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ORIGINAL ARTICLE

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ABSTRACT

Both germline and somatic variants contribute to the genetic background and pathogenesis of melanoma. Germline variants include the presence of rare pathogenic or likely pathogenic variants of high, medium, and low penetrance melanoma-predisposing genes. Rare variants of high penetrance melanoma-predisposing genes are associated with melanoma development, whereas the medium and low penetrance predisposing genes can significantly increase melanoma risk. In this study, we clarified the germline genetic background of a Hungarian melanoma cohort ($n = 17$). Using a gene panel of 30 melanoma-predisposing genes, germline genetic variants were identified in 10 of the 17 patients (58.82%). A novel, likely pathogenic, missense variant (p.Y143C) in a medium penetrance melanoma-predisposing gene, *melanocortin 1 receptor gene* (*MC1R*), and two novel, likely pathogenic nonsense variants in low penetrance genes, p.Q218Ter in caspase 8 (*CASP8*) and p.Q40Ter in the fat mass- and obesity-associated (*FTO*) gene were detected. This study highlights the importance of elucidating the germline genetic background of melanoma, which may improve prediction of individual risk and the risk of family members and to optimize preventive, screening, and therapeutic measures for each patient and melanoma-prone families.

1 | Introduction

Malignant melanoma is considered a complex disease, and its development is influenced by genetic, environmental, and lifestyle factors (Read, Wadt, and Hayward 2016; Soura et al. 2016; Nan et al. 2009). It is one of the most commonly diagnosed malignant tumors worldwide. According to the data from the American Cancer Society, the overall lifetime risk of developing malignant melanoma is approximately 3% in Caucasian people. Approximately 330,000 new cases of malignant melanoma were diagnosed worldwide in 2022, leading to 47,000 deaths (Soura et al. 2016).

Accumulating evidence suggests that variants of both predisposing and susceptibility genes significantly contribute to the germline genetic background of melanoma. Rare germline pathogenic, or likely pathogenic variants are located in high, medium, or low penetrance melanoma-predisposing genes. Rare variants of high penetrance melanoma-predisposing genes (*CDKN2A, CDK4, BAP1, POT1, ACD, TERF2IP*, and *TERT*) are associated with a very high melanoma susceptibility, resembling a monogenic disease with autosomal dominant inheritance. Rare variants in medium penetrance melanoma-predisposing genes (*MC1R, MITF*, and *SLC45A2*) and low penetrance genes (*TYR, OCA2, ASIP, PL2G6, FTO, PARP1, ATM, CDKAL1,*

Summary

- Three novel, likely pathogenic, germline variants were detected in a Hungarian melanoma cohort, including a missense variant in a medium penetrance gene, melanocortin 1 receptor (*MC1R*), and two nonsense variants in low penetrance genes, in *caspase 8* (*CASP8*) and in the fat mass- and obesity-associated (*FTO*) gene.
- This study highlights the importance of elucidating the genetic background of malignant melanoma.
- This provides a better understanding of the disease and improves the estimation of individual risk and the risk to family members.
- It supports value of preventive screening and effective therapeutics for melanoma patients and melanoma-prone families.

CCND1, and *CYP1B1*) are alone unable to cause the development of melanoma, but they provide a polygenic genetic susceptibility and can significantly increase the risk (Read, Wadt, and Hayward 2016).

Of note, some melanoma susceptibility genes were not only associated with the development of melanoma, but also with other malignant tumors (Read, Wadt, and Hayward 2016; Soura et al. 2016; Zocchi et al. 2021). The high penetrance melanoma-predisposing gene *CDKN2A* is associated with melanoma-pancreatic cancer syndrome (OMIM 606719) and melanoma astrocytoma syndrome (OMIM 155755). The low penetrance melanoma-predisposing gene *ATM* is associated with breast cancer susceptibility (OMIM 114480).

A positive family history is associated with approximately 10% of melanoma patients, which indicates an elevated risk for further melanoma development. For familial melanoma cases, approximately 50% carry rare germline, disease-causing variants of high penetrance melanoma-predisposing genes. For the other half of these cases, the genetic background is unknown (Read, Wadt, and Hayward 2016). To gain insight into the germline genetic background of melanoma, we determined the predisposing variants in a Hungarian melanoma cohort with increased risk ($n = 17$) using a 30-gene melanoma panel with known high, medium, and low penetrance genes.

2 | Materials and Methods

2.1 | Patients and Samples

In this study, 17 Hungarian, unrelated, melanoma patients were enrolled. We considered this cohort with increased risk since all of them had at least three dysplastic naevi diagnosed by expert dermato-oncologists and proved by dermatohistological examinations (Table 1). Ten patients were women and 7 were men. Fourteen patients were diagnosed with malignant melanoma, and three patients had dysplastic naevus syndrome. The mean age at the first diagnosis of malignant melanoma was 49.5 years. After genetic counseling and obtaining written informed consent, peripheral blood samples were collected and genomic DNA was isolated using QIAGEN DNeasy kit.

Genetic testing for melanoma was carried out according to the recommendations. The geographical area of Hungary (Central Europe), where the patients were located, is a low or moderate melanoma incidence area. A diagnosis was made in patients with two primary (synchronous or metachronous) melanomas, in families with one case of invasive melanoma, and one or more diagnoses of melanoma and/or pancreatic cancer in a first- or second-degree relative on the same side of the family (Zocchi et al. 2021). We also performed genetic testing in patients with one malignant melanoma and multiple dysplastic nevi, based on the suspicion of familial atypical multiple mole melanoma syndrome. Three patients had a positive family history of malignant melanoma, two patients were diagnosed with two or more primary melanomas, and 12 patients had multiple dysplastic nevi removed. Tumors other than melanoma were diagnosed in six patients: Five patients had an additional tumor (prostate cancer, basal cell carcinoma, thyroid cancer, adenomatous polyp of the transverse colon felxurae lienalis et sigmoidal with dysplasia grade 3, and lung adenocarcinoma), whereas one patient had two primary cancers other than melanoma (adenocarcinoma of the large intestine, basal cell carcinoma infiltrating the skin). One patient had lymphatic metastases at the time of diagnosis, and one patient developed a second primary malignant melanoma that was metastatic at the time of diagnosis in multiple organs (multiplex metastases in the brain, lien, hepar, lung, cholecysta, and bladder, with lymphadenomegalia colli l.s. et hilii l.d.). This study was approved by the Hungarian National Public Health Centre and was conducted according to the Helsinki guidelines.

2.2 | Targeted Next-Generation Sequencing With a Virtual Melanoma Gene Panel

Patients' genotypes were determined using a targeted next-generation sequencing (NGS) approach. Libraries were prepared using the SureSelectQXT Reagent Kit (Agilent Technologies, Santa Clara, CA). Pooled libraries were sequenced on the Illumina NextSeq 550 NGS platform using the 300-cycle Mid Output Kit v2.5 (Illumina Inc. San Diego, CA, USA). Adapter-trimmed and Q30-filtered paired-end reads were aligned to the hg19 human reference genome using the Burrows-Wheeler Aligner (BWA). Duplicates were marked using the Picard software package. The Genome Analysis Toolkit (GATK) was used for variant calling (BaseSpace BWA Enrichment Workflow v2.1.1. with BWA 0.7.7-isis-1.0.0, Picard: 1.79 and GATK v1.6-23-gf0210b3).

Sequencing revealed that the mean on-target coverage was 71× per base with an average percentage of targets covered greater than or equal to 30×, respectively. Variants passed through the GATK filter were used for downstream analysis and annotated using the ANNOVAR software tool (version 2017 July 17). Single-nucleotide polymorphism testing was performed as follows: High-quality sequences were aligned with the human reference genome (GRCh37/hg19) to detect sequence variants, which were analyzed and annotated. Variants were filtered according to read depth, allele frequency, and prevalence reported in genomic variant databases, such as ExAc (v.0.3) and

TABLE 1 | Summary of the clinical characteristics of the 17-member Hungarian malignant melanoma cohort with increased risk.

Patients	Sex	Age of onset	Number of primary melanomas	Number of dysplastic naevi	Family history for melanoma	Additional cancers
1	Female	58	1	> 5	Negative	None
2	Male	76	1	> 3	Positive	Prostate caner
3	Female	82	1	> 10	Negative	None
4	Male	55	1	> 3	Negative	None
5	Female	26	2	> 10	Negative	None
6	Female	51	3	> 10	Negative	Adenocarcinoma nvasivum intestini crassi, flexura lieanlis; carcinoma basocellulare
7	Female	44	0	> 10	Negative	None
8	Female	57	0	> 10	Negative	Carcinoma basocellulare 2x
9	Female	42	1	< 3	Positive	None
10	Female	42	1	> 5	Negative	Thyroid carcinoma
11	Male	51	1	> 100	Negative	2 colon polyps with grade 3 dysplasia
12	Male	50	1	> 10	Negative	Lung adenocarcinoma, prostate hypertrophy
13	Male	42	1	> 10	Negative	None
14	Female	43	1	> 5	Negative	None
15	Male	31	2	> 5	Negative	Multiplex metastases
16	Female	53	0	> 5	Positive	None
17	Male	40	1	> 10	Negative	None

Kaviar. Variant prioritization tools (PolyPhen-2, SIFT, LRT, Mutation Assessor) were used to predict the functional impact of the mutation. We interpreted the sequencing results using the Franklin Genoox website, which creates and uses a virtual malignant melanoma panel that includes 30 genes associated with melanoma susceptibility based on a review by Read, Wadt, and Hayward (2016). Secondary findings were screened and reported based on the current ACMG guidelines (ACMG SF v3.2) (Miller et al. 2023). VarSome and Franklin bioinformatic platforms (https://franklin.genoox.com) were used based on the guidelines of the American College of Medical Genetics and Genomics. The candidate variants were confirmed by bidirectional capillary Sanger sequencing.

3 | Results

Using a 30-gene melanoma panel, melanoma-predisposing germline genetic variants were identified in 10 (58.82%) patients in the Hungarian cohort (Table 2). We identified rare germline heterozygous variants ($n=11$; Table 2) in a high penetrance melanoma susceptibility gene (*CDKN2A*), in a medium medium penetrance gene (*MC1R*), and seven variants in low penetrance genes (*ATM, TYR, OCA2, SETDB1, FTO, CAS8, PARP1*). Of the 11 identified rare variants, 3 were novel and first identified by this study and 8 were recurrent variants.

Regarding the three novel variants, all of them were likely pathogenic, including the p.Y143C missense variant in a medium penetrance melanoma-predisposing gene, melanocortin 1 receptor (*MC1R*), two nonsense variants in low penetrance genes: the p.Q218Ter in caspase 8 (*CASP8*) and the p.Q40Ter in the fat mass- and obesity-associated (*FTO*) gene were detected.

The novel p.Y transcript 143C (c.428A>G) of *MC1R* (16q24.3; NM_002386.4) is a missense variant in exon 1 that results in a tyrosine to cysteine amino acid change in the 143rd position of the protein. Based on the ACMG classification guidelines, this variant is classified as likely pathogenic considering the extremely low frequency of the variants in the gnomAD population databases (PM2). Since it is a missense mutation, most computational prediction tools predicted benign effect.

