essential prognostic in breast cancer. It has a significantly higher expression in *BRCA*-positive breast cancer [48,49]. It implies its potential as an efficient prognostic factor in *BRCA*-positive breast cancer and future therapeutic implications in the context of emerging data suggest it might be advantageous to promote, rather than hinder, cell proliferation for immunotherapy to be optimally effective [50,51].

4.3. Cohort Particularities

We consider that our study group is representative of Romania since the women enrolled came from different geographic areas and ethnicities (multidimensional scaling analysis supported the genetic similarity of the Wallachia, Moldavia, and Dobruja groups with the Balkans, while the Transylvanian population was closely related to Central European groups) [52].

An expected finding was that we had a higher mutation prevalence than other studies. Most patients addressing genetic counseling services had at least two or three NCCN-based

testing criteria. We noticed that most patients had at least two or more NCCN-based eligibility criteria for genetic testing. This particularity implicitly associates a higher positivity rate than other groups where patients were tested with only one criterion. The over-selection is mainly due to inappropriate screening and testing guidelines in hereditary cancers. Among the factors contributing to the underutilization of genetic testing services in Romanians, we mention lower awareness of testing among patients and medical staff and support for obtaining genetic counseling and testing, particularly in resource-limited settings representing 60% of the general population. Genetic counseling, testing, and after-testing discussions are often described as complicated and inaccessible for many women in Romania. Paradoxically, approximately 40% of patients with eligibility testing criteria did not have the genetic test because of objective financial impediments. Genetic testing in Romania must be supported by the government or reimbursed by health insurance to avoid significant discrepancies between socio-economic groups.

Recurrent mutations in our study were c.3607C>T, c.181T>G, c.5266dupC in the *BRCA1* gene, c.9371A>T in the *BRCA2* gene, c.172_175delTTGT in the *PALB2* gene, c.1564_1565delGA in the *ATM* gene, c.470T>C in *CHEK2* gene, and c.650G>A in the *MUTYH* gene. For high-penetrant genes such as *BRCA1*, *BRCA2*, *PALB2*, and moderate-high penetrant genes such as *ATM*, recurrent mutation frequency converges to already published data for Eastern European populations. We observed a high frequency for *CHEK2* variants, the most frequent moderate-risk breast cancer predisposition gene. Despite contradictory data on the c.407T>C pathogenicity, this variant may have more than a polygenic role model in breast carcinogenesis and deserves further large-data analysis. An unexpected finding in our cohort was the presence of heterozygous *MUTYH* pathogenic variants, among which c.650G>A was recurrent and already in the attention of various investigators for the association with invasive breast carcinomas [41].

We also evaluated the attitude regarding surgical prophylaxis among women with a high risk of bilateralization. If most patients with pathogenic mutations in genes with increased penetrance opted for prophylactic mastectomy in favor of conservative imaging methods, paradoxically, more patients with pathogenic mutations in genes with low or moderate penetrance requested genetic or surgical consult to perform radical surgical prophylaxis. In this regard, prophylactic mastectomy recommendations were always preceded by the Tumour Board assessment and psychological counseling to avoid unnecessary interventions.

Genetic counseling has been challenging due to insufficient genetic screening programs and medical education. For example, an extensive pre-pandemic report revealed that even in developed countries, 50% of women diagnosed with breast cancer do not receive genetic counseling [53]. If genetic counseling was available without significant impediments for educated patients younger than 40 years of age, in older women or women with low education, we confronted issues related to understanding the information, advantages, and the medical use of genetic testing. In this case, more than one genetic counseling session or integration of another family member was necessary. Since most of our subjects were submitted to genetic testing at diagnosis, a critical timing in patients' medical management also focused on identifying subjects at higher risk of psychological distress to address them for psychological support and give them the appropriate coping strategy. We observed that a positive genetic test still creates a significant emotional stigma for the patient and other family members and therefore requires professional assistance to avoid further emotional distress.

5. Conclusions

The genetic characteristics of HBC in Romania are similar to those reported for the East Caucasian and Slavic populations. *BRCA* carriers and patients with a family history of cancer are diagnosed with breast cancer earlier than carriers of other mutations. TN tumors are associated with *BRCA1* mutations, while Luminal B subtype tumors are associated with *BRCA2* mutations. Further attention is recommended for moderate-penetrant genes

like *CHEK2* to determine the role of pathogenic variants in assessing BC predisposition. Romania, part of the EU, requires the implementation of genetic screening programs and proper genetic counseling services to reduce the number of women with hereditary genetic components and late diagnosis.

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Data Availability Statement: Data is available on request to the corresponding author.

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REVIEW

Molecular basis and therapeutic targets in prostate cancer: A comprehensive review

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Prostate cancer is one of the most significant causes of morbidity and mortality in male patients. The incidence increases with age, and it is higher among African Americans. The occurrence of prostate cancer is associated with many risk factors, including genetic and hereditary predisposition. The most common genetic syndromes associated with prostate cancer risk are *BRCA*-associated hereditary breast and ovarian cancer (HBOC) and Lynch syndrome. Local-regional therapy, i.e., surgery is beneficial in early-stage prostate cancer management. Advanced and metastatic prostate cancers require systemic therapies, including hormonal inhibition, chemotherapy, and targeted agents. Most prostate cancers can be treated by targeting the androgen-receptor pathway and decreasing androgen production or binding to androgen receptors (AR). Castration-resistant prostate cancer (CRPC) usually involves the PI3K/AKT/mTOR pathway and requires targeted therapy. Specific molecular therapy can target mutated cell lines in which DNA defect repair is altered, caused by mutations of *BRCA2*, partner and localizer of *BRCA2* (*PALB2*), and phosphatase and tensin homolog (*PTEN*) or the transmembrane protease serine 2-*ERG* (*TMPRSS2-ERG*) fusion. Most benefits were demonstrated in cyclin-dependent-kinase 12 (*CDK12*) mutated cell lines when treated with anti-programmed cell death protein 1 (PD1) therapy. Therapies targeting *p53* and AKT are the subject of ongoing clinical trials. Many genetic defects are listed as diagnostic, prognostic, and clinically actionable markers in prostate cancer. AR splice variant 7 (AR-V7) is an important oncogenic driver and an early diagnostic and prognostic marker, as well as a therapeutic target in hormone-resistant CRPC. This review summarizes the pathophysiological mechanisms and available targeted therapies for prostate cancer.

Keywords: Prostate cancer, pathways, genetic syndromes, targeted therapy.

Introduction

Prostate cancer is the second most frequently diagnosed cancer in males, after the lung cancer, according to statistics. Furthermore, regarding mortality rates, prostate cancer seems to be the fifth cause of cancer-related deaths in males worldwide [1]. The incidence of prostate cancer appears to be correlated with older age. The average age at which males are diagnosed is 66 years. Race is also a critical consideration, as African Americans have a higher incidence of prostate cancer, while Asian males have a lower incidence than Caucasians. African Americans also have a much higher mortality rate associated with prostate cancer. Genetic factors and lifestyle might explain the differences. Dietary factors, body mass index (BMI), and physical activity must also be considered when determining the risk of developing prostate cancer [2]. Malignant tumors of the prostate can often be asymptomatic, making early diagnosis more difficult. More common symptoms are difficulty urinating or emptying the bladder, urgent urination, bloody urine, and nocturia. Sometimes it can present with lower back pain due to bone

metastasis [3]. Hereditary prostate cancer is suspected if there is a family history of prostate cancer in at least three generations, either in the maternal or paternal lineage, if at least three first-degree relatives have been diagnosed with prostate cancer, if at least two relatives were diagnosed before the age of 55, or even if there are documented cases of other cancers in the family, such as breast, ovarian, or pancreatic cancer. Genetic testing is performed through next-generation sequencing (NGS), which allows whole genome or whole exome sequencing and thus finds the various genes or nucleotides which may present genetic alterations. Identifying germinal mutations can also play a part in choosing the correct treatment protocol for the patients and offering proper genetic counseling [4].

Risk factors

Several risk factors are known to be associated with prostate cancer, some of which are either somatic or germline genetic mutations. Age is considered a major risk factor, as most

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prostate cancers occur after the age of 65, while diagnosis before the age of 40 is rare. Ethnicity and lifestyle also play a role in prostate cancer risk assessment. A family history of prostate cancer also increases the risk of developing malignant prostate tumors. For example, it seems that having brothers diagnosed with prostate cancer represents a higher risk than having a father who has had a disease. Obesity, smoking, exposure to toxic chemicals, vasectomies, prostatitis, and sexually transmitted infections are also considered risk factors, as studies demonstrated their correlation to higher morbidity and mortality rates [5]. Increased BMI is linked to high-grade prostate cancer and overall mortality. Obesity and metabolic syndrome induce dysregulation of the insulin axis, promote inflammatory cytokine signaling, and result in DNA-damaging, oxidative stress, and subsequent carcinogenic effect [6].

A significant proportion of prostate cancer susceptibility has been attributed to an inherited predisposition. Approximately 15%–20% of all cases occur in a hereditary, familial context and include high to moderate penetrant genes also reported as a genetic risk factor for other cancers: breast cancer gene 1 (*BRCA1*), *BRCA2*, ataxia telangiectasia mutated (*ATM*), checkpoint kinase 2 (*CHEK2*), partner and localizer of *BRCA2* (*PALB2*), mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), *MSH6*, *PMS2*. *BRCA1* and *BRCA2* genes are mainly discussed for hereditary breast and ovarian cancers, although there is a higher risk for other malignancies like colon cancer, melanoma, thyroid cancer, and prostate cancer [7]. Germline mutations in *BRCA*, especially the *BRCA2* gene, are a well-known genetic risk factor for developing malignant prostate tumors. *BRCA1* carriers have a 1.8-fold to 3.8-fold increased relative risk (RR) of diagnosis by the age of 65. The risk is even higher for *BRCA2* carriers, from 2.5-fold to 8.6-fold by the age of 65, more frequently with a younger onset, more aggressive phenotype, and higher mortality rates.

Lynch syndrome is also considered a risk factor for several cancer types, including prostate cancer. Lynch syndrome appears due to genetic alterations of DNA mismatch repair genes, leading to genetic material defects and a higher risk for tumor development. While this syndrome is primarily associated with gastrointestinal cancers, it also plays a part in prostate cancer. Epithelial cellular adhesion molecule gene (*EPCAM*) mutations lead to increased methylation of the *MSH2* promoter, eventually leading to *MSH2* protein loss, thus resembling characteristics of Lynch syndrome and defective DNA mismatch repair [8]. The *HOX* genes play an essential role in the embryologic development of different tissues. Mutations in the *HOXB13* gene have been associated with highly increased risk of prostate cancer, although they are responsible for only a small fraction of prostate cancer cases worldwide [9]. Studies showed a correlation between *CHEK2* missense mutations and a higher risk for prostate cancer, but familial clustering had not been demonstrated. Due to its role in encoding a G2 checkpoint kinase, the *CHEK2* gene plays a crucial role in DNA repair [10]. Similarly to *BRCA2* mutations, *NBS1* gene mutations affect tumor suppression. In addition, *NBS1* mutations have been linked to immunodeficiency, chromosomal instability,

and predisposition to developing cancers. Prostate cancers in which *NBS1* mutation is identified tend to be more aggressive and have a poor prognosis even when conventional therapy is administered [11].

Several somatic mutations have been linked to prostate cancer, usually indicating a higher risk of developing metastatic disease and potential resistance to treatment. Mutations in the *PIK3CA* oncogene, in which the function is associated with cell differentiation and migration, have been described in multiple human cancers, including prostate cancer. Other somatic mutations, such as those occurring in *KIT*, *BRAF*, or *TP53* genes, were correlated with cell overproliferation, more advanced stages of the disease, and a poor prognosis. While tumor cells with *BRAF* mutations may be candidates for *BRAF* inhibitors, and those with *KIT* mutations seem to respond to tyrosine kinase inhibitors, *TP53* mutated cells exhibit resistance to multiple therapies, including cisplatin, alkylating agents, antimetabolites, or anthracyclins [12–14].

A limited role in developing hereditary prostate cancer was attributed to zinc phosphodiesterase ELAC protein 2/histone promoter control protein 2 (*ELAC2/HPC2*) gene mutations. In addition, from the identified genetic variants, the Glu216Stop nonsense mutation seemed responsible for some familial prostate cancer cases [15]. Studies were performed to determine if macrophage scavenger receptor 1 (*MSR1*) mutations can be linked to hereditary prostate cancer. However, these mutations appear to have moderate penetrance and are detected in a limited number of cases, therefore, the correlation between hereditary prostate cancer and *MSR1* mutations is not currently significant [16]. RNase L gene (*RNASEL*) plays a role in cancer prevention by degrading RNA, determining cellular stress response and apoptosis. Genetic mutations in this gene lead to defective apoptosis and an increased risk of developing malignant tumors. In prostate cancer, *RNASEL* mutations are associated with younger age of onset and more aggressive course of the disease [17]. Loss of retinoblastoma 1 (*RB1*) is also an essential factor in prostate cancer. Together with *TP53*, it increases cell proliferation and decreases androgen receptor (AR) signaling. Tumors that present combined loss of *RB1* and *TP53* are very aggressive, do not respond to AR antagonist treatment and are associated with a limited survival rate. Therefore, targeted molecular therapy must be considered in these cases [18]. Genetic alterations in the *ATM* gene follow a similar mechanism to those linked with *BRCA2*, *BRCA1*, or *CHEK2* mutations. In addition, higher tumor progression rates were described in patients with *ATM* mutations and increased sensitivity to ionizing radiation as a risk factor for tumorigenesis [19]. *PALB2* mutations were associated with more aggressive forms of prostate cancer and a higher mortality rate [20]. *CDK12* inactivation, a DNA damage response gene, leads to aggressive prostate cancers that do not respond to treatment with hormone therapy, taxanes, or poly ADP-ribose polymerase inhibitors (PARPi). However, immunotherapy with programmed cell death protein 1 (PD-1) inhibitors seems effective for these patients and represents a viable option for their therapeutical management [21].

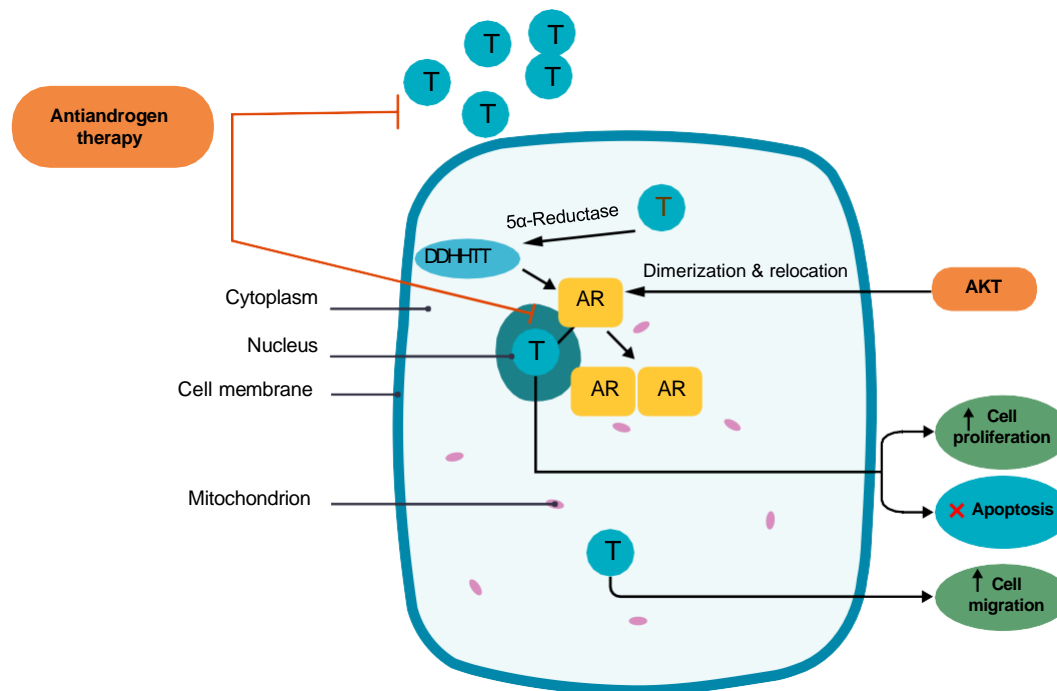


Figure 1. Androgen receptor pathway [24]. DHT: Dihydrotestosterone; T: Testosterone; AR: Androgen receptor.

Single nucleotide polymorphisms (SNPs) are reported to have a lower to moderate effect on prostate cancer progression compared to combinations of SNPs, leading to more severe progression patterns. There are more than 100 common SNPs described in association with prostate cancer. These genetic alterations involve multiple mechanisms leading to tumor progressions, such as oxidative stress or impaired steroid metabolism, defective DNA repair, increased angiogenesis, and cell adhesion. In addition, SNPs can determine a higher risk of relapsing in prostate cancer patients treated with androgen deprivation therapy. Correctly identifying SNPs, alongside somatic and germline gene mutations, and knowing the pathophysiological mechanism they imply, represent the future of precision medicine and individualized targeted treatment options [22].

Pathophysiology of prostate cancer

While metabolism alterations are not considered a cause of malignant lesions, they promote tumor cell survival and proliferation. Typically, cells rely on aerobic pathways to convert metabolites into the energy they need, but tumor cells use anaerobic pathways. Thus, glycolysis is less efficient, but ATP is produced faster. Increased glucose intake leads to increased production of endogenous fatty acids, which is not usually observed in healthy cells. Most tumor cells express higher levels of Acetyl-CoA carboxylase, fatty acid synthase (FASN), and lipogenic enzymes. Tumors with increased FASN levels lead to poor prognosis. Treating cancer cells with cerulenin, which inhibits FASN, showed promising effects, as it inhibited cell proliferation and even induced cell death [23].

The AR pathway is the most frequently responsible for tumorigenesis and tumor growth in prostate cancers. Testosterone and 5α-dihydrotestosterone are crucial in tumor development and progression. When entering the nucleus, sex steroids bind to promoter regions of genes, regulate cell proliferation, and provide the means to escape apoptosis. Sex steroids are also involved in cell cycle control and migration by binding to molecules outside the nucleus. The AKT serine/threonine kinase plays a role in the dimerization and relocation of AR on the nucleus membrane (Figure 1). Approximately 80%–90% of malignant tumors in the prostate depend on androgens for their development, which is why anti-androgen therapy represents an essential pillar in prostate cancer management. Some prostate cancers, called castration-resistant prostate cancers (CRPC), will not respond to androgen ablation therapy. These tumor cells seem to have an intrinsic androgens production [24].

AR splice variant 7 (AR-V7) genetic alterations affect the *AR* gene located on the X chromosome and produce splice-variant ARs. Although results are debatable, recent studies have shown more aggressive growth patterns and decreased overall survival in patients with AR-V7 mutations. Correlations have also been demonstrated between AR-V7 mutations, abiraterone, and enzalutamide resistance. Thus, the AR-V7 mutation status can be a prognostic marker for metastatic CRPC [25]. Non-coding RNAs have essential regulatory roles in different molecular pathway levels, such as transcription and translation. They are involved in malignant cell proliferation, decreased apoptosis, metastasis formation, and drug resistance. In prostate cancer, they regulate AR activity. Because they are characterized by high stability in biological fluids, non-coding RNAs may be good

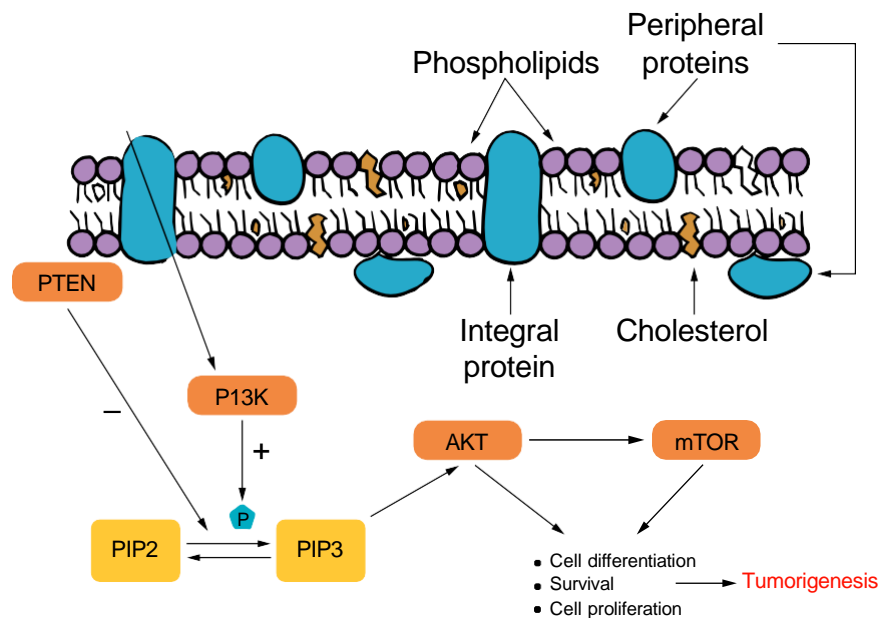


Figure 2. PI3K pathway in tumorigenesis [28, 29]. PTEN: Phosphatase and tensin homolog; PI3K: Phosphatidylinositol 3-kinase; mTOR: Mechanistic target of rapamycin; PIP3: Phosphatidylinositol-3,4,5-triphosphate.

candidates for markers in liquid biopsies. Targeting them as a therapeutic approach could inhibit AR expression and the disease progression [26, 27].

Phosphatidylinositol 3-kinase (PI3K) signaling is responsible for most cases in which prostate cancers are castration-resistant. The PI3K pathways are associated with more aggressive tumor growth and poor outcome. This pathway plays a part in obtaining phosphatidylinositol-3,4,5-triphosphate (PIP3) and AKT activation. PIP3 formation is inhibited by phosphatase and tensin homolog (PTEN). This step is strictly linked to PTEN's binding to the plasma membrane. Out of the advanced cases of prostate cancer, 70%–100% show aberrations in PI3K/AKT/mTOR signaling pathways. Loss of PTEN, typically membrane-localized, leads to overactivation of PI3K pathway and, therefore, tumorigenesis in the prostate glandular tissue. Inhibiting AKT signaling shows promising results in stopping tumor formation and growth and can result in tumor shrinkage. The PI3K pathway is widely involved in castration-resistant tumor pathophysiology and interacts with androgen signaling (Figure 2) [28, 29].

Autophagy is a complex process affecting cancer cells, as it promotes both cell death in the early stages of tumor development and cancer-cell proliferation once the mass is formed. There are three main types of autophagy: chaperone-mediated autophagy (CMA), microautophagy, and the primary form, macroautophagy. The last one plays a significant role in cellular homeostasis [30]. The autophagy mechanism has different phases from initiation to elongation and, finally, fusion with the lysosome, implying that various molecular pathways are involved. 5'AMP adenosine monophosphate-activated protein kinase (AMPK), mechanistic target of rapamycin (mTOR), and ATGs are the most well-known regulators of autophagy [31]. The mechanism has yet to be entirely understood, but a

particular role is attributed to the PI3K/AKT/mTOR signaling pathway. PTEN and AMPK downregulate the pathway and promote autophagy. Some data showed that autophagy could be associated with chemotherapy resistance of prostate cancer cells. Further studies are needed to determine whether autophagy modulators can represent a therapeutic option for prostate cancer and how to use this complex process for the patient's benefit [32].

Transmembrane serine protease 2 (TMPRSS2) is a transmembrane protein primarily found in prostate's secretory epithelium, prostate cancer cells, and in pancreatic and colon cancer specimens. *TMPRSS2-ERG* gene fusions can diagnose prostate cancer on a molecular level. Tumors that express *TMPRSS2-ERG* gene fusions are considered fusion-positive prostate cancers. These tumors present genetic alterations with oncogene overexpression and inactivation of tumor suppressor genes. The ERG protein has been proven to have a role in regulating the AR pathway. Research shows that ERG overexpression is more significant in the peripheral zone of the prostate (Figure 3). *TMPRSS2-ERG* fusions are responsible for checkpoint impairment in the cell cycle and for tumor proliferation, but also tumor cell migration and invasiveness by overexpression on metalloproteinase 9 (MMP9) and plexin A2. The gene fusion affects chromosome 21 and seems most frequent in the Indian and Caucasian populations [33, 34].

Iron homeostasis also plays an essential role in prostate cancer. Increasing transferrin receptor protein 1 (TfR1) levels and progressive ferroportin downregulation have been associated with transforming primary tumors to metastatic cancer in prostate cancer [35]. While physiologic iron levels favor the proliferation of prostate cancer cells, excess iron causes deleterious reactive oxygen species (ROS) to increase, subsequently activating cell death programs. Interestingly, tumor

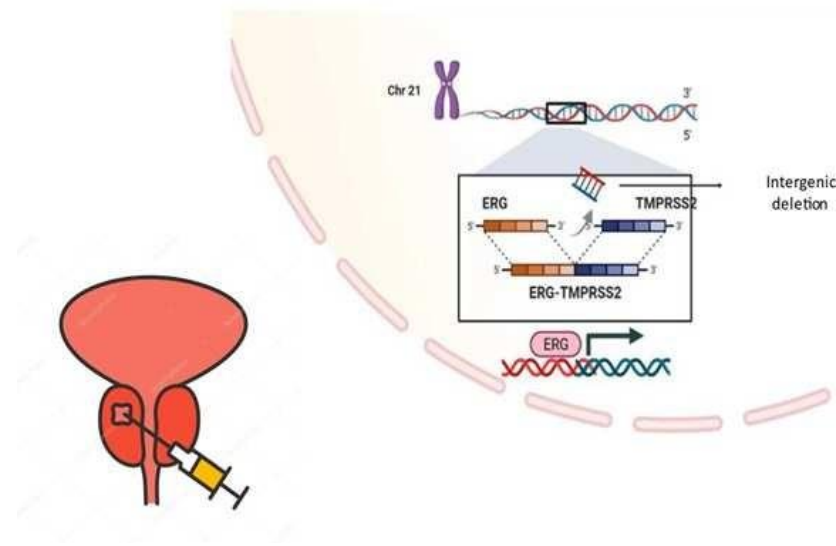


Figure 3. Role of *TMPRSS2-ERG* gene fusion in prostate cancer [33, 34]. *TMPRSS2*: Transmembrane protease serine 2.

microenvironment (TME)-associated macrophages loaded with iron seem to have a pro-inflammatory effect on tumor cells [36], and propose iron supplementation as a potential targeted treatment for prostate cancer [37].

More recent studies showed that viruses linked to respiratory infections, including SARS-CoV-2, need the *TMPRSS2-ERG* complex to enter cells, and it has been discussed if prostate cancer patients are more exposed to these infections due to *TMPRSS2-ERG* overexpression. However, gene fusion represents an essential diagnostic tool and a potential therapy target [38, 39].

As the most critical TME component, cancer-associated fibroblasts (CAFs) influence tumorigenesis and impact metastasizing and therapeutic resistance for prostate cancer. CAFs might be used as a prognostic factor. CAFs are prostate cancer's major cellular stromal component and promote cancer progression through lactate metabolism. The alteration of NAD⁺/NADH ratio through lactate uptake, further sirtuin 1 (SIRT1)-dependent progastricin (PGC)-1 α activation, and subsequent excessive mitochondria activity results in oncometabolite superoxide accumulation [40]. Further studies are yet needed to determine to what extent therapy can target CAFs to stop tumor growth and extension [41].

