University of Szeged

Albert Szent-Györgyi Medical School

Doctoral School of Experimental and Preventive Medicine

The complexities of genetic testing and counseling in multifactorial and familial diseases

PhD Thesis

Barbara Anna Bokor M.D.

Supervisors:

Nikoletta Nagy M.D., Ph.D., D.Sc.

Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged

Department of Medical Genetics, University of Szeged, Szeged

Balázs Németh, M.D., Ph.D.

Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged

Department of Internal Medicine, University of Szeged, Szeged

List of publications forming the basis of the thesis

1. Bokor BA, Abdolreza A, Kaptás F, Pál M, Battyani Z, Széll M, Nagy N. Novel Variants in Medium and Low Penetrance Predisposing Genes in a Hungarian Malignant Melanoma Cohort With Increased Risk. Pigment Cell Melanoma Res. 2025 Jan;38(1):e13214. doi: 10.1111/pcmr.13214. Epub 2024 Nov 28. PMID: 39609110.

   Pigment Cell & Melanoma Research, Q1, IF: 2.6, 2025.01. (accepted: 2024.11.)

2. Bokor BA, Abdolreza A, Kaptás F, Pál M, Battyani Z, Széll M, Nagy N. Novel FANCI and RAD54B Variants and the Observed Clinical Outcomes in a Hungarian Melanoma Cohort. Int J Mol Sci. 2024 Dec 24;26(1):23. doi: 10.3390/ijms26010023. PMID: 39795882; PMCID: PMC11719457.

   International Journal of Molecular Sciences, Q1, IF: 4.9, 2024.12.

3. Bokor BA, Abdolreza A, Pál M, Battyani Z, Széll M, Nagy N. A Novel Germline Frameshift Variant in the Tumor Suppressor Gene OBSCN in a Melanoma Patient. Int. J. Mol. Sci. 2025, 26, 10553. https://doi.org/10.3390/ijms262110553

   International Journal of Molecular Sciences, Q1, IF: 4.9, 2025.10.

4. Bokor BA, Váncsa Sz, Patai ÁV, Hegyi P, Németh BCs. Longitudinal clinical characteristics of misfolding-induced hereditary pancreatitis caused by PRSS1 p.L104P variant in a Hungarian family. Pancreatology, 2026, ISSN 1424-3903, https://doi.org/10.1016/j.pan.2026.02.003.

   Pancreatology, Q1, IF: 2,7, 2026.02.

INTRODUCTION

Our research work centered around two diseases, which are both considered primarily complex diseases: melanoma malignum and chronic pancreatitis. While the genetic background of these diseases is considered primarily multifactorial and polygenic, both of these diseases are unique in the way, that they also have monogenic forms, that can be inherited with a Mendelian inheritance in an autosomal dominant manner.

Genetic testing and genetic counseling of rare Mendelian diseases is a part of the routine patient care, with clear professional guidelines and regulations in case of many inheritable diseases. With the increasing knowledge about the genetic background of complex diseases and the paradigm shift in helathcare towards personalized medicine, there is also an increasing need for genetic testing and counseling in case of more common complex diseases to improve patient care.

The germline genetic background of melanoma malignum

Melanoma malignum is considered as a complex disease, its development is influenced by genetic, environmental and lifestyle factors. Regarding its frequency, it is one of the most commonly diagnosed malignant tumors worldwide.

While the majority of genetic alterations found in melanoma malignum are somatic mutations, there is increasing evidence that underlying germline mutations play an important role in the disease development. Rare variants of high penetrance melanoma predisposing genes (*CDKN2A, CDK4, BAP1, POT1, ACD, TERF2IP* and *TERT*) can cause a very high melanoma susceptibility resembling to a monogenic disease with autosomal dominant inheritance. Rare variants in medium penetrance melanoma predisposing genes (*MC1R, MITF* and *SLC45A2*) and low penetrance genes (T*YR, OCA2, ASIP, PLA2G6, FTO, PARP1, ATM, CDKAL1, CCND1* and *CYP1B1*) are unable to cause the development of melanoma alone, but they are inherited in a polygenic manner and can significantly increase the personal melanoma risk.