The novel p.Gln218Ter (c.652C>T) variant of *CASP8* (2q33.1; NM_033358.4) is a nonsense variant in exon 7, which results in the formation of a premature termination codon after the 218th amino acid in the polypeptide. Based on the ACMG classification guidelines, this variant is considered likely pathogenic. However, most *in silico* prediction tools suggest a benign effect (BayesDel noAF, BayesDel addAF, EIGEN, FATHMM, DANN), and one prediction tool indicated an uncertain effect (MutationTaster).

The p.Gln40Ter (c.118C>T) variant of the *FTO* gene (16q12.2; NM_001080432.3) is a nonsense variant in exon 2, which results in the formation of a premature termination codon after the 40th amino acid of the polypeptide chain. This change may either cause severely impaired protein function or lead to mRNA-mediated decay of the transcript. Based on the ACMG classification guidelines, this variant is classified as likely pathogenic. The predictions of *in silico* computational tools are conflicting: some support a pathogenic effect (BayesDel addAF, BayesDel noAF, EIGEN), others support a benign effect (FATHMM, LRT), and some tools report an uncertain result (Dann, Mutationtaster).

Of the identified recurrent rare germline heterozygous variants ($n=8$), a pathogenic one was identified in the low penetrance melanoma-predisposing tyrosinase (*TYR*) gene. Four of the eight recurrent variants are classified as leaning pathogenic VUS and located in a high penetrance cyclin-dependent kinase inhibitor 2a (*CDKN2A*) gene, and in the low penetrance genes, oculocutaneous albinism type 2 (*OCA2*), and set domain protein bifurcated 1 (*SETDB1*). Three rare recurrent germline VUS was detected in the ataxia-telangiectasia mutated gene (*ATM*), poly (ADP-ribose) polymerase 1 (*PARP1*) gene, and *OCA2* genes.

Regarding frequent variants, the A risk allele for melanoma of the p.R402Q missense polymorphism (rs1126809) of the low penetrance melanoma-predisposing *TYR* gene was detected in 14 (82.35%) of the 17 melanoma patients (Table 2). The A allele of the p.R402Q variant forms a risk haplotype with the C allele of the c.-301C>T promoter SNP (rs4547091) of the *TYR* gene for oculocutan albinism (Zocchi et al. 2021). The segregation analysis did not detect a CA risk haplotype for albinism in any of our melanoma patients. We identified the *BRCA1* (17q21.31 NM_007294.4):c.181T>G p.Cys61Gly pathogenic variant as a secondary finding, which is a known disease-causing variant for hereditary breast and ovarian cancer.

4 | Discussion

The global landscape of germline genetic variants associated with melanoma susceptibility is becoming more clear. This study further augments the spectrum of identified germline predisposing variants by adding data from a 17-member Hungarian cohort affected by melanoma.

The pathomechanisms by which different variants can lead to elevated melanoma susceptibility vary. High penetrance melanoma genes and some of the medium and low penetrance genes disrupt the function of genes that play important roles in the cell cycle, differentiation, and division, thus leading to an elevated risk for melanoma and other additional tumors. Most of the medium and low penetrance genes result in an increased melanoma risk by forming a phenotype more prone to the development of malignant melanoma (e.g., increased number or density of nevi, decreased pigmentation of the skin and hair, and increased sensitivity to UV radiation) (Read, Wadt, and Hayward 2016). This is consistent with the results of the present study as most patients with low penetrance mutations had a very high number of nevi, fair hair and skin, and a general disposition that was prone to skin cancer formation.

While we identified predisposing variants in most of the patients, considering the multifactorial nature of the disease, it is also noteworthy that some patients reported lifestyle factors that could contribute to the development of malignant melanoma, mainly high sun exposure (5 patients), frequent sunburns in childhood and early adulthood (8 patients), and the frequent use of tanning salons (1 patient) (Table 3).

TABLE 2 | Summary of the identified germline variants in a 17-member cohort of Hungarian melanoma patients with increased risk.

Patient No.	Gene	Variant	ACMG classification	Frequency (Rare: MAF < 0.01)	Novel/ Recurrent
1	*ATM*	c.8734A > G p.Arg2912Gly	VUS	Rare	Recurrent
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
2	*TYR*	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
3	*OCA2*	c.2433G > T p.Arg811Ser	Leaning pathogenic VUS	Rare	Recurrent
4	*CDKN2A*	c.343G > T p.Val115Leu	Leaning pathogenic VUS	Rare	Recurrent
	SETDB1	c.1744A > G p.Thr582Ala	Leaning pathogenic VUS	Rare	Recurrent
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
5	*TYR*	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
6	*TYR*	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
7	***FTO***	**c.118C > T p.Gln40Ter**	**Likely pathogenic**	**Rare**	**Novel**
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
8	*SETDB1*	c.3226C > T p.Arg1076Cys	Leaning pathogenic VUS	Rare	Recurrent
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
9	***CASP8***	**c.652C > T p.Gln218Ter**	**Likely pathogenic**	**Rare**	**Novel**
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
10					
11	*TYR*	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
12	*OCA2*	c.1817C > T p.Thr606Ile	VUS	Rare	Recurrent
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
13	*TYR*	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
14					
15	*PARP1*	c.523G > A p.Glu175Lys	VUS	Rare	Recurrent
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent

(Continues)

TABLE 2 | (Continued)

Patient No.	Gene	Variant	ACMG classification	Frequency (Rare: MAF < 0.01)	Novel/ Recurrent
16	***MC1R***	**c.428A > G p.Tyr143Cys**	**Likely pathogenic**	**Rare**	**Novel**
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
17	*TYR*	c.325G > A p.Gly109Arg	Pathogenic	Rare	Recurrent
	TYR	c.1205G > A p.Arg402Gln	VUS/risk factor	High	Recurrent
	*BRCA1**	c.181 T > G p.C61G	Pathogenic	Rare	Recurrent

Note: All variants were detected in heterozygous form. The three novel likely pathogenic variants identified by this study is highlighted with bold.
**BRCA1* c.181T > G p.Cys61Gly pathogenic variant, a known disease-causing variant for hereditary breast and ovarian cancer, was identified as a secondary finding.

TABLE 3 | Presence of environmental and lifestyle factors in the 17-member Hungarian malignant melanoma cohort with increased risk (NR, not reported).

Patient No.	High sun exposure	Frequent sunburns in childhood	Use of tanning salons
1	+	+	−
2	+	−	−
3	NR	NR	−
4	+	+	−
5	+	+	NR
6	NR	NR	NR
7	+	+	−
8	NR	NR	NR
9	NR	NR	NR
10	−	+	+
11	NR	NR	−
12	NR	NR	−
13	−	+	−
14	NR	NR	NR
15	NR	NR	−
16	−	+	−
17	−	+	−

This study has identified three novel variants in genes associated with the development of melanoma. These variants not only broaden the variant spectrum of these genes, but we also describe their association with the melanoma phenotype. Among these novel variants, the likely pathogenic missense variant, p.Y143C, was detected in *MC1R*, which is a medium penetrance melanoma-predisposing gene. The likely pathogenic nonsense variants, p.Q218Ter and p.Q40Ter, were present in the *CASP8* and *FTO* low penetrance genes, respectively. A GWAS study found association between FTO and melanoma, and another study reported that the overexpression of FTO can promote melanoma development (Iles et al. 2013; Yang et al. 2019). Further studies are needed to confirm the putative role of these variants in the development of melanoma.

Regarding the identified recurrent variants, the p.Arg2912Gly variant of the *ATM* gene was described in association with different cancer types, including breast, ovarian (Jarhelle et al. 2019), brain, pancreatic (Young et al. 2018), and colorectal (Randon et al. 2019) cancers. The p.Val115Leu variant of the *CDKN2A* gene was reported in Ewing sarcoma (Zhang et al. 2016), laryngeal squamous cell carcinoma (Todorova et al. 2015),

cholangiocarcinoma, and acute lymphoblastic leukemia (Li et al. 2022). The p.Arg811Ser variant of the *OCA2* gene appeared in two publications associated with oculocutaneous albinism (Strauss et al. 2018; Rooryck et al. 2008). The p.Arg1076Cys variant *SETDB1* was associated with intestinal epithelial homeostasis (Južnić et al. 2021). Finally, the p.Gly109Arg of *TYR* was associated with oculocutan albinism (Camand et al. 2001; Lasseaux et al. 2018). Based on the literature, we conclude that the previously published variants identified in our study were not yet associated with malignant melanoma susceptibility, except for the *TYR* c.1205G > A polymorphism, which has a significant association with melanoma risk.

Among the frequent variants of the melanoma-predisposing genes, the *TYR* c.1205G > A variant is associated not only with oculocutan albinism, but is also a risk factor for malignant melanoma. We determined whether the reported risk of the *TYR* haplotype for albinism (Michaud et al. 2022) was present in our melanoma cohort; however, we could not identify any patients carrying either of the *TYR* risk haplotypes.

We also identified a pathogenic BRCA1 c.181 T > G variant in our cohort as a secondary finding in one male patient with malignant melanoma. Although the *BRCA1* gene is not listed as a susceptibility gene for malignant melanoma, there is some evidence indicating that the presence of pathogenic *BRCA1* or *BRCA2* mutations can result in an increased risk of malignant melanoma, particularly in males, as well as several other common BRCA-associated tumors (Ibrahim et al. 2018; Sun et al. 2020). For this reason, we considered this mutation as a pathogenic finding in our patient, rather than an actual secondary finding. Thus, we would recommend considering the addition of the *BRCA1* and *BRCA2* genes to the list of melanoma-associated genes, genetic counselling for family members and screening of carriers of this variant.

In conclusion, we identified 11 rare variants in melanoma-associated genes from a 17-member Hungarian melanoma cohort with increased risk, whereas three had not been previously reported in the literature. Our findings support the presence of germline melanoma susceptibility genes in patients with melanoma and highlight the importance of genetic testing in cases where there is a suspicion of hereditary melanoma. Identifying the genetic background for elevated melanoma risk can help us better understand the individual risk of the patient and estimate the risk for family members. It will also improve the prognosis for this disease and lead to better screening, preventive, and treatment options for melanoma patients. Our results further broaden our knowledge of the genetic prediction of melanoma and will help clinical geneticists and dermatologists improve patient care.

Author Contributions

B.A.B. wrote the manuscript. A.A., F.K., and M.P. performed the molecular biology investigations. Z.B. performed the clinical investigations. M.S. and N.N. supervised and contributed to the writing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Novel *FANCI* and *RAD54B* Variants and the Observed Clinical Outcomes in a Hungarian Melanoma Cohort

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Abstract: Accumulating evidence suggests that inherited melanoma is not rare and approx. one in seven individuals with melanoma has clinically relevant hereditable cancer-predisposing and/or -susceptibility variant(s). Concerning its germline genetic background, genetic screening aims to identify either variants of predisposing genes with high penetrance or variants of susceptibility genes with medium or low penetrance. However, less attention is paid to genetic testing of germline variants of genes influencing patients' survival outcomes or enhancing the design of new therapies. We aimed to investigate whether the germline genetic background of a Hungarian melanoma cohort (n = 17) contains any pathogenic or likely pathogenic variants of the *BRCA2*, *POLE*, *WRN*, *FANCI*, *PALB2*, and *RAD54B* genes and if the presence of these variants correlate with the clinical findings of the patients, including the advanced stage of melanoma, poor prognosis, and poor survival. We identified three novel variants in the *FANCI* gene and one novel variant in the *RAD54B* gene. We detected rapid disease progression, unfavorable outcome, and therapeutic resistance in the patient carrying the likely pathogenic *FANCI* variant. Our study highlights the importance of screening germline variants of genes influencing melanoma progression, therapy resistance, and survival of patients.