Long non-coding RNAs could play an essential role in prostate cancer pathogenesis [42]. Dysregulation of long non-coding RNA leads to either a tumor suppressor or oncogenic effect in cancerous cells and influences tumor proliferation and metastasis. For example, growth arrest specific 5 (*GAS5*), a potent tumor inhibitor, interferes with AKT/mTOR signaling pathway by targeting microRNA mir-103. Maternally expressed 3 (*MEG3*), another tumor-inhibiting factor, inhibits the sponge miR-9-5p cycle and induces gene silencing. On the other hand, tumor growth promoters like prostate cancer-associated transcript 1 (PCAT1), PVT1, and urothelial cancer associated 1 (UCA1) act as transcriptional repressors, a mir-145-5P sponge, and a P13K/AKT pathway activator [43].

NK3 homeobox 1 (NKX3.1) protein is normally expressed in healthy prostate cells. In mice, NKX3.1 has growth-suppressing and differentiation-promoting effects, but its role as a tumor suppressor could not be demonstrated in human prostate cancers. With disease severity, NKX3.1 levels are decreasing. Complete loss can be documented in most metastatic prostate cancers. This phenomenon might be explained by the NKX3.1 protein being expressed only by normal cells of the prostate. The most critical role of the protein is currently as a prognostic marker. However, it might be a helpful tool for developing targeted therapies that are tissue-specific to treat the malignant tumors of the prostate and prevent as many side effects as possible (Figure 4) [44].

Malignant prostate tumors are biochemically characterized by much-decreased citrate and zinc levels compared to unaffected prostate tissue. This fact might be explained by the inability of malignant cells to accumulate and deposit zinc due to partial or total loss of the genes which encode the zinc uptake transporter. Restoring zinc levels can be an essential prophylactic or even therapeutic measure in the approach to prostate cancer. Usually, zinc levels are high, especially in the peripheral zone of the prostate. Since zinc is vital for citrate production, the citrate levels will also be low. This biochemical manifestation often occurs in the premalignant stages before the development of malignant tumors. Studies did not show that dietary zinc supplements help prevent prostate cancers [45].

Screening and prevention

Correctly implemented screening protocols are an important tool in cancer management, as they can help identify cancers early when patients can benefit the most from the treatment. Nevertheless, as the psychological impact of a cancer diagnosis is significant, it is essential to choose wisely the screening methods and their target groups. Regarding prostate cancer, the issue is delicate, as not all prostate cancers require treatment [46].

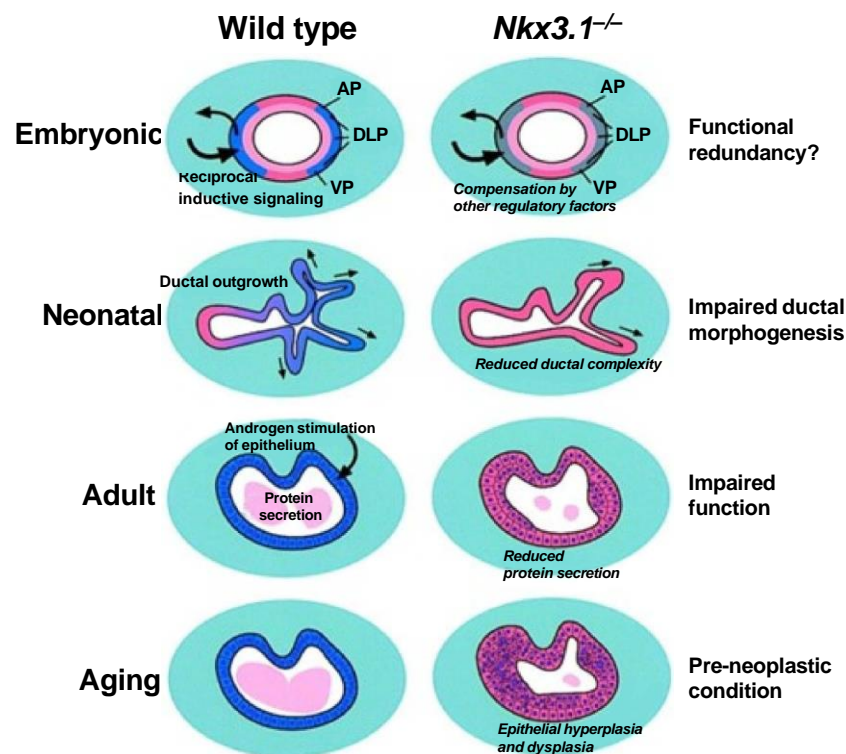


Figure 4. Effects of NKX3.1 loss in tissue development and tumorigenesis [44]. NKX3.1: NK3 homeobox 1.

The currently used screening method consists of prostate-specific antigen (PSA) level testing. Magnetic resonance imaging (MRI) testing is not feasible for the whole population of male patients who might be at risk. In addition, the most significant limitation of PSA testing is that it cannot discriminate between aggressive cancers and clinically indolent tumors. It is still discussed if men who are not considered at higher risk should undergo screening for prostate cancer, but it is established that those with a life expectancy lower than 10 years do not benefit from PSA-level screening [47].

Screening is recommended for people with a family history of prostate cancers, African Americans, and those with documented germline mutations in the *BRCA2* gene, starting at 40–45 years of age, depending on each guideline. *BRCA1* mutations are starting to be considered as an indication for screening at a younger age. However, more genetic alterations should be included in the guidelines in the near future to offer proper care for these patients [48].

Although studies were conducted on prostate cancer prevention, there were not any clear guidelines about active steps that can be taken to prevent it from occurring. However, some studies showed that besides having a healthy, balanced lifestyle and avoiding toxic exposure, specific diets and vitamin supplements can either increase or decrease the risk of developing prostate cancer. A slight increase in risk has been described for people whose diets include lots of dairy foods and calcium. Vitamin E and folic acid seem also to increase the risk of developing prostate cancer. Opposite to folic acid, folate has been listed as a protective factor against malignant prostate tumors [49].

As most prostate cancers rely on androgens for tumor growth and proliferation, drugs that lower testosterone levels, such as finasteride and dutasteride, lower the risk of developing malignant prostate tumors. However, a medication that lowers testosterone and thus 5α -dihydrotestosterone causes side effects that affect the patient's quality of life, such as lower libido, gynecomastia, or erectile dysfunction. Therefore, considering all the facts, administering the medication as a primary prophylactic measure is not part of the clinical practice. Furthermore, it remains unclear if finasteride and dutasteride also lower mortality if the patients develop prostate cancers [50].

Diagnostic strategies

If high PSA or prostate cancer antigen 3 gene (PCA3), levels are found during the screening for prostate cancer, or if the clinical rectal examination raises any suspicions, a core needle biopsy is recommended as a diagnostic method. The biopsy can be either transrectal or transperineal and samples should be collected from different prostate parts. The procedure is guided by transrectal ultrasound (TRUS) or MRI. The standard systematic diagnostic biopsy of the prostate using TRUS is slowly being replaced by MRI-based techniques. In-bore MRI-guided biopsy provides high-quality enhanced images for detection and biopsy guidance but requires MR-compatible facilities and limited access [51]. Cognitive TRUS-targeted biopsy requires a two-step procedure with the initial MRI localization of the tumor followed by an ultrasound-guided biopsy. Multiparametric MRI and fusion-guided biopsy have demonstrated clinical

benefits over systematic biopsy alone, leading to improved diagnosis, risk stratification, and treatment [52].

The urologist and morphopathologist are important in the multidisciplinary team, ensuring correct patient management [53]. For a complete diagnosis, calculating the Gleason score is necessary. Based on the cellular atypia grade described during the morphological examination, a score is assigned to the two most dominant cell types, from one to five, in which one represents little or no cellular atypia and five highly atypical prostate cells. The sum of the two established grades represents the Gleason score. A score above seven associates a high risk for cancer development and aggressive spreading. Thus, the Gleason score also indicates the prognosis of the patient [54]. If prostate cancer is diagnosed, it is essential to determine whether metastasis exists. Potential metastasis most commonly affects the lymph nodes, bones, lungs, and liver. It can be diagnosed using CT scans, MRIs, or PET scans. Prostate-specific membrane antigen (PSMA)-PET scan can also detect prostate cancer cells in case of biochemical recurrence, using the radioactive properties of PSMA [55].

Liquid biopsy is a powerful, minimally invasive tool to detect clinically actionable molecular targets. Still, there are many challenges to overcome until liquid biopsies are implemented as routine analysis in screening and early diagnosis of prostate cancer. The multi-cancer early detection test (GRAIL) study based on Circulating Cell-free Genome Atlas (CCGA) [56] validated a pan-cancer methylation targeted-based assay, among others, in prostate cancer early diagnosis. The test showed an excellent overall sensitivity of early detection, however, detecting prostate cancer using the multi-cancer assay was lower and similar to other screening programs.

Treatment

There are several treatment options for prostate cancer, but not all require aggressive approaches, so choosing the proper management is essential. In addition, it must be considered whether the patient is symptomatic, how advanced the cancer is, the life expectancy, and the quality of life of the patient, and whether the patient has important comorbidities [57]. Prostate cancer treatment can cause adverse effects, such as incontinence or erectile dysfunction, which affect the patient's quality of life. Therefore, it needs careful consideration, as sometimes the risks outweigh the benefits of being treated [58]. For tumors found in the early stages, which show a slow progression, watchful waiting is an option for patients whose life expectancy is less than five years. Active surveillance is recommended for low-risk cancers which have not been spread beyond the prostate and have a Gleason score of up to six. They will undergo periodic PSA-level testing, digital rectal examinations, and biopsies at specific intervals [59].

Local treatment options include surgical approaches, focal therapies, and radiotherapy. Surgical removal of the prostate can be done either laparoscopically or in open surgery. Open retropubic or perineal radical prostatectomy was commonly used until laparoscopic and robotic-assisted radical prostatectomies became routine surgeries for prostate cancer-diagnosed

patients. Transurethral resection of the prostate (TURP) is also a viable option for symptom management but not for treating cancer, as it removes prostate tissue to relieve some urinary manifestations, such as nocturia and infections. It is therefore reserved for adenomas [60].

Interestingly, despite the type of procedure done, the risk and severity of adverse effects of a prostatectomy seem to remain the same, with urinary incontinence and sexual dysfunction affecting the quality of life [61]. Focal therapies include cryoablation and high-intensity focused ultrasound (HIFU), which can remove small, low-risk tumors from the prostate but are still a topic of several studies, to determine whether they are as effective as surgery or radiation therapy [62]. Radiation treatment can be external-beam radiation therapy or brachytherapy, in which the radioactive source is implanted into the prostate, affecting a smaller amount of healthy tissue. Proton therapy has not been proven to be a more viable option for prostate cancer treatment. Radiotherapy can cause urinary and bowel dysfunctions and sexual dysfunction [39, 40].

Systemic treatment can be administered individually or as part of a treatment plan, including surgery or radiation therapy. One of the essential systemic treatments is hormone therapy, which aims to lower sex hormone levels and thus block the AR pathway. Most prostate cancers are androgen-dependent, so low androgen levels should significantly slow tumor growth [63]. Castration can be surgical by performing bilateral orchiectomy and thus stopping androgen production in the testicles, or chemical, by administering a medication that blocks the AR pathway. Chemical castration is often reversible [64]. Luteinizing hormone-releasing hormone (LHRH) agonists and antagonists effectively reduce androgen production in the testicles. Androgen production in the adrenal gland or even some prostate cells can be blocked with androgen synthesis inhibitors, such as ketoconazole or abiraterone acetate, which is a CYP17A1 inhibitor and affects testosterone production from progesterone. However, ketoconazole is no longer widely used due to its many drug interactions [42]. AR inhibitors, such as enzalutamide, block androgens binding to the corresponding receptors and therefore prevent them from exerting their effects [43]. Hormone therapies have several significant side effects which affect sexual function, cause weight gain, gynecomastia, cardiovascular involvement, or even depression and memory impairment [42]. An important issue for patients treated with hormone therapy is bone health. In many cases, they can develop osteopenia or osteoporosis. Bone-modifying drugs, such as zoledronic acid, alendronate, or risedronate, can be administered to those at high risk of developing lower bone density. These drugs do not seem to prevent bone metastasis of prostate cancers, however, they have a role in preventing complications related to bone health in patients who already have metastatic tumors in the bones [65]. Around 10%–20% of prostate cancers develop mechanisms that help them progress despite low testosterone levels, including CRPCs [39]. Bipolar androgen therapy (BAT) is a new treatment for men whose prostate cancer has become resistant to standard hormone-blocking therapy [66] and involves administering high levels of testosterone. The mechanism of BAT is

based on excessive testosterone ability to induce DNA damage, activate cell stress pathways, stimulate immune-activating proteins, and even deregulate the activity of growth-promoting genes. Recent clinical studies revealed that BAT could be safely administered to asymptomatic patients with metastatic CRP, inhibits disease progression, produces sustained PSA in 30%–40% of patients, and resensitizes and prolongs response to subsequent antiandrogen therapy [67].

Chemotherapy is used to treat advanced, metastatic prostate cancers. Unfortunately, the well-known side effects seriously affect the patient's quality of life. Standard chemotherapy regimens start with docetaxel, a taxane that inhibits microtubular depolymerization. Taxanes are the only chemotherapeutic agents with demonstrated benefits on the survival of patients with metastatic CRPCs [68]. Although platinum-based chemotherapy is not widely used in prostate cancers, studies showed that patients with mutations that alter DNA damage repairs, such as defective mismatch repair in Lynch syndrome or defects caused by *BRCA2* mutations, respond well to platinum-based cytostatic drugs. However, multiple studies are needed to determine if platinum-based chemotherapy should be a standard approach in prostate cancer treatment for patients with specific genetic findings [69]. Most patients present with prostate adenocarcinoma, an androgen-driven luminal type tumor typically associated with elevated PSA and are sensitive to AR inhibition therapy. However, poorly differentiated neuroendocrine carcinomas (NEPC) similar to small-cell carcinoma histology are more aggressive and require platinum-based chemotherapy using small-cell-lung carcinoma (SCLC) regimens [70]. About 20% of patients treated with hormonal therapies develop small-cell carcinomas as a resistance mechanism (t-NEPC) and coexist with adenocarcinoma as a heterogeneous mixed tumor. It is currently updated in the National Comprehensive Cancer Network (NCCN) guidelines to include consideration of metastatic biopsy and subsequent *RB1* and *TP53* somatic analysis [71].

Using iron as a complementary treatment measure for antiandrogenic therapy seems to increase the efficacy of prostate cancer cell death. Iron was proven toxic for cancer cells, causing oxidative stress and protein damage [72].

Current treatment options for bone metastases which occur in more than 60% of patients with advanced prostate cancer are palliative and not curative, therefore, the search for potential therapeutics is stringent, but still challenging [73]. Bisphosphonate and denosumab reduce bone fragility and delay metastases progression. Tyrosine kinase inhibitors like cabozantinib (XL184) and dasatinib have also been investigated in bone metastasized prostate cancer with promising outcomes in clinical trials. Atrasentan, an endothelin-A receptor (ETAR) antagonist, lowers the level of PSA and the incidence of bone pain in metastatic patients [74].

Novel treatment options in oncology target-specific disease progression mechanisms, offering both better results and limited impact on healthy cells. Genetic testing is essential in finding the appropriate targeted therapy based on each patient's specific cancer and molecular characteristics [75]. Anti-PD1 antibody therapy appears to be effective in a limited

number of prostate cancers in which defective mismatch repair and microsatellite instability have been demonstrated, but the results are not consistently promising. However, it is notable that *CDK12* mutated cell lines showed favorable responses to anti-PD1 therapy [76]. Patients with metastatic CRPCs, who do not respond to AR inhibitors and androgen synthesis inhibitors and have DNA repair defects, are candidates for targeted therapy with PARP inhibitors. Currently used PARP inhibitors are olaparib or rucaparib, which are effective in particular prostate cancer cases [77]. The role of PARPi is demonstrated especially in treating cancer cells with defective homologous recombination, which are susceptible to the alteration of the base excision repair pathway. The most important example of such cell lines is *BRCA*-mutated cancer cells. *TMPRSS2-ERG* fusion, *PALB2* mutations, and *PTEN* loss also make the cell lines good candidates for PARPi therapy. On the other hand, *ATM*, *CHEK2*, and *CDK12* mutations were associated with lower response to PARP inhibitor treatment [78].

Immunotherapy has not been of great importance in the treatment of prostate cancer and has not significantly improved clinical outcomes. Immunotherapy with sipuleucel-T, an active cell-based autologous immunotherapy, has been attempted for metastatic prostate cancer. Although it does not lead to tumor shrinkage or lower PSA levels, it prolongs patients' life by up to four months in advanced diseases. Immunotherapy in prostate cancer can be used only if patients are asymptomatic. However, its benefits could not be clearly stated. Although showing principal activity, sipuleucel-T is no longer used in routine practice [79]. Vaccination is not yet available in clinical practice. PROSTVAC, a PSA recombinant vaccinia vector, showed promising activity in metastatic CRPC patients [80].

PSMA levels are much increased in cancerous cells compared to healthy prostate tissue. PSMA represents an important diagnostic tool and can also be the target of a humanized monoclonal antibody, J591, for metastatic prostate cancer patients. It remains to be studied how to correctly select the target groups which qualify for this novel treatment. PSMA-antibody-drug conjugates and PSMA-based chimeric antigen receptor-therapy (CAR)-T is the subject of several studies which target PSMA as a treatment option for CRPC [81]. PSMA BiTE is a bispecific CD3 and PSMA antibody construct, which re-directs and activates T-cells to PSMA-expressing cells already used in treating other malignancies and showing promising results in prostate cancer [82]. In clinical trials, three other bispecific T-cell engagers, glypican-1 and disintegrin and metalloproteinase 17 (ADAM 17) and six-transmembrane epithelial antigen of the prostate 1 (STEAP-1) are under evaluation [83–85].

TP53 inactivation plays an important role in second-generation anti-androgens drug resistance and neuroendocrine differentiation, therefore, tumor suppressor reactivation by blocking the interaction of MDM2-p53 is a promising target in metastatic CRPC [86]. Idasanutlin is currently the subject of several ongoing clinical trials. It targets *p53* altered cell lines, which in case of prostate cancer, together with alterations in *RB1*, leads to castration-resistant, aggressive tumors with a poor prognosis. However, available studies referred to hematological

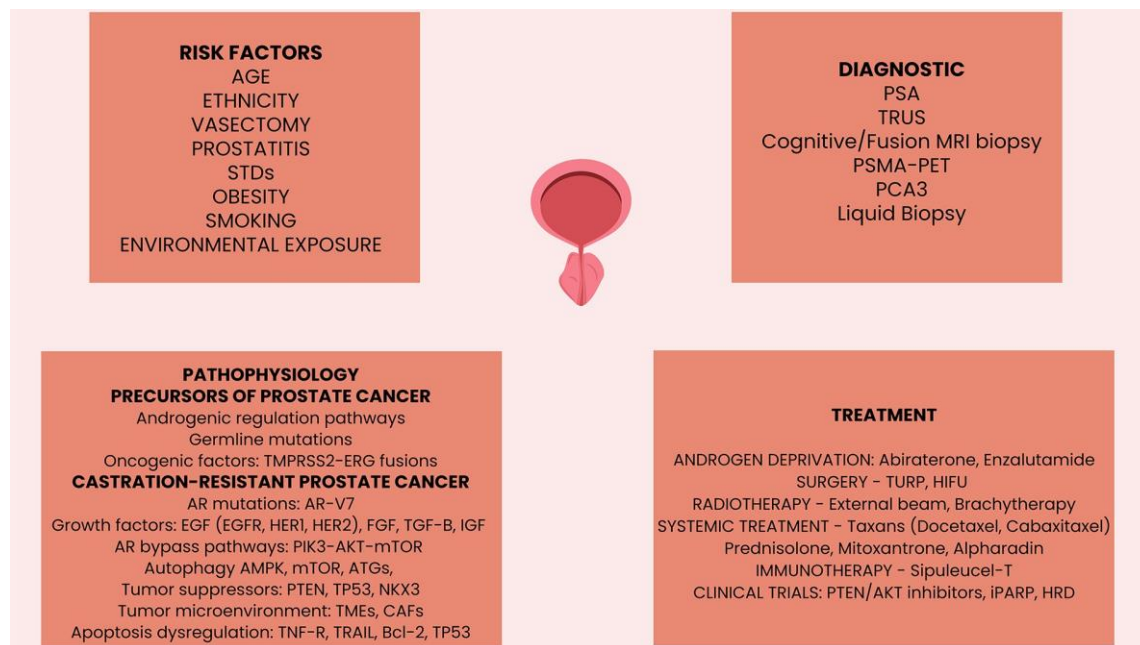


Figure 5. Graphical abstract. AR: Androgen receptor; AR-V7: Androgen receptor splice variant 7; AMPK: 5'AMP adenosine monophosphate-activated protein kinase; ATGs: Autophagy-related genes; Bcl-2: B-cell lymphoma; CAFs: Cancer-associated fibroblasts; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; HER: Human epidermal growth factor receptor; HIFU: High intensity focused ultrasound; IGF: Insulin growth factor; MRI: Magnetic resonance imaging; NKX3-1: NK3 Homeobox 1; iPARP: Poly (ADP-ribose) polymerase inhibitors; PCA3: Prostate cancer antigen 3; PIK3-AKT-mTOR: Phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway; PTEN: Phosphatase and tensin homolog; PSA: Prostate specific antigen; TRUS: Transrectal ultrasound scan; PSMA-PET: Prostatic specific membrane antigen-positron emission tomography; TMPRSS2-ERG: Transmembrane protease serine 2 v-ets to erythroblastosis virus E26 fusion; TGF- β : Transforming growth factor beta; TMEs: Tumor microenvironment immune elements; TNF-R: Tumor necrosis factor receptor; TP53: Tumor suppressor protein 53; TRAIL: Tumor necrosis factor-ligand apoptosis-inducing ligand; TURP: Transurethral resection of the prostate.

malignancies, and clinical trials specific to prostate cancers are needed in the future [87].

As *AKT* is a crucial component of the PI3K pathway, PI3K inhibitors demonstrated only partial responses in some early-phase clinical trials [7]. AKT inhibitors that reach the clinical trial phase can be either ATP-competitive inhibitors, such as ipatasertib or capivasertib, or allosteric inhibitors, such as perifosine or MK-2206. Ipatasertib showed some prolonging of the progression-free interval in some phase 2 trials. The combination of docetaxel and capivasertib is the subject of ongoing clinical trials. Ipavasertib and abiraterone, administered in combination, resulted in significantly better progression-free survival, demonstrated radiologically [88]. However, several studies are needed before their potential inclusion in clinical practice as treatment options for prostate cancers [89].

Genetic counseling

Given the importance of genetic testing in the clinical management of several pathologies, genetic counseling has a rising role in patient care. For example, in case of prostate cancer, it aims to assess hereditary cancer risk, recommend genetic testing for the patients that could benefit from it, and explain all the possible outcomes of undergoing genetic testing so that the patient can make a fully informed decision. Furthermore, once the genetic test is performed, medical geneticists should interpret the results with the patient and explain what they imply for

the patient and his family members, who might also need further investigations to assess their hereditary cancer risk [90]. The results of any genetic testing must be interpreted, considering the personal and family history previously discussed with the patient. Genetic testing might guide the treatment plan for prostate cancer, as some mutations make the patient a good candidate for targeted therapy, thus offering better outcomes and minimizing the side effects of the treatment [91]. Some of the obtained results might require further investigations or research, and the patient must be informed about these possibilities before the testing. Such results can be variants of uncertain significance (VUS) or even negative results in patients with a significant family history of cancers [43, 92]. The psychological impact of being diagnosed with cancer is significant, with psychological support being a crucial part of patient care. Hereditary cancers imply an even more significant impact, as family members might also be affected [93]. Germline mutations in *BRC1* and *BRC2* genes were reported in about 15% of metastatic prostate cancers [94, 95]. Detecting germline mutations in prostate cancer is important from the perspective of oncological risk assessment, personalized therapy, and family genetic counseling. According to the NCCN guidelines (Version 1.2021) [96], germline molecular testing is recommended for men with any one of the following: family history of a relative with a germline mutation, metastatic prostate cancer, regional or node-positive disease, high-risk prostate cancer (defined by grade group, T staging, and PSA levels at

diagnosis), intraductal or cribriform histology, and Ashkenazi Jewish ancestry. All men carrying *BRCA1* or *BRCA2* mutations should start cancer screening at the age of 40 with yearly PSA and consider the same in men with *BRCA1*, *ATM*, *HOXB13*, and DNA MMR mutations. Also, men with metastatic CRPC and mutations in DNA repair genes, such as *BRCA1*, *BRCA2*, and *ATM* might indicate PARPi therapy. Prophylactic prostatectomy is not indicated for mutation carriers [97].

Conclusion

Prostate cancer significantly contributes to increased cancer-related mortality rates in men globally. Although most cases have an early diagnosis, the disease invariably evolves toward advanced disease. Periodic PSA-based screening remains the most commonly used screening method for early detection of prostate cancer, closely followed by diagnostic TRUS, MRI, and PSMA-positron emission tomography (PSMA-PET). Radical prostatectomy or ablative radiotherapy are curative approaches in localized cases. In relapsed cases, radiotherapy or androgen deprivation therapy combined with chemotherapy or novel androgen signaling-targeted agents are used to control the systemic disease evolution. CRPC is targeted with AR agents, chemotherapy, radionuclides, and PARPi. Despite therapies that have improved survival rates, metastatic prostate cancer remains incurable. Even if prostate cancer does not have molecular characteristics that would make it an ideal candidate, immunotherapy remains to be an engaging treatment option for prostate cancer (Figure 5).

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Case Report

A New Frameshift Mutation of *PTEN* Gene Associated with Cowden Syndrome – Case Report and Brief Review of the Literature

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Abstract: Cowden syndrome (CS) is a rare disease that was first described in 1963 and later included in the large group of genodermatoses. It is the most common syndrome among the *PTEN*-associated hamartomatous tumor syndromes (PHTS). CS has an autosomal dominant inheritance pattern, with increased penetrance and variable expressivity, making early diagnosis difficult. Mutations in the *PTEN* gene (phosphatase and TENsin homolog) are involved in its pathogenesis, involving many organs and systems originating in the three embryonic layers (ectodermum, endodermum, and mesodermum). The consequence is the development of hamartomatous lesions in various organs (brain, intestines, thyroid, oropharyngeal cavity, colon, rectum, etc.). Multiple intestinal polyps are common in patients with CS, being identified in over 95% of patients undergoing colonoscopy. The authors describe the case of a patient who presented the first signs of the disease at 3 1/2 years (tonsil polyp) but was diagnosed only at the age of 20 following a colonoscopy that revealed hundreds of intestinal polyps, suggesting further molecular testing. A heterozygous frameshift mutation was identified in the *PTEN* gene, classified as a potentially pathogenic variant (c.762del.p(Val255*)). The authors present this case to highlight the path taken by the patient from the first symptoms to the diagnosis and to emphasize the clinical aspects of this mutational variant that have still not been identified in other patients with this syndrome.