In approximately 10% of melanoma patients there is a positive family history, which in itself means an elevated risk for further melanoma development. Concerning familial melanoma cases, approximatelly 50% of them are carrying rare, germline, disease-causing variants of high penetrance melanoma predisposing genes. Regarding the other half of these cases, the genetic background is unelucidated.

Besides the germline variants of the melanoma-predisposing and melanoma-susceptibility genes, accumulating evidence suggest that germline variants of other genes, involved in DNA

repair mechanisms, have been implicated in rendering melanoma patients more susceptible to tumor progression and affecting their response to treatments.

In the subset of patients negative for pathogenic or likely pathogenic variants in established melanoma susceptibility genes, the question remains whether genes not traditionally associated with melanoma, but linked to other cancer types, might harbor relevant variants.

The genetic backround of chronic pancreatitis

Chronic pancreatitis (CP) is a continuing inflammatory disease of the pancreas, by definition characterized by irreversible morphological changes, typically causing chronic abdominal pain and permanent loss of function. The etiology of pancreatitis is heterogenous. While it is clear, that genetic factors play an important role in the disease development, as a multifactorial disease, environmental, lifestyle factors, morphological variants of the pancreas and other risk factors also contribute to the development of chronic pancreatitis. To our current knowledge, genetic factors may act as causative genetic variants in the *PRSS1* gene, while variants in the *CFTR*, *SPINK1*, *CTRC*, *CPA1*, *TRPV6* genes and *CEL*-MODY variants and *CEL-HYB1* haplotype in the *CEL* gene can cause susceptibility to pancreatitis.

The *PRSS1* gene encodes human cationic trypsinogen, the zymogen form of the pancreatic digestive enzyme trypsin. Most disease-causing mutations in the *PRSS1* gene lead to increased autoactivation or decreased degradation of trypsinogen, causing increased intrapancreatic trypsin activity. Pathogenic variants in the *PRSS1* gene cause hereditary chronic pancreatitis in an autosomal dominant manner with incomplete penetrance.

The *CTRC* gene encodes the serine protease chymotrypsin C, which, based on functional studies, plays an important role in regulating trypsinogen activation, acting as a protective mechanism for intrapancreatic trypsin activation. Loss-of-function mutations in the *CTRC* gene lead to diminished degradation of trypsinogen, therefore leading to increased intrapancreatic trypsin activation, which can predispose to the development of pancreatitis by the trypsin-dependent pathway.

In the last decade misfolding-causing *PRSS1* variants that result in chronic pancreatitis independently from trypsinogen activation were published. The rare *PRSS1* p.L104P variant was first observed in sporadic cases of chronic pancreatitis, however, later it was linked to the development of hereditary chronic pancreatitis in case of 3 unrelated families.

AIMS

The aim of our studies was to better understand the genotype-phenotype associations of two complex diseases, melanoma malignum and chronic pancreatitis.

In order to get more insight into the germline genetic backgroung of melanoma, we first aimed to investigate 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 melanoma predisposing genes.

In our second study, we aimed to investigate whether the patients of the Hungarian melanoma cohort with increased risk (n=17) carry any pathogenic or likely pathogenic germline variants in genes associated with melanoma survival and response to therapy, and whether the presence of these variants correlate with the clinical findings of the patients.

The aim of our third 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.

In case of our investigations into the genetic background of chronic pancreatitis, our aim was to expand the genetic testing of a Hungarian family carrying the *PRSS1* p.L104P variant published previously by our study group, and follow the clinical manifestations of misfolding-induced hereditary pancreatitis in the same family to observe and better understand the disease course.