Keywords: melanoma; germline; progression; therapy resistance; patients' survival; likely pathogenic variant; novel variant

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1. Introduction

Malignant melanoma is recognized as a complex disease, its development being influenced by genetic, environmental, and lifestyle factors [1–3]. Accumulating evidence suggests that inherited melanoma is not rare and approx. one in seven individuals with melanoma has clinically relevant hereditable cancer-predisposing or -susceptibility variant(s) [4]. Concerning the germline genetic background of melanoma, genetic screening aims to identify either variants of predisposing genes with high penetrance (*CDKN2A*, *CDK4*, *BAP1*, *POT1*, *ACD*, *TERF2IP*, and *TERT*) or variants of susceptibility genes with medium or low penetrance (*MC1R*, *MITF*, *SLC45A2*, *TYR*, *OCA2*, *ASIP*, *PL2G6*, *FTO*, *PARP1*, *ATM*, *CDKAL1*, *CCND1*, and *CYP1B1*), which are known to play a major role in the genetic background of melanoma [1,5].

We have recently reported a Hungarian melanoma cohort (n = 17) with increased risk [5]. All of them had at least three dysplastic naevi diagnosed by expert dermatologists

and proved by dermatohistological examinations [5]. Fourteen patients were diagnosed with malignant melanoma and three patients had dysplastic naevus syndrome [5]. Using a gene panel of the melanoma-predisposing and melanoma-susceptibility genes described above, germline genetic variants of genes were identified in 10 of the 17 patients (58.82%) [5].

In addition to the germline variants of the melanoma-predisposing and melanoma-susceptibility genes, the accumulating evidence suggests that germline variants of genes, involved in DNS repair mechanisms, have been implicated in rendering melanoma patients more susceptible to tumor progression and affecting their response to treatments [6]. Here, our aim was to investigate whether patients in the Hungarian melanoma cohort (n = 17) with increased risk carry any pathogenic or likely pathogenic germline variants of the *BRCA2*, *POLE*, *WRN*, *FANCI*, *PALB2*, and *RAD54B* genes associated with melanoma survival and response to therapy. We also investigated whether the presence of these variants correlates with the clinical findings of the patients, including the advanced stage of melanoma, poor prognosis, and poor survival.

2. Results

2.1. In Silico Variant Analysis

We identified mutations using a six-gene panel in four of the 17 patients (23.5%). None of them overlaps with the variants reported by Amaral et al. (2020) on the *BRCA2*, *POLE*, *WRN*, *FANCI*, *PALB2*, and *RAD54B* genes [6]. However, we identified three novel variants in the Fanconi anemia, complementation group I gene (*FANCI*) in three patients (patients 9, 15, and 16), and one novel variant in the RAD54 homolog B gene (*RAD54B*) in one melanoma patient (patient 14) (Figure 1).

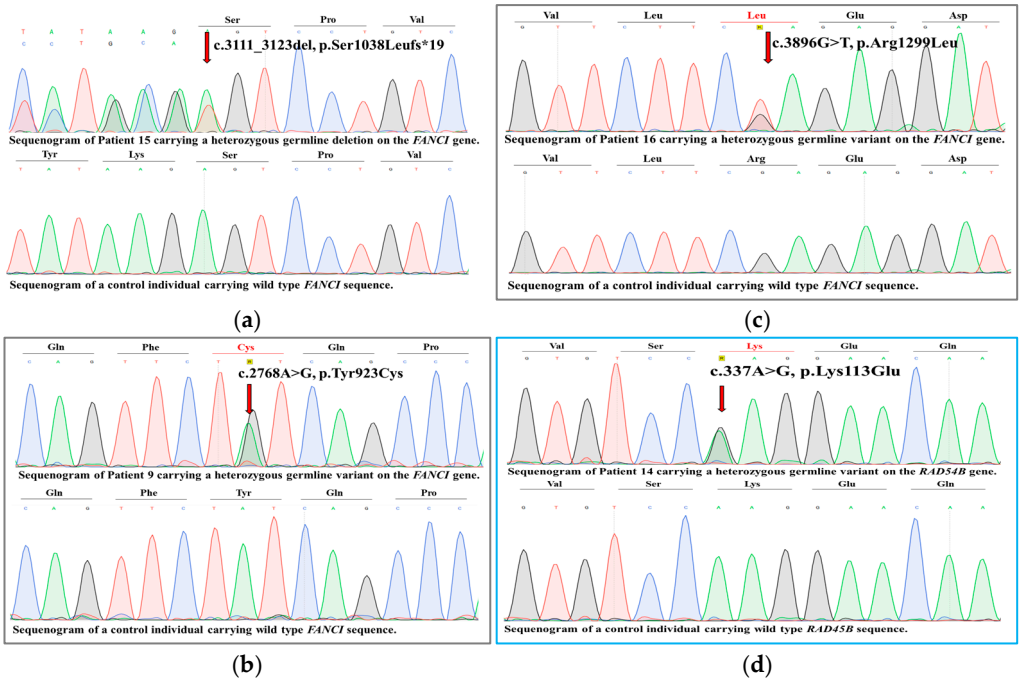

Figure 1. Sequenograms of the novel *FANCI* and *RAD54B* variants identified in the Hungarian melanoma cohort (n = 17). (**a**) The novel likely pathogenic *FANCI* variant c.3111_3123del, p.Ser1038LeufsTer19 is carried by patient 15. (**b**) Among the novel missense VUS FANCI variants, the c.2768A > G, p.Tyr923Cys is present in patient 9, and (**c**) the c.3896G > T, p.Arg1299Leu is present in patient 16. (**d**) The novel likely pathogenic *RAD54B* variant is detected in patient 14 (*FANCI* sequenograms are surrounded by grey, while *RAD54B* sequenograms are surrounded by light blue frames).

The novel c.3111_3123del, p.Ser1038LeufsTer19 variant of the *FANCI* gene (15q26.1; NM_001113378.2) is a nonsense variant in exon 29 resulting in the formation of a premature termination codon after the 1038th amino acid of the polypeptide (Figure 2). Based on the ACMG classification guideline, this variant is classified as likely pathogenic, considering that this is a null variant in a gene where loss of function is a known mechanism of disease (PVS1) and has an extremely low frequency in the gnomAD database (PM2).

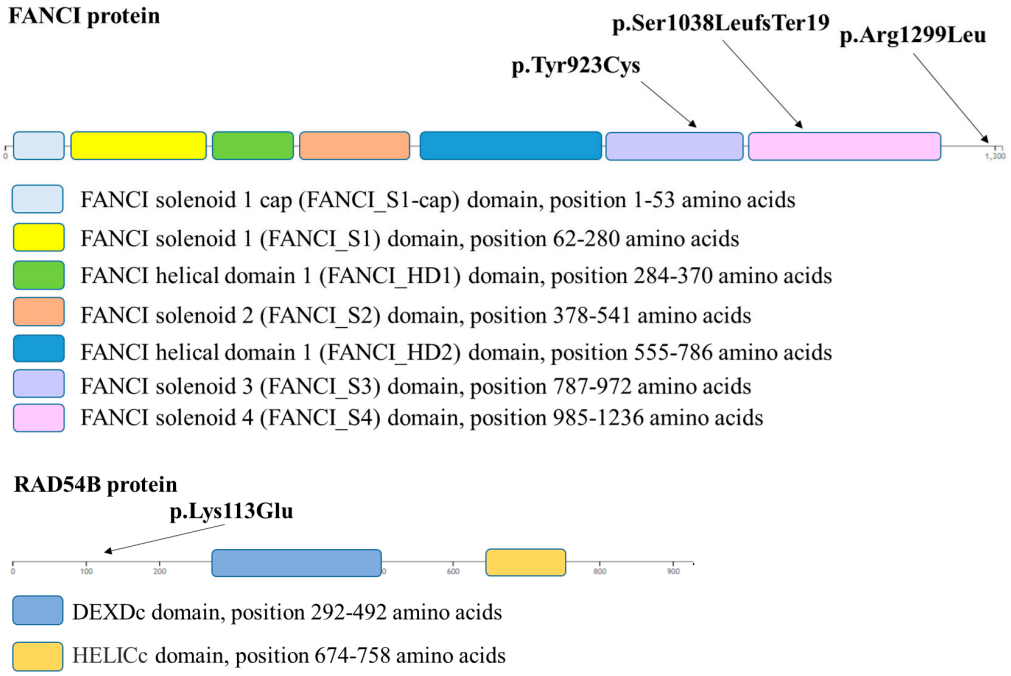

Figure 2. Position of the identified variants on the FANCI and on the RAD54B proteins (https://www.rcsb.org/sequence/3s51; accessed on 14 November 2024).

The novel c.2768A > G, p.Tyr923Cys variant of the *FANCI* gene (15q26.1; NM_001113378.2) is a missense variant in exon 25 causing a tyrosine-to-cysteine amino acid change in the 923th position of the protein (Figure 2). According to the ACMG classification guideline, this variant is classified as a variant of unknown significance, considering the extremely low frequency of the variant in the gnomAD population databases (PM2). EVE (evolutionary model of variant effect; https://evemodel.org/; accessed on 14 November 2024) suggests pathogenic effect (Figure 3a) and other in silico prediction tools also support a deleterious effect of the variant (MT, DANN, Canonym, fitCons), while others report an uncertain effect (REVEL, MUT Assessor, SIFT, FATHMM, BayesDel).

The c.3896G > T, p.Arg1299Leu variant of the *FANCI* gene (15q26.1; NM_001113378.2) is a missense variant in exon 37 causing an arginine-to-leucine amino acid change at the 1299th position of the protein (Figure 2). According to the ACMG classification guideline, this variant is classified as a variant of unknown significance, considering the extremely low frequency of the variant in the gnomAD population databases (PM2). Some of the in silico prediction tools support a deleterious effect of the variant (MT, DANN, GenoCanyon, fitCons), while other tools such as EVE (Figure 3b), SIFT, FATHMM, and MetaLR predict an uncertain effect. This variant was previously only published in one paper as a candidate for susceptibility to ovarian cancer [7].

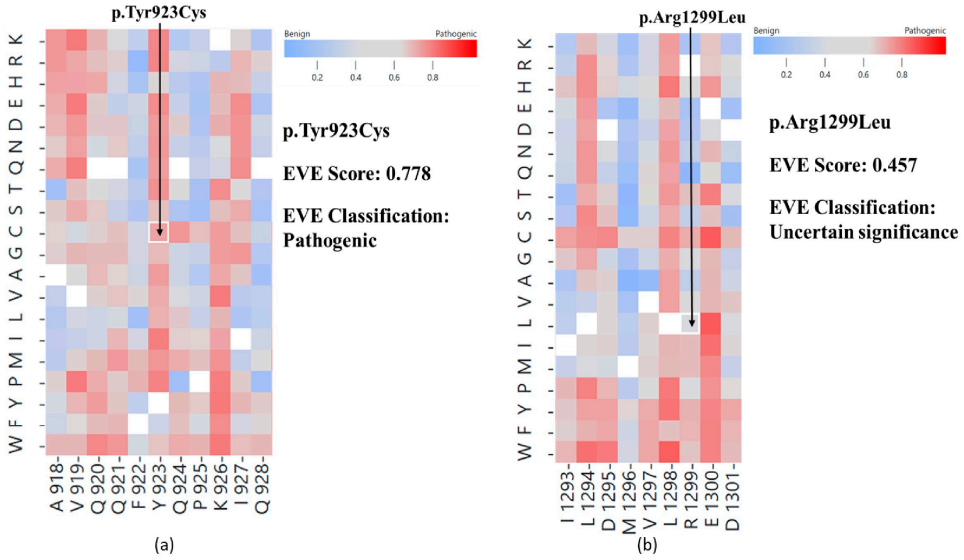

Figure 3. Heat maps of the evolutionary model of variant effect (EVE) scores of the missense variants of the FANCI protein. (**a**) Regarding the high EVE score (0.778) of the p.Tyr923Cys missense variant, it is classified as pathogenic according to the EVE classification. (**b**) Based on the medium EVE score (0.457) of the p.Arg1299Leu missense variant, it is classified as uncertain significance according to the EVE classification (https://evemodel.org/; accessed on 14 November 2024).