Keywords: Cowden syndrome; *PTEN* gene; intestinal polyps; hamartomas



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1. Introduction

Cowden syndrome (MIM/ =158350) is a monogenic disease with autosomal dominant transmission; it is part of the PHTS group (*PTEN*-associated hamartoma tumor syndrome), which also includes Bannayan–Riley–Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and *PTEN*-related Proteus-like syndrome [1]. In 1963, Lloyd and Denis described this condition and named it after their patient, Rachel Cowden. She presented a complex phenotype that included craniofacial dysmorphism (macrocephaly, mandibular hypoplasia, microstomia, ogival palatine vault), papillomatosis of the tongue and oropharynx, skeletal anomalies, multiple thyroid adenomas, and fibrous cysts in the breasts [2]. CS is the most common syndrome in this group, and it is usually diagnosed in adulthood; in

contrast to BRRS, which has an earlier onset, these patients frequently present symptoms from the autistic spectrum [3], one of the first signs that alarm and direct parents to a specialist medical assessment. The gene responsible for the occurrence of this syndrome is *PTEN*, which is located on the long arm of chromosome 10 (10q23) and was identified and located by Nelen in 1996 [4]. It is a gene with dual action; on the one hand, it acts as a lipid phosphatase, and on the other hand, it acts as a protein phosphatase [5]. The molecular diagnosis of pathogenic variants is critical, although gene mutations are not identified in over 20% of cases [6]. Although it has a dominant inheritance pattern, most cases are isolated. The syndrome has a prevalence of 1:200,000, predominantly reported in the Caucasian population (96%), with the sex ratio slightly favoring the female sex (women are affected in 60% of cases). Only 500 cases are currently reported in the literature [7]. Due to the variable expressivity, it is often diagnosed late. The specific signs of the syndrome are often categorized as belonging to other pathologies. The average age of diagnosis is 39 years [8]. Gastrointestinal involvement is common in CS, and the appearance of intestinal polyps is the pathognomonic sign; polyps are present in 85% of affected people and have various histologies: hamartomatous, inflammatory, adenomatous, hyperplastic, ganglioneuromatous [9].

In 1996, the group working for the International Consensus for CS established the major and minor criteria for diagnosing patients with CS [10]. Subsequently, the criteria underwent a critical revision, especially at the level of the major criteria. The US National Comprehensive Cancer Network later accepted this revision [11]. The diagnostic criteria are summarized in Table 1.

Table 1. Diagnostic criteria of CS (adapted after the International Cowden Consortium) [12].

Pathognomonic lesions:		
Mucocutaneous lesions:		
Facial trichilemmomas.		
Acral keratoses		
Papillomatous lesions		
Mucosal lesions		
Major criteria	Minor criteria	Observations
Breast cancer	Other thyroid lesions.	
Endometrial cancer	Mental retardation (IQ ≤ 75).	
Thyroid cancer (mainly follicular thyroid carcinoma)	Gastrointestinal hamartomas.	
Gastrointestinal hamartomas	Fibrocystic disease of the breast.	
Lhermitte–Duclos disease, adult	Lipomas and fibromas.	
Macrocephaly	Genitourinary tumors or malformations.	
For a person with a negative family history of CS, the following criteria must be taken into account:		
1. Pathognomonic mucocutaneous lesions: trichilemmoma or cutaneous facial papules and oral mucosal papillomatosis or oral mucosal papillomatosis and acral keratoses palmoplantar keratoses.		
2. Two major criteria.		
3. One major and three minor criteria.		
4. Four minor criteria.		
In the case of a person affected by CS, the diagnostic criteria for his relatives are:		
1. A pathognomonic mucocutaneous lesion.		
2. Any one major criterion with or without minor criteria.		
3. Two minor criteria.		
4. One of which must be macrocephaly or Lhermitte–Duclos disease.		

This article emphasizes the importance of the early diagnosis of Cowden syndrome to facilitate better monitoring of its clinical evolution and rapid therapeutic intervention. The case presented by the authors had a whole odyssey, from the first sign of the disease (the peritonsillar polyp) to the diagnosis almost 15 years later. At that time, he (the

patient) already had hundreds of intestinal polyps. The mutational variant identified in the presented case has not been reported in the specialized literature.

2. Materials and Methods

2.1. Case Report

The authors present the case of a 20-year-old patient based on the records of CRGM Bihor from April 2023; the patient was referred by a gastroenterology medical specialist for genetic advice. He is the second child in the family born from pregnancy with physiological evolution, spontaneous birth, cranial presentation at 40 W with a weight of 4600 g, and normal neonatal evolution. **Family history:** organized family, no consanguineous relationship. The paternal grandfather died of colon cancer at 74. The maternal grandmother had a thyroidectomy (2006) for multinodular goiter and a left nephrectomy. **Past medical history:** From the age of 3 1/2 years, repeated referrals to different medical specialists (all tumor-related) as follows: 2007 (3 1/2 years) a tonsillar papilloma surgically removed at the age of 4; 2012 at 9 years, plantar tumor (diagnosed as a plantar hemangioma) surgically excised; 2015 at 12 years of age, an adenomatous lesion of the left thyroid lobe was identified, and a left thyroid lobectomy was performed (the patients was also receiving dermatological care for juvenile acne); 2018 at 15 years old, an ultrasound identified a nodular lesion in the right thyroid lobe that is still under follow-up; 2023 at 20 years of age, the exacerbation of dyspeptic syndrome resulting in the patient becoming unresponsive to medication with digestive enzymes and antispasmodics, leading to gastroenterology consultation followed by a colonoscopy that revealed multiple polypomatous lesions with a suspected genetic-associated polyposis syndrome—a medical genetic assessment was recommended. Furthermore, in 2023, a dermatological consultation revealed a papular lesion on the forehead, with the clinical appearance of trichilemmoma on the forehead and facial acne; a biopsy of the papular formation was recommended.

2.2. Laboratory Investigations

Laboratory tests were focused on the assessment of thyroid hormones and hematological parameters.

2.3. Molecular Investigations

The total genomic DNA was extracted from the biological sample using a bead-based method. After the fluorometric assessment of DNA quantity, the qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Molecular tests were performed at Blueprint Genetics. The patient was tested using next-generation sequencing (NGS) with a multi-gene panel of 43 genes that were selected based on their associations (according to what has been reported in the medical literature) with Hereditary Gastrointestinal Cancer Panel Plus [13], which includes sequence analysis and copy number variation analysis of the following genes: *APC*, *ATM*, *AXIN2*, *BLM*, *BMPR1A**, *BRCA1**, *BRCA2*, *BUB1B*, *CDH1*, *CDKN2A*, *EPCAM*, *FANCC*, *GALNT12*, *GREM1*, *KIT*, *MEN1*, *MLH1*, *MLH3*, *MSH2*, *MSH3*, *MSH6*, *MUTYH*, *NF1**, *NTHL1*, *PALB2*, *PDGFRA#*, *PMS2**, *POLD1*, *POLE*, *PTEN**, *RHBDF2*, *RPS20*, *SDHB*, *SDHC*, *SDHD#*, *SMAD4*, *SMARCB1*, *STK11*, *TMEM127*, *TP53*, *TSC1*, *TSC2*, and *VHL*. **The sequencing library** was prepared by ligating sequencing adapters to both ends of the DNA fragments. Sequencing libraries were size-selected using a bead-based method to ensure optimal template size and subsequently amplified via polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using a hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and eliminating the presence of leftover primers and adapter–adapter dimers. Readily available sequencing libraries that passed the quality control stage were sequenced using Illumina’s sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). **Bioinformatics and quality control:** Burrows–Wheeler Aligner software was used for read alignment. The variant classification follows the modified

Blueprint Genetics Variant Classification Schemes from the ACMG guideline 2015. The patient's sample was subjected to thorough quality control measures, including contamination and sample mix-up assessments. Copy number variations (CNVs) were detected from the sequence analysis data using a proprietary bioinformatics pipeline. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data were adjusted to account for the effects of varying guanine and cytosine content. All available evidence of the identified variants was compared to the classification criteria. Sequence variants classified as pathogenic, likely pathogenic, and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a confirmed positive call. The molecular analysis revealed the heterozygous c.762del, p.(Val255*) mutation in the *PTEN* gene, a likely pathogenic frameshift mutation. Written consent was obtained in order to include case details and all images.

3. Results

3.1. Clinical Evaluation of the Patient

Phenotypical traits: height 177 cm (75th percentile), weight 97 kg (over 95th percentile); craniofacial dysmorphism (macrocephaly with a head circumference of 64 cm) (over 99th percentile), round face, multiple folliculitis lesions, a 2/3 mm scar affirmatively after the excision of a comedo and a papillomatous lesion (trichilemmoma) on the forehead; anterior thoracic, especially posterior, shows multiple pustular lesions—suprasternal keloid scar after surgical removal of the left thyroid lobe. (Figures 1 and 2).

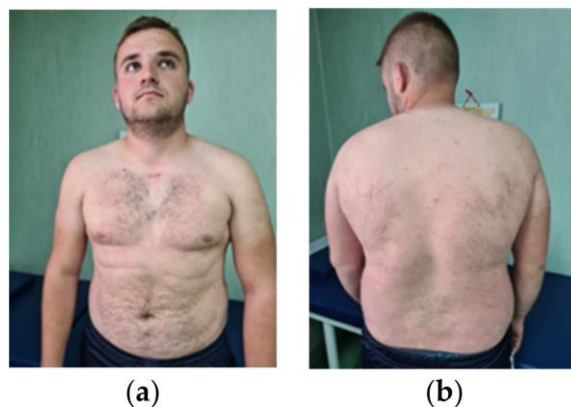


Figure 1. (a) Anterior and posterior chest shows multiple pustular lesions; (b) keloid scar after thyroid lobectomy; a gynoid pattern of subcutaneous cellular tissue.

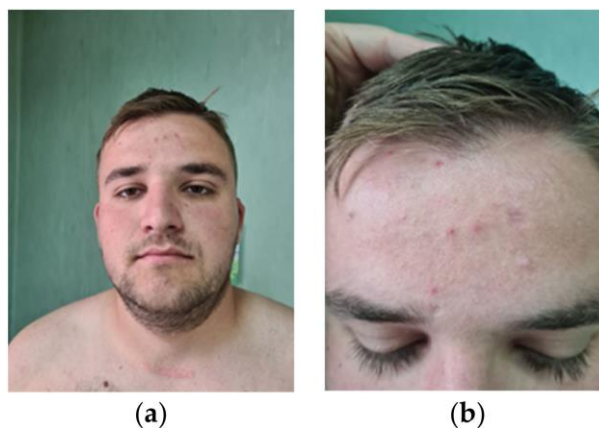


Figure 2. (a) Macrocephaly; round face. (b) Folliculitis lesions (approx. 2/3 mm scar after removal of a comedo and one skin-colored papillomatous lesion (trichilemmomas) underlying the scar).

3.2. Laboratory Investigations

Our laboratory analyses focused mainly on thyroid hormones. The TSH level was slightly increased in the year of diagnosis of the left thyroid follicular nodules, with a value of 4.1 mIU/L (reference values: 0.40–4.00 mIU/L). After the left thyroid lobectomy, the TSH and thyroid hormone levels remained normal.

3.3. Interdisciplinary Consultations

Table 2 summarizes the interdisciplinary consultations.

Table 2. Interdisciplinary management.

		Description	Recommendations
ENT (Ear-Nose-Throat)	2006 3 1/2 years	Tonsillar polyp, approx. 0.5/0.5 cm, surgically removed.	<i>Pathological anatomy:</i> tonsillar papilloma.
	2007 4 years	Tonsillar polyp significantly increased in size, approx. 4/5 cm, associating respiratory distress and oral breathing.	<i>Tonsillectomy.</i>
Surgery	2012 9 years	Left plantar hemangioma.	<i>Plantar ultrasound:</i> Slightly hypoechoic structure of approx. 6.5/3.1 cm in the continuity of the subcutaneous cellular tissue. <i>Doppler examination:</i> Well-represented vasculature with high velocities and extremely variable impedance indices. The appearance suggests plantar hemangioma. <i>Surgical excision of the hemangioma.</i>
	2018 15 years	Left thyroid lobe nodules.	<i>Left thyroid lobectomy.</i>
	2020 17 years	Contusion wound with partial section of right Achilles tendon.	Pathological examination confirms a follicular adenoma.
Endocrinology	2015 12 years	Left multinodular goiter.	<i>Thyroid ultrasound:</i> Right lobe without changes; left lobe: 3 nodular lesions of approx. 3.5/2.7/2 cm, 2.5/2/1.5 cm, and 2/1.5/1.5 cm, respectively. <i>Ultrasound of the right thyroid lobe:</i> Average volume, echogenic, homogeneous structure. Inferior, a hyperechoic restricted lesion with minimal proliferation on the anterior wall, non-vascularized cc 0.5 cm (cyst?); vascularization of the thyroid parenchyma of normal appearance.
	2018 15 years	Right thyroid lobe nodule (cyst).	
	2020 17 years	Right thyroid nodule.	
Gastroenterology	2023 April 20 years	Colonic polyposis under observation.	Lower digestive endoscopy: Internal hemorrhoids gr II. Suspected inflammatory bowel disease; a colonoscopy was recommended. <i>Colonoscopy:</i> At the level of the ileum, colon, and rectum, multiple polyps (hundreds of sessile polyps); sigma: pedunculated polyp with dysplastic appearance – excision; anal canal: polypoid poly-lobed formation of approx. 2.5 cm – biopsy (Figure 3a–d). Pathological anatomy: Hyperplastic polyps at the level of the rectum; juvenile hamartomatous polyps at the level of the anus (Figure 4a,b).
Dermatological	2023, May, 20 years	Facial acne and papular lesion on the forehead with the appearance of trichilemmoma.	A biopsy of the papillomatous lesion was recommended.

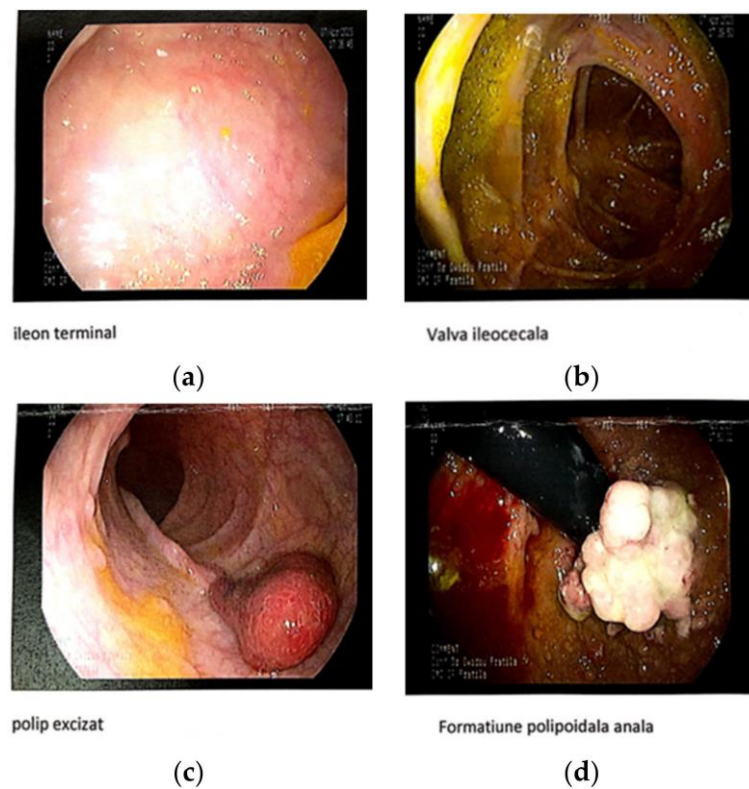


Figure 3. Lower digestive endoscopy: (a) terminal ileum; (b) ileocecal valve; (c) rectal polyp; (d) anal polypoid formation.

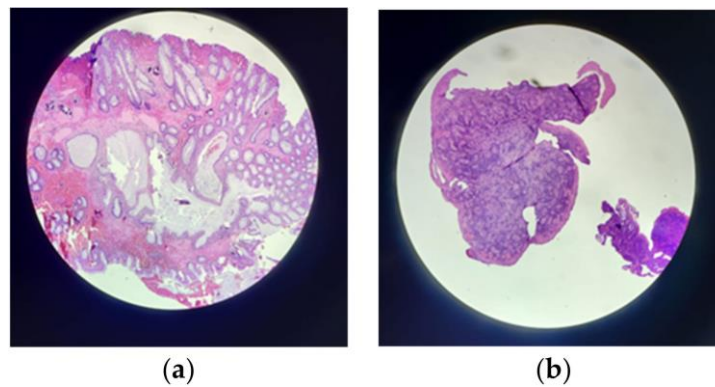


Figure 4. Pathological anatomy: microscopy; (a) juvenile polyp; (b) anal polypoid formation.

3.4. Molecular Investigations

Extensive germline testing (43 genes) to assess predisposition to hereditary gastrointestinal and colorectal syndromes was positive. The c.762del.p(Val255*) heterozygous mutation was identified in the *PTEN* gene. This variant is absent in gnomAD, an extensive reference population database ($n > 120,000$ exomes and $>15,000$ genomes) that aims to exclude individuals with severe pediatric diseases. This variant generates a frameshift in exon 7 (of 9 exons), resulting in a premature stop codon. This is predicted to lead to a loss of normal protein function, either through protein truncation or nonsense-mediated mRNA decay. Loss of function is an established disease mechanism in this gene (HGMD). In silico prediction algorithms (POLYPHEN, SIFT, MUTTASTER) support the pathogenicity of the identified variant, thereby also supporting the negative effect on the phenotype. To our knowledge, this variant has not been reported in the medical literature or in disease-related variation databases.

4. Discussion

4.1. Clinical Aspects

4.1.1. Craniofacial Dysmorphism

Patients with CS present macrocephaly, broad forehead with frontal bossing, a widened nose, mandibular hypoplasia, microstomia, and ogival palatine vault. The presented case associates macrocephaly with a cranial circumference of 64 cm, a prominent forehead, anteverted ears, and a wide-rooted nose. Macrocephaly is most commonly reported to be identified in 80–100% of patients with PHTS [14,15]. Macrocephaly is defined as an occipitofrontal circumference (OFC) 2 SD or higher than the mean for age, gender, and ethnicity measured over the greatest frontal circumference. Macrocephaly is found in more than 100 OMIM syndromic or non-syndromic entities like Fragile X, Nevroid Basal Cell Carcinoma (NBCCS), and Sotos syndrome, and requires complex clinical assessment and further molecular analysis for diagnosis.

4.1.2. Thyroid Involvement

Thyroid involvement is a constant presentation in CS. There is a wide range of manifestations, from benign tumor lesions (adenomatous follicles, nodular goiter) to malignant or autoimmune anomalies (Hashimoto or lymphocytic thyroiditis) [16,17]. Thyroid damage occurs in early childhood and can be diagnosed via thyroid ultrasounds [18]. Thyroid function should be constantly monitored in terms of the risk of malignant transformation (TSH, Tg, TgAc) and to identify functional changes in this gland [19]. Clinicians should consider that the presence of thyroid adenomatous follicles could be an important indication of this disease. In the case of our patient, thyroid nodules were diagnosed at the age of 12 clinically, sonographically, and paraclinically via assessing whether their TSH values were outside of the reference values. At the age of 15, a left lobectomy was performed. Two years after the surgical intervention, the patient developed a nodular lesion in the right thyroid lobe.

4.1.3. Lymphoid Involvement

Autoimmune-related phenotypes and lymphoid hyperplasia can be observed in 40% of PHTS patients [20]. These patients show dysregulated immune function with lymphopenia, CD4+ T cell depletion, and changes in T and B lymphocyte subsets. Tonsillar hypertrophy following the expansion of lymphoid tissue has been described in CD and PHTS [21–23]. This can be about varying degrees of hypertrophy, from small asymptomatic sizes to large sizes that cause functional respiratory disorders (difficult oral breathing) and sleep apnea, more commonly found in BRRS [24]. The appearance of tonsillar nodules can also cause upper airway obstruction [25,26]. In the case presented by the authors, the tonsillar polyp was the first warning sign of the disease. It appeared at the age of 3 1/2 years and was surgically excised. The postoperative evolution was uneventful. Six months after the resection of the tonsillar papillomatous formation, the boy showed an increase in the size of the papilloma, causing severe functional disorders, including difficult oral breathing, having a permanently open mouth, and eating disorders (feeding only with liquid foods), meaning that emergency tonsillectomy was required at the age of 4.

4.1.4. Gastrointestinal Manifestations

Intestinal polyps represent the most essential and pervasive feature of patients with CS and other PHTS patients. Additionally, >90% of *PTEN* mutation carriers who had a colonoscopy had colorectal polyps, typically with mixed histologies [27]. Polyps can be present at any level of the digestive tract, starting from the stomach to the level of the anus; from an anatomopathological point of view, polyps can be inflammatory, hamartomatous, adenomatous, or ganglioneuromatous [27]. Their presence is often detected due to rectal bleeding or intestinal blockage through intussusceptions [28]. There is a 9 to 16% lifetime risk for malignant transformations for polyps, with the average age of diagnosis being between 44 and 48 years [29]. In patients with mutations in the *PTEN* gene, the prevalence

of colorectal polyps can reach 90% [30,31]. Our patient, at the age of 17 1/2 years, began to present anal bleeding, initially interpreted as being due to hemorrhoids. In May 2023, following a lower digestive endoscopy and a colonoscopy, more than 100 intestinal polyps were identified from the ileum to the anus. A biopsy was performed for five polyps, four of which had inflammatory histopathological characteristics, and one adenoma.

4.1.5. Skin Changes

Characteristic skin lesions identified in CS include trichilemmomas, acral keratosis, mucocutaneous neuromas, oral papillomas, and macular pigmentation of the penis gland, all of which are often the first clues for clinical diagnosis [32]. The most common changes that can occur are trichilemmomas-type lesions that present more frequently in the ears, forehead, and perinasal area. However, they can appear in any area of the body. Trichilemmomas are benign tumors originating from the outer cells of hair follicles; usually asymptomatic, they appear as solitary or multiple soft, smooth, skin-colored papules. Their biopsy is usually necessary for diagnosis. Palmoplantar keratoses, gingival papilomas, and hemangiomas may also occur [33,34]. A tongue with a scrotal appearance, lingual nodule, ogival palatine vault, and cutaneous neuromas can also be spotted [35]. Identifying skin lesions in childhood can significantly impact the disease's evolution, being an alarm sign for a hereditary tumor syndrome, thus allowing its rapid identification and the application of early cancer screening [36]. The presented case was located on the upper part of the forehead and chest (multiple pustular lesions and a skin-colored papular lesion with the appearance of trichilemmomas); the dermatologist recommended the biopsy of this lesion, which also revealed a surgically excised plantar hemangioma.

4.1.6. Cancer Risk

Patients with *PTEN* germline variants have a higher risk for malignant events compared to the general population [37]. A recent literature data review reported higher risks for breast cancer (67% to 85%), endometrium cancer (19% to 28%), thyroid cancer (6% to 38%), renal cancer (2% to 24%), colorectal cancer (9% to 32%), and melanoma (0% to 6%), with a Cumulative Lifetime Cancer Risks (CLTRs) of 81–90% and a median age at diagnosis of 36 years [38]. Another extensive study revealed a higher incidence of cancer in *PTEN* carriers, providing a guide for risk-management strategies with enhanced surveillance approaches to improve clinical outcomes regardless of their initial clinical presentation [39]. Breast cancer is the most common malignancy in *PTEN* female carriers; the tumors are more commonly triple negative (loss of progesterone, estrogen, and HER2 expression), are more aggressive, and could affect both breasts [40]. Despite being a rare occurrence, breast cancer has also been reported in male *PTEN* carriers [41].

4.1.7. Neurodevelopmental Anomalies

Neurodevelopmental anomalies have also been reported in *PTEN* carriers. Along with macrocephaly, research in the literature indicates potentially increased rates of developmental delay and autism spectrum disorder (ASD) and other behavioral and psychological manifestations [42]. A representative percentage of approximately 25% of people with germline *PTEN* mutations also have characteristic criteria of ASD [43]. As mentioned above, macrocephaly is an endophenotypic trait in CS; interestingly, these patients have increased brain mass and white matter volumes [44] that might be linked to various intellectual disabilities and distinctive cognitive profiles (delayed speech, poor working memory, and processing speed) [45]. Other structural anomalies of the central nervous system, such as meningiomas, arteriovenous malformations, large perivascular spaces, cortical dysplasia, and gray matter heterotopias, have also been reported [46]. In addition to being pathognomonic for CS, Lhermitte–Duclos disease is diagnosed in adults and is characterized by a hamartomatous overgrowth of the cerebellum and large neuronal cells with a “tiger-striped” appearance expanding into the granular and molecular layers of the cerebellar cortex upon imaging assessment [47].

4.2. Genetics

The *PTEN* Gene

The *PTEN* gene is located on the long arm of chromosome 10 (10q23) and consists of 9 exons that encode a protein consisting of 403 amino acids [48]. Germline mutations occur most frequently in exons 5,7,8 and are found more frequently in cancers under the PHTS umbrella [49]. On the other hand, somatic mutations also occur in other tumors, such as prostate cancer, glioblastoma multiform, and even uncommon localizations [50]. More than 300 pathogenic variants in the *PTEN* gene have been described [51]. The *PTEN* gene codes for a dual-activity phosphatase with a tumor suppressor effect that, under normal conditions, inhibits the phosphatidylinositol 3-kinase (PI3K) signaling pathway through its lipid phosphatase activity but simultaneously negatively regulates the MAPK pathway through its protein phosphatase activity. *PTEN* activity occurs at both the cytoplasmic and nuclear levels [52].

Cytoplasmic activity. As a lipid phosphatase, *PTEN* plays a negative feedback role in the PIP3/AKT/mTOR signaling pathway. Typically, the cascade is initiated by binding signal molecules to growth factor tyrosine kinase (RTK) receptors. Activated growth factors will recruit and activate phosphatidylinositol 3-kinase. Activated PI3K will downstream phosphorylate phosphatidylinositol 4,5-diphosphate (PIP2) to the second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3), which will activate serine/threonine protein kinase B (AKT) both directly and by recruiting lipamide pyruvate dehydrogenase kinase isozyme 1 (PDK1). In turn, AKT will inhibit the tuberous sclerosis complex (TSC), inhibiting the mTOR (mechanistic target of rapamycin) complex [53–56]. Through its lipid phosphatase function, *PTEN* dephosphorylates the secondary messenger molecule PIP3 to PIP2 (basically causes PIP3 to convert back to PIP2), effectively inhibiting AKT activation; consequently, the mTOR complex will super-activate, causing cell proliferation, cell transformation, and tumorigenesis. *PTEN* has a negative feedback role in the RAS/MAPK pathway by inhibiting the adapter protein Grb2 (Growth factor receptor bound protein 2) through its protein phosphatase activity. Grb2 connects growth factor receptors and the Ras-MAPK signaling pathway through SH domains in its structure (it has one SH2 domain and two SH3 domains). Through the SH2 domain, it attaches to the activated RTK receptor, and through the SH3 domains, it interacts with one end of the SOS (son-of-sevenless) protein; the other end of the SOS protein binds to a domain called Ras-GEF, through which it interacts with the Ras protein. Later, RAS will activate the RAF molecule downstream, phosphorylating MERK 1 and 2 and activating ERK1 and 2; thus, it plays an essential role in cell growth and cell proliferation [48,57].

At the nuclear level, *PTEN* plays a significant role in genomic stability, chromosomal architecture maintenance, and cell cycle control [58].

4.3. Genotype–Phenotype Correlation

Cowden syndrome and BRRS are two conditions in this group that occur due to mutations in this gene. Despite the fact that, initially, the two syndromes were considered two different entities, nowadays, the two represent a single disease but with different penetrance and variable expressivity caused by the same mutation in the *PTEN* gene [59].