PATIENTS AND METHODS

Patients and samples of the Hungarian melanoma cohort with increased risk

In our studies regarding melanoma malignum, 17 unrelated Hungarian melanoma patients were enrolled. 10 patients were women and seven were men. 14 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.

The diagnosis for enrollment 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.

The studies were approved by the Hungarian National Public Health Centre and were conducted according to the Helsinki guidelines.

Targeted Next-Generation Sequencing With Virtual Melanoma Gene Panels

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 hu-man 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).

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 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 (https://franklin.genoox.com). In our first study, we created and used a virtual malignant melanoma panel that includes 30 genes associated with melanoma susceptibility (*ACD, AGR3, ARNT, ASIP, ATM, BAP1, CASP8, CCND1, CDK4, CDKAL1, CDKN2A, CYP1B1, FTO, HERC2, MBD4, MC1R, MITF, MX4, OCA2, PARP1, PLA2G6, POT1, SETDB1, SLC45A2, STN1, TERF2IP, TERT, TMEM38B, TYR* and *XRCC3)*. Secondary findings were screened and reported based on the current ACMG guidelines (ACMG SF v3.2).

In our secon study, we interpreted the sequencing results using a virtual panel that included 6 genes (*BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B*) influencing melanoma prognosis and survival.

In our third study of the Hungarian melanoma cohort, we interpreted the sequencing results using a virtual gene panel comprising 19 genes associated with multiple other tumor types (*ABCA1, ADAMTSL3, ATP8B1, CUBN, DIP2C, EGFL6, EPHA3, EPHB6, FBXW7, FLNB, GNAS, MACF1, MLL3, OBSCN, PKHD1, SPTAN1, SYNE1, TECTA,* and *ZNF668*).

Variants were interpreted based on the guidelines of the American College of Medical Genetics and Genomics. The candidate variants were confirmed by bidirectional capillary Sanger sequencing in all three of our studies.

Patients and samples of the Hungarian family with hereditary chronic pancreatitis

Follow-up investigations of family members with known hereditary pancreatitis from our previous communication were carried out and further family members were recruited. Clinical data were obtained.

Genomic DNA was extracted from whole blood or, in case of children, buccal swab samples. De-identified genomic DNA samples were obtained from the Hungarian National Pancreas Registry (ethical approval: 22254-1/2012/EKU and TUKEB 36305-1/2016/EKU and NNK 17787- 8/2020/EUIG, biobanking approval: IF702-19/2012). All participants gave informed consent according to the ethical guidelines of the Declaration of Helsinki.

PCR and DNA sequencing

Target sequences were amplified by conventional polymerase chain reaction (PCR). PCR was performed using 1.0 U HotStarTaq DNA Polymerase (Qiagen), 0.2 µM dNTP, 0.5 µM primers, 10x PCR buffer (Qiagen) and 10-50 ng of genomic DNA template in an end-volume of 20 µL. PCR amplicons were sequenced by the Sanger method.

Fecal elastase measurement

Semiquantitative meaurement of stool elastase concentration was performed based on the manufacturer's instructions (ScheBo ® Pancreatic Elastase 1 Stool Test).

RESULTS

Germline melanoma predisposing variants

Using a 30-gene panel, melanoma predisposing germline genetic variants were identified in 10 patients (58.82%) of the 17 member Hungarian cohort.

We identified rare germline heterozygous variants (n=11), one in a high penetrance melanoma susceptibility gene (*CDKN2A*), one in a medium penetrance gene (*MC1R*) and nine variants in low penetrance genes (*ATM, TYR, OCA2, SETDB1, FTO, CASP8, PARP1*). Among the 11 identified rare variants, six are novel ones, first identified by this study and five are recurrent ones.

Regarding the six novel variants, three of them are likely pathogenic ones: the p.Y143C missense variant in the *MC1R* medium penetrance melanoma predisposing gene, and two nonsense variants in a low penetrance genes: the p.Q218Ter variant in *CASP8* and the p.Q40Ter variant in the *FTO* gene were detected.