The p.Tyr923Cys variant affects the functional domain FANCI solenoid 3 (position 787–972 amino acids) and the variant p.Ser1038LeufsTer19 affects the functional domain FANCI solenoid 4 (position 985–1236 amino acids). The p.Arg1299Leu variant does not affect any known functional domain of the FANCI protein (SMART Protein, https://smart.embl.de/smart/show_motifs.pl?ID=Q9NVI1-1&DO_PFAM=DO_PFAM&; accessed on 14 November 2024) (Figure 2).

The novel c.337A > G p.Lys113Glu variant of the *RAD54B* gene (8q22.1; NM_012415.3) is a missense variant in exon 4 causing a lysine-to-glutamine amino acid change in the 113th position of the protein (Figure 2). Based on the ACMG classification guideline, this variant is classified as a variant of unknown significance, considering the extremely low frequency of the variant in the gnomAD population databases (PM2), and also the fact that in silico prediction tools unanimously support a benign effect on the gene (BP4) (Revel, MUT Assessor, MT, PrimateAI, BayesDel, SpliceAI).

The identified variants do not affect any non-coding RNA regions (Ensemble Genome Browser; Ensembl release 113, October 2024).

2.2. Clinical Outcomes

Amaral et al. (2020) identified an association between the reported variants of the *BRCA2*, *POLE*, *WRN*, *FANCI*, *PALB2*, and *RAD54B* genes and the patients' clinical outcomes as well as the therapy resistance [6]. Therefore, in the case of the four variants (three on the *FANCI* gene and one on the *RAD54B* gene) identified by this study, we also analyzed the clinical characteristics of the patients who harbor these variants.

The likely pathogenic variant in the *FANCI* gene is present in patient 15, who was first diagnosed with melanoma malignum at the age of 31 years (1999) [5]. The staging examinations showed no signs of metastases; consequently, only the excision of the melanoma was performed, without any additional treatment. After treatment for malignant melanoma, the patient attended follow-up examinations yearly, which did not show signs of late metastases, relapse, or second primary melanomas in the following years. In March 2023, a neck, thorax, abdominal, and pelvic CT scan was performed on the asymptomatic patient, where suspicion of multiplex cerebral and lung metastases, multiplex metastases in the liver and

spleen, as well as lymphadenomegalia colli l.s. et hilii, and contrast accumulation in the gall bladder and bladder were reported. Contrast cranial MRI confirmed the presence of multiplex brain metastases. The tissue biopsy taken from the liver and subsequent histopathology showed a liver metastasis of melanoma malignum. A careful clinical examination did not show any signs of the primary tumor, but the presence of a second primary melanoma malignum was suspected behind the disseminated multiplex metastases.

In patient 15, a genetic examination of the tissue biopsy showed BRAF positivity; however, considering the advanced and disseminated nature of the disease, especially the presence of brain metastases, palliative whole-brain radiation therapy (WBRT) was performed and a request for ipilimumab + nivolumab immunotherapy was submitted. Meanwhile, dabrafenib + trametinib targeted molecular therapy was administered for 3 months between April and July 2023, leading to a clinical improvement in the patient's condition. In July 2023, ipilimumab + nivolumab therapy was initiated and after three months it was switched to nivolumab monotherapy. With the continuous administration of ipilimumab + nivolumab combined immunoterapy, and then nivolumab monotherapy, the condition of the patient remained stable until the end of November 2023, when rapid clinical progression was observed, indicating resistance to immunotherapy. The treatment of the patient was once again switched to dabrafenib + trametinib combined molecular targeted therapy, but the therapy could not control the further progression of the disease leading to the patient's exit in March 2024.

The advanced nature of metastatic disease at the time of diagnosis in November 2023, the unfavorable prognosis of the disease, and resistance to immunotherapy and targeted molecular therapy in the presence of a likely pathogenic *FANCI* variant in the patient support the possible disease-modifying role of the *FANCI* gene in patients with malignant melanoma (Table 1).

Table 1. Clinical characteristics and therapies administered in the Hungarian cohort (n = 17). Patients carrying either *FANCI* or *RAD54B* variants are highlighted with a gray background.

Patient No.	Age of Onset	No. of Primary Melanomas	Lymphatic Metastasis	Other Metastasis	Therapy				
					Excision	Targeted Molecular Therapy	Immuno-Therapy	Radio-Therapy	Chemo-Therapy
1	58	1	no	no	yes	no	no	no	no
2	76	1	no	no	yes	no	no	no	no
3	82	1	no	no	yes	no	no	no	no
4	55	1	no	no	yes	no	no	no	no
5	26	2	no	no	yes	no	no	no	no
6	51	3	yes	no	yes	no	yes	no	no
7	44	0	no	no	no	no	no	no	no
8	57	0	no	no	no	no	no	no	no
9	42	1	no	no	yes	no	no	no	no
10	42	1	no	no	yes	no	no	no	no
11	51	1	no	no	yes	no	no	no	no
12	50	1	no	no	yes	no	yes	no	no
13	42	1	no	no	yes	no	no	no	no
14	43	1	no	no	yes	no	no	no	no
15	31	1	yes	yes, multiple	yes	yes	yes	yes	no
16	53	0	no	no	no	no	no	no	no
17	40	1	no	no	yes	no	no	no	no

In the case of patient 9, we identified another novel *FANCI* variant, the c.2768A > G, p.Tyr923Cys. The female patient was diagnosed with melanoma malignum at the age of 42 years and in the absence of lymphatic or other metastases, only an excision of the melanoma was performed in 2020, without adjuvant therapy. The patient had a positive family history of malignant melanoma (aunt on the father's side). Patient 9 is under regular dermatological care, and her condition is unchanged as of December 2024.

We also identified the c.3896G > T, p.Arg1299Leu variant in the *FANCI* gene in a 53-year-old female patient with dysplastic naevus syndrome, who had multiple dysplastic naevi removed, but in her case no melanoma malignum was observed yet. She also had a positive family history of melanoma malignum, as her father was affected by the disease. The patient is under regular dermatological care and her condition is unchanged as of December 2024.

In the case of the two novel VUS variants we identified in the *FANCI* gene, we were unable to establish any disease-modifying role based on the available clinical data of our patients, so further studies and careful follow-up of these patients are needed to determine their role in melanoma disease progression and therapeutic response.

Additionally, we identified a VUS variant in *RAD54B* in a 43-year-old female patient (patient 14), who had a stage-pT4 melanoma malignum at the time of diagnosis, without lymphatic involvement or other metastases. After excision of the cutaneous melanoma in 2019, no other therapy was administered, and after 5 years of follow-up the patient remains in remission. Based on this, we could not identify any evidence supporting the disease-modifying role of the germline *RAD54B* c.337A > G p.Lys113Glu variant regarding the unfavorable outcome, progression of malignant melanoma, or resistance to immunotherapy. However, the fact that she carries a VUS variant in a gene (RAD54B) that has been implicated in disease progression alerts us that she needs careful follow-up.

3. Discussion

Here, we report the genetic examination of a Hungarian melanoma cohort with increased risk. In our previous publication, we have summarized the germline variants of melanoma-predisposing and melanoma-susceptibility genes [5]. However, less attention is paid to genetic testing of germline variants of genes influencing patients' survival outcomes or enhancing the design of new therapies [6]. Here, we investigated whether melanoma patients in this published cohort harbor pathogenic or likely pathogenic germline variants in genes associated with unfavorable clinical outcomes [6].

The germline variants of *BRCA2*, *POLE*, *WRN*, *FANCI*, *PALB2*, and *RAD54B* genes, involved in DNS repair mechanisms, have been implicated in rendering melanoma patients more susceptible to tumor progression and affecting their response to treatments [8]. BRCA2 protein is involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair. In *BRCA2* mutation carriers, both uveal melanoma and cutaneous melanoma were found at significantly increased frequency [9–11]. Additionally, the germline variants in *BRCA2* have been found to increase the risk of melanoma and affect survival rates [12]. POLE and WRN are involved in maintaining genomic integrity through DNA replication and repair. Germline variants in these genes may impair these functions, contributing to higher levels of genomic instability in melanoma cells [13–15].

Additionally, variants of *FANCI*, *PALB2*, and *RAD54B* are associated with altered survival outcomes in melanoma patients [8,16,17]. *FANCI* is part of the Fanconi anemia pathway, which is vital for interstrand cross-link repair. Variants of *FANCI* may enhance DNA damage accumulation in melanoma cells, which may promote more aggressive cancer characteristics [17,18]. PALB2 protein partners with BRCA2 in homologous recombination,

and mutations in *PALB2* are similarly implicated in an increased melanoma risk and poorer survival [7].

FANCI has four distinct alpha solenoid segments (S1–S4). Regarding the three novel *FANCI* variants identified by this study, the p.Ser1038LeufsTer19 variant affects the solenoid 4 domain, and the p.Tyr923Cys variant is located within the solenoid 3 domain on the FANCI protein (Figure 3). Our results correlate well with previous findings, as heterozygous germline deletion in exon 9 reported by Amaral et al. (2020) is also located within the solenoid 3 domain of the FANCI protein [6].

Accumulating evidence suggests that genes associated with oncogenic pathways are identified as potential mini-drivers in tumor development [19]. Patients with rare pathogenic or likely pathogenic variants in mini-driver genes are association with worse tumor prognosis [19]. Further studies are needed to investigate the putative mini-drivers in melanoma.

In accordance with the observations about germline pathogenic and likely pathogenic *FANCI* variants in the literature, in the case of the novel, likely pathogenic c.3111_3123del, p.Ser1038LeufsTer19 variant in the *FANCI* gene, we detected a strong link with rapid disease progression, unfavorable outcome, and therapeutic resistance based on available clinical and genetic data of the patient. In the case of the other VUS variants in the *FANCI* and *RAD54B* genes in our Hungarian cohort, we observed no effect on disease progression or therapeutic response. Further studies are needed to report and highlight the clinical importance and relevance of genetic screening of putative germline variants that influence disease progression or therapeutic response in patients with melanoma.

4. Materials and Methods

4.1. Patients

In our current analysis, we included 17 Hungarian, unrelated melanoma patients, 10 females and 7 males. 14 patients were diagnosed with melanoma malignum, while 3 patients were diagnosed with dysplastic naevus syndrome. The histological characteristics of the tumors are summarized in Table 2.

Table 2. Histological characteristics of the melanoma in the Hungarian cohort (n = 17).