The correlation between genotypes and phenotypes needs to be better defined to personalize the screening tests. However, some studies emphasize that missenses are rare in thyroid neoplasms with mutational variants, and frameshifts are much more common [60,61]. Nonsense variants would be associated more frequently with colorectal cancer, missense variants would be associated more frequently with autism spectrum disorders, and those in the promoter region would be associated more frequently with breast cancer [62]. Other studies, such as [63], have demonstrated no genotype–phenotype correlation; however, it should be noted that their sample included only 13 cases with PHTS. Estimating the risk for organ-specific cancer, as with other hereditary cancers, is challenging, if not impossible; therefore, it is relative to determining who and what type of cancer will develop during their lifetime. Without a clear genotype–phenotype correlation, it is suitable for

patients diagnosed at a young age with a pathogenic or likely pathogenic variant to be monitored according to the recommendations made by international cancer surveillance guidelines [64]. Since the defect identified in the patient was not reported in other patients with *PTEN*-related syndromes, it is impossible to make genotype-to-genotype associations; thus, we had to make assumptions about the phenotype based on a defect with the same molecular characteristics. New findings suggest that frameshift mutations are more damaging (affecting the c2 domain) and that they could also be associated with a higher risk for a malignant lesion in carrier patients [65].

4.4. Treatment

There is no specific treatment for the disease, as no international consensus exists on this aspect. Considering the evolutionary course with multiple tumor involvement, the treatment is complicated; practically every tumor form has a specific treatment. Diagnosing and treating each tumor process as early as possible is essential to intervene medically and surgically.

Treatment with inhibitors of the mTOR pathway (Sirolimus), which restore the *PTEN* pathway, is a promising prospect. Since many signs have an early onset [66], mTOR inhibition may represent a suitable chemopreventive strategy to halt Cowden's disease progression. The prognosis of the disease is unfavorable due to the increased risk of cancer; the correct monitoring of these patients is essential for the survival of these patients. The presented case benefited from surgical treatment only (tonsillectomy, left thyroid lobectomy), with total colectomy and right thyroid lobectomy currently being recommended due to the increased risk of malignancy [67].

PTEN loss-of-function mutations, which are oncogenic driver events in CS-related breast cancers, result in dominant AKT activation. Preclinical evidence suggests that cancers with AKT activation have increased sensitivity to AKT inhibition and may improve the outcome of patients with somatic and germline *PTEN* mutations [68].

4.5. Screening Methods and Genetic Counseling

Monitoring and screening CS patients is challenging for clinicians; currently, opinions are divided. The American College of Gastroenterology Guidelines (A.G.G.) recommend a colonoscopy every two years starting at the age of 15; they also recommend periodic specialist clinical consultations regarding the thyroid, breasts, kidneys, and uterus in the event of the appearance of tumors or the malignant transformation of already present nodules. On the other hand, the NCCN (National Comprehensive Cancer Network) guidelines recommend a colonoscopy from age 35 and subsequent colonoscopies every five years or sooner if polyps are already present. Prophylactic colectomy is also recommended in the presence of multiple polyps with a high risk of malignancy [69]. The screening and prophylactic measures recommended for Cowden syndrome are synthesized in Table 3.

Screening and prophylaxis recommendations are highlighted in Table 3. Guideline Genturis [70]

Table 3. Screening methods.

System	Screening and Prophylaxis
Thyroid	<ul style="list-style-type: none"> From the age of diagnosis (first thyroid cancer reported at 7 years): annual thyroid ultrasound.
Kidneys	<ul style="list-style-type: none"> Starting at the age of 40, renal ultrasound every 1–2 years. Renal imaging (CT or, preferably, MRI).
Colon	<ul style="list-style-type: none"> Starting at the age of 35, a colonoscopy every 5 years and more frequently if there are polyps or suggestive symptoms.
Breast	<ul style="list-style-type: none"> Starting at the age of 30, annual MRI or mammography every 2 years.

Table 3. Cont.

System	Screening and Prophylaxis
Dermatological	<ul style="list-style-type: none"> An annual dermatological examination is recommended (including dermatoscopies for skin melanoma). Due to the tendency to form keloid scars associated with <i>PTEN</i>, the excision of skin lesions is recommended, but only if they show signs of malignancy or generate significant symptoms.
Growth	<ul style="list-style-type: none"> From the age of diagnosis and at the recommendation of the doctor, psycho-motor evaluations of the child are recommended. Brain M.R.I. scans are recommended if the patient is symptomatic. Patient education and assessment for early intervention as needed.
Genetic counseling	<ul style="list-style-type: none"> Family genetic counseling. Psychological support is recommended in all cases (e.g., communicating the diagnosis, family planning, prophylactic mastectomy).

Pathogenic *PTEN* germline mutations are characteristic of *PTEN*-associated hamartomatous syndromes, with different localizations and an increased risk of malignant transformation. Cowden syndrome, an autosomal dominant pathology with high penetrance and the most common *PTEN*-opathy, is often difficult to diagnose due to the variability in its characteristic phenotype. Pathogenic *PTEN* variants associated with excessive cell proliferation and implicit tumorigenesis are transmitted according to the autosomal dominant inheritance pattern, with a 50% recurrence risk for the offspring of an affected parent. Still, approximately 45% of mutations are “de novo” or a consequence of parental mosaicism. Frameshift mutations and loss-of-function truncated proteins appear more commonly in thyroid cancer [60]. The benefits and implications of asymptomatic relatives of a patient diagnosed with CS must be discussed on a case-by-case basis, especially if children are involved.

5. Conclusions

CS is the most prevalent pathology among the syndromes under the umbrella of PHTS. The importance of early diagnosis, along with patient education, genetic counseling, and periodic follow-up, plays a vital role in the evolution and prognosis of this syndrome, with the multidisciplinary team making a significant contribution. The presented case raises awareness regarding the importance of diagnosing this heterogenous syndrome as quickly as possible since, although an early onset, we still need help to establish a diagnosis.

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Conflicts of Interest: The authors declare no conflict of interest.

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Review

Breast Cancer Screening and Prophylactic Mastectomy for High-Risk Women in Romania

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Abstract: Breast cancer remains a significant contributor to morbidity and mortality within oncology. Risk factors, encompassing genetic and environmental influences, significantly contribute to its prevalence. While germline mutations, notably within the BRCA genes, are commonly associated with heightened breast cancer risk, a spectrum of other variants exists among affected individuals. Diagnosis relies on imaging techniques, biopsies, biomarkers, and genetic testing, facilitating personalised risk assessment through specific scoring systems. Breast cancer screening programs employing mammography and other imaging modalities play a crucial role in early detection and management, leading to improved outcomes for affected individuals. Regular screening enables the identification of suspicious lesions or abnormalities at earlier stages, facilitating timely intervention and potentially reducing mortality rates associated with breast cancer. Genetic mutations guide screening protocols, prophylactic interventions, treatment modalities, and patient prognosis. Prophylactic measures encompass a range of interventions, including chemoprevention, hormonal inhibition, oophorectomy, and mastectomy. Despite their efficacy in mitigating breast cancer incidence, these interventions carry potential side effects and psychological implications, necessitating comprehensive counselling tailored to individual cases.

Keywords: breast cancer; prophylactic mastectomy; genetic factors; BRCA; high risk



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1. Introduction

Breast cancer (BC) remains the most common type of cancer diagnosed in women. Although in recent decades, there have been fewer cases diagnosed in advanced, metastatic stages, breast cancer remains a major public health issue worldwide [1].

One important characteristic of breast cancer lies in the multiple subtypes that have been described, each presenting its challenges in terms of diagnosis and treatment. These subtypes, from hormone receptor-positive to HER2-positive and triple-negative, guide patient management and prognosis. A complete and correct diagnosis involves understanding the molecular mechanisms behind these subtypes, and it is essential for developing targeted therapies and improving patient outcomes [2].

There are multiple risk factors known for breast cancer besides gender and age, including genetic predisposition, hormonal influences, environmental exposures, and lifestyle variables. Defining these predisposing factors is vital in understanding the aetiology and

devising personalised prevention strategies. Treatment options vary from conventional approaches, such as surgery, chemotherapy, and radiotherapy, to immunomodulation and targeted therapy.

Prophylactic measures are essential factors in limiting the incidence of BC, especially for individuals at high risk due to genetic predispositions. Prophylactic mastectomy stands as one of the most important measures, but due to its significant psychological implications, the decision to undergo this type of intervention is often complex. This procedure has become more important in patient management over the past few years. A mastectomy is often followed by reconstructive procedures, which can significantly reduce the psychological impact on a patient [3].

Although there is continuous progress regarding the treatment options in oncology, the prevention and early detection of cancers still represent the key elements for ensuring better outcomes for patients, together with the necessary access to rehabilitation methods and psychological supports in a multidisciplinary approach.

2. Prevalence

Epidemiological data provided by GLOBOCAN (2018 and 2020) [4] suggest an increasing incidence of BC. If in 2018 approximately 2.1 million cases were diagnosed, in 2020, the number increased to 2.3 million. The latest report describes breast cancer as the primary type of cancer diagnosed (11.7%), surpassing lung cancer (11.4%), considering both sexes. Mortality rates have been higher in less-developed countries [4–6].

Breast cancer exhibits distinct epidemiological characteristics in East European countries, presenting both challenges and opportunities for public health interventions. It represents a significant health concern in Romania, with notable prevalence and associated mortality rates. The incidence of breast cancer in Romania has been on the rise, reflecting a global trend; in 2020, it was 12.2% for both sexes and 26.9% for women. The mortality rates for BC were reported to be 7.2%. The region faces a unique combination of risk factors, including genetic predispositions, lifestyle choices, and socio-economic factors that contribute to the prevalence of breast cancer. Late-stage diagnoses are unfortunately common, often due to limited access to screening programs and healthcare resources. Additionally, certain East European countries may face healthcare infrastructure challenges, impacting early and effective treatment. The burden of breast cancer in these nations is also influenced by cultural perceptions, which may affect prevention efforts, awareness, and the seeking of medical care [7–10].

Despite the well-known benefits and the current practice in most countries in the EU, genetic diagnosis is not covered by the national health system in Romania, thus making genetic testing and proper diagnosis significantly more difficult for patients with low incomes. Furthermore, Romania lacks a national register in which diagnosed genetic mutations can be reported, and so proper statistical analysis can be performed on the genotypic characteristics of the Romanian population [9].

Our country also needs medical professionals specializing in oncological genetics, and genetic counselling is only available for needy patients. Multidisciplinary boards to discuss oncological cases, especially with a significant genetic background, are available in very few institutions, affecting proper patient care [9].

The public health system is often overwhelmed, especially in the bigger hospitals, since the smaller ones do not have the proper equipment to offer cancer treatment. Although progress in the field is sometimes achieved more quickly, access to the private health system is often limited, implying more significant costs for the patients [9].

3. Risk Factors

3.1. Genetic Risk Factors in Breast Cancer

Hereditary breast cancers represent approximately 5–10% of all diagnosed breast cancers [11]. In approximately half of the hereditary cases, the defect is inherited from one of the parents according to the dominant model; in the other half, the defect is secondary to

a de novo mutation [12], and no significant family histories are reported in these cases. On the other hand, a positive family history is not necessarily synonymous with the carrier status of germline mutations. Polygenic determinism, characterised by multiple molecular defects with additive, convergent effects accompanied by other risk factors, also plays a role in familial aggregation and cancer development [12–14].

3.1.1. BRCA Mutations

BRCA1 and BRCA2 play crucial roles as tumour suppressors in repairing double-strand DNA breaks (induced by natural and medical-use radiation or other environmental hazards) through the process of homologous recombination (HR) [15]. Aberrant BRCA1 and BRCA2 activities lead to the accumulation of mutations and abnormal cell divisions. Although most of these cells are genetically unstable and do not survive, some acquire malignant potential, thus leading to tumour formation. BRCA1 is also involved in cell-cycle regulation, transcription, and chromatin remodelling [16].

The role of the BRCA2 gene is to regulate the activity of *RAD51*, another gene involved in the DNA repair process [16,17]. Both BRCA genes are associated with a high risk of breast and ovarian cancer, with most of the BC cases being non-special-type ductal carcinomas [18]. The most common histopathological subtype associated with BRCA1-positive breast cancers is typically the “basal-like” or “triple-negative” subtype. Triple-negative breast cancer (TNBC) is characterised by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. The basal-like subtype shares similarities with triple-negative breast cancer and is characterised by the expression of basal cytokeratins (such as CK5/6 and CK14). These tumours often have a high histological grade, pushing margins, and a higher likelihood of presenting lymphocytic infiltrates [17–19].

DNA repair mechanism deficiencies make cells more susceptible to specific therapeutic agents. Poly ADP-ribose polymerase (PARP) inhibitors have emerged as a promising class of drugs for BRCA-associated cancers. These inhibitors exploit the impaired DNA repair in BRCA-mutated cells, leading to enhanced cancer cell death. BRCA-associated breast and ovarian cancers have been shown to be more sensitive to specific chemotherapeutic agents, such as platinum-based drugs. This knowledge can guide treatment decisions and optimise therapeutic outcomes [20–22].

As patients with BRCA mutations present significantly higher risk for breast cancer development, enhanced surveillance methods and proper genetic counselling are necessary. Prophylactic measures may include double mastectomy and oophorectomy [23,24].

3.1.2. Non-BRCA Mutations

Other high-penetrance genes associated with breast cancer have been described, such as *TP53* (Li-Fraumeni syndrome), *PTEN* (Cowden syndrome), *PALB2*, and *STK11* (Peutz-Jeghers syndrome) [24]. Pathogenic germline mutations in these genes contribute to an increased risk of various cancers, including breast cancer.

PALB2 (a partner and localiser of BRCA2) plays a crucial role in DNA repair. *PALB2* mutations impair the function of the BRCA protein complex, leading to compromised DNA repair mechanisms and increased genomic instability. Biallelic germline loss-of-function mutations in the *PALB2* gene can lead to Fanconi’s anemia [25], while monoallelic impairments increase the risk of breast and pancreatic cancer [26,27]. *PALB2* mutations are often associated with the development of the triple-negative breast cancer (TNBC) subtype, similar to BRCA1 mutations. Similar to BRCA mutations, *PALB2*-associated breast cancers may exhibit sensitivity to PARP inhibitors. Additionally, understanding the molecular subtypes guides therapeutic decisions, as TNBC often requires tailored treatment approaches, such as chemotherapy [26,27].

TP53, known as the “guardian of the genome”, regulates cell cycle progression and prevents the growth of cells with damaged DNA. Thus, mutations result in the loss of this tumour-suppressive function, contributing to uncontrolled cell proliferation. Somatic

acquisitions in the *TP53* gene are the most observed alterations in cancer patients, occurring in approximately 30% of all breast cancer cases. Carriers of germline mutations in the *TP53* gene have a risk of breast cancer of up to 85% by the age of 60 years. Most of these breast cancers are early onset, with the median age at diagnosis being 34 years [28].

TP53 mutations are observed across various breast cancer subtypes, including luminal B and HER2-positive, but they are more commonly associated with the basal-like subtype. Breast cancers with *TP53* mutations may resist multiple available therapies, and treatment decisions need to consider specific molecular characteristics [28,29]. The overall prognosis for these patients is poor compared to those with wild-type *TP53* [29].

Germline mutations in the *PTEN* gene are associated with Cowden syndrome, a hereditary hamartomatous syndrome with a heterogeneous phenotype. *PTEN* acts as a tumour suppressor by regulating division and cell growth. *PTEN* mutations result in uncontrolled cell growth and contribute to cancer development. Thus, they may be involved in the development of diverse breast cancer subtypes, including hormone receptor-positive and HER2-positive subtypes. The lifetime risk with a *PTEN* mutation is approximately 40–60% compared to 12.5% for the general population. *PTEN*-mutated breast cancers may exhibit altered signalling pathways, impacting responsiveness to targeted therapies, but other therapies targeting molecular pathways, such as PI3K inhibitors, represent an area of active research [30,31].

E-cadherin (*CDH1* gene) germline mutations are more often linked to diffuse gastric cancer, but they also come with an inherited predisposition to develop lobular breast carcinoma (LBC). *CDH1* encodes E-cadherin, a cell adhesion molecule. *CDH1* mutations disrupt cell adhesion, promoting invasiveness and metastasis. Prophylactic measures, such as preventive mastectomy, may be considered for individuals with *CDH1* mutations [32,33].

The ataxia-teleangiectasia mutated (*ATM*) gene has a prevalence of 40% in BC patients. Pathogenic germline variants of *ATM* are associated with an increased risk of BC [34] and a worse prognosis since the tumour itself is more aggressive. There is a higher rate of lymph node involvement. *ATM* missense variants have a similar effect as *BRCA1* mutations on cancer cells, sensitizing the cancer cells to platinum-derived drugs. There seems to be a higher risk of chemo- and radio-therapy resistance and of developing secondary tumours in both breasts [35].

In addition to high-penetrance genes, researchers have identified several moderate and low-penetrance genes that influence breast cancer risk. Germline defects in the *RAD51*, *CHEK2*, and *BARD1* genes may have a more subtle impact individually but can collectively contribute to an increased susceptibility [11–14].

The genetic characteristics of hereditary BC in Romania do not differ much from those reported after observing the Slavic and East Caucasian populations. *BRCA* mutations and a positive family history of cancer are linked to earlier diagnoses of BC compared to other mutations. Non-*BRCA* mutations have been described most frequently in the *CHEK2*, *ATM*, and *PALB2* genes [36].

3.2. Non-Genetic Risk Factors

Breast cancer is associated with various risk factors, such as female sex and advanced age. Among the modifiable risk factors, the most important are obesity, chronic consumption of oral contraceptives and hormone replacement therapies, radiation exposure, alcohol consumption, smoking, a diet rich in saturated fats and synthetic sugars, and lack of physical exercise [37].

The early onset of menstruation and late menopause, advanced reproductive age, and lack of breastfeeding can also cause prolonged exposure to higher estrogen levels and, thus, a higher risk of developing BC. Variations in breast tissue density and architecture contribute to the complexity of breast cancer risk stratification. Dense breast tissue, characterised by a higher proportion of glandular and fibrous tissue relative to fatty tissue, is recognised as a risk factor for breast cancer. Dense breasts not only make mammographic detection more challenging but are also associated with an increased likelihood of develop-

ing cancer. This heightened risk is attributed to the increased cellular activity and potential for developing abnormal cells within denser tissue. Conversely, breasts with a higher proportion of fatty tissue tend to have a lower cancer risk [38–41].

3.3. Risk Stratification

Several breast cancer risk assessment models are used in clinical practice and research, and we will focus on some of the more commonly used ones.

The Gail Model is a widely employed tool for estimating the risk of developing invasive breast cancer in women. It calculates risk based on several factors, including age, race, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, and the presence of atypical hyperplasia on breast biopsy. The Gail Model provides a 5-year and lifetime risk estimate, aiding in clinical decision-making regarding screening and preventive interventions. The simplicity and accessibility of the Gail Model contribute to its extensive use in both research and clinical settings [42].

An updated version of the Gail Model, the Breast Cancer Risk Assessment Tool (BCRAT), incorporates additional risk factors to enhance precision. It includes race/ethnicity, personal history of breast cancer or ductal carcinoma in situ (DCIS), and certain benign breast diseases as part of its risk calculation. As with its predecessor, the BCRAT provides 5-year and lifetime risk estimates. Its online availability through the National Cancer Institute's website facilitates widespread use. The BCRAT is particularly valuable in assessing risk in diverse populations and aids in identifying individuals who may benefit from intensified screening or preventive measures [43].

The Tyrer–Cuzick model, also known as the International Breast Cancer Intervention Study (IBIS) model, is a comprehensive risk assessment tool that incorporates many risk factors. It calculates breast cancer risk by considering factors such as age, family history of breast cancer, hormonal factors (e.g., age at menarche and age at first birth), breast density, and the presence of specific genetic mutations (e.g., BRCA1 and BRCA2). This model is precious for assessing risk in women with a family history of breast cancer and provides a more refined risk estimate. The IBIS model aids in personalised risk management and decision-making related to surveillance and preventive measures [44].

4. Investigations

Systematic exploration consists of documenting the clinical presentation, imagistic characteristics, histological variations, molecular genetics, and specific biomarkers, each contributing to the comprehension of the underlying pathophysiology [45].

4.1. Germline Genetic Testing

As breast cancer often exhibits familial patterns, germline testing is a key component of genetic counselling and precision medicine. The focus primarily lies in identifying pathogenic variants in specific genes, such as *BRCA1*, *BRCA2*, *TP53*, *PTEN*, and others, which have been associated with hereditary breast cancer predisposition. These genetic mutations, when present in the germline, significantly elevate the lifetime risk of developing breast cancer [15,16,24,28].

Identifying the carriers of pathogenic variants is valuable for affected individuals and their family members who may also be at risk. The early detection of these mutations enables risk reduction and tailored screening strategies. Germline testing can also influence treatment decisions. High-risk individuals may opt for preventive measures, such as enhanced screening, prophylactic surgeries, and lifestyle modifications, to mitigate their risk of breast cancer [17,25,29].

The revelation of hereditary risk may have profound emotional and social implications. As such, comprehensive genetic counselling is an integral component of germline testing, providing individuals and their families with the necessary information and support to make informed decisions [24].

4.2. Imaging

Imaging methods are essential both as screening options and as part of the diagnostic procedures for breast cancer. The screening methods rely on mammary ultrasounds at a younger age and mammography later in life, when the breast tissue is better seen with this method.

Ultrasounds are used to distinguish between solid and cystic masses and guide the biopsies once the tumours are found. Mammography is highly effective in finding early signs of cancer, such as microcalcifications, especially in asymptomatic cases. Three-dimensional mammography enhances diagnostic accuracy. Molecular breast imaging (MBI) can be more effective for scanning dense breast tissue [46].

MRIs provide detailed images of any potential lesions and can also evaluate the extent of the disease or monitor the response to neoadjuvant therapy. CT and PET-CT are especially useful in assessing the extent of the disease's spread.

While still an evolving technology, thermography has been explored for its potential as a screening tool. It measures temperature variations in breast tissue, and although it is not a standalone diagnostic tool, it may provide additional information for further evaluation [47,48].

In Romania, there have been some attempts to increase health literacy and the general population's access to mammography as a screening procedure covered by national health programs and through projects implemented by NGOs, but the impact remains very low. Recent studies show that 79% of the women at risk from Romania have never undergone mammographies for breast cancer screening [9].

4.3. Biological Markers

Biomarker assessment in breast cancer plays a crucial role in proper diagnosis, predicting prognosis, and guiding treatment decisions. The presence or absence of an estrogen receptor (ER) and a progesterone receptor (PR) indicates a tumour's hormone receptor status, predicting the response to hormonal therapies. Hormone receptor-positive tumours may respond to endocrine therapies such as tamoxifen or aromatase inhibitors [49].

Human Epidermal Growth Factor Receptor 2 (HER2) is a protein that promotes cancer cell growth. Thus, HER2-positive breast cancers are associated with aggressive behaviour, but they can be targeted with therapies such as trastuzumab (Herceptin). Tumours lacking both hormone receptors and HER2 are called triple-negative [49].

Ki-67 is a marker of cell proliferation and tumour aggressiveness and can predict the response to chemotherapy [49,50].

CDK4/6 amplification can also be evaluated in hormone receptor-positive breast cancers. Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, such as palbociclib and ribociclib, target cell-cycle regulation [50].

Serum markers, such as CA 15-3 and CA 27.29, while not diagnostic on their own, aid in monitoring disease progression and treatment response in advanced stages.

These biomarkers altogether enable personalised treatment approaches and provide critical insights into the biological characteristics of breast cancer [49–51].

5. Prophylactic Measures

Together with controlling the modifiable risk factors, self-assessment as part of a rigorous screening protocol should be practised by all women, documenting any masses that occur and are not related to regular modifications during the menstrual cycle. A specialist should assess any palpable mass to rule out the presence of a malignant tumour [47].

A study conducted in Romania assessed the rates of self-examination for breast cancer screening to measure medical literacy on this topic. Meagre rates of monthly self-examination were reported in the age groups of 15–24 and above 50, marking these groups as vulnerable. The most cases of correct self-examination were described in the age group of 25–49 [52].

5.1. Chemoprevention and Hormonal Inhibition

Chemoprophylaxis involves the use of certain drugs or natural compounds to reduce the risk of cancer occurrence or its progression [53]. At a cellular and molecular level, it aims to control protein activities during cancer development initiation, promotion, or progression stages. Ball et al. collected data on the following main chemoprevention methods: selective estrogen receptor modulators (SERMs), aromatase inhibitors, and aspirin [53,54].

SERMs are the most used chemoprevention method among high-risk women. They work by modulating the estrogen response. The prominent representatives of this class are Tamoxifen, Raloxifen, and Lasofoxifene [54–59]. The National Surgical Adjuvant Breast and Bowel Project P-1 (NSABP-P1) study proved that Tamoxifen use (20 mg/day for five years) led to a 49% risk reduction in invasive breast cancer compared with a placebo, with even higher risk reductions observed in elders [60].

Comparatively, Raloxifene was proven to be equally effective as Tamoxifene in decreasing the risk of invasive breast cancer [55–57]. Lasofoxifene is a third-generation SERM with greater potency. Studies have shown that Lasofoxifene can reduce the risk of total BC incidence by 79% and ER+ invasive breast cancer incidence by 83% compared to a placebo administration [59,60].

Raloxifene has been associated with a minor risk of developing thromboembolic events, whereas the incidences of ischemic heart disease, stroke, and osteoporotic fractures were similar for both Tamoxifen and Raloxifene. Endometrial cancer was reported less frequently in association with Raloxifene use compared to Tamoxifen use [58–61].

Aromatase inhibitors, such as Exemestane and Anastrozole, are primarily used by postmenopausal women. They inhibit the production of estrogen, which can promote certain types of breast cancers, by inhibiting the aromatase enzyme. As potential side effects, aromatase inhibitors may lead to bone density loss and musculoskeletal issues [62,63].

Aspirin may also have chemopreventive effects against breast cancer. While relatively safe, it can also have side effects such as gastrointestinal bleeding [64].

Multiple natural phenolic compounds have been proven to play a potential role in breast cancer chemoprevention by modifying several epigenetic factors involved in carcinogenesis [65].

Curcumin and its analogues can represent potential chemoprevention agents by exerting their antiproliferative and anti-inflammatory properties [66]. Diindolylmethane, a bioactive compound found in cruciferous vegetables, promotes changes in estrogen metabolism and may also aid in chemoprophylaxis [67].

While chemoprevention can bring benefits in preventing breast cancer development, it is essential to weigh the potential benefits against the risks and side effects of these agents.

5.2. Oophorectomy

Oophorectomy, also known as ovarian removal or ovariectomy, is a surgical procedure which implies the removal of one or both ovaries. This procedure can have significant implications for breast cancer prevention, particularly in women who are considered at high risk of developing breast cancer due to genetic or other risk factors [68,69].

The primary goal of oophorectomy in this context is to reduce estrogen and progesterone levels, which can stimulate the growth of hormone receptor-positive breast cancer cells. Risk-reducing bilateral salpingo-oophorectomy (RRBSO) is recommended by international guidelines for healthy women carrying germline *BRCA1* and *BRCA2* mutations, starting at the age of 35–40 years for *BRCA1* and 40–45 years for *BRCA2* mutation carriers. The intervention is recommended after proper family planning because of its consequent premature menopause. RRBSO potentially causes other side effects related to estrogen deprivation [70].

While it reduces the risk of developing breast cancer without eliminating it, the decision to undergo an oophorectomy is complex, and it involves significant psychological challenges, especially for young women [71].