Three of the six novel rare germline hetrerozygous variants are classified as variants of uncertain significance (VUS) and located in low penetarnce genes. The identified novel rare VUS variants are the following: the *SETDB1* p.T582A missense variant is a leaning pathogenic VUS, the *PARP1* p.E175L and the *OCA2* p.T606I are VUS variants.

Among the identified recurrent rare germline heterozygous variants (n=5), a pathogenic one was identified in a low penetrance melanoma predisposing gene, the *TYR* gene. Three of the five recurrent variants are classified as leaning pathogenic VUS variants, one is located in the *CDKN2A* high penetrance gene, and two in low penetrance genes, *OCA2* and *SETDB1*. One rare recurrent germline VUS was detected in the *ATM* gene.

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. It has been recently described that the A allele of the p.R402Q variant is forming a risk haplotype with the C allele of the c.-301C>T promoter SNP (rs4547091) of the *TYR* gene for oculocutan albinism. After performing the segregartion analysis, we did not detect the CA risk haplotype in any of our melanoma patients.

In one melanoma patient, we identified the *BRCA1* p.C61G pathogenic variant as a secondary finding, which is a known disease-causing variant for hereditary breast and ovarian cancer.

Germline variants influencing melanoma prognosis

We identified mutations using a 6-gene panel in 4 of the 17 patients (23,5%). None of them overlaps with the variants reported by Amaral et al. (2020) in the *BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B* genes. However, we identified three novel variants in the FANCI gene in three patients, and one novel variant in the *RAD54B* gene in one melanoma patient.

The novel c.3111_3123del, p.S1038LfsTer19 variant of the *FANCI* gene is classified as likely pathogenic, while the novel c.2768A>G, p.Y923C and the novel c.3896G>T, p.R1299L variants of the *FANCI* gene are classified as variants of unknown singnificance. The novel c.337A>G p.K113E variant of the *RAD54B* gene is also classified as a variant of unknown significance.

The p.Y923C variant affects the *FANCI* solenoid 3 functional domain (position 787-972 amino acids) and the p.S1038LfsTer19 variant affects the FANCI solenoid 4 domain (position 985-1236 amino acids). The p.R1299L 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&).

We also analyzed the clinical characteristics of the patients harboring these variants.

The likely pathogenic variant in the *FANCI* gene is present in a patient, who was first diagnosed with melanoma malignum at the age of 31 years. He was diagnosed later with a second, occult melanoma with multiple metastases at the age of 55 years, and passed away 11 months after the diagnosis in spite of multiple therapeutic attempts.

The advanced nature of the metastatic disease at the time of diagnosis, the unfavourable prognosis of the disease, and the resistance to immunotherapy and targeted molecular therapy in the presence of a likely pathogenic *FANCI* variant in the patient supports the possible disease-modifying role of the *FANCI* gene in melanoma malignum patients.

In the case of the two novel VUS variants we identified in the *FANCI* gene, we could not establish any disease-modifying role based on the available clinical data of our patients, so further studies and the 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 the *RAD54B* in a 43 years old female patient with pT4 melanoma malignum at the time of diagnosis. After the excision of the cutaneous melanoma, no other therapy was administered, and after 5 years of follow-ups the patient remains in remission. Based on this, we could not identify any evidence supporting the disease-modifying role of the germline *RAD54B* p.K113E variant.

Germline variants of other tumor predisposing genes in melanoma patients

Within the expanded gene panel analysis, we identified the novel p.G7108AfsTer10 frameshift germline variant of the tumor suppressor gene *OBSCN* in a 58-year-old female patient with a

history of one primary cutaneous melanoma. According to the ACMG guidelines, the variant was classified as likely pathogenic.

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. One third-degree relative (cousin ont he mother's side) was affected by breast cancer, however, this family member declined genetic testing.