Patient No.	pTNM Stage	Breslow Thickness (mm)	Clark Level	Mitotic Rate (mm^2)	Presence of Ulceration/ Regression
1	pT2a	1.28	III	1–26	no
2	pT1a	0.5	II	0	regression
3	NA	NA	NA	NA	NA
4	pT4a	6	IV	2	ulceration
5	pT1a	0.35	II	2	no
	pT1a	0.32	II	1	no
6	pT3a	3.15	IV	2–3	regression
	pT2a	2	III	2–3	regression
	in situ superficially spreading melanoma				
7	patient is affected by dyspastic naevus syndrome				
8	patient is affected by dysplastic naevus syndrome				
9	pT3a	0.78	IV	2–3	ulceration

Table 2. *Cont.*

Patient No.	pTNM Stage	Breslow Thickness (mm)	Clark Level	Mitotic Rate (mm^2)	Presence of Ulceration/ Regression
10	pT1a	0.75	III	2	no
11	pT1b	0.6	IV	2	ulceration
12	pT2a	1.9	IV	1	no
13	pT1a	0.58	III	0	no
14	pT4a	4.7	III	3–4	no
15	pT1a	0.8	II	0	no
16	patient is affected by dyspastic naevus syndrome				
17	pT2a	1.21	IV	2	no

The mean age of the melanoma patients at the time of diagnosis of the first melanoma malignum was 49.5 years. Family history, the presence of any other immune system disease, and the detected *CDKN2A* variants are summarized in Table 3.

Table 3. Clinical features of and the presence of any *CDKN2A* variants in the patients (n = 17).

Patient No.	Immune System Disease	Family History of Melanoma	Presence of Any Germline *CDKN2A* Variant
1	unknown	negative	no
2	unknown	positive	no
3	unknown	negative	no
4	unknown	negative	yes, a leaning-pathogenic VUS
5	unknown	negative	no
6	unknown	negative	no
7	unknown	negative	no
8	ulcerative colitis	negative	no
9	unknown	positive	no
10	unknown	negative	no
11	unknown	negative	no
12	unknown	negative	no
13	unknown	negative	no
14	unknown	negative	no
15	unknown	negative	no
16	unknown	positive	no
17	unknown	negative	no

Further detailed clinical characteristics of the 17 members of the Hungarian malignant melanoma cohort with increased risk are summarized in our previous publication [5]. After genetic counseling and obtaining the written informed consent of the enrolled individuals, peripheral blood samples were taken, and genomic DNA was isolated using the QIAGEN DNeasy kit (Qiagen, Hilden, Northrine-Westfalia, Germany).

Of the 17 patients, 3 patients had more than one primary melanoma (patients 5, 6, and 15). Two patients had lymphatic metastases (patients 6 and 15), and only one patient (patient 15) had additional multiple metastases (multiplex brain, spleen, lung, liver metastases, contrast accumulation in the gallbladder and bladder). Each patient diagnosed with melanoma

malignum underwent excision of the melanotic lesion, three patients received immunotherapy (patients 6, 12, and 15), one patient received combined targeted molecular therapy (patient 14), while none of the patients required traditional chemotherapy. Patient 15 underwent palliative radiotherapy for brain metastases (whole-brain radiation therapy, WBRT) (Table 1).

4.2. Targeted Next-Generation Sequencing with a Virtual Gene Panel

Patients' genotypes were determined using a targeted next-generation sequencing (NGS) approach. Libraries were prepared using the SureSelectQXT Reagent Kit (Agilent Technologies, Santa Clara, CA, USA). Pooled libraries were sequenced on the Illumina NextSeq 550 NGS platform using the 300-cycle Mid Output Kit v2.5 (Illumina, Inc., San Diego, CA, USA). Adapter-trimmed and Q30-filtered paired-end reads were aligned to the hg19 human reference genome using the Burrows–Wheeler Aligner (BWA). Duplicates were marked using the Picard software package. The Genome Analysis Toolkit (GATK) was used for variant calling (BaseSpace BWA Enrichment Workflow v2.1.1. with BWA 0.7.7-isis-1.0.0, Picard: 1.79, and GATK v1.6-23-gf0210b3).

Sequencing revealed that the mean on-target coverage was 71× per base with an average percentage of targets covered greater than or equal to 30×, respectively. Variants passed through the GATK filter were used for downstream analysis and annotated using the ANNOVAR software tool (version 17 July 2017). Single-nucleotide polymorphism testing was performed as follows: high-quality sequences were aligned with the human reference genome (GRCh38/hg19) to detect sequence variants, which were analyzed and annotated. Variants were filtered according to read depth, allele frequency, and prevalence reported in genomic variant databases, such as ExAc (v.0.3) and Kaviar. Variant prioritization tools (PolyPhen-2, SIFT, LRT, Mutation Assessor) were used to predict the functional impact of the mutation. We interpreted the sequencing results using the Franklin Genoox website, which creates and uses a virtual panel that includes 6 genes (*BRCA2*, *POLE*, *WRN*, *FANCI*, *PALB2*, and *RAD54B*) influencing melanoma prognosis and survival [6].

The candidate variants were confirmed by bidirectional capillary Sanger sequencing carried out according to the standard protocol with an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Regions of 500 nucleotides upstream and downstream from the identified variants were picked from the Ensemble Genome Browser (https://www.ensembl.org/index.html?redirect=no; accessed on 3 July 2024) and used for the designing of the primers on the online website Blast Primer Designer (https://www.ncbi.nlm.nih.gov/tools/primer-blast/; accessed on 3 July 2024). The sequences of the primers used for Sanger sequencing are listed in Table 4.

Table 4. Primers used for the confirmation of the candidate variants with Sanger sequencing.

FANCI exon 25 forward primer	TTGTGGGGAGATTACACAACC
FANCI exon 25 reverse primer	TCTCAAGTGTCTTCTGGTAGGT
FANCI exon 25 forward primer	CAATACCACTTTCTCCTGCTTC
FANCI exon 25 reverse primer	CAGCCACTCTTTGTGGTTGA
FANCI exon 37 forward primer	GTGCGTGCTTGCTTTAGGTA
FANCI exon 37 reverse primer	ATCAAACAAGTCGGGGCAAC
RAD54B exon 4 forward primer	TGTGCCTTTTGGTTTTGTTTGAAT
RAD54B exon 4 reverse primer	AGATTGTCAGGCTCACTAACCA

Information regarding non-coding elements was searched by the Ensemble Genome Browser.

Author Contributions: Conceptualization, M.S. and N.N.; methodology, A.A.; software, F.K.; validation, M.P., A.A. and F.K.; formal analysis, Z.B. and B.A.B.; clinical investigation, N.N.; resources, M.S.; data curation, M.P.; writing—original draft preparation, B.A.B.; writing—review and editing, M.S. and N.N.; visualization, N.N.; supervision, N.N.; project administration, M.P.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the UNIVERSITY OF SZEGED (58523-4/2017/EKU).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper and pre- and post-test genetic counseling have been carried out.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors. The data are not publicly available because they are genetic data.

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

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Communication

A Novel Germline Frameshift Variant in the Tumor Suppressor Gene *OBSCN* in a Melanoma Patient

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Abstract

Malignant melanoma is a complex malignancy with genetic, environmental, and lifestyle factors in its etiology. While germline variants in melanoma predisposition genes have been described, many patients remain genetically unexplained after panel testing. We previously analyzed a Hungarian melanoma cohort (n = 17), identifying variants in predisposing or susceptibility genes in 58.82% of patients. For individuals negative on this melanoma-specific panel, we expanded testing to a 19-gene panel associated with multiple cancer types. Next-generation sequencing was performed, followed by Sanger sequencing for confirmation. Variants were classified according to ACMG guidelines. In a 58-year-old female patient with a history of primary cutaneous melanoma, we identified a novel heterozygous frameshift variant in the tumor suppressor gene *OBSCN* (c.21322_21323insCTGG, p.G7108AfsTer10; NM_001386125.1). This insertion introduces a premature stop codon in exon 89 within the immunoglobulin-like domain, predicting protein truncation. Classified as likely pathogenic (PVS1, PM2), the variant is absent from population databases. To date, somatic *OBSCN* mutations have been reported in melanoma. This first report of a germline *OBSCN* frameshift variant in melanoma expands the genetic landscape of melanoma predisposition and suggests that *OBSCN* may represent a candidate gene contributing to melanoma risk.

Keywords: melanoma; OBSCN; germline variant; cancer predisposition; next-generation sequencing

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1. Introduction

Malignant melanoma is a complex disease with a multifactorial etiology, involving genetic, environmental, and lifestyle-related factors that jointly contribute to its development and progression. The incidence of melanoma has been steadily rising worldwide, with approximately 331,722 new cases and 58,667 deaths estimated globally in 2022, according to GLOBOCAN data [1]. The burden is particularly high in populations of European ancestry, and the mortality rate remains substantial due to the tumor's high metastatic potential. A large body of evidence has accumulated over the last three decades supporting that inherited predisposition contributes to a proportion of melanoma cases, particularly in families with multiple affected members or patients presenting with melanoma at a relatively young age [2].

From the perspective of germline genetic susceptibility, melanoma can arise due to variants in high-penetrance predisposing genes, as well as medium- or low-penetrance susceptibility alleles [3]. Classical examples of high-penetrance melanoma predisposition genes include *CDKN2A* and *CDK4*, both of which regulate critical pathways of cell cycle control and senescence. In addition, genes involved in telomere maintenance (*TERT*, *POT1*, *ACD*, *TERF2IP*) and DNA repair pathways (*BAP1*, *BRCA1*, *BRCA2*, *TP53*) have been implicated in rare but strongly predisposing familial cases. On the other end of the spectrum, common variants of genes such as *MC1R*, *TYR*, *OCA2*, *SLC45A2*, and others contribute modestly to melanoma risk, often in the context of pigmentation phenotype and UV sensitivity [4]. The identification of pathogenic variants in such genes allows for individualized risk assessment, genetic counseling, and tailored surveillance of patients and their family members. Genetic testing panels have become increasingly important in clinical practice, as they allow simultaneous screening of multiple candidate genes associated with melanoma predisposition [5].

In our previous study, we analyzed a Hungarian cohort of 17 melanoma patients with increased risk and a personal or family history suggestive of genetic predisposition. Using a targeted next-generation sequencing panel comprising known melanoma-predisposing and melanoma-susceptibility genes, we identified germline genetic variants in 10 of the 17 patients (58.82%) [6]. These findings underscore the clinical relevance of panel testing in uncovering actionable germline variants in a significant proportion of high-risk melanoma patients. However, in the subset of patients who tested negative for pathogenic or likely pathogenic variants in this established melanoma panel, the question remained whether genes not traditionally associated with melanoma, but linked to other cancer types, might harbor relevant variants. This prompted us to broaden our analysis.

For those patients who were negative on the melanoma-specific panel, we expanded our investigation to include a broader spectrum of cancer-associated genes. To explore potential germline contributors beyond well-known germline melanoma genes, we analyzed a virtual panel of 19 genes selected based on their reported involvement in multiple tumor types and putative tumor suppressor or DNA repair roles. This strategy allowed the inclusion of genes implicated in other cancers, but not systematically investigated in melanoma. Specifically, we designed a virtual panel of 19 genes previously associated with the development of multiple different cancer types: *ABCA1*, *ADAMTSL3*, *ATP8B1*, *CUBN*, *DIP2C*, *EGFL6*, *EPHA3*, *EPHB6*, *FBXW7*, *FLNB*, *GNAS*, *MACF1*, *MLL3*, *OBSCN*, *PKHD1*, *SPTAN1*, *SYNE1*, *TECTA*, and *ZNF668* [7]. Targeted next-generation sequencing was performed, followed by rigorous bioinformatics analysis. Candidate variants of potential clinical relevance were subsequently validated through bidirectional capillary Sanger sequencing, ensuring high confidence in the detected variants.