It is essential for individuals considering oophorectomy to discuss their fertility preservation options and concerns with their healthcare providers. In the case of a unilateral oophorectomy (the removal of one ovary), the remaining ovary may continue to produce eggs, preserving fertility to some extent. However, a bilateral oophorectomy (the removal of both ovaries) results in the loss of egg production, rendering a woman unable to conceive naturally. The hormonal changes which follow this procedure may also impact fertility. Before undergoing an oophorectomy, a woman who wishes to preserve her fertility may explore options such as oocyte (egg) or embryo cryopreservation, which involves harvesting and freezing eggs or embryos for future use in assisted reproductive technologies such as in vitro fertilization (IVF) [72].

5.3. Mastectomy

The optimal surgical management of BRCA-mutation carriers remains a debatable subject. Although surgical prophylaxis is the preferred option in a majority of the BRCA-positive cases, other studies suggest that BRCA mutation carriers treated with BCT present similar oncological outcomes compared to mastectomy, and young BRCA patients with incipient BCs may not need up-front mastectomy, though prophylactic surgery might be performed when ovarian cancer risk epidemiologically rises and potential reproductive desire is fulfilled [73].

Prophylactic mastectomies, preemptive surgical measures for those at heightened risk of breast cancer, involve diverse techniques tailored for risk reduction and aesthetic outcomes [72].

A bilateral mastectomy, the removal of both breast tissues, offers options such as simple mastectomy, skin-sparing mastectomy (preserving skin for future reconstruction), and nipple-sparing mastectomy (preserving the nipple-areola complex). A contralateral prophylactic mastectomy (CPM) addresses the unaffected breast in the case of unilateral breast cancer, employing techniques similar to those used in a bilateral mastectomy [74,75].

Immediate breast reconstruction frequently accompanies a prophylactic mastectomy, presenting choices such as autologous tissue (e.g., DIEP flap) or implants. This reconstruction may co-occur with the mastectomy or during a later intervention. Individuals carrying *BRCA1* or *BRCA2* mutations, associated with elevated breast cancer risk, often opt for risk-reducing mastectomies, significantly diminishing the likelihood of future breast cancer occurrences [76–78].

Emerging techniques include robotic-assisted surgery for precision, especially in nipple-sparing mastectomies, and oncoplastic approaches that blend aesthetics with risk reduction [79].

Prophylactic mastectomy, while a potent risk reduction strategy, profoundly affects individuals psychologically. The decision involves complex considerations, impacting body image, self-esteem, and intimate relationships. Emotional responses vary, encompassing anxiety, grief, and relief. Decision-making stress is common, and post-surgical adjustment takes time [80,81].

Genetic counselling is integral to the decision-making process. It provides individuals with a comprehensive understanding of their genetic risk, the implications of testing positive for specific mutations, and potential alternatives to prophylactic mastectomies.

Counselling offers a supportive space for discussing the psychological aspects, allowing individuals to make informed decisions aligned with their values and preferences [82,83].

In Romania, there are only a few professionals who specialise in both oncological and reconstructive surgery. Thus, access to both proper treatment and aesthetic results is often difficult.

Psychological Impact of a Mastectomy

Women with mastectomies have shown high satisfaction rates, reaching 70% after 14.5 years from a bilateral mastectomy and ranging between 83% and 90% after 10.3–20 years from contralateral mastectomies [84]. However, positive body image was

significantly affected, especially with bilateral mastectomies, due to many factors, such as self-consciousness, feeling less sexually attractive, and dissatisfaction with the scars. Decreased sexual satisfaction was linked to both body image issues and a loss of sensation in the breast [85,86].

Women who underwent unilateral mastectomies were less satisfied with their appearance than those who underwent bilateral mastectomies. Some data suggest that reconstruction is associated with lower long-term satisfaction, explained by more frequent surgical complications and concerns about implants [87]. Ha et al. studied insurance coverage across the United States and discovered the following: Preauthorised coverage for prophylactic mastectomies was assured by 39% of insurance policies (n = 39). There was a consensus amongst these policies to cover prophylactic mastectomies for *BRCA1* and *BRCA2* mutations (n = 39, 100%), but the coverage was variable for other genetic mutations (15–90%). In the United Kingdom, according to the NHS, prophylactic mastectomies are eligible without additional funding for all high-risk women [88,89].

Studies conducted in Romania, which have included women diagnosed with breast cancer as a target group, have shown that support groups and psychotherapy offered both before and after undergoing treatment were very helpful in improving a patient's mental health status and overall quality of life. Furthermore, a better quality of life was described even following online group sessions, which implied open discussions about their fears and the diagnosis's impact on their personal and social lives. Thus, the psychological impact of this diagnosis appears to be reduced by proper psychological support, to which breast cancer patients in Romania have access in some instances [90].

6. Conclusions

Breast cancer remains one of the leading causes of morbidity and mortality among oncological pathologies. Various risk factors have been linked to a higher prevalence of breast cancer, among which genetic and environmental factors play essential roles.

Germline mutations associated with higher risk for BC are most commonly found in the *BRCA* genes, but various other variants have been described in affected individuals. The diagnoses rely on imaging methods, biopsies, biological markers, and genetic testing. The cancer risk for each individual can be assessed using specific scores and evaluations.

Potential genetic mutations guide the screening protocols and prophylactic measures, as well as the treatment options and prognoses, of breast cancer patients. Prophylactic interventions involve chemoprevention, hormonal inhibition, oophorectomy, and mastectomy. While these measures can help prevent breast cancer development, they also come with potentially significant side effects or psychological implications, and proper counselling is required in each case.

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Review

Insights into Clinical Disorders in Cowden Syndrome: A Comprehensive Review

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Abstract: PTEN Hamartoma Tumour Syndrome (PHTS) encompasses diverse clinical phenotypes, including Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome (BRRS), Proteus syndrome (PS), and Proteus-like syndrome. This autosomal dominant genetic predisposition with high penetrance arises from heterozygous germline variants in the PTEN tumour suppressor gene, leading to dysregulation of the PI3K/AKT/mTOR signalling pathway, which promotes the overgrowth of multiple and heterogenous tissue types. Clinical presentations of CS range from benign and malignant disorders, affecting nearly every system within the human body. CS is the most diagnosed syndrome among the PHTS group, notwithstanding its weak incidence (1:200,000), for which it is considered rare, and its precise incidence remains unknown among other important factors. The literature is notably inconsistent in reporting the frequencies and occurrences of these disorders, adding an element of bias and uncertainty when looking back at the available research. In this review, we aimed to highlight the significant disparities found in various studies concerning CS and to review the clinical manifestations encountered in CS patients. Furthermore, we intended to emphasize the great significance of early diagnosis as patients will benefit from a longer lifespan while being unceasingly advised and supported by a multidisciplinary team.

Keywords: Cowden syndrome; PTEN; clinical disorders; review



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1. Introduction

1.1. Definition

PTEN Hamartoma Tumour Syndrome (PHTS) presents a range of clinical phenotypes, including Cowden syndrome (CS, OMIM 158350), Bannayan–Riley–Ruvalcaba syndrome (BRRS, OMIM 153480), Proteus syndrome (PS, OMIM 176920), and Proteus-like syndrome (PS-like) [1,2].

1.2. Pathophysiology of the PTEN

PHTS is an autosomal dominant genetic predisposition caused by heterozygous germline variants in the PTEN tumour suppressor gene, localised on 10q23.31 [3,4]. The PTEN gene is endowed with a double activity: lipid phosphatase and protein phosphatase at the cytoplasmic level. Concomitantly, PTEN has a paramount role in genomic stability when acting at the nuclear level by maintaining the chromosomal architecture as well as supervising the cell cycle. Regarding its action at the cytoplasmic level, as a lipid phosphatase, PTEN plays a negative feedback role in the PIP3/AKT/mTOR signalling pathway [5]. Typically, the cascade is initiated by the binding of signal molecules to growth factor tyrosine kinase (RTK) receptors. When activated, the growth factors activate phosphatidylinositol 3-kinase, which phosphorylates phosphatidylinositol 4,5-diphosphate (PIP2) to

the second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3), switching on the serine/threonine protein kinase B (AKT) [6]. Successively, AKT suppresses the tuberous sclerosis complex (TSC), inhibiting the mTOR (mechanistic target of rapamycin) complex. Through its lipid phosphatase function, PTEN dephosphorylates the secondary messenger molecule PIP3 to PIP2, effectively impeding AKT activation. Therefore, the mTOR complex eagerly activates, causing abnormal cell proliferation and transformation [7].

Regarding its protein phosphatase activity, PTEN also possesses a negative feedback role in the RAS/MAPK pathway by inhibiting Grb2 (growth factor receptor bound protein 2), which connects growth factor receptors and the RAS/MAPK signalling pathway by recognizing the SH (Src homology) domains: one SH2 domain and two SH3 domains [7,8]. Through the SH2 domain, which may be recognized as the prototypical modular protein-protein interaction domain, it attaches to the activated RTK receptor [9]. Moreover, through SH3, a small protein domain, defined as a conserved sequence in the viral adaptor protein v-Crk [10], PTEN interacts with one end of SOS (son-of-sevenless), a dual specificity guanine nucleotide exchange factor (GEF) that regulates the Ras and Rho family, promoting the integrity of DNA; the other end of the SOS protein connects with a domain called Ras-GEF, which leads to RAS and RAF molecule activation and downstream phosphorylation of MERK 1 and 2, thus activating ERK1 and ERK2 with a significant function in cell growth and cell proliferation [9–11].

1.3. Pathophysiology of the PHTS

PTEN plays a crucial role in suppressing the PI3K/AKT/mTOR signalling cascade, regulating cell growth. Dysfunctional PTEN leads to overgrowth through pathway dysregulation. It leads to multi-system tissue and cell overgrowth with highly diverse phenotypic presentations, encompassing both benign and malignant tumours, macrocephaly, developmental delay, autism spectrum disorders, thyroid abnormalities, and skin lesions [4,11]. The syndrome is associated with elevated risks of various cancers, with reported increased lifetime risks and earlier onset for breast, endometrial, thyroid, and other cancers [1,2]. Hereditary cancers carry unique significance as they tend to develop earlier and confer a heightened risk of aggressive, multifocal, and bilateral cancers [12]. About 5–10% of malignancies result from hereditary predisposition syndromes, highlighting their substantial impact [13,14]. These syndromes, often stemming from mutations in key genes involved in cell regulation and DNA repair, elevate cancer risk, as exemplified by CS and PTEN mutations [15]. The median age of cancer diagnosis in patients diagnosed with CS is around 36 years, with cumulative lifetime risks of 85–90% in females and 81–88% in males [1]. Also, the mean age of patients diagnosed with CS varies in the literature, being reported between 36 and 50.5 years [11,16–19].

Early diagnosis is critical for inclusion in PHTS-specific surveillance programs, aiding in timely cancer detection, improved prognosis, and extended life expectancy [1,2,11]. However, PHTS remains under-recognized due to its complex and variable clinical presentation, and its low prevalence of approximately 1 in 200,000 is believed to be underestimated [20,21].

The rate of de novo pathogenic PTEN variants exhibits variability across different literature sources, with certain authors citing rates ranging from 10% to 40% [22], while others report figures of 10% to 44% [2,23]. Moreover, there are indications from some sources of a broader range spanning from 10% to 47% [1].

Research findings indicate that a substantial portion, approximately 80% [11] and potentially even as high as 85% [2,12], of individuals fitting the stringent criteria for CS exhibit germline PTEN mutations. Furthermore, closer examination reveals that around 30–35% of patients fulfilling the diagnostic criteria carry these mutations [24]. Additionally, investigations suggest that the proportion of patients who possess these mutations while adhering to the less stringent diagnostic criteria could be as much as 25% [2,12,23]. Germline PTEN mutations are also associated with BRRS and PS [2,25].

After the identification of the PTEN gene in 1997 [26], it became clear that not every patient with PHTS possesses pathogenic PTEN mutations in their germline. Roughly 15% of individuals with classic CS and about 95% of those with CS-like (not fulfilling complete diagnostic criteria) presentations do not exhibit detectable PTEN mutations [12]. Patients diagnosed with CS/CS-like presentations who exhibit nonmutant PTEN alleles may carry mutations in alternative genes, including SDHx, KLLN, AKT1, PIK3CA, PIK3R1/2, WWP1, SEC23B, and USF3 [12,13,23,27].

In the subsequent sections, we offer a comprehensive overview of both malignant and benign manifestations of CS, as well as delve into the genotype–phenotype relationship.

2. Clinical Disorders

CS gives rise to a multitude of disorders affecting nearly all body systems. Given its intricate nature, the upcoming sections provide insight into and outline the array of clinical issues experienced by individuals diagnosed with CS.

2.1. Breast Disorders

Individuals affected by CS face an increased susceptibility to both benign and malignant breast tumours [23]. While breast cancer (BC) was initially not considered a typical part of the syndrome, it has since emerged as the predominant malignancy within the CS spectrum [24].

The onset of BC in CS patients typically occurs at an age range spanning from 38 and 46 years [15,23,28], though some reports indicate a broader span of 38 to 50 years [24], and others even extend to 52 years [20]. Notably, certain authors suggest an even earlier onset around the age of 30 [2]. In comparison, the average age of BC diagnosis in the general population is 63 years [20].

CS women are reported to exhibit a 25% to 50% risk of developing BC [11,23], which is a notable contrast to the 12% risk observed among women in the general population [11]. Recent studies, however, have revealed a significantly greater risk of approximately 77–85% [24,28,29]. The prevalence of bilateral BC in CS females ranges from 25% to 48%, markedly surpassing the 0.8–3% incidence in the general population. Moreover, CS patients are at a remarkable 9-fold higher risk of developing BC as a second primary malignancy [20], with a substantial 29% risk of a secondary BC within a decade of the initial diagnosis [29]. Females affected by CS face a potential risk of up to 67% for the development of benign breast conditions [2], such as fibrocystic breast disease [23]. This vulnerability is not exclusive to female CS patients, as male individuals with PTEN mutations have also been reported to develop BC [23]. However, while several instances of male BC with PTEN mutations have been documented [30–32], its association with CS remains unproven, with the largest reported cohorts not observing such an association [24].

It is essential to acknowledge the potential selection bias inherent in the cited studies due to cohort construction methods. Therefore, despite the unequivocal elevation of BC risk in individuals with PTEN mutations, the precise degree of this risk remains subject to debate, with a need for future studies designed to mitigate bias.

2.2. Gastrointestinal Disorders

Although the initial perspective suggested that PTEN mutations did not confer an elevated risk of colon cancer (CC), recent data have challenged this notion [24]. The prevalence of CC occurrences among CS patients has been reported to range from approximately 9% to 17% [2,24,27,29], while its manifestation is often marked by an early onset [24]. In contrast, the general population exhibits a much lower prevalence of CC, standing at only 5% [2].

The median age at which CC is diagnosed in CS patients exhibits variance across the literature, with some studies citing medians of 46 to 58 years [20], while others propose a lower median of 44 years [29]. Nonetheless, the youngest documented case of a CS patient with CC was only 28 years old [29]; this case is a notable contrast to the mean age

of CC diagnosis in the general population, which stands at 73 years [20]. This disparity underscores the heightened CC risk associated with PTEN mutations, revealing a 2- to 3-fold increased risk in comparison to the general population [29]. Furthermore, CS patients face a potential up to 6-fold increased risk of secondary CC development [20].

Notably, individuals diagnosed with CC often exhibit pre-existing or concomitant colonic polyposis [2,23,24]. Intriguingly, a significant proportion of individuals who present at least one mutant PTEN allele, ranging from 90% to 95%, display colorectal polyps upon undergoing colonoscopy [2,23,24,33]. These polyps encompass diverse histologies, including hamartomas (present in 65.8% of cases), juvenile polyps, ganglioneuromas (hamartomatous tumours that originate in enteric nervous system cells [34]), adenomatous polyps, inflammatory polyps, leiomyomas, lipomas, lymphoid polyps, and hyperplastic polyps, even though the last ones are common in the general population [2,23,24,29,35]. Synchronous histologies can be observed within the gastrointestinal polyps of individuals with CS [24]. While polyps can be identified in various gastrointestinal locations, studies suggest that those in the colon notably escalate the risk of malignancy in CS patients [29]. Although three cases of gastric cancers in CS patients have been documented, they occurred in individuals over 60 years old, and the connection between these two pathologies remains uncertain [29].

In adult CS patients, the presence of oesophageal glycogenic acanthosis has been reported in 20% of cases, forming part of the diagnostic criteria [23,24,36,37].

2.3. Endometrial Disorders

Endometrial cancer (EC) is acknowledged as a significant component of CS, but its prevalence and clinical characteristics remain poorly defined. In the general population, the risk of developing EC ranges from 2% [29] to 2.6% [2,11,23]. However, among CS patients, the prevalence varies considerably, with reported risks spanning from 5–10% [11], 19–28% [13], to 21–28% [2,15,23]. Consequently, the overall risk of EC in CS patients is approximately 40 times higher than in the general population [20]. In individuals carrying PTEN mutations, even without a formal CS diagnosis, EC was detected in 7.6% to 17% of cases [24].

The relationship between age and EC risk presents conflicting findings. Some studies suggest a 1% risk at age 20, rising to 2% at age 30 [20]. Conversely, others indicate a 30% risk at age 60 [2,23] and a 19–28% risk at age 70 [13,24,29]. Notably, a statistically significant increase in risk is observed around age 25 [2,23], persisting beyond age 50 when compared to the general population [11]. This is supported by the median age of EC diagnosis in CS patients, which is 48 years, an age significantly younger than the general population's median age of 68 years [13,20,29]. There are also case studies in the literature reporting the discovery of EC in adolescent CS patients [38].

Screening for EC in CS patients is a subject of controversy, as it has not demonstrated a reduction in mortality [13].

Additionally, individuals who present at least one mutant PTEN allele may experience benign gynaecological conditions, such as uterine fibroids [2,23].

2.4. Dermatological Disorders

Dermatological manifestations are a distinctive feature of CS. These skin and mucosal characteristics play a crucial role as clinical indicators for diagnosing CS. However, the prevalence and specific attributes of these dermatological signs have evolved and may exhibit variations among individual patients. The array of dermatological manifestations observed in individuals with CS encompasses a wide spectrum, including multiple mucocutaneous lesions, melanoma, papilloma, acral keratosis, lipomas, trichilemmomas, penile freckling, sclerotic fibromas, café-au-lait spots, vitiligo, acrochordons, and even acanthosis nigricans [4,23,24,29,39–41].

While earlier studies suggested that all CS patients displayed dermatological manifestations, contemporary perspectives within the scientific community lean towards viewing

this as a potential overestimation. Furthermore, these studies indicated that dermatological manifestations were prevalent among paediatric CS patients, yet they often lacked specific age-related details [24]. According to existing literature, approximately 99% of CS patients exhibit dermatological lesions by the age of 30 [21]. However, the accuracy of this percentage remains uncertain due to discrepancies in the literature, with prevalence rates varying between 90% and 100% before the age of 30 [16,39]. Nonetheless, what remains evident is that most CS patients do develop dermatological disorders by the conclusion of their third decade of life.

It is noteworthy that certain authors have reported a skewed distribution among CS patients in middle age or older with orofacial manifestations, noting that a ratio of 2.3 to 1 favours women over men in this subgroup. However, older studies did not explicitly highlight any discernible disparities between the sexes in terms of dermatological features in CS [16].

Papillomatosis stands out as a distinctive feature of CS [16]. These papillomatous growths can manifest in various oral and pharyngeal locations, including the buccal, lingual, gingival, and labial mucosa, as well as the pharynx and larynx [16,24,36]. Remarkably, oral papilloma associated with CS tend to remain asymptomatic, and their dimensions are typically limited to a maximum diameter of 3 mm [21,24]. Notably, papillomatosis can even impart a scrotal appearance to the tongue [21,36]. However, it is important to note that the reported prevalence of oral papilloma exhibits a wide range, varying from 15.2% to 85% across different studies [21]. Given this substantial variation, it is imperative to exercise caution in interpreting these statistics, as biases may be present, necessitating further research to establish more accurate figures. Additionally, CS patients may also present with oral fibromas, albeit with a prevalence that ranges from 14% to 76%, demonstrating a similarly broad range of reported prevalence rates [24].

Acral keratosis, although less frequently documented, is another dermatological characteristic associated with CS [16]. These keratotic lesions exhibit a maximum diameter of 4 mm and primarily appear in palmoplantar regions, affecting both paediatric and adult CS patients, presenting with a verrucous appearance [24,36]. Importantly, acral keratosis may also occur in other areas of the body, such as the face and trunk [21,24,36]. While initial reports suggested a prevalence of 63–73% for acral keratosis, recent studies have indicated a broader prevalence range of 10.2% to 82%, reinforcing the need for caution when interpreting prevalence data due to the variability in reported figures [21].

Lipomas serve as a significant clinical criterion for diagnosing CS [24,41]. In the general population, these fatty tumours have an estimated prevalence of approximately 1%. However, when considering CS patients, recent studies have revealed a substantially higher prevalence, ranging from 34.6% to 56.7% [21]. Notably, the first reported instance of testicular lipomas in a CS patient was documented as recently as 2003, emphasizing the rarity of this type of lipoma in the absence of testicular neoplasms [24]. Furthermore, subcutaneous lipomas have also been described in the context of CS, although specific prevalence data for this manifestation remain unreported [41].

Mucosal neuromas, which represent hamartomas of the peripheral nerve sheath, have been documented in CS [21,24]. These neuromas can manifest in various locations, including the mouth, face, extremities, and trunk [21,24,36]. A notable characteristic of mucosal neuromas is their tendency to cause discomfort, particularly impacting oral hygiene [36]. While earlier reports suggested a prevalence range of 5–10.9% for these neuromas, more recent studies have reported a prevalence of 0%, highlighting the need for further investigation and potential variations in prevalence among different patient populations [21].

Penile pigmentation, characterized by pigmented speckled macules on the genitalia, is another dermatological manifestation frequently encountered in CS patients [41]. In the general population, the prevalence of such pigmentation is reported to be up to 15% [24]. However, in CS patients, the prevalence is significantly elevated, estimated to be approximately three times higher than that of the general population, with reported prevalence

rates ranging from 48% to 53% [24]. Other studies have also corroborated these findings, reporting similar prevalence figures of 41% [21] or 48% [40]. Intriguingly, penile freckling can manifest at a very young age, even before one year of age [36].

The presence of multiple trichilemmomas is strongly indicative of a PTEN mutation [24]. These lesions can appear on various parts of the body, including the face, neck, axillae, hands, abdomen, and other regions [24,36,41]. Notably, trichilemmomas can be observed in patients under 18 years of age, with prevalence rates ranging from 6% to 25% in some studies [24] or even higher, reaching up to 38% in adults [21]. The clinical similarity of these lesions to other dermatological manifestations necessitates histopathological examination for accurate diagnosis [24].

Although generally rare, the literature includes case reports describing sclerotic fibromas of the skin in CS patients [24].

Melanoma has been observed in individuals diagnosed with CS, although the association and prevalence remain subject to limited available data in the literature, warranting further investigation for a more comprehensive understanding.

In the general population, melanoma prevalence ranges from 2% [23,42] to 2.8% [23]. Among CS patients, the reported prevalence of melanoma varies, with estimates of around 6% [2,4,23,39]. Nevertheless, some studies suggest different figures, such as 1% [23], 2–6% [42], 5% [15], or even as high as 28.3% [29]. It is important to note that the latter prevalence figure comes with a wide 95% confidence interval of 7.6% to 35.4% [29], suggesting potential bias in the cohort data. Age appears to influence melanoma prevalence among CS patients. At the age of 20, the prevalence is approximately 0.4%, but this risk increases significantly, multiplying by 15 times by the age of 70 [20].

The median age for diagnosing melanoma in CS patients is approximately 40 years, which is notably younger than the general population, where the median age is 63 years [20]. However, there have been reports of melanoma in very young CS patients, with one case documented in a 3-year-old [2,4,23,43–45].

The risk of developing melanoma as a second cancer in CS patients appears to be seven times higher than in the general population, emphasizing the importance of yearly dermatological evaluations as recommended by specialists [44,45].

2.5. Thyroid Disorders

CS patients exhibit various thyroid manifestations, encompassing both benign and malignant disorders [46]. Among the benign thyroid disorders observed in CS patients are multinodular follicular adenomas, nodular hyperplasia, adenomatous nodules, Hashimoto thyroiditis, lymphocytic thyroiditis, C cell hyperplasia, and goitre [2,4,23,24,27,36,46]. Some studies suggest that approximately three-quarters of adult CS patients exhibit these manifestations [2,23], while others report a lower prevalence ranging from 30% to 68% [24]. In paediatric patients with CS, the prevalence of these benign thyroid manifestations is even lower, ranging from 2% to 14% [24]. Hashimoto thyroiditis, for instance, is found in 3% to 21% of individuals with PTEN mutations, in contrast to a 2% prevalence in the general population [24]. Thyroid nodules are a common occurrence in CS patients, with reported rates of up to 65%, and multinodular goitres have been found in up to 4% of cases [24]. Remarkably, the youngest CS patient diagnosed with benign thyroid manifestations was only 5 years old [36].

As previously mentioned, CS presents malignant manifestations, particularly in the form of epithelial thyroid neoplasia rather than medullary thyroid cancer [2]. Intriguingly, the medullary thyroid cancer type, which is found in the general population and multiple endocrine neoplasia syndrome, specifically type 2, has not been identified in CS patients to date [2,4].

Epithelial thyroid neoplasia, particularly the follicular and papillary types, is considered characteristic of CS [2,4,29]. Notably, the papillary form of epithelial thyroid neoplasia is twice as common as the follicular form [23,24]. CS patients face a lifetime risk of developing thyroid cancer, estimated at approximately 35% [2,4,15,23,27], or potentially even

slightly higher at around 38% [11,20,24,36]. Some authors provide risk estimates with large 95% confidence intervals, ranging from 6% to 38% [2,42], or even 14% to 35% [4,29]. Despite the variations in risk estimates, it is evident that CS patients face a significantly increased lifetime risk of developing thyroid cancer, considering the general population's estimated risk is only 1% [2,42,47].

Individuals with CS tend to be diagnosed with thyroid neoplasia at a much younger age, with a median age ranging from 31 to 37 years [4,11,20], which is significantly earlier than the general population, where the median age is 53 years [20]. Moreover, the risk of developing thyroid cancer in CS as a second cancer is six times higher than in non-CS individuals [20]. Some of the youngest CS patients diagnosed with thyroid neoplasia were just 6 and 7 years old [4,36], underscoring the importance of considering CS in paediatric patients, especially in cases involving males and a history of benign manifestations [2,23,36].

The presence of benign thyroid disorders in CS significantly increases the risk of developing thyroid neoplasia. In adult CS patients, the presence of these benign disorders contributes to a higher risk compared to the general population and accounts for 55.6% of thyroid neoplasia cases in CS patients [16].

Notably, CS can result from mutations in various genes. Interestingly, patients with mutations in the SDHx gene exhibit a higher prevalence of thyroid cancer than those with PTEN mutations, although the prevalence is lower when patients have mutations in both SDHx and PTEN genes [42].

2.6. Renal Disorders

Kidney complications have a recognized association with CS [3,47]. The literature reports both benign manifestations, such as renal cysts [22], and malignant ones, notably renal cell carcinoma (RCC) [15,29]. RCC is considered a minor diagnostic criterion for CS [3,29], and in the general population, the risk of developing RCC is approximately 1.6% [2,23,42]. However, in CS patients, the lifetime risk is significantly elevated, with estimates ranging from 34% to 35% [4,24,29]. A recent study reported a risk of 33.6% [27]. Nevertheless, some articles present a wide 95% confidence interval of 2% to 34% [2,20,23,42], underscoring the need for further research. In this context, individuals with CS have an elevated risk, approximately 30 times higher than non-CS individuals [20].

The median age for RCC diagnosis in CS patients is reported to be around 49 to 55 years [20]. However, some studies suggest a median age of 39 years [29], signifying a noteworthy 10-year difference. This discrepancy necessitates additional investigations. Nevertheless, RCC tends to manifest earlier in CS patients compared to the general population, where the median age for RCC diagnosis is 68 years [20]. The risk of developing RCC begins to increase notably around the age of 40 [2], but cases of RCC have been documented at earlier ages.

Regarding the histology of RCC in CS patients, the literature identifies two predominant types [2,29]. The papillary type is more prevalent than the chromophobe type [2,4,24]. Notably, ultrasonography is not particularly sensitive in detecting RCC, especially when the mass is small, with CT or MRI preferred in these situations [2,23].

2.7. Nervous System Disorders

Cerebral abnormalities have been established as potential manifestations in individuals diagnosed with CS [42,48]. These abnormalities encompass Lhermitte-Duclos disease (LDD) and meningiomas, both of which have been documented in the literature as clinical features associated with this syndrome, although they may also manifest in non-syndromic cases [2,24,27,49]. Notably, gliomas have not been reported in CS patients [42].

LDD is a rare, benign condition characterized by the gradual hamartomatous overgrowth of the cerebellum, involving the expansion of neurons into the granular and molecular layers [2,11]. In adult individuals, the presence of LDD can raise suspicion of CS and is considered a major diagnostic criterion [2,11,24]. However, the association between paediatric CS patients and LDD is not as robust as it is in adults [24]. Radiologically, this

condition exhibits a distinct appearance often described as “tiger-striped” or “tigroid” [2,11]. The documented prevalence of LDD varies in the literature, with reports ranging from 1.8% to 6% [24]. Additionally, there are inconsistencies in the age of LDD diagnosis, with some studies suggesting onset between ages 20–39 or 30–49 [5,24,50]. However, cases of LDD have been reported in CS patients ranging from 4 to 75 years old [51,52]. Gender does not appear to influence the presence or absence of LDD, although these tumours tend to develop more frequently on the right side of the cerebellum [50]. Typically, LDD affects only one part of the cerebellum, but instances involving the vermis have also been described [11,27]. Patients with LDD may exhibit symptoms such as headaches, visual disturbances, nausea, increased intracranial pressure, ataxia, or cranial nerve palsies, with symptom manifestation dependent on the size of the cerebellar tumour mass [11,49,50,52]. These masses can remain stable over time or exhibit unpredictable rapid growth, and the sole treatment option is complete surgical removal to prevent potential recurrence [11].

Meningiomas have been described in individuals with CS, as mentioned earlier. However, given their relatively common occurrence in the general population, it remains unclear whether meningiomas are characteristic manifestations of CS [24,27]. The prevalence of meningiomas in CS individuals is approximately 8.25%, and there seems to be a gender-related aspect, with these tumours more commonly identified in male CS patients. In contrast, meningiomas are generally more prevalent in females in the non-CS population [27].

2.8. Vascular Disorders

CS patients exhibit numerous multifocal vascular disorders, specifically hamartomatous vascular malformations, as reported in the literature [24,27,53]. These manifestations encompass arteriovenous fistulas and haemangiomas, including cavernous haemangiomas, and can be observed in both paediatric and adult patients. They are considered minor diagnostic criteria for CS [4,11,24,54,55]. These vascular disorders have the potential to affect various organs, including the brains dura, skin, muscles, and adrenal glands [4,21,49,56,57]. Remarkably, one-third of mutant-PTEN patients present with vascular malformations [24]. Several studies report comparable findings in CS patients, with prevalence rates for vascular disorders ranging from 18% to 34% [11]. These figures are two to three times higher than the prevalence of such malformations in the general population, where the range is typically 5% to 10% [11]. Nevertheless, one study suggested that the prevalence of these malformations in CS patients is nearly equivalent to that in the general population, with rates ranging from 6.4% to 11.4% [21].

These malformations are believed to arise due to the influence of proteins encoded by PTEN, which regulate the function of vascular endothelial growth factor [11,27,58–60].

2.9. Neurodevelopmental and Autism Spectrum Disorders

In CS patients, neurodevelopmental disorders such as macrocephaly, developmental delay, and autism spectrum disorder (ASD) are prevalent manifestations from early childhood [11,23,40,61,62]. Studies indicate that individuals with ASD and macrocephaly have a notably increased likelihood of carrying a mutant PTEN gene, with 1 to 2 out of every 10 such cases linked to PTEN mutations [63,64]. While macrocephaly is recognized as a major diagnostic criterion, ASD is considered a minor criterion [11,41]. Neurodevelopmental features, including ASD, developmental delay, intellectual disability, and epilepsy, are observed in nearly all CS patients, with some presenting all these characteristics [23,29]. However, it is worth noting that certain studies report that the exact prevalence of these features remains undetermined [36].

Macrocephaly is highly prevalent, affecting 80% to 100% of CS patients [23,27]. This condition results from generalized megalencephaly, with the size of both neuron and glial cells contributing to this enlargement [65,66]. Some authors have noted the presence of nonspecific white matter abnormalities in the brains of CS patients, characterized by enlarged perivascular spaces. [36] Additionally, it has been observed that the severity of

macrocephaly in ASD patients with mutant PTEN is greater than in ASD patients with wild-type PTEN [23].

2.10. Immune Disorders

Immune disorders are reported in mutant PTEN patients [22]. However, these conditions are a distinct feature of CS patients, affecting anywhere from one-quarter to half of them [22,67]. These immune disorders encompass a range of manifestations, including lymphopenia, aberrant B and T cell homeostasis and function, hypogammaglobulinemia, and dysgammaglobulinemia [2,22,36]. These immune irregularities can result in various clinical presentations, such as colitis, thymic lymphoid hyperplasia leading to airway obstruction and gastric tube-related anaemia, vasculitis, Hashimoto thyroiditis, haemolytic anaemia, pulmonary cysts, and eosinophilic esophagitis [36,68–72]. Elevated blood levels of lactate and other inflammatory markers have also been observed in CS patients [22].

2.11. Metabolic Disorders

CS patients commonly exhibit a range of metabolic disorders, spanning from obesity to insulin dysregulation [73]. These individuals often display heightened insulin sensitivity, which contributes to the development of obesity and an elevated body mass index (BMI) [2,23,36]. Paradoxically, this heightened insulin sensitivity results in a substantially reduced risk of developing type 2 diabetes, while increasing the susceptibility to obesity and cancer [2,36]. Researchers have linked this phenomenon to the loss of inhibition on the PI3K-AKT signalling pathway [23].

3. Discussions

3.1. Overview of the CS Clinical Disorders

CS highlights a broad spectrum of clinical disorders as outlined previously. Table 1 provides a reference list of the aforementioned manifestations. For deeper insights into their development risks, prevalences, and other factors, we advise referring to the preceding text; the table serves merely as a concise overview of the manifestations documented in existing CS literature.

Table 1. CS manifestations encountered in the literature.

Affected Organ/System	Disorders Encountered	Affected Organ/System	Disorders Encountered
Breast disorders	<ul style="list-style-type: none"> - Breast cancer - Fibrocystic breast disease 	Haematological and vascular disorders	<ul style="list-style-type: none"> - Arteriovenous fistulas - Haemangiomas (e.g., cavernous ones) - Vasculitis - Gastric tube-related anaemia - Haemolytic anaemia
Endometrial disorders	<ul style="list-style-type: none"> - Endometrial cancer - Uterine fibroids 	Immune disorders	<ul style="list-style-type: none"> - Lymphopenia - Aberrant B and T cell homeostasis and function (e.g., thymic lymphoid hyperplasia) - Hypogammaglobulinemia - Dysgammaglobulinemia

Table 1. *Cont.*

Affected Organ/System	Disorders Encountered	Affected Organ/System	Disorders Encountered
Gastrointestinal disorders	<ul style="list-style-type: none"> - Colon cancer - Colorectal polyposis (different histologies) - Papillomatosis (buccal, lingual, gingival, labial mucosa, and pharynx) - Oesophageal glycogenic acanthosis - Gastric cancer - Colitis - Eosinophilic esophagitis 	Neurodevelopment disorders	<ul style="list-style-type: none"> - Macrocephaly - Developmental delay - Autism spectrum disorders - Intellectual disability - Epilepsy
Dermatological disorders	<ul style="list-style-type: none"> - Melanoma - Papillomatosis - Oral fibromas - Acral keratosis - Lipomas - Trichilemmomas - Penile freckling - Neuromas - Sclerotic fibromas - Café-au-lait spots - Vitiligo - Acrochordons - Acanthosis nigricans 	Respiratory disorders	<ul style="list-style-type: none"> - Airway obstruction due to thymic lymphoid hyperplasia - Pulmonary cysts - Papillomatosis (larynx)
Thyroid disorders	<ul style="list-style-type: none"> - Multinodular follicular adenomas - Nodular hyperplasia - Adenomatous nodules - Hashimoto thyroiditis - Lymphocytic thyroiditis - C cell hyperplasia - Goitre - Epithelial thyroid cancer (follicular and papillary types) 	Metabolic disorders	<ul style="list-style-type: none"> - Obesity - Insulin dysregulation - Elevated blood levels of lactate and inflammatory markers
Renal disorders	<ul style="list-style-type: none"> - Renal cysts - Renal cell carcinoma 	Nervous system disorders	<ul style="list-style-type: none"> - Lhermitte–Duclos disease - Meningiomas

It is crucial to note that not all manifestations listed above serve as diagnostic criteria for CS. Therefore, please consult the subsequent section, where the accepted major and minor criteria for diagnosing PHTS/CS are described.

3.2. Diagnostic criteria for PTHS/CS

Over time, the diagnostic standards for CS have changed. To make the diagnosis of CS easier, the International Cowden Consortium Criteria were created in 1996. To help doctors identify patients with CS, these criteria listed four major and eight minor criteria (Table 2) [11]. A diagnosis of CS is verified using the 1996 criteria if an individual meets one of the following conditions: adult LDD; a particular number of mucocutaneous features; macrocephaly combined with another major criterion; one major criterion plus three minor criteria; or the presence of four minor criteria. Additionally, patients must meet the requirement of two key criteria to be diagnosed with CS, one of which must be macrocephaly or Lhermitte–Duclos disease [11,24].

Table 2. International Cowden Syndrome Consortium Criteria for PHTS/CS.

Major Criteria	Minor Criteria
Lhermitte–Duclos disease	Genitourinary tumours (RCC) or malformations
Thyroid cancer	Lipomas
Macrocephaly	Fibromas
Breast cancer	Mental retardation
	Fibrocystic disease of the breast
	Gastrointestinal hamartomas
	Other thyroid lesions, such as goitre
	Mucocutaneous lesions or palmoplantar keratosis can meet the criteria for CS alone if six or more lesions are present

The initial criteria were revised in 2013 because of several changes that researchers put into place, adding new components to both the major and minor criteria. Novel criteria were presented, including macular pigmentation of the glans penis and epithelial endometrial cancer as major criterion, and vascular anomalies or malformations, autistic disorder, colon cancer, oesophageal glycogenic acanthoses, testicular lipomatosis, and oesophageal glycogenic acanthoses as minor criteria. Additionally, certain criteria were elevated from the minor criteria to the major criteria category, such as mucocutaneous lesions and gastrointestinal hamartomas (Tables 2 and 3).

Table 3. NCCN Criteria for PHTS/CS, version 3.2024.

Major Criteria	Minor Criteria
Breast cancer	Autism disorder
Endometrial cancer (epithelial)	Colon cancer
Thyroid cancer (follicular)	Oesophageal glycogenic acanthoses
Gastrointestinal hamartomas (includes ganglioneuromas but excludes hyperplastic polyps, ≥ 3)	Lipomas
Lhermitte–Duclos disease (adult)	Intellectual disability ($IQ \leq 75$)
Macrocephaly (≥ 97 th percentile)	Renal cell carcinoma
Macular pigmentation of the glans penis	Testicular lipomatosis
Multiple mucocutaneous lesions (any of the following):	Thyroid cancer (papillary or follicular variant of papillary)
- Multiple trichilemmomas (≥ 3 , at least one biopsy-proven)	
- Acral keratosis (≥ 3 palmoplantar keratotic pits and/or acral hyperkeratotic papules)	Thyroid structural lesions (e.g., adenoma, multinodular goiter)
- Mucocutaneous neuromas (≥ 3)	
- Oral papillomas (particularly on tongue and gingiva), ≥ 3 , or at least one biopsy-proven or dermatologist-diagnosed	Vascular anomalies or malformations (including multiple developmental venous anomalies)

The National Comprehensive Cancer Network (NCCN) has embraced these updated criteria, incorporating the revised PTEN hamartoma syndrome clinical criteria as the

foundation for its version 3.2024 CS/PTEN hamartoma syndrome management guidelines (Table 3).

Individuals or families whose one member satisfies the updated criteria or has a PTEN mutation can be diagnosed using these new criteria. For a person to be diagnosed with CS in this situation, they must meet at least three major criteria, with one of them being one of the following: macrocephaly, gastrointestinal hamartomas, or Lhermitte–Duclos illness OR present two major and three minor criteria (Table 3). It is necessary to have the presence of any two major criteria, with or without minor criteria, OR one major and two minor criteria, OR three minor criteria (Table 3) for a diagnosis in families where one member meets the revised PHTS clinical diagnostic criteria or has a PTEN mutation [24].

3.3. Surveillance Cancer Guidelines for Patients with PHTS/CS

As mentioned earlier, individuals diagnosed with CS face a heightened risk of developing cancers at a younger age than the general population. Consequently, diligent surveillance of these patients is crucial for early detection and treatment of malignancies. To address this need, the European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS) and NCCN have developed cancer surveillance guidelines specifically tailored for CS patients (Tables 4 and 5) [74–76].

Table 4. ERN GENTURIS Cancer Surveillance Guidelines for individuals with PHTS/CS.

Cancer	Surveillance Method	Interval	FROM AGE
Breast	MRI	Annually	30 years
	Mammography	Every 2 years	40 years
Thyroid	Ultrasound	Annually	18 years ¹
Renal	Ultrasound	Every 2 years	40 years
Colorectal	Baseline colonoscopy	-	35–40 years
Melanoma	Baseline skin examination ²	-	30 years
Endometrial ³	Not recommended	-	-

¹ Moderate evidence for the age of commencement of surveillance. ² Consider further surveillance as required.

³ Consider surveillance as part of clinical trials.

Both sets of cancer surveillance guidelines focus on the same six cancers: breast, thyroid, renal, colorectal, melanoma, and endometrial. Setting aside this commonality, the two guidelines exhibit variations in their recommendations regarding the approach to supervision.

Therefore, concerning breast cancer, NCCN suggests commencing screening at 18 years of age with self-examination, followed by clinical evaluations starting at 25, or earlier if familial history warrants. It is notable that NCCN advises initiating mammography and MRI by age 30, a decade earlier than ERN GENTURIS recommends for mammography. Additionally, the variance in timing for these diagnostic examinations to be performed merits acknowledgement.

Another significant contrast in the initiation age for surveillance is observed in thyroid monitoring. NCCN advises beginning surveillance at age 7, whereas ERN GENTURIS advocates for commencement from age 18.

Regarding melanoma, colorectal, and renal cancers, both consortia offer largely similar recommendations. Minor disparities exist in the commencement age for colon cancer follow-up, with NCCN emphasizing the significance of familial history once more. It is noteworthy that for melanoma, ERN GENTURIS suggests initiating dermatology consultations at age 30, whereas NCCN does not specify a particular age, leaving the timing to the discretion of individual cases.

Table 5. NCCN Cancer Surveillance Guidelines Version 3.2024 for individuals with PHTS/CS.

Cancer	Surveillance Method	Interval	FROM AGE
Breast	Self-examination	Monthly	18 years
	Clinical examination	Semiannually	25 years OR 5–10 years earlier than the earliest known breast cancer in the family (whichever comes first)
	MRI and mammography	Annually	30 years OR 10 years earlier than the earliest known breast cancer in the family (whichever comes first)
Thyroid	Ultrasound	Annually	7 years
Renal	Ultrasound	Annually or every 2 years	40 years
Colorectal	Baseline colonoscopy	Every 5 years	35 years OR 5–10 years earlier than the earliest known colorectal cancer in the family (whichever comes first)
Melanoma	Baseline skin examination	Annually	-
Endometrial	Patient education regarding the symptoms and the evaluation of these symptoms should include an endometrial biopsy.	-	35 years
	Endometrial biopsy	Annually or every 2 years	

Discrepancies emerge when comparing the two guidelines, particularly regarding endometrial cancer surveillance. ERN GENTURIS does not advocate for specific surveillance measures, delegating the monitoring of this cancer to physicians during clinical assessments. Conversely, NCCN recommends educating patients to recognize signs of endometrial cancer, prompting them to seek medical attention, followed by a biopsy if necessary. Additionally, NCCN suggests conducting annual or biennial biopsies starting at age 35, emphasizing the importance of proactive screening rather than relying solely on patient recognition of warning signs.

While the visions of the two consortia may not align perfectly, it is essential to recognize that both emphasize the importance of proactive surveillance for CS patients. Despite any differences, the overarching goal is to raise awareness and address serious pathologies associated with this condition, with the potential for significant positive outcomes.

While ERN GENTURIS and NCCN guidelines primarily address monitoring the mentioned pathologies, physicians should also prioritize surveillance of other conditions linked to CS (Table 1). The decision regarding their supervision plan should be left to the discretion of physicians overseeing CS patients, who should evaluate risks and benefits on a case-by-case basis.

3.4. Screening and Psychological Implications of CS

The goal of CS cancer surveillance is to identify cancer early to provide better curative treatments. A multidisciplinary approach and patient commitment are required by recommendations [74]. The possibility of developing numerous cancers emphasises the necessity of ongoing surveillance, even when the illness carries a low financial cost to health [74]. For those who are 50% at risk, genetic testing is advocated, and for those who have been diagnosed, personalised surveillance is advised based on personal and family history [36]. But baseline risk evidence quality is judged inadequate, emphasising the necessity of worldwide and national registries for prospective data collecting [74]. Understanding cancer risk factors, individualised risk assessments, prophylactic medications, and customised treatments should be the main goals of research. Information groups play a crucial role in patient education, which is essential for early detection and prevention [36]. Liquid biopsy technologies and other new surveillance techniques are being investigated, highlighting

the significance of continuous assessment and international partnerships for improved care and research prospects in this high-risk group [74].

Approximately two-thirds of patients who are suffering from hereditary cancer syndromes like CS express severe difficulty, and they frequently have high levels of worry, sadness, and distress. Female patients with cancer are especially vulnerable to depression. Among them, anxiety and depression are linked to factors like age, unemployment, and previous treatment. It is advised that distress screening be included in national guidelines. Raising awareness of the psychological challenges experienced by patients and their families is essential as genetic diagnostics develop [75].

4. Conclusions

CS is a disorder caused by mutations in the PTEN gene, which functions as a tumour suppressor gene. Mutations in this gene disrupt the normal regulation of cell growth and division, generating a wide array of clinical presentations and giving rise to diverse manifestations that span from benign to malignant, affecting nearly every system within the human body, particularly the skin, the mucous membranes, the breast, the thyroid, the gastrointestinal tract, and the central nervous system. Management of CS involves regular surveillance and screening for associated malignancies, with the multidisciplinary team playing a major role.

Genetic counselling and testing are recommended in case of a suggestive family history to assess the risk and provide appropriate guidance, as early detection and comprehensive management strategies are crucial in improving outcomes and quality of life for patients with CS.

This syndrome is considered rare, and its precise incidence remains unknown among other important factors, which affects individuals experiencing challenges in obtaining an accurate diagnosis and accessing appropriate medical care.

We urge the academic community to persist in researching all aspects related to CS/PHTS. The literature is notably inconsistent in reporting the frequencies and occurrences of the disorders, as mentioned above, adding an element of bias and uncertainty when looking back at the available research. With this review, our aim is to highlight the varied presentations seen in CS patients, along with the absence of standardized data regarding CS.

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Review

The Impact of Chromosomal Mosaicisms on Prenatal Diagnosis and Genetic Counseling – A Narrative Review

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Abstract: Genetic disorders represent a high-impact diagnosis for both patients and their families. Prenatal screening methods and, when recommended, genetic testing allow parents to make informed decisions about the course a pregnancy is going to take. Although offering certainty about the potential evolution and prognosis of the pregnancy, and then the newborn, is usually not possible, genetic counseling can offer valuable insights into genetic disorders. Chromosomal mosaicisms are genetic anomalies that affect only some cell lines in either the fetus or the placenta or both. They can affect autosomal or heterosomal chromosomes, and they can be either numerical or structural. The prognosis seems to be more severe if the genetic alterations are accompanied by malformations visible in ultrasounds. Several genetic techniques can be used to diagnose certain mosaicisms, depending on their nature. A novel approach in prenatal care is non-invasive prenatal screening (NIPS), also known as non-invasive prenatal testing (NIPT), which, although it does not always have diagnostic value, can provide valuable information about potential genetic anomalies, especially numerical, with high sensitivity (Se).

Keywords: chromosomal mosaicism; prenatal diagnosis; genetic counseling; NIPS/NIPT



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1. Introduction

Prenatal diagnosis is a crucial aspect of modern obstetrics, allowing for the early detection of malformations and genetic disorders [1]. Prenatal genetic testing is indicated in cases of ultrasound or biochemical screening abnormalities, a family history of genetic disease, advanced maternal age, and in vitro fertilization (IVF) [2].

Chromosomal mosaicism, the presence of distinct cell lines with different chromosomal complements, can arise from errors in meiosis and/or mitosis [3]. Depending on which chromosome is affected, the mosaicism can be autosomal or heterosomal. The aneuploid cells can be present only in the fetus or only in the placenta or it can affect both, depending

on the differentiation stage at which the mosaicism occurs [4]. The most common form of chromosomal mosaicism involves monosomy X [5].

The detection and interpretation of chromosomal mosaicism in cytogenetic diagnostics can be challenging due to the presence of cultured artifacts, pseudomosaicisms, and other factors [6]. A range of techniques are used in the diagnosis of chromosomal mosaicism, each with its advantages and limitations. Karyotyping, chromosomal microarray analysis (CMA), and fluorescence in situ hybridization (FISH) are commonly used in prenatal diagnosis [5]. Next-generation sequencing (NGS) platforms, such as Miseq™ Veriseq and Ion Torrent Personal Genome Machine PGMTM ReproSeq, have been shown to accurately detect chromosomal mosaicisms and segmental aneuploidies in embryos preimplantation [7].

A range of samples can be analyzed for chromosomal mosaicism in prenatal diagnosis, including amniotic fluid, chorionic villi, and peripheral blood cells [8]. The samples can sometimes be contaminated with maternal tissue fragments, as well as blood or placental cells. When mosaicism is diagnosed using amniotic fluid, the relative proportion of abnormal amniotic cells cannot be precisely correlated with that in fetal tissues [9].

NIPT, or non-invasive prenatal testing, is a method for detecting fetal chromosomal abnormalities by analyzing cell-free fetal DNA, which represents 3–13% of the total circulating DNA in the maternal plasma. The fetal fraction is influenced by biological factors: it increases with gestational age and decreases with a higher maternal BMI and age but is not significantly affected by fetal aneuploidy [10–12]. It is a highly sensitive and specific screening tool with a low false positive rate, making it an attractive alternative to traditional serum screenings and invasive tests [13,14].

2. Autosomal Mosaicism

Prenatal genetic diagnosis and clinical management in cases of chromosomal mosaicism (CM) should be performed with caution because the level of mosaicism and the distribution patterns of abnormal cell lineages can be shifting and unpredictable. For these reasons, different specimens or testing methods should be analyzed and compared in order to formulate a clear overview of the current knowledge on this matter [14].

Zhang et al. [15] performed a single-center retrospective study to evaluate the occurrence and clinical importance of CM in prenatal genetic diagnosis using G-banding karyotyping and chromosomal microarray analysis (CMA). The frequency and clinical features of CM were analyzed in fetuses with and without phenotypic anomalies in ultrasounds by CMA and G-banding karyotyping. The results showed that the most common type of CM was mosaic autosomal trisomy (19.23%, 20/104), and its frequency was greater in fetuses presenting an unusual phenotype (28.85%, 15/52) compared to fetuses with a normal phenotype (9.62%, 5/52). The mosaic fractions were similar between cases with or without phenotypic abnormality based on the general classification or specimen sources. Conflicting mosaic results were obtained in 16 cases (15.38%, 16/104) from distinct specimens or using various testing methods [15].

Even though quantitative fluorescence polymerase chain reaction (QF-PCR) using short tandem repeat (STR) markers is specialized in detecting whole chromosome trisomies of chromosomes 13, 18, 21, X, and Y, partial deletion or chromosomal mosaicism may also be identified. Therefore, QF-PCR can facilitate the identification of deletions or duplications in STR loci, but additional analysis using next-generation sequencing (NGS) or CMA is mandatory to validate the diagnosis [16].

Trisomy 16 (T16) is frequently encountered in the case of first-trimester abortion. Since complete T16 is not compatible with life, the majority of cases are mosaic trisomies. In this context, non-invasive prenatal testing (NIPT) offers both a quick and prompt prenatal screening for chromosome abnormalities and therefore may guide pregnancy management. Peng et al. [17] conducted a retrospective assessment of 14 cases that presented a high risk of T16 by NIPT evaluation (Table 1). Out of all cases, 12 cases were identified as T16 and 2 cases as T16 mosaicism. Using invasive prenatal diagnosis (karyotyping and CMA), five true positive cases and nine false positive cases were confirmed. All nine false positive

case pregnancies were continued, and eight out of nine newborns presented low weights (less than 2.5 kg) at birth. There were also two premature deliveries. In conclusion, by combining both cytogenetic techniques and molecular methods, T16 mosaicism can be accurately identified [17].

Multiple genetic tests can be used in detecting fetal trisomy 9 mosaicism. Ma et al. [18] reported two cases of trisomy 9 mosaicism that were initially suggested by NIPT. An elevated level (from 42% to 50%) of mosaicism was obtained after karyotype analysis of the amniocytes in both cases. Uncultured amniocytes were analyzed by CMA, and no CNV was found, besides a large fragment loss of heterozygosity. The ultrasound findings revealed no phenotypic abnormalities except for small size for gestational age. In case 1, CMA and fluorescent in situ hybridization (FISH) performed on uncultured fetal cord blood confirmed trisomy 9 mosaicism. After comprehensive genetic counseling, the parents requested the termination of both pregnancies. Further molecular genetic tests of tissue samples from the aborted fetus and the placenta were performed. The tests revealed the presence of fetoplacental mosaicism. In various tissues, the levels of trisomy 9 mosaicism ranged from 76% to not present [18].

Trisomy 8 mosaicism (T8M), also called Warkany syndrome, represents a rare chromosomal disorder. Cell-free fetal DNA (cffDNA) screening was used to initially identify T8M in a 17-week pregnancy. Karyotype analysis, single-nucleotide polymorphism array (SNP-array), FISH, and BACs-on-Beads™ (Bobs™) assay were performed to evaluate the fetal sample and to confirm the diagnosis. After karyotyping, the cultured amniocytes, trisomy 8 was found in 1 of 73 metaphases. SNParray was performed further on cultured amniocytes and neonatal cord blood cells to reveal the existence of T8M. Interphase FISH carried out on native neonatal cord blood cells validated the T8M percentage as 10%. The Bobs™ assay supported the previous findings. The parents were informed about the possibility of fetal defects later on, but they decided to continue the pregnancy. At birth, the baby was normal. A follow-up was carried out at 3 years old. He had language retardation, facial asymmetry, and low-set ears and had experienced periodic fever [19].