The aim of this study was to investigate whether an expanded multi-cancer gene panel could identify novel germline variants potentially contributing to melanoma predisposition in patients negative for established high, medium and low-penetrance melanoma genes, and to report the discovery of a novel *OBSCN* truncating variant in these patients.

2. Results

2.1. Identification of a Novel OBSCN Variant

Within this expanded gene panel analysis, we identified a novel heterozygous germline variant of the tumor suppressor gene obscurin (*OBSCN*) in a 58-year-old female patient with a history of one primary cutaneous melanoma (Figure 1).

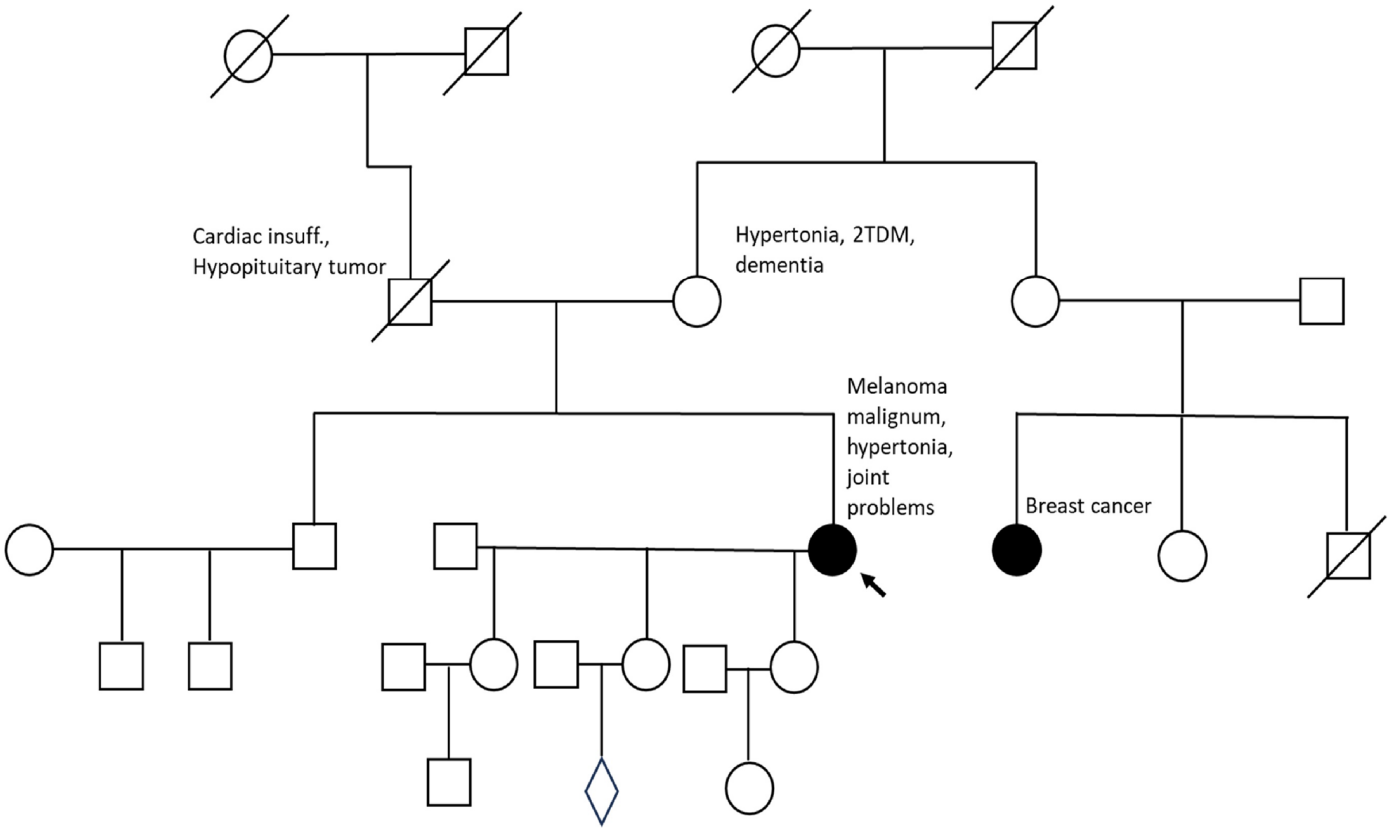

Figure 1. Pedigree of the Hungarian melanoma patient carrying the novel germline *OBSCN* variant. The proband (arrow) was diagnosed with a primary cutaneous melanoma and found to carry the heterozygous frameshift variant c.21322_21323insCTGG (p.G7108AfsTer10). The patient's mother also carries the same variant but has no cancer history to date. The father was not available for testing (deceased). Squares and circles represent males and females, respectively. Filled symbols denote individuals affected by cancer. Unfilled symbols denote individuals with no known cancer diagnosis. Diagonal lines indicate deceased individuals. The arrow marks the proband, the individual in whom the genetic evaluation was initiated. A horizontal line connecting a square and a circle represents a biological parental relationship, and vertical lines show offspring.

The detected variant is described as c.21322_21323insCTGG, p.G7108AfsTer10 (NM_001386125.1) (Figure 2).

This frameshift insertion occurs in exon 89, introducing a premature stop codon after amino acid position 7118 and leading to a truncated protein (Figure 3).

The patient's personal cancer history included only melanoma, with no evidence of other tumor types. She reported no known family history of melanoma, though her mother was found to carry the same *OBSCN* variant upon genetic testing. The patient's father was not available for testing, having passed away previously. At present, no additional cancer diagnoses have been recorded among her first-degree relatives (Figure 1).

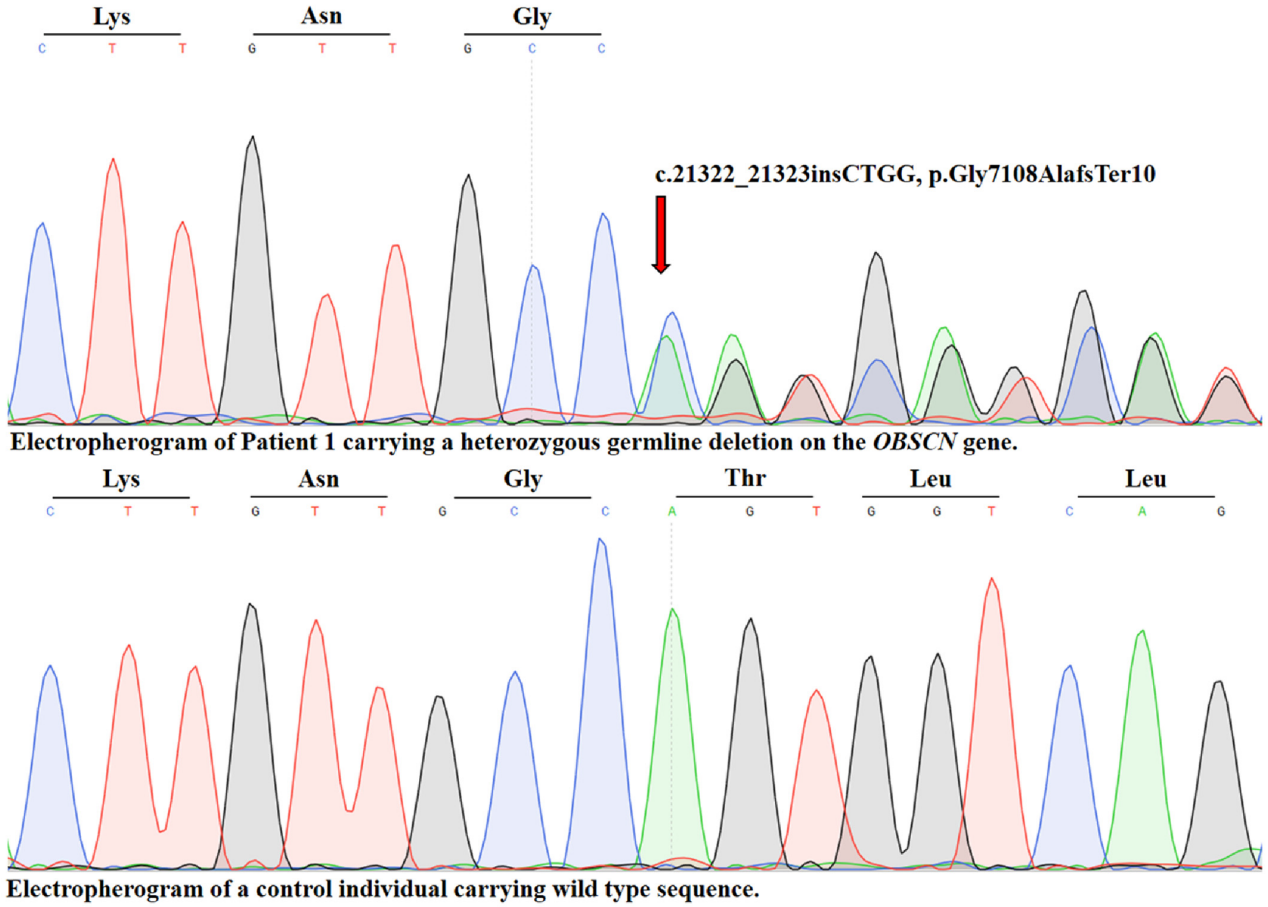

Figure 2. Sanger sequencing confirmation of the novel *OBSCN* variant. Bidirectional capillary sequencing verified the heterozygous frameshift insertion (c.21322_21323insCTGG, p.G7108AfsTer10) in exon 89 of the *OBSCN* gene (NM_001386125.1). The electropherogram demonstrates the site of insertion (arrow), confirming the presence of the variant in the proband. Colored peaks: represent the four nucleotides detected during capillary electrophoresis: Adenine (A) = green, Cytosine (C) = blue, Guanine (G) = black and Thymine (T) = red.