When it comes to prenatal counseling for autosomal mosaicism, a clear understanding of cell-based chromosome tests' performance in contrast with CMA and DNA-based copy number variation sequencing (CNV-seq) analyses is necessary to obtain an accurate assessment of autosomal mosaicism levels, especially in detecting low-level mosaicism. In this regard, Ma et al. [4] conducted a retrospective analysis on 5367 pregnancies, and 72 fetuses presented with mosaic chromosomal aneuploidy, including 22 cases with autosomal mosaicism and 5 fetuses with large cryptic genomic rearrangements. Low-level mosaicism was identified in 13 out of 22 cases using CMA, while CNV-seq analyses identified mosaicism levels down to 5% in 19 of the 22 fetuses with autosomal mosaicism (Table 1). Moreover, in all 19 cases confirmed by CNV-seq, the percentages of trisomic cells for autosomal trisomy 21, 18, and 13 were in accordance with the karyotyping results. Nevertheless, the percentage of aneuploidy for cases 16, 17, 18, 19, and 22 was considerably lower in the culture samples in comparison with the uncultured ones. Although trisomy 8 was not identified by karyotyping methods in case 18, CNV-seq, along with CMA, showed 18% and 24% mosaic trisomy 8. Lastly, for cases 11, 20, and 21, the CMA and CNV-seq results were normal in the uncultured amniotic fluid cells, but karyotyping revealed mosaicism for trisomy 9, trisomy 21, and trisomy 20 in cultured amniotic fluid cells. Although CNV-seq proved superior in its effectiveness in identifying further and clinically relevant information concerning autosomal mosaicism compared with CMA, prenatal genetic evaluation of autosomal mosaicism remains a demanding process. According to Ma et al. [4], if karyotype analyses show low-level mosaic aneuploidy, the results should be corroborated with DNA-based tests, preferably CNV-seq, if possible [4].

Table 1. Genetic techniques and clinical impact in autosomal mosaicism cases [4,14,17].

Reference	No. of Cases	Tissue Sample	Analysis	Abnormal Type	Phenotype Severity
Ma et al. [4].	22 cases with autosomal aneuploidy mosaicism: 11 cases with T21 2 cases with T18 2 cases with T15 Other (T13, T9, T8, T22, T20, T2)	amniotic fluid or fetal cord blood	Karyotyping	Mosaicism (21 cases)	No information available
			CMA	Low-level mosaicism down to 20% (13 cases)	
			CNV-seq	Mosaicism level down to 5% (19 cases)	
Peng et al. [17]	14 cases with high risk of T16: 12 cases of T16 2 cases of T16 mosaicism	maternal blood	NIPT		
		amniotic fluid or cord blood	Karyotyping and CMA (following NIPT)	T16 mosaicism (five true positive cases)	Three cases had ultrasound abnormalities (a) Four fetuses died (induced labor, intrauterine death, or death after birth)
				Nine false positive cases	Eight babies had birth weights less than 2.5 kg (b) Two premature babies
Wang et al. [14]	Eight cases with fetal CR				
	Six NMI cases with no previous variants of TSC1/TSC2	umbilical cord and CR tissue	NGS and ddPCR	Low-level mosaic variants (four cases) (c) Somatic mosaic variants in the CR tissue (two cases) (d)	Five fetuses presented single tumors Three fetuses presented multiple tumors One fetus had tricuspid regurgitation
	Two cases suspected of familial gonadal mosaicism	umbilical cord, CR tissue, seminal fluid, and parental blood		TSC1/TSC2 gene variants (e)	

Abbreviations: T21, trisomy 21; T18, trisomy 18; T15, trisomy 15; T13, trisomy 13; T22, trisomy 22; T20, trisomy 20; T8, trisomy 8; T9, trisomy 9; T2, trisomy 2; CMA, chromosomal microarray analysis; CNV-seq, copy number variation sequencing; NIPT, non-invasive prenatal testing; CR, cardiac rhabdomyoma; NMI, no mutation identified; NGS, next-generation sequencing; ddPCR, droplet digital polymerase chain reaction. (a) Ultrasound examination of case 1 revealed intrauterine growth restriction, a persistent right umbilical vein, and abnormal umbilical blood flow. The newborn had a low birth weight (1.9 kg), and no other abnormalities were present. In case 2, the ultrasound examination showed inconsistency with the gestational age, small limbs, and a cardiac defect. Echocardiography revealed total abnormal pulmonary venous drainage, a ventricular septal defect, and a left aortic arch with the right descending aorta. The newborn died due to congenital heart disease 13 days after birth. In case 3, the ultrasound scan showed a butterfly vertebral anomaly in T3, and the pregnancy was terminated. (b) In newborn screening, one case presented with cerebral edema and anemia. The mother had preeclampsia. (c) The allelic frequencies were higher within the cardiac rhabdomyoma tissue than in the umbilical cord tissue. (d) The allelic frequencies were absent in the umbilical cord tissue (0%). (e) Both fathers had low-level mosaicism and presented gonadal mosaic variants. The allele frequencies of these variants in the seminal fluid were higher than 30%.

Liu et al. [20] investigated both the precision and depth evaluation of clinical CNV-seq, also known as low-pass genome sequencing (LP GS), in the identification of chromosomal mosaicism and copy number variants (CNVs). They demonstrated that the uniquely aligned high-quality reads (UAHRs) influenced the detection sensitivity of LP GS for CNVs and mosaic aneuploidies. Consequently, exactly 30 million UAHRs (single-end 35 bp) were suggested to detect mosaic aneuploidies and to identify the majority of mosaic CNVs with values over 1.48 Mb containing mosaicism levels greater than 30%. Therefore, they concluded that CNV size had an impact on the accuracy of LP GS in detecting CNVs and especially in identifying mosaic aneuploidies [20].

Concerning the prenatal detection of small supernumerary marker chromosomes (sSMCs), CMA and fluorescence in situ hybridization (FISH) have proven to be key instruments in discovering the origin and the genetic structure of sSMCs. Traditional cytogenetic methods, such as G-banding analysis, provide scarce information regarding the origin of sSMCs. Sun et al. [21] presented the case of an sSMC(15) female fetus who inherited the mosaic sSMC(15) from her mother. G-banding analysis was performed through amniocentesis, and the karyotype of the fetus was 47,XX,+mar/46,XX. Through molecular genetic tests in both the mother and the fetus, the sSMC was identified as *inv dup(15) (D15Z1+, SNRPN-, PML-)*, which proved the maternal inheritance of sSMC(15). The female infant was born with no phenotypic signs of abnormality at 39 weeks of gestational age. Therefore, when it comes to prenatal sSMCs cases, prenatal genetic counseling can be performed effectively using molecular genetic techniques [21].

Prenatal genetic counseling in cases of de novo balanced reciprocal translocation continues to be challenging. Chen et al. [22] reported the incidental identification of a familial 8p23.2 microduplication including *CSMD1* correlated with a karyotype of 46,XY,t(7;8)(q31.2;p23.1)/46,XY diagnosed by amniocentesis in a normally evolving pregnancy. The male baby had no phenotypical modifications at birth. A follow-up checkup was performed at the age of six months, and the infant presented no phenotypic or developmental abnormalities [22].

Although Optical Genome Mapping (OGM) is recognized for its ability to detect chromosomal anomalies, various aberrations are not detected by OGM. In this regard, a study has been conducted aiming to identify the aberrations overlooked by OGM and evaluate the factors involved. The results showed that OGM may miss structural variations (SVs) including CNVs, balanced translocations, and inversions that have breakpoints placed in large repetitive sequences, apart from Robertsonian translocations. According to the authors, GRCh38 is considered the appropriate option as the reference genome when OGM genome assembly is used. In certain circumstances, different genetic techniques used in combination with OGM may improve the detection rate of the method [23].

One study assessed eight fetuses with fetal cardiac rhabdomyoma (CR) and their families. Six fetuses out of eight had not previously presented any *TSC1/TSC2* variants, and the other two families were suspected of gonadal mosaicism. NGS was performed using tissue from the umbilical cord, CR tissue, and parental blood. All positive results, including two paternal semen samples, were confirmed using droplet digital polymerase chain reaction (ddPCR). The results showed that the fetuses carried low-level somatic mosaic variants, and the CR tissue obtained from one fetus presented a second-hit variant (Table 1). Thus, hybrid capture NGS attained by associating NGS with ddPCR improved the accuracy of prenatal tuberous sclerosis complex (TSC) diagnosis [24].

Although there are only a few cases of mosaic trisomy 12 reported prenatally and postnatally in the literature, prenatal genetic diagnosis of this mosaic aneuploidy can be of great importance [25]. Bonasoni et al. [26] presented a case of mosaic trisomy 12 discovered by amniocentesis, associated with a negative prenatal ultrasound. The family decided to terminate the pregnancy at 22 weeks of gestational age. Postmortem, the fetus displayed non-lethal morphological characteristics not reported previously in the literature. Although the fetal ultrasound may be normal, prenatal genetic counseling should focus on identifying

minor abnormalities and the widespread presence of trisomic cell lines in different internal organs, especially in cases of advanced maternal age [26].

3. Heterosomal Mosaicism

Sex chromosome abnormalities range from 0.5 to 0.71% of all prenatal diagnoses and can cause fetal gonadal dysgenesis and structural and functional abnormalities of other organs, as well as intellectual disability of varying severity [27,28].

Compared to prenatal diagnosis of the common trisomies (21, 18, and 13), the diagnosis of sex chromosome aneuploidies (SCAs), excluding 45,X0 and its mosaic variants, remains problematic due to the lack of clear clinical and ultrasound abnormalities [29,30].

In a large study comprising 17,428 singleton pregnancies with no structural abnormalities seen in fetal ultrasounds that underwent non-invasive prenatal testing (NIPT), 202 samples were positive for chromosomal anomalies. Out of these, 91 samples showed positive results for SCAs. Most cases were represented by the following chromosomal formulas: 45,X0 (41), followed by 47,XXY (17), mosaic SCAs (12), 47,XXX (11), and lastly 47,XYY (10). As a confirmation procedure, amniocentesis was performed in 78 of the 91 SCA cases, showing there were only 38.46% true positive SCAs. The most frequent true positive rate was seen for mosaic sex chromosome aneuploidies (83.33%), followed by 47,XYY (57.14%), 47,XXY (37.50%), 47,XXX (36.36%), and 45,X0 (28.95%) [31].

Xu et al. [32] conducted a study including 32,931 women with singleton pregnancies who underwent NIPT screening, out of which 140 results were positive for SCAs. Prenatal diagnosis through amniocentesis or cordocentesis was performed in 103 cases. The general PPV (positive predictive value) for NIPT in SCAs was 55.34%, with the individual PPVs ranging from 85% for 47,XXX and 85% for 47,XXY to 68.75% for 47,XYY and only 26.09% for 45,X0. The authors concluded that NIPT should only be used as a screening method, and SCAs observed on NIPT only should not be used as an indication for pregnancy termination [32]. Similar results were obtained by Yung et al. [33], showing a 50% PPV for positive SCA cases observed by NIPT in 170 out of 238 high-risk cases (47,890 total cases). The individual PPV was 70.58% for 47,XXX, 81.13% for 47,XYY/47,XXY, and 30% for 45,X0 [33].

In a study conducted by Reiss et al. [34], NIPT had a false positive rate of 91% (10/11 cases) and missed 4 true positive cases, all with cystic hygroma [34]. Suboptimal cell culture and fetoplacental and maternal mosaicism have been consistently reported as error sources in SCA detection [29,35,36].

Sun et al. [37] examined 3387 cases of pregnant women through cordocentesis, all of whom were considered at high risk of chromosomal abnormalities. A total of 182 abnormal karyotypes were identified, of which 37 cases were SCAs. A total of 16 of the 37 cases were sex chromosome mosaïcisms. Equally, 4 out of 16 pregnant patients chose to continue the pregnancy, delivering 3 phenotypically normal children and 1 with ambiguous genitalia (46,X,i(Y)(q10) [20]/45,X [6]). Considering this outcome, the authors indicated that sex chromosome mosaïcism should not be taken as a clear indication of pregnancy termination [37].

To differentiate between true SCA mosaïcism and pseudomosaïcism, novel techniques are being developed. Fan et al. [38] used segmental duplication quantitative fluorescent PCR (SD-QF-PCR) to show true mosaïcism and mosaic proportions in 20 control samples and 14 amniotic fluid mosaic samples previously validated by first- and second-line karyotype analysis. Among the 14 mosaic samples, the numbers of samples showing true mosaïcism and pseudomosaïcism detected by this method were 6 and 8, respectively. Compared to karyotyping and FISH alone, this method can also be used on tissues from the chorionic villus or fibroblasts from aborted fetuses or stillborn babies, which would otherwise take a long time to cultivate. Mosaïcism was completely detectable for proportions above 10%, but a larger sample size was considered necessary to validate the detection limit [38].

Zhang et al. [39] used BACs-on-Beads™ (BoBs) on 31 samples of amniotic fluid and umbilical cord blood from high-risk pregnancies that were already confirmed through karyotyping, single-nucleotide polymorphism microarray (SNP array), and copy number variation sequencing (CNV-seq) as cases of SCA mosaicism. Prenatal BoBs had a 74.2% sensitivity for detecting SCA mosaicism, with a detection limit of 6% [39].

Table 2 summarizes the positive predictive value of NIPT in detecting SCA.

Table 2. Total number, singular, and total PPV% obtained from cordocentesis of amniotic fluid for the most frequent SCAs [31–34,37].

Study	Cases Studied (nr.)	SCAs	45,X0	47,XXX	47,XXY	47,XYY	Mosaic SCAs	Other SCAs	Total PPV %
Dai et al. [31]	17,428	NIPT	41	11	17	10	12	-	38.46
		PPV (%)	28.59	36.36	37.50	57.14	83.33	-	
Xu et al. [32]	32,931	NIPT	62	29	28	20	0	One lower X case	55.34
		PPV (%)	26.09	85	85	68.75	-	0	
Yang et al. [33]	47,800	NIPT	137	27	47	-	-	-	50.00
		PPV (%)	30.00	70.58	81.13	-	-	-	
Reiss et al. [34]	2,851	NIPT	11	5	2	0	0	0	44
		PPV (%)	9	100	100	-	-	-	
Sun et al. [37]	3,387	Cordocentesis	2	5	7	6	19	48,XXXX	

PPV – positive predictive value. * While for other SCAs the prediction was more accurate, the number of false positives for monosomy X was high (91%).

4. Structural Chromosomal Mosaicisms

Structural chromosomal mosaicisms, which represent only a fraction of all chromosomal mosaicisms, can be identified by various methods, including karyotyping, chromosome microarray, SNP array, BACs-on-Beads (BoBs) assay, and multicolor FISH [40].

The use of the QF-PCR technique was also attempted, but it can only detect mosaicism when the abnormal cell line represents at least 10% of the whole sample and cannot detect inversions, translocations, or deleted/duplicated regions that are not within the STR marker range. A case study showed that the QF-PCR technique successfully identified the loss of the Yq11.2 region, subsequently confirmed by two additional techniques. Chromosome microarray analysis detected a 10.1 Mb deletion and a 16 Mb mosaic deletion (representing a mosaicism proportion of 20%) [16].

SNP array can provide important characteristics of complex mosaicisms, such as their content, origin, and mechanism. These details are significant for precise prenatal prognostic assessment and genetic counseling. A retrospective analysis of SNP array testing on 4512 prenatal diagnosis samples identified 15 cases of mosaic segmental duplication/deletion, involving both autosomal chromosomes and sex chromosomes. All of the cases resulted in intrauterine fetal death [41].

According to a study that included a total of 1409 pregnant women at high risk of chromosomal aberrations, the BoBs technique has a reduced detection rate for mosaicisms compared with karyotype analysis. More specifically, from a total of 6 cases of chromosomal mosaicism, 4 of them were missed by BoBs assay (including a case of a balanced translocation mosaicism) [42].

Among cytogenetic techniques, multicolor FISH (M-FISH) can detect mosaic complex chromosomal rearrangements when molecular techniques (MLPA, array-CGH) fail to give a conclusive result. This was the case in a clinical report that described the application of the M-FISH technique to finding the additional segment on chromosome 4 in a case of very low-level mosaicism for the cell line with the der(4)t(4;17) chromosome (5%). Only M-FISH

led to the identification of the translocated DNA fragment, corresponding to additional chromosome 17q material on chromosome 4q [43].

Most cases of structural chromosomal mosaicisms detected prenatally have been described as deletions or duplications, as well as translocations at a lower frequency. A recent study using multiple techniques for the prenatal detection of chromosomal aberrations described a structural anomaly, which was found not to influence fetal development, most likely because the mosaicism was distinguished as a distal 5 p deletion in a single colony of amniocytes [44].

22q11.2 deletion syndrome (22q11.2DS) refers to a syndrome that results from the deletion of the 22q11.2 region, affecting approximately 1 in every 4000 to 6000 newborns and 1 in every 1000 unselected fetuses [43]. A rare case of 22q11 deletion syndrome (DS) was reported in China, where a healthy female with a history of two pregnancies with conotruncal defects was recruited in a clinical study. The mother had nearly none of the clinical characteristics that are related to 22q11.2 DS, except for a few insignificant abnormal results in her research facility examinations, such as hypocalcemia (2.21 mmol/L), hypophosphatemia (0.99 mmol/L), and a moderately low percentage of CD4+ T helper cells (percentage: 23.86%, absolute counts: 404.36 μ L). It was supposed that the cell load with 22q11.2 deletion within the gonads may be higher than that in the blood. Performing interphase FISH, the results for the mother showed that 164 (82%) of her analyzed cells had normal signals and 36 (18%) of the cells presented a deletion of one copy of the 22q11.2 region. To confirm the 22q11.2DS mosaicism of the mother, metaphase FISH was performed, and hemizygous deletion signals were shown in more than 10% of the cells. The same structural chromosomal defect was also identified in the fetus, along with the presence of tetralogy of Fallot (ToF) and right renal agenesis, which was suggestive of multiple malformations. The pregnancy was interrupted, and labor was induced at approximately 28 weeks of gestation at the couple's will [45].

A false positive result has proven to be a significant finding as part of the Dutch TRIDENT study. Using genome-wide non-invasive prenatal screening (NIPS), the research team detected a 20-megabase specific deletion starting at 10q25 in eight pregnancies. Since all the deletions started close to the *FRA10B* fragile site in 10q25, they investigated whether the pregnant women were indeed carriers of *FRA10B*. To confirm the presence of the deletion in the fetus, routine array analysis was performed on DNA isolated from amniotic fluid in four cases, but the deletion could not be confirmed in any of them. Therefore, they concluded that the result could have been caused by a maternal low-level mosaic deletion associated with *FRA10B* expansions, with no consequences for fetuses [46].

Taiwanese Journal of Obstetrics & Gynecology published a case of a chromosomal deletion involving the *NBEAP1* and *POTEB* genes located on chromosome 15 (15q11.1-q11.2), associated with diffuse lymphangiomatosis. Because of abnormal fetal ultrasound findings, a 33-year-old woman underwent amniocentesis at 22 weeks of gestation. Karyotyping revealed 46,XX,del(15)(q11.1q11.2)/46,XX mosaicism. The parents decided to have an abortion; therefore, it was not possible to obtain additional data on the evolution of the pregnancy [47].

Multiple Xq duplication syndromes have been reported, such as Opitz-Kaveggia syndrome, FG syndrome 5, Xq25 duplication syndrome, Xq26.3 duplication syndrome, Xq27.3-q28 duplication syndrome, and Xq28 duplication syndrome. A varying degree of intellectual disability or organic dysfunction characterizes each of these syndromes, but it was noted that a mosaic Xq duplication with the following chromosomal formula – 46,X,der(X)dup(X)(q22.1q22.2)dup(X)(q25q22.3)/46,XX – may lead to pregnancies with a favorable outcome. Such was the case for a 40-year-old woman who underwent amniocentesis at 16 weeks of gestation due to her advanced maternal age. As a result of the culture artifacts, the in vitro culture process for the amniocytes caused an overestimated mosaic level, but the parents decided to continue the pregnancy, and a healthy female baby was delivered at 39 weeks of gestation [48].

Another case of structural mosaicism with a favorable outcome was reported as an association between a familial 8p23.2 microduplication encompassing the *CSMD1* gene and a balanced reciprocal translocation. A 38-year-old pregnant woman, with no phenotypical changes, underwent amniocentesis at 19 weeks of gestation due to her advanced maternal age. Amniocentesis revealed a karyotype of 46,XY,t(7;8)(q31.2;p23.1)/46,XY. It has been suggested that array comparative genomic hybridization (aCGH) is advisable in all carriers of balanced complex chromosomal rearrangements. aCGH analysis on the DNA extracted from both cultured amniocytes and parental blood cells revealed a 2.178 Mb 8p23.2 microduplication encompassing *CSMD1* in the fetus and the mother. At 38 weeks of gestation, a healthy male baby was delivered, with no developmental delays by the age of 6 months. All the analyzed cells in the cord blood, umbilical cord, and placenta had the karyotype of 46,XY [22].

A false positive result upon non-invasive prenatal testing due to a maternal structural chromosomal aberration has also been reported for duplications. A 37-year-old woman underwent amniocentesis at 19 weeks of pregnancy to ensure that fetal development was not affected by her advanced maternal age. Amniocentesis showed a karyotype of 46,XY. Simultaneous array comparative genomic hybridization (aCGH) revealed a 1.3 Mb duplication of 17p12. The mother did not have any phenotypical findings, but she also turned out to be a carrier of the same 17p12 microduplication. Prenatal ultrasounds found no anomalies, and the parents decided not to terminate the pregnancy [49].

Structural chromosomal mosaicisms involving translocations have been less frequently described in clinical studies. Prenatal diagnosis and postnatal follow-up in a case of mosaicism for a Robertsonian jumping translocation showed a favorable fetal outcome. Jumping translocations refer to a rare type of mosaicism in which the same chromosomal segment is translocated to different chromosomes in different cell lines. In this case, the newborn was a phenotypically normal male. The result of the first amniocentesis performed during the pregnancy was 45,XY,der(15;22)(q10;q10)/46,XY,i(15)(q10)/46,XY. The maternal karyotype was 45,XX,der(15;22)(q10;q10), and the paternal karyotype was 46,XY. The mother was referred for genetic counseling, and repeated amniocentesis performed at 23 weeks of gestation revealed 45,XY,der(15;22)(q10;q10)mat/45,XY,-22. Fluorescence in situ hybridization (FISH) analysis using a chromosome 15q-specific probe and a chromosome 22q-specific probe detected three 15q signals in 4/104 cells (3.8%), encompassing a trisomy. She was advised to continue the pregnancy, and it proved to be a case of transient mosaicism for a Robertsonian jumping translocation associated with a familial Robertsonian translocation, inherited from the mother by the child [50].

More information about the most common structural anomalies detected in mosaic forms can be found in Table 3.

Table 3. Most common structural anomalies associated with mosaicism in prenatal diagnosis [45–47,51–53].

Structural Anomaly	22q11.2 Microdeletion	10qter Deletion	15q11.2 Deletion
Frequency	1 in 4000–6000 newborns, 1 in 1000 fetuses	1 in 1263	57–127 in 10,000
Type of tissue	amniotic fluid peripheral blood	amniotic fluid placental biopsies umbilical cord and blood maternal blood lymphocytes	amniotic fluid placental tissue umbilical cord maternal peripheral blood paternal peripheral blood
Type of test	amniocentesis fluorescence in situ hybridization (FISH) chromosomal microarray analysis	amniocentesis fluorescence in situ hybridization (FISH) array comparative genomic hybridization	amniocentesis array comparative genomic hybridization
Clinical implications	cardiovascular anomalies, immunodeficiency, endocrine abnormalities, renal abnormalities, developmental delays, and behavioral and mental disorders	facial dysmorphism, pre- and postnatal growth retardation, cardiac and genital anomalies, and developmental delay	neuropsychiatric or neurodevelopmental disorders dysmorphic features may be associated with diffuse lymphangiomatosis when involving the NBEAP1 and POTE genes

5. Confined Placental Mosaicism

Confined placental mosaicism (CPM) is defined by the presence of two or more distinct cell lines with different chromosomal compositions within the placenta while the fetus maintains a uniform chromosomal formula. This can lead to complications in interpreting prenatal screening results, as the placental cells might not accurately represent the fetal genetic status. [54–56].

CPM typically arises early in embryonic development. Its causes are not fully understood, but there are several mechanisms by which it might occur, as summarized in Table 4:

Table 4. Possible causes of confined placental mosaicism [57,58].

Postzygotic Mutation	A mutation occurring in one of the early cell divisions can lead to two distinct cell lines: one with a normal set of chromosomes and one with an abnormal set. If the abnormal cells are confined to the placenta, CPM occurs.
Trisomy Rescue	Initially, the embryo might have three copies of a particular chromosome (trisomy). During subsequent cell divisions, one of these extra chromosomes might be lost in some cells. If this loss occurs predominantly in the fetal cell line, leaving the trisomic cells in the placenta, it results in CPM.
Placental Origin of Mutation	Sometimes, the mutation causing the chromosomal discrepancy occurs specifically in the trophoblast cells, which form the placenta, rather than in the cells destined to become the fetus. This leads to the placenta having a different chromosomal makeup than the fetus.

Confined placental mosaicism (CPM) can be categorized into three main subtypes based on the location and type of chromosomal abnormalities within the placenta. These subtypes are essential for understanding the implications and origins of the mosaicism and are summarized in Table 5:

Table 5. CPM subtypes [55–57].

Subtype	Affected Placental Layer	Pregnancy Outcome
Subtype 1	This subtype involves mosaicism confined to the cytotrophoblast layer of the placenta. In this type, abnormal cells are present only in the cytotrophoblast, which is the outer layer of the placenta that makes direct contact with maternal blood.	Subtype 1 CPM often has less impact on fetal development because the abnormal cells do not infiltrate into the chorionic villi where nutrient exchange primarily occurs.
Subtype 2	This subtype includes mosaicism confined to the mesenchymal core of the chorionic villi. Abnormal cells are found within the mesenchymal (connective tissue) core of the placental villi but not in the cytotrophoblast.	Subtype 2 CPM might have more significant implications for fetal development because it can affect the structural integrity and function of the placental villi, potentially leading to issues like intrauterine growth restriction (IUGR).
Subtype 3	This subtype involves mosaicism in both the cytotrophoblast and the mesenchymal core of the chorionic villi. Abnormal cells are present in both major compartments of the placenta, indicating a widespread distribution of mosaicism.	Subtype 3 CPM is generally considered the most severe form, with a higher likelihood of impacting fetal development and pregnancy outcomes due to the extensive involvement of the placenta.

The detection of CPM typically involves chorionic villus sampling (CVS), which includes short-term culture of the villi and long-term culture of the villi and non-invasive prenatal testing (NIPT)—a positive NIPT finding is followed by CVS or amniocentesis. Studies show that NIPT is more sensitive to CPM compared to CVS. [56,58–60].

Because CPM affects only the placental chromosomal formula and not the fetal chromosomal number, chromosomal analysis of placental cells might not match the true chromosomal status of the fetus.

There are three main outcomes of CVS testing:

1. True positive: Both the placenta and the fetus have the same chromosomal abnormality.
2. True negative: Both the placenta and the fetus have normal chromosomes.
3. False positive/negative: The placenta shows a chromosomal abnormality that is not present in the fetus, or vice versa, indicating CPM.