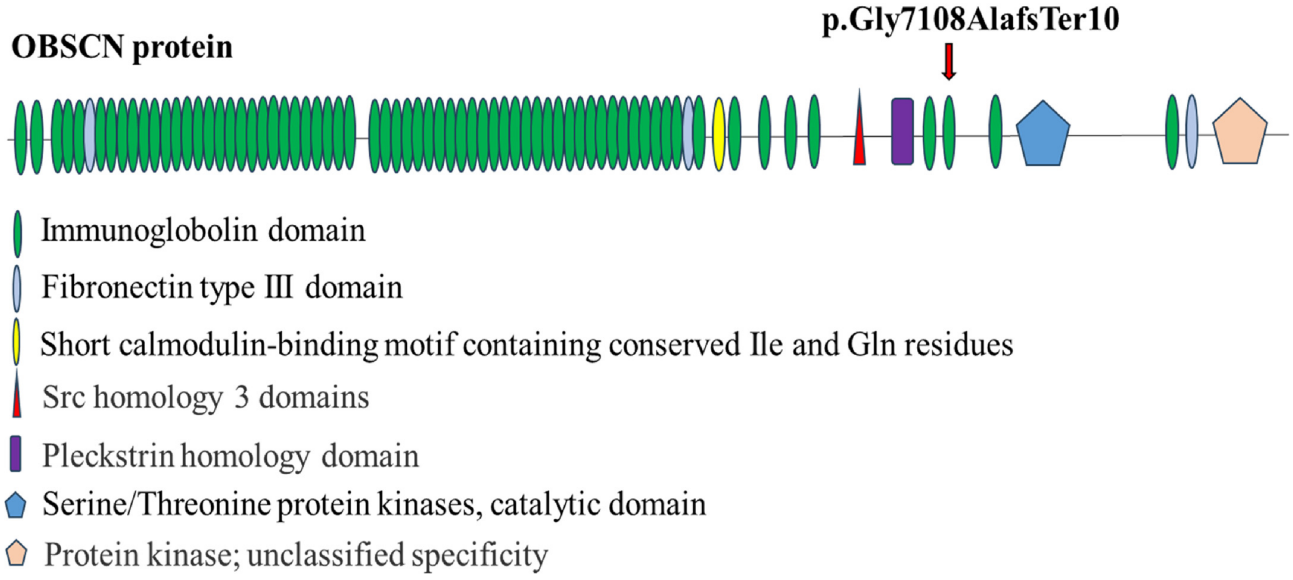

Figure 3. Schematic representation of the OBSCN protein domains and the location of the novel variant. The OBSCN protein comprises multiple structural and signaling domains, including immunoglobulin-like repeats, fibronectin type III domains, RhoGEF, and kinase domains. The novel c.21322_21323insCTGG,

p.G7108AfsTer10 germline frameshift variant (arrow) identified in the proband is indicated in exon 89, within the immunoglobulin-like domain (amino acids 7077–7146). The resulting premature stop codon is predicted to truncate the protein, potentially disrupting cytoskeletal organization and cellular signaling (SMART https://smart.embl.de/smart/show_motifs.pl, accessed on 20 March 2025).

2.2. Figures, Tables and Schemes

According to the American College of Medical Genetics and Genomics (ACMG) guidelines, the variant was classified as likely pathogenic, based on the following criteria:

- PVS1 (very strong evidence): A null variant (frameshift/nonsense) in a gene where loss of function is a known disease mechanism.
- PM2 (moderate evidence): The variant is absent, or extremely rare, in large population databases such as gnomAD, suggesting it is not a common benign polymorphism.

"In silico prediction tools supported the deleterious effect of the frameshift variant. The CADD PHRED-scaled score was 37, indicating a high likelihood of pathogenicity. MutationTaster predicted the variant as 'disease-causing,' and PROVEAN classified it as 'deleterious.' These computational data further support the classification of the OBSCN c.21322_21323insCTGG, p.G7108AfsTer10 variant as likely pathogenic according to ACMG guidelines."

No functional studies on this particular variant exist to date; however, its predicted truncating nature strongly supports a deleterious effect on protein function.

3. Discussion

The *OBSCN* gene, located at 1q42.13, encodes obscurin, a very large cytoskeletal protein that belongs to the family of giant sarcomeric signaling proteins [8]. Obscurin contains multiple immunoglobulin-like (Ig-like) and fibronectin type III (FnIII) domains, in addition to signaling motifs such as RhoGEF and kinase domains [9]. It plays a fundamental role in cytoskeletal organization, cell adhesion, cell–cell recognition, and intracellular signaling pathways [10]. OBSCN is one of the largest genes in the human genome, and its extensive length inherently increases the probability of acquiring both somatic and germline mutations [11].

The identified frameshift variant (p.G7108AfsTer10) is located within the Ig-like domain spanning residues 7077–7146 (Figure 1). Given the truncation within the immunoglobulin-like domain, it is plausible that the variant may compromise cytoskeletal stability and intracellular signaling organization, consistent with previously observed roles of obscurin in maintaining structural integrity and signal transduction. Although the truncating variant results in the loss of C-terminal signaling and kinase regions, several N-terminal domains, including the pleckstrin homology (PH) and Src homology 3 (SH3) domains, remain intact. The retention of these interaction motifs could allow partial binding to cytoskeletal partners but may result in an aberrant or non-functional protein complex. Alternatively, a dominant-negative effect cannot be excluded, whereby the truncated obscurin competes with the full-length protein for binding sites, potentially perturbing cytoskeletal and adhesion-related pathways.

Increasing evidence indicates that *OBSCN* acts as a tumor suppressor gene across multiple cancer types [12–14]. Loss or mutation of *OBSCN* has been implicated in: brain tumors, oral squamous cell carcinoma, gastrointestinal tract cancers, Wilms tumor, renal cell carcinoma, female reproductive cancers (ovarian, endometrial), prostate cancer, breast cancer and melanoma. The mechanism of tumorigenesis is thought to involve impaired cytoskeletal stability, altered cell–cell adhesion, and dysregulation of intracellular signaling, ultimately promoting invasive and metastatic phenotypes. Loss of heterozygosity (LOH) at

the *OBSCN* locus has been reported in several tumor contexts, supporting its role as a bona fide tumor suppressor.

To date, the literature on *OBSCN* in melanoma remains limited. Previous studies have reported only a somatic missense mutation (p.E4574K), located in the Fn-III 60 domain of the OBSCN protein, in melanoma tumor tissue [7]. No germline *OBSCN* variants have been previously associated with melanoma predisposition. Our finding therefore represents the first report of a nonsense germline likely pathogenic *OBSCN* variant in a melanoma patient. In the present case, no other relevant germline variant was identified in any of the known high, medium or low-penetrance melanoma genes, suggesting that the identified *OBSCN* variant may represent the only identified germline genetic factor, from which we can suppose that it might contribute to melanoma. The proband's mother, who carries the same variant but remains cancer-free, may reflect incomplete penetrance or the influence of protective genetic and environmental modifiers. This highlights the need for future segregation and functional studies to clarify *OBSCN*'s contribution to melanoma susceptibility. To note, OBSCN expression is not prognostic in melanoma according to TCGA and Protein Atlas data; rare truncating germline OBSCN variants could still contribute to melanoma susceptibility.

Given the truncating nature of this variant, its impact on protein function is expected to be more severe than the previously reported somatic missense substitution. The germline occurrence raises the possibility that *OBSCN* may play a role not only in tumor progression but also in individual susceptibility to melanoma development.

The identification of a novel germline *OBSCN* variant in a melanoma patient has several potential implications:

Expanded gene panels: This case supports the clinical relevance of using expanded multi-cancer panels in melanoma patients with negative standard results, as it enabled the identification of a novel OBSCN variant that would otherwise remain undetected.

1. Genetic counseling: Long-term dermatologic and oncologic follow-up is warranted for both the proband and her mother, considering the germline nature of the variant and its potential association with multi-tumor risk.
2. Surveillance: Given the involvement of OBSCN in breast, gastrointestinal, and gynecologic cancers, carriers might benefit from comprehensive surveillance protocols including annual dermatologic examinations, mammography or breast MRI starting at age 40, and colonoscopic screening per standard population guidelines.
3. Research directions: Future research should focus on both predictive and functional validation. Immediate steps include comprehensive in silico modeling and gene network analyses to identify pathways potentially affected by OBSCN loss. Definitive insights, however, will require in vitro studies assessing cytoskeletal organization and cell adhesion in melanoma cells expressing truncated OBSCN.

4. Materials and Methods

Genomic DNA was extracted from venous blood mixed with the anticoagulant EDTA using the DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany), as described in the manufacturer's instructions. For quantification Qubit Fluorometric Quantification instrument was used according to the manufacturer's instructions.

In this study, all samples were investigated using whole-exome sequencing (WES). The analysis presented in this manuscript, however, focuses on a 19-gene panel extracted from the WES data. This is a virtual panel and was designed to investigate genes with known relevance across multiple cancer types. Thus, the reported findings highlight variants within this virtual gene panel. Targeted next-generation sequencing (NGS) was performed with a virtual cancer gene panel comprising 19 genes associated with multiple tumor types

(*ABCA1*, *ADAMTSL3*, *ATP8B1*, *CUBN*, *DIP2C*, *EGFL6*, *EPHA3*, *EPHB6*, *FBXW7*, *FLNB*, *GNAS*, *MACF1*, *MLL3*, *OBSCN*, *PKHD1*, *SPTAN1*, *SYNE1*, *TECTA*, and *ZNF668)* [4]. Genotypes of patients were determined using next-generation sequencing. Library preparation was carried out using the SureSelectQXT Reagent Kit (Agilent Technologies, Santa Clara, CA, USA). Pooled libraries were sequenced on an Illumina NextSeq 550 NGS platform using the 300-cycle Mid Output Kit v2.5 (Illumina, Inc., San Diego, CA, USA). Adapter-trimmed and Q30-filtered paired-end reads were aligned to the hg19 Human Reference Genome using the Burrows–Wheeler Aligner (BWA). Duplicates were marked using the Picard software package. The Genome Analysis Toolkit (GATK) was used for variant calling (BaseSpace BWA Enrichment Workflow v2.1.1, with BWA 0.7.7-isis-1.0.0, Picard: 1.79 and GATK v1.6-23-gf0210b3). The mean on-target coverage achieved from sequencing was 71 per base, with an average percentage of targets covered greater or equal to 30 of 96% and 90%, respectively. Variants passed by the GATK filter were used for downstream analysis and annotated using the ANNOVAR software tool (version 2017 July 17). Single-nucleotide polymorphism testing was performed as follows: high-quality sequences were aligned with the human reference genome (GRCh37/hg19) to detect sequence variants, and the detected variations were analyzed and annotated. Variants were filtered according to read depth, allele frequency and prevalence in genomic variant databases, such as ExAc (v.0.3) and Curr. Issues Mol. Biol. 2023, 45 5302 Kaviar. Variant prioritization tools (PolyPhen2, SIFT, LRT, Mutation Taster, and Mutation Assessor) were used to predict the functional impact. For variant filtering and interpretation, VarSome and Franklin bioinformatic platforms [https://franklin.genoox.com, accessed on 18 May 2023] were used according to the guidelines of the American College of Medical Genetics and Genomics (ACMG), and population frequencies were checked in the gnomAD database. Candidate variants were confirmed by bidirectional capillary Sanger sequencing.

5. Conclusions

In summary, we identified a novel germline frameshift variant in the OBSCN gene (c.21322_21323insCTGG, p.G7108AfsTer10) in a Hungarian patient with malignant melanoma. The variant introduces a premature stop codon within an immunoglobulin-like domain of obscurin, likely resulting in protein truncation and loss of function. Given the established tumor suppressor role of OBSCN and its involvement in cytoskeletal organization, cell adhesion, and intracellular signaling, this alteration may have functional consequences relevant to melanomagenesis.

While OBSCN mutations have been described in several malignancies, including breast, gastrointestinal, and genitourinary cancers, no germline variants have previously been associated with melanoma predisposition. Our observation therefore broadens the mutational and functional spectrum of OBSCN and raises the possibility that its disruption contributes to melanoma susceptibility through impaired cytoskeletal signaling and cellular homeostasis.

Further studies in larger patient cohorts, as well as functional assays, will be necessary to confirm the pathogenicity and penetrance of OBSCN germline variants in melanoma. Nevertheless, this case underlines the value of using extended multi-cancer gene panels in genetically unexplained melanoma cases. Such approaches enhance the detection of rare but potentially relevant variants, thereby advancing our understanding of melanoma genetics and informing future risk assessment, genetic counseling, and precision oncology efforts.

Author Contributions: Conceptualization, M.S. and N.N.; methodology, A.A.; software and validation, M.P., and A.A.; formal analysis, B.A.B.; investigation, N.N.; resources, Z.B. and M.S.; data curation, M.P.; writing—original draft preparation, B.A.B.; writing—review and editing, M.S. and

N.N.; visualization, N.N.; supervision, N.N.; project administration, M.P.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of the University of Szeged (58523-4/2017/EKU, 12 September 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from the patients to publish this paper and pre- and post-test genetic counselling have been carried out.

Data Availability Statement: The data presented in this study are available from the corresponding author upon request. The data are not publicly available because they are genetic data.

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

Abbreviations

The following abbreviations are used in this manuscript:

ACMG	American College of Medical Genetics and Genomics
CDKN2A	Cyclin Dependent Kinase Inhibitor 2A
CDK4	Cyclin Dependent Kinase 4
TERT	Telomerase Reverse Transcriptase
POT1	Protection of Telomeres 1
ACD	Adrenocortical Dysplasia protein homolog (also known as TPP1)
TERF2IP	Telomeric Repeat Binding Factor 2 Interacting Protein
BAP1	BRCA1 Associated Protein 1
BRCA1/2	Breast Cancer Gene 1/2
TP53	Tumor Protein p53
MC1R	Melanocortin 1 Receptor
TYR	Tyrosinase
OCA2	Oculocutaneous Albinism II
SLC45A2	Solute Carrier Family 45 Member 2
NGS	Next Generation Sequencing
LOH	Loss of Heterozygosity
OBSCN	Obscurin
gnomAD	Genome Aggregation Database
Ig-like	Immunoglobulin like domain
FnIII	Fibronectin type III domain

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Research Letter

Longitudinal clinical characteristics of misfolding-induced hereditary pancreatitis caused by *PRSS1* p.L104P variant in a Hungarian family

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Chronic pancreatitis (CP) is a continuing inflammatory disease of the pancreas, characterized by irreversible morphological changes, typically causing chronic abdominal pain and permanent loss of function [1]. CP is a multifactorial disease; genetic, environmental, lifestyle factors, morphological variants of the pancreas and other risk factors also contribute to the disease development [1,2]. Genetic factors may act as causative genetic variants in the *PRSS1, CPA1 and CEL* genes, while other variants in the *CFTR*, *SPINK1*, *CTRC*, *CPA1*, *TRPV6* genes and CLDN2-MORC4 locus, CEL-MODY variants and *CEL-HYB1* haplotype may act as susceptibility factors for chronic pancreatitis [1–3].

The *PRSS1* gene (7q34; NM_002769.5) encodes human cationic trypsinogen, the zymogen form of the pancreatic digestive enzyme trypsin. Most disease-causing mutations in the *PRSS1* gene lead to increased intrapancreatic trypsin activity [4,5], however, in the last decade misfolding-causing *PRSS1* variants that result in sebsequent ER stress were published [6,7]. Pathogenic variants in the *PRSS1* gene cause hereditary chronic pancreatitis in an autosomal dominant manner with incomplete penetrance, 80% in case of the most common p.R122H variant [2–4].

In a previous communication, our study group published the data of a Hungarian family, with multiple family members carrying the rare misfolding-causing *PRSS1* p.L104P variant, most of whom developed chronic pancreatitis by adulthood [8]. In our current longitudinal study, we followed the clinical manifestations of misfolding-induced hereditary pancreatitis in the same family to observe and better understand the disease course.

Follow-up investigations were carried out [8] and further family members were recruited. Genetic testing of the index patient (Fig. 1., IV./2.), and family members III./2., IV./4., V./1., V./2. V./4., V./5., V./6. and V./7 (Fig. 1.). were carried out during our initial investigations [8], while genetic testing of family members VI./1., VI./2 (Fig. 1.). were carried out during our current longitudinal study. We obtained clinical data and performed stool elastase test of the participants. The patients received pretest and posttest genetic counseling from a clinical geneticist. All participants gave informed consent.

We identified the pathogenic *PRSS1* p.L104P variant in nine out of eleven investigeted family members. At the time of our initial study, only three family members were diagnosed with CP (IV./2., III./2. and IV./4.). However, during the follow-up period, two

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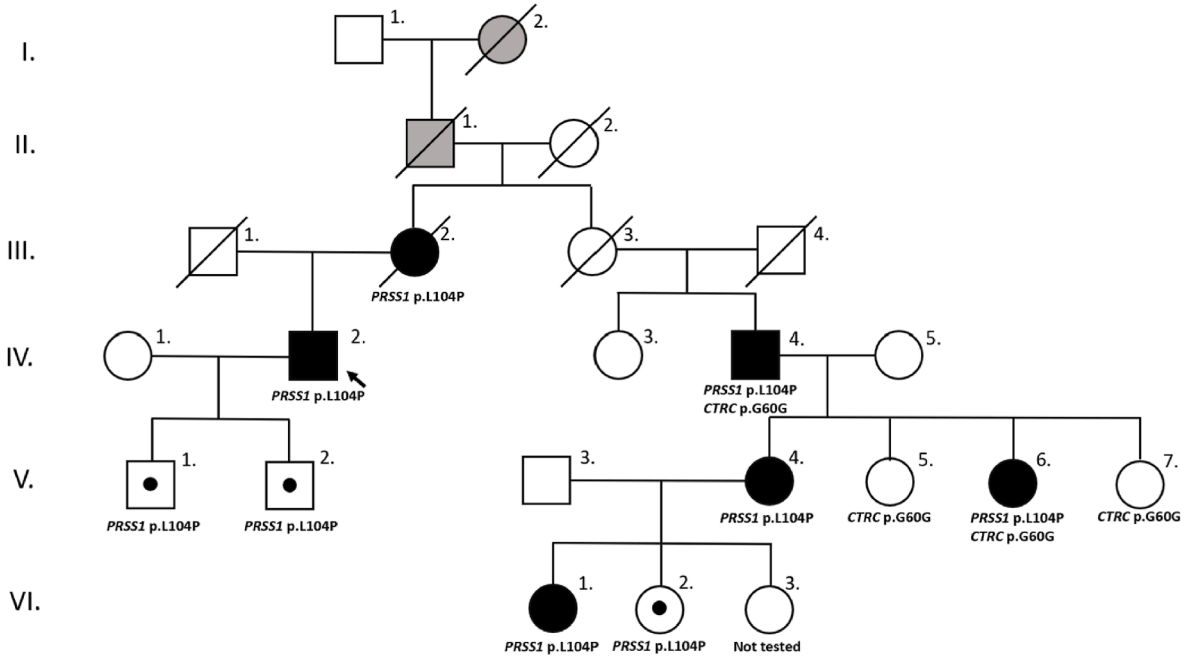

Fig. 1. Pedigree of the family with the *PRSS1* p.L104P variant. The empty circles represent the healthy females and the squares represent the healthy males on the pedigree, the black circles represent females diagnosed with chronic pancreatitis, the black squares represent males diagnosed with chronic pancreatitis. The empty circles and squares with a black dot in the middle represent asymptomatic females and males, respectively, carrying the pathogenic p.L104P variant. The gray circle and square represent family members with a suspected chronic pancreatitits. The crossed out circles and squares represent family members who have passed away. The arrow marks the index patient.

previously asymptomatic carriers (V./4. and V./6.) also developed CP, and a 5 year old girl (VI./1.) was also identified as a carrier of the p.L104P variant, and was simultaneously diagnosed with CP shortly after developing 3 episodes of severe AP.

The collected clinical data, the results of the imaging tests and the fecal elastase tests of symptomatic patients can be found in Supplementary Tables 1, 2 and 3. The age at diagnosis of CP was quite variable in the family, between 5 and 58 years. All symptomatic family members developed multiple episodes of AP before or after the diagnosis of CP. The imaging tests showed characteristic signs of CP, atrophy and calcification of the pancreas, every family member had dilation of the Wirsungian duct, five out of six patients had Wirsungolithiasis and two family members also developed pancreatic pseudocysts (Suppl. Table 2). The fecal elastase tests showed severe exocrine dysfunction in case of all symptomatic family members (except VI./1., whose fecal elastase test was carried out before the first acute episode), while the fecal elastase tests of all asymptomatic carriers showed normal exocrine function. Only patients IV./2. and IV./4. developed Type 3c diabetes mellitus to this date. It is also noteworthy, that while patient V./1. remained an asymptomatic carrier, he showed diminished growth rate during the follow-up period (10th pc height).

None of the family members developed pancreatic cancer to this date.

In our previous communication, we observed a reduced penetrance of the *PRSS1* p.L104P variant, however, we also hypothesized, that it can be due to the later age-of-onset of hereditary CP caused by the p.L104P variant [8]. In accordance with this hypothesis, some of the family members, who were previously identified as asymptomatic carriers developed chronic pancreatitis. The new observed penetrance is ~67% that is higher, than the ~43% penetrance previously reported [8] in the same family.

During our investigations, a young girl (Fig. 1., VI./1.) carrying only the pathogenic p.L104P variant also developed CP at the age of 5. This development is in accordance with the observations of Enea et al. [9], who identified the p.L104P variant in two children, who developed CP also at a young age. These observations point to a very variable age-of-onset caused by the p.L104P variant.

It is also noteworthy, that most family members were diagnosed with chronic pancreatitis at the time of the first documented acute episode, or shortly after, which leads to the assumption, that the morphological changes of the pancreatic tissue are present before the patients become symptomatic. However, we could not prove the presence of exocrine dysfunction with the fecal elastase tests in asymptomatic carriers.

The morphological presentation of the disease also showed some characteristic features. The imaging tests detected pancreatic stones and dilatation in the ductus Wirsungianus in almost each symptomatic patient. Based on these observations, we assume that the *PRSS1* p.L104P variant causes calcifying chronic pancreatitis with pancreatic stone formation and dilation of the Wirsungian duct.

In conclusion, based on our observations about the family carrying the *PRSS1* p.L104P variant, we can suggest that the pathogenic, misfolding-causing p.L104P variant is not only a strong susceptibility factor, but a causative genetic variant for hereditary chronic pancreatitis, with an incomplete penetrance and a variable age-of-onset compared to other *PRSS1* variants causing increased intrapancreatic trypsin activity.

Based on the autosomal dominant inheritance and incomplete penetrance of the p.L104P variant, we suggest carrier testing of both symptomatic and asymptomatic family members, if this variant is identified in a patient with recurrent acute or chronic pancreatitis. Careful genetic counseling is also recommended before and after the genetic testing, considering the diverse nature of the severity of the disease, age-of-onset and the incomplete penetrance, and the psychological burden caused by identifying a

pathogenic disease-causing variant in an asymptomatic family member.

Informed consent statement

All family members or their legal guardian (in case of family members under the age of 18 years) gave written informed consent to the data collection and genetic analysis.

Ethical and biobanking approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board and Ethics Committee of National Centre for Public Health and Pharmacy (protocol code 22254-1/2012/EKU (391/PI/2012), date of approval: 15 August 2012; and and TUKEB 36305-1/2016/EKU and NNK 17787-8/2020/EÜIG, date of approval: 18 May 2020).

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2026.02.003.

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