Understanding the subtypes of CPM is crucial for assessing the potential impact on pregnancy and fetal development. The location and extent of mosaicism within the placenta determine the severity and implications of the condition.

Clinical studies show conflicting findings when it comes to how CPM influences pregnancies. Most pregnancies associated with CPM are uneventful, but some studies show that CPM can lead to fetal growth restrictions (FGRs). The exact type of CPM influences the placental function—types 2 and 3 are considered to have significant implications for FGRs, low weight at birth, or preterm delivery, being associated with adverse pregnancy outcomes such as low levels of first-trimester serum pregnancy-associated plasma protein A, preterm births, and newborns small for gestational age [61]. CPM is more common in spontaneous abortions than in viable pregnancies, and it is associated with an increased frequency of second- and third-trimester pregnancy loss or intrauterine fetal growth retardation [58,60,61]. The placental genome plays an important role in its development and optimal function. In cases of CPM, placental development and maternal–fetal exchanges may be affected; however, the precise mechanisms by which the placental genome impacts fetal development remain unclear [62].

The effects of CPM on the fetal phenotype and intrauterine development depend on the chromosomes involved and the distribution of abnormal cells among tissues. The most published placental abnormality is trisomy 16, which can have significant implications for pregnancy outcomes and fetal development. Placental trisomy 16 can be associated with intrauterine growth restriction and preeclampsia, miscarriage, and stillbirth due to its severe impact on placental function [63].

Other abnormalities associated with higher-risk pregnancies are trisomies 2, 3, 7, 13, 15, and 22. If a high-risk CPM is diagnosed through genetic testing, clinicians are advised to perform regular ultrasounds and fetal growth assessments [58–65].

Besides its effects on fetal development, CPM can also lead to false positive or false negative results in prenatal screenings: a positive NIPT test can result from a CPM and also from a fetal chromosomal abnormality. Genetic counseling is crucial to interpret the results, understand the implications, and guide the decision-making process [40,62–65]. In cases of suspected CPM, CVS or amniocentesis is the next step for validating the NIPT results, confirming the fetal genotype, and distinguishing between true fetal abnormalities and confined placental mosaicism. NIPT result validation through CVS or amniocentesis ensures that expectant parents receive accurate information, enabling them to make well-informed decisions about their pregnancy and prepare for any necessary medical interventions. It is important to acknowledge that amniocentesis and CVS, being invasive procedures, carry a small risk of infection, bleeding, or even miscarriage. However, in the context of CPM, the benefits of obtaining a definitive diagnosis often outweigh the risks [57].

6. Discussion

The development of advanced techniques, such as high-throughput sequencing, has significantly improved the standards of prenatal diagnosis [1]. This is particularly important in the context of genetic disorders, where prenatal and preimplantation genetic diagnosis can provide valuable information and the opportunity to make informed decisions regarding the outcome of the pregnancy. Up to 5% of pregnancies are affected by genetic disorders [66]. Among the most common chromosomal disorders found in prenatal diagnosis are chromosomal mosaicisms, with an incidence ranging from 0.5% to 0.6% [5].

Mosaicisms can affect human development and can lead to genetic abnormalities, miscarriages, stillbirths, or live births with various malformations but also healthy newborns with no phenotypical modifications. The clinical consequences of mosaicism depend on the chromosome involved, as well as the timing and location of the error [67]. Somatic

mosaicism, a related concept, refers to the occurrence of two genetically distinct cell populations within an individual, derived from a postzygotic mutation. It has been found to be involved in various disorders, including cancer and neurodegenerative diseases [68]. The dynamic nature of chromosomal mosaicism throughout ontogeny and its association with human diseases highlight the need for further research in this area [69].

Chromosomal mosaicism in fetuses can lead to a range of outcomes, from severe microcephaly and growth deficiency to normal fetal growth and no evidence of intrauterine growth retardation. The prognosis appears to be more optimistic in cases without structural anomalies observed by ultrasound [5,70], although there have been cases reported with autosomal trisomy mosaicism showing that, when autopsied, the fetuses had several abnormalities that were not visible in the previous ultrasounds: facial dysmorphism, hypertelorism, intestinal malrotation, and partial anomalous pulmonary venous return [26]. However, the presence of mosaicism in embryos transferred during assisted reproductive technology (ART) can result in healthy babies, suggesting that the prognosis may vary depending on the specific chromosomal abnormalities and the extent of mosaicism [70]. An important factor for the phenotype is the mosaic fraction [15]. Further research is needed to fully understand the prognosis of fetuses with chromosomal mosaicism.

Confined placental mosaicism is a unique condition that underscores the complexity of prenatal genetics. Understanding the causes and implications of CPM is essential for accurate prenatal diagnosis and management. As prenatal testing technologies continue to advance, our ability to detect and interpret CPM will improve, ultimately enhancing the care and support provided to expectant parents [62–64].

While NIPT provides a high probability of detecting certain chromosomal abnormalities, it does not confirm them definitively. Positive results should be followed up with certain diagnostic tests, which implies obtaining fetal cells. Two primary methods for obtaining fetal cells for genetic analysis are amniocentesis and chorionic villi sampling (CVS). These procedures provide the material needed for various molecular genetic tests [71].

Amniocentesis can be performed between 15 and 20 weeks of pregnancy and involves collecting amniotic fluid, which contains fetal cells. These cells are cultured and then karyotyped. CVS is a diagnostic test that involves obtaining a small sample of placental tissue, which can provide more direct and detailed genetic information. CVS can be performed between 10 and 13 weeks of pregnancy, allowing for early diagnosis [72].

The genetic material obtained through amniocentesis or CVS can be analyzed using a variety of cytogenetic and molecular genetic tests. Each test has its specific applications, advantages, and limitations, and the choice of test depends on the clinical context and the specific information required (Table 6) [57,71–74].

In prenatal diagnosis, chromosomal karyotyping analysis and single-nucleotide polymorphism-based microarray (SNP array) are valuable for detecting chromosomal mosaicism in amniotic fluid samples, with FISH used for further verification [8]. The MrMosaic method, which uses deviations in the allele fraction and read coverage from next-generation sequencing data, has been developed to detect structural mosaic abnormalities [42]. High-resolution full-genome analysis methods, such as single-cell array-based comparative genomic hybridization, have been developed to address these challenges and have revealed significant fractions of cells with unique chromosomal abnormalities in human somatic and embryonic stem cell cultures [75].

Karyotyping plays a crucial role in the prenatal diagnosis of chromosomal mosaicism. For the past 50 years, it has been considered the golden standard for detecting chromosomal abnormalities [4]. It has been found to be an accurate and convenient method for detecting sex chromosome mosaicisms, which can help in making informed decisions about pregnancy continuation [76]. However, it is important to note that karyotyping has its limitations, and when used in combination with other techniques, such as single nucleotide polymorphism-based microarray (SNP array) and FISH, it can provide more precise information for genetic counseling [8]. Despite its sensitivity, karyotyping may also have limitations due to artifacts and bias resulting from cell cultivation, particularly for

sex chromosomal abnormalities, where combining it with uncultured FISH or DNA-based methods are necessary [4,5].

Research has shown that the use of FISH can be valuable in the diagnosis of chromosomal mosaicism, especially in identifying mosaicism caused by postzygotic mutations and in characterizing small supernumerary marker chromosomes, respectively [77]. Also, the utility of FISH in detecting low-level mosaicism for chromosomal rearrangements has been highlighted [78]. These studies collectively underscore the significance of FISH in the diagnosis of chromosomal mosaicism, particularly in cases where other molecular techniques may be insufficient [77,78].

Array analysis, particularly array comparative genomic hybridization (aCGH), has been shown to be effective in detecting chromosomal mosaicism in prenatal diagnosis [5,8,79,80]. This method has been used in over 1600 cases, with a high detection rate of mosaicism in chorionic villus and amniotic fluid samples [79]. It has also been compared to other techniques such as karyotyping and SNP array, with aCGH showing promising results [8]. Furthermore, a custom-designed, exon-targeted whole-genome oligonucleotide array has been used to detect somatic mosaicism in a significant number of cases [81]. Despite the challenges in detecting mosaicism, array analysis is beneficial in prenatal diagnosis, particularly when combined with other techniques [5].

Another method for analyzing chromosomal mosaicism is quantitative fluorescence polymerase chain reaction (QF-PCR). The main advantage of this method is the low cost and speed at which results are available (24–48 h) [16]. It is particularly useful for detecting trisomies 21, 18, and 13, as well as sex chromosome aneuploidies [16,81,82]. However, it may not be able to detect chromosomal rearrangements and some mosaic samples (when the normal cell line is below 10% of the whole sample), which require cytogenetic analysis [16,82].

NIPT is typically performed from around 10 weeks into a pregnancy and has been shown to have a high screening capacity for chromosomal abnormalities, particularly trisomy 21 (Down’s syndrome) [10]. The process of NIPT involves the quantification of copy number alterations from the sequencing of cell-free DNA, with various tools and software packages available for data analysis [83,84]. Despite its potential, the implementation of NIPT presents challenges, including the need for regulation and oversight, particularly in areas where sex-based abortions are prevalent [13].

NIPT has revolutionized prenatal diagnosis by enabling the detection of chromosomal mosaicism, a phenomenon that can lead to false positive and false negative results with traditional testing methods. The use of advanced bioinformatics algorithms in NIPT has allowed for the identification of fetoplacental mosaicism, which can influence risk estimation and improve genetic counseling [85]. Additionally, the development of a novel analysis pipeline for NIPT has improved the detection of all autosomal fetal aneuploidies, including mosaic trisomies, thereby enhancing prenatal management [86]. However, it is important to note that maternal mosaicism of sex chromosomes can cause discordant sex chromosomal aneuploidies in NIPT, highlighting the need for confirmatory testing [35].

Table 6. Genetic tests used in prenatal diagnosis [5,16,57,71–74,87–92].

Technique	Description	Indications	Genetic Defect Detected	Limitations
Karyotyping	The process of pairing and ordering all the chromosomes of an organism, providing a genome-wide snapshot of an individual’s chromosomes.	<ul style="list-style-type: none"> - advanced maternal age - abnormal maternal serum screening - known balanced translocations in the family - fetal abnormalities in ultrasound 	<ul style="list-style-type: none"> - chromosome aneuploidies - balanced chromosomal rearrangements - deletions and duplications (>5–10 Mb) - chromosomal mosaicism 	<ul style="list-style-type: none"> - artifacts, particularly for sex chromosomal abnormalities - long processing time - low resolution

Table 6. *Cont.*

Technique	Description	Indications	Genetic Defect Detected	Limitations
Fluorescence In Situ Hybridization (FISH)	FISH uses fluorescent probes that bind to specific parts of the chromosome. This allows for the detection of specific genetic abnormalities.	<ul style="list-style-type: none"> - uncultured samples can be used - specific microdeletion suspected, too small to be identified on karyotyping 	<ul style="list-style-type: none"> - chromosome aneuploidies - submicroscopic deletions and duplications - complex chromosomal rearrangements - low-level mosaicism for chromosomal rearrangements 	<ul style="list-style-type: none"> - has a targeted approach - structural chromosomal aberrations can be missed - faster but less comprehensive than karyotyping
Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR)	Rapid method for detecting aneuploidies by amplifying DNA regions containing specific short tandem repeats (STRs).	<ul style="list-style-type: none"> - quicker results than using conventional karyotyping 	<ul style="list-style-type: none"> - aneuploidies - microdeletions/duplications 	<ul style="list-style-type: none"> - detects chromosomal mosaicism when the abnormal cell line represents at least 10% of the whole sample - inversions, translocations, or deleted/duplicated regions must be within the STR marker range
Polymerase Chain Reaction (PCR)	Technique used to amplify small segments of DNA, allowing for detailed analysis.	<ul style="list-style-type: none"> - specific genetic conditions by targeting known mutations 	<ul style="list-style-type: none"> - single gene mutations 	<ul style="list-style-type: none"> - cannot be used to identify unknown targets - prone to errors during multiplication
Chromosomal Microarray Analysis (CMA)	CMA, also known as array comparative genomic hybridization (aCGH), detects copy number variations (CNVs) across the genome.	<ul style="list-style-type: none"> - low-risk pregnancies - fetuses with structural abnormalities - stillbirths 	<ul style="list-style-type: none"> - deletions/duplications 	<ul style="list-style-type: none"> - detects variants of unknown significance - does not detect balanced chromosomal rearrangements - low-level mosaicism can be missed
Multiplex Ligation Probe Amplification (MLPA)	MLPA is a technique used to detect CNVs and methylation abnormalities in specific genomic regions.	<ul style="list-style-type: none"> - uniparental disomies - imprinting errors - small deletions and duplications 	<ul style="list-style-type: none"> - CNVs and methylation abnormalities in specific genomic regions 	<ul style="list-style-type: none"> - cannot be used to identify unknown targets - prone to errors during multiplication
Next-Generation Sequencing (NGS)	NGS involves sequencing millions of small fragments of DNA in parallel, providing a comprehensive analysis of the genome.	<ul style="list-style-type: none"> - single-nucleotide mutations when not looking for a specific target region 	<ul style="list-style-type: none"> - monogenic disorders - CNVs, small insertions/deletions 	<ul style="list-style-type: none"> - can often detect variants of uncertain significance (VUSs) - expensive

As each situation will be unique, every diagnosed case of chromosomal mosaicism needs to be assessed carefully by clinicians. Genetic counseling plays a crucial role for these families, as it can provide the necessary information for them to decide how they proceed in the pregnancy and for future family planning [9]. Prenatal and postnatal phenotypical findings can vary significantly, even within the same genotype, and sometimes the phenotypes can present significant overlaps, even for completely different genotypes [93]. Although usually the exact prognosis for the patient is impossible to predict, proper diagnosis and

checkups can provide the insights needed to make an informed decision about continuing or interrupting a pregnancy.

Low-level mosaicism is even harder to diagnose, especially when facing a rare chromosomal anomaly [9]. Special focus needs to be placed on the psychological impact on the families when genetic anomalies are discovered, and multidisciplinary teams are needed for the proper management of these cases, where the parents need to make informed decisions keeping in mind the potential quality of life of their future child [94].

7. Conclusions

Chromosomal anomalies, and especially mosaic variants, remain a great challenge for prenatal diagnosis, both for clinical geneticists and families. Proper genetic counseling is often difficult and often requires multiple discussions with family members. Wider access to innovative genetic testing will provide better opportunities for higher accuracy regarding prenatal testing results.

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O'Donnel-Luria-Rodan Syndrome: New gene variant identified in Romania (A case report)

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Abstract. O'Donnel-Luria-Rodan (ODLURO) syndrome is a neurodevelopmental disorder with autosomal dominant inheritance. It appears more frequently in males during the first decade of life and is associated with developmental delay, low intelligence quotient, autism spectrum disorder-like behavior, epilepsy, speech delay, aggression, facial and skeletal deformities, gastrointestinal symptoms and hypotonia. Although few cases have been documented, it appears that the phenotype spectrum may vary, especially between the two biological sexes. The present study reported a case of a 5-year-old male patient who was diagnosed with ODLURO at the age of 4 years using whole-exome sequencing. Molecular analysis identified a new mutation in the lysine methyltransferase 2E (inactive) (KMT2E) gene, which was classified as a variant with unknown significance. The father, who presented with non-specific and undiagnosed psychiatric manifestations, presented the same KMT2E variant. The case described in the present study is not only interesting because there are <40 cases described in the literature, but also because a new inherited mutation in the KMT2E gene, present in both father and son, that resulted in different phenotypic manifestations was identified.

Introduction

O'Donnel-Luria-Rodan (ODLURO) syndrome was first reported in 2019 in 30 patients, with the majority of patients being under the age of 10 years. To date, there have been 38 reported cases worldwide (1). It is a neurodevelopmental

disorder that leads to delayed development, low intelligence quotient (IQ), speech delay, autism spectrum disorders, anxiety and seizures. Other manifestations include hypotonia, feeding difficulties and mild physical deformities (2). The inheritance of ODLURO is autosomal dominant, although the majority of cases appear *de novo* (3). The clinical manifestations develop at an early age and tend to have variable expressivity that is primarily dependent on the biological sex of the patient (4).

The lysine methyltransferase 2E (inactive) (KMT2E) gene encodes a member of the lysine N-methyltransferase-2 family, a group of enzymes that serve an important role in chromatin remodeling through transcriptional regulation. ODLURO is caused by mutations in the KMT2E gene that result in pathogenic enzyme variants responsible for regulating histone 4 methylation on lysine 3 (H3K4). Lack of proper methylation leads to abnormalities in the molecular activity of neurons. As methylation maintains the open chromatin state, any dysregulation affects proper transcription regulation. H3K4 methylation undergoes dynamic changes during the neurodevelopmental phase, impacting both the environment of neural and glial cells in the brain and the molecular activity of the neurons (5,6).

O'Donnell-Luria identified 31 different mutations in the heterozygous state for the KMT2E gene in a group of 34 individuals with ODLURO syndrome (OMIM 618512) (4). All patients received clinical and molecular evaluation via exome or genome sequencing through a collaboration of four research centers. The majority of the identified mutations were consistent with haploinsufficiency due to truncated proteins, except four patients with missense mutations affecting highly conserved residues. As expected, the majority of the mutations occurred *de novo*, dominant variant inheritance was observed in three affected siblings who may have inherited the variant from the affected father. Functional studies of the identified variants were not performed, but the authors speculated that the pathogenic mechanism linked to altered KMT2E binding function was the result of haploinsufficiency.

Case report

The present study reports the case of a 5-year-old male patient diagnosed with ODLURO syndrome at the age of 4 years, who was the first reported patient with ODLURO in Romania. The

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Key words: O'Donnel-Luria-Rodan syndrome, lysine methyltransferase 2E (inactive), variant of uncertain significance, autism spectrum disorder, molecular analysis

patient was the only child of non-consanguineous parents and the family history was negative. The patient was born at term after an uncomplicated pregnancy and delivery, and displayed normal parameters at birth.

Gross motor development was delayed, as the patient began sitting at 7 months and walking at 15 months of age. Verbal skill communication was never acquired and the mother noticed behavioral anomalies, including avoidance of gaze, retreating into repetitive, self-centered games, and no interaction with other children of his age. After the age of 2 years, the patient was consulted by several pediatric neurologists and psychiatrists, but in the absence of specific magnetic resonance imaging anatomical and electrophysiological functional abnormalities of the CNS, the patient was diagnosed with an idiopathic autistic spectrum disorder. At the age of 5 years, the patient was referred for medical genetic assessment. Overall development showed the-standard deviation values of height and weight for the age. The patient recognized only a few basic, short words, but could not reproduce them or verbally communicate in any way. The patient's father also faced challenges with social interactions, living isolated from the family and displaying autistic behaviors, but had not undergone a psychological evaluation.

Intellectual disability was objectified by the psychologist using specific evaluation tools, and the patient presented with an IQ of 52. The patient also displayed the following characteristics: Self-isolation, repetitive and unusual body movements, paroxysmal crying, reduced attention span to 6-7 sec and difficulty making eye contact. All of these, in addition with the presentations of anxiety and a tendency to self-harm indicated an autism spectrum disorder.

The patient had light, sensitive skin, blonde hair, blue eyes, and presented macrocephaly and mild facial dysmorphism, including antimongoloid slanting palpebral fissures, prominent nasolabial folds and frontal bossing. Another interesting finding was the narrowing of the distal phalanx of the patient's fingers (Fig. 1).

Among the most debilitating symptoms, severe gastroesophageal reflux with recurrent nausea, vomiting and abdominal pain was reported. Nutrigenomic testing was recommended, and the results revealed a tendency for compulsive eating behaviors, especially for calorie-rich foods. Moreover, the specific HLA DQ 2.5 haplotype defines a predisposition to developing celiac disease due to gluten intolerance.

Due to the heterogeneous, non-specific clinical manifestations, whole-exome sequencing (WES) was performed with parental informed consent. The investigator reported a heterozygous mutation in the KMT2E gene (KMT2E NM_018682.3: c.498-11T>C), which was characterized by a deletion that resulted in the inclusion of a pathogenic exon in the mature mRNA, thus altering the structure and function of the synthesized polypeptide. Targeted genetic testing was performed in both parents, and the variant was identified in the father (Table I).

Discussion

ODLURO is a newly defined genetic condition, and as with most rare genetic diseases, there is little information in the literature on its etiopathogenesis. The authors who defined this

syndrome revealed an association between KMT2E gene mutations and a clinical phenotype with several common elements, including neurodevelopmental delay with autistic behavior and intellectual disability, non-specific facial dysmorphism and various functional abnormalities (4).

The patient reported in the present study was a 5-year-old male who was diagnosed with ODLURO based on clinical manifestations and a heterozygous mutation in the KMT2E gene (KMT2E NM_018682.3: c.498-11T>C), which is currently classified as a variant of uncertain significance (VUS) due to the lack of reported cases with this genetic variant in the medical literature related to ODLURO. The same variant was identified in the patient's father, who also presented with psychiatric symptoms, but not to the same severity as the patient. In this case, VUS management and evaluating a possible etiopathogenic link with the phenotype was challenging. The label VUS emerges if no reports connect the variant to the specific disease. Also, if a variant is extremely rare, it might take time to conduct comprehensive studies and catalog enough cases that associate it with a medical condition. The arguments that the identified variant may be pathogenic are due to its molecular characteristics; According to the investigator, *in silico* analysis shows that the mutation is predicted to disrupt the highly conserved acceptor splice site and thus may have a negative effect on the phenotype, as it alters the AG-3' intronic acceptor site, with the synthesis of an incomplete mRNA and defective unstable polypeptide synthesis (7).

Although the majority of cases of ODLURO are a consequence of a *de novo* mutation, inheritance was identified in a family with three affected siblings (8) Parental WES analysis identified the same variant in the father. Interestingly, the father presented with autistic-like behavior and his wife stated that he has a childhood history for a psychiatric disorder, but could not provide details related to the diagnosis. Phenotypic differences may be explained by incomplete penetrance and variable expressivity, an already well-known aspect for dominant inheritance (9).

Autism spectrum disorder is common in patients with ODLURO, especially in males, together with speech delay, intellectual disability, anxiety and aggressive behavior (8). The KMT2E gene is known to serve an important role in neurodevelopment, and mutations in this gene have been linked to several cases of epilepsy, autism spectrum disorder, intellectual disability and schizophrenia (10). The patient described in the present study also presented a complete lack of verbal communication, as well as delayed speech.

Facial dysmorphic features like dolichocephaly, large forehead, deep-set eyes, antimongoloid palpebral fissures, peri-orbital fullness, prominent cheeks and prominent nasolabial folds have been described in patients with ODLURO (11). The patient described in the present study presented with macrocephaly, frontal bossing, antimongoloid palpebral fissures, prominent cheeks and prominent nasolabial folds.

Gastrointestinal symptoms have also been reported in numerous patients with ODLURO (4). However, predisposition to developing coeliac disease and being positive for HLA DQ 2.5 have not yet been reported in correlation with ODLURO, and might be independent traits. Although eating disorders, such as compulsive overeating, have not previously been reported to be linked to ODLURO, the patient reported

Table I. Clinical features of the presented patient in comparison with other reported patients with ODLURO syndrome (12-14).

Clinical features in ODLURO syndrome	Manifestations in previously reported cases	Manifestations in the presented case
Facial dysmorphic features	Dolichocephaly, large forehead, deep-set eyes, antimongoloid palpebral fissures, periorbital fullness, prominent cheeks and prominent nasolabial folds	Macrocephaly, frontal bossing, antimongoloid palpebral fissures, prominent cheeks and prominent nasolabial folds
Height, weight, and developmental abnormalities	Short stature and delayed development	Delayed physical development
Osteoarticular abnormalities	Tapering fingers	Tapering fingers
Neurological and psychiatric involvement	Hypotonia, seizures, intellectual disability, delayed speech, anxiety and autism	Autism, intellectual disability, lack of verbal communication, anxiety and eating disorders
Cardiovascular involvement	Septal defects	N/A
Gastroenterological involvement	Nausea, vomiting, motility disorder and gastroesophageal reflux	Nausea, vomiting, motility disorder and gastroesophageal reflux
Immunological involvement	N/A	Positive for HLA DQ 2.5, which results in a predisposition to developing celiac disease

ODLURO, O'Donnel-Luria-Rodan.

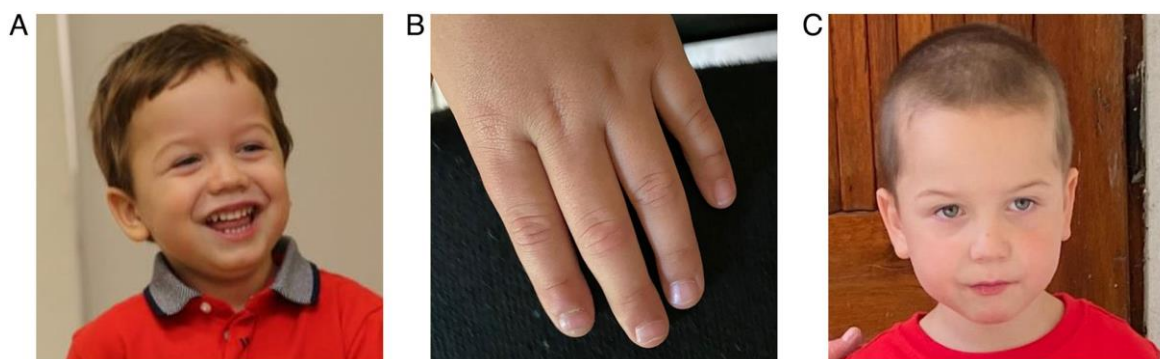


Figure 1. Facial phenotype and tapering fingers. (A and C) Facial phenotype of the patient with dolichocephaly, prominent forehead and antimongoloid palpebral fissures. (B) Tapering fingers of the patient.

in the present study required nutritional and psychological guidance for this condition to restore proper nutritional values.

In conclusion, based on the aforementioned genetic findings and taking into consideration the phenotype of the patient, the c.498-11T>C, KMT2E VUS was a potential cause for the patient's symptoms. However, further studies are required to confirm the pathogenicity of the variant. It appeared that the mutation was inherited from the father and decreased penetrance may explain the milder phenotype, restricted to autistic-like behavior, therefore further functional and clinical studies are necessary and will allow precise pinpointing of the pathologic significance of this variant. The case reported in the present study is an example of rare disease challenges for diagnostic uncertainty when considering the unknown clinical significance of a specific variant. Until further evidence for the identified variant has been identified, the case reported in

the present study should remain in focus as the first case of ODLURO diagnosed in Romania.

ODLURO is a rare neurodevelopmental disorder caused by a germline mutation in the KMT2E gene that is primarily *de novo*, but also inheritable. Common symptoms include delayed development, low IQ, poor verbal communication, autism spectrum disorders, anxiety and seizures. Other manifestations include hypotonia, feeding difficulties and mild physical deformities. The present study reported a 5-year-old male patient who presented with macrocephaly and mild facial dysmorphia, including antimongoloid slanting palpebral fissures, prominent nasolabial folds and frontal bossing, tapering fingers, and severe gastroesophageal reflux with recurrent nausea, vomiting and abdominal pain in association with KMT2E NM_018682.3: c.498-11T>C, heterozygous variant, with gluten intolerance as an atypical finding.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AC and MSM conducted the genetic consult and counselling. AC, ZCB, EK, DM and II interpreted the genetic test results and confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

The patient's family provided consent for publication.

Competing interests

The authors declare that they have no competing interests.

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