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

Hungarian family with hereditary chronic pancreatitis

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. However, during the follow-up period, two previously asymptomatic carriers also developed CP, and a 5 year old girl was also identified as a carrier of the p.L104P variant, and was simultaniosly diagnosed with CP shortly after developing three episodes of severe AP.

The age at diagnosis of CP was quite variable within 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. The fecal elastase tests showed severe exocrine dysfuntion in case of all symptomatic family members, while the fecal elastase tests of all asymptomatic carriers showed normal exocrine function. Only two patients developed Type 3c diabetes mellitus to this date. It is also noteworthy, that while a 13-year-old patient 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.

DISCUSSION

Germline melanoma malignum predisposing variants

Our study further widens the spectrum of the identified germline melanoma predisposing variants adding the data of a 17-member Hungarian cohort affected by familial melanoma.

The pathomechanism by which the different variants can lead to an elevated melanoma susceptibility are different. 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 of cells, while most of the medium and low penetrance genes can lead to an increased melanoma risk by forming a phenotype more prone to the develpoment of melanoma malignum (e.g. increased number or density of naevi, decreased pigmentation of the skin and hair, increased sensitivity to UV radiation). The results of our study seem to be in accordance with this.

While we identitfed predispoding variants in most of the patients, considering the multifactiorial nature of the disease, it is also noteworthy that some patients reported lifestyle factors that could contribute to the development of melanoma malignum (high sun exposure, frequent sunburns in childhood and early adulthood and the frequent use of tanning salons).

This study has identified six novel variants in genes associated with the development of melanoma. These variants not only further widen the variant spectrum of these genes but here we also describe their association with the familal melanoma phenotype. Among these novel variants the likely pathogenic, missense variant, the *MC1R* p.Y143C was detected in a medium penetrance melanoma predisposing gene. The likely pathogenic, nonsense variants *CASP8* p.Q218Ter and *FTO* p.Q40Ter were present in low penetrance genes. Three novel VUS variants were detected in the *SETDB1, OCA2* and *PARP1* low penetrance genes. Regading these VUS variants, further studies are needed to confirm their putative role in the development of melanoma.

Regarding the identified recurrent variants, the *ATM* p.R2912G variant, the *CDKN2A* p.V115L variant and the *SETDB1* p.R1076C variant were publisched in association with multiple other types of cancer, while the *OCA2* p.R811S and the *TYR* p.G109R variants were previously reported in association with oculocutan albinism.

As it can be seen above, after searching the literature, we can conclude, that the previously published variants identified in our study were not published in association with melanoma malignum susceptibility as to this date, except for the *TYR* c.1205G>A polymorphism.

Among the frequent variants of the melanoma predisposing genes, we highlight the *TYR* c.1205G>A variant, which is associated not only with oculocutan albinism, but also reported as a risk factor for melanoma malignum. However, we could not identify any patients in our cohort

carrying the reported *TYR* risk haplotypes, which could also be attributed to the relatively small sample size in our cohort.

We also identified the pathogenic *BRCA1* c.181T>G variant in our cohort as a secondary finding in one male patient with melanoma malignum. Altough the *BRCA1* gene is not listed as a susceptibility gene for melanoma malignum, there is some evidence in the literature indicating that pathogenic *BRCA1* or *BRCA2* mutations can lead to an increased risk of melanoma, especially in males. For this reason, in this patient, we considered this mutation as a pathogenic finding, and we would recommend considering the addition of the *BRCA1* and *BRCA2* genes to the list of melanoma-associated genes.

Germline variants influencing melanoma prognosis and survival

Germline variants of *BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B* genes, involved in DNA repair mechanisms, have been implicated in rendering melanoma patients more susceptible to tumor progression and affecting their response to treatments. The BRCA2 protein is involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair. *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. Additionally, variants of *FANCI, PALB2,* and *RAD54B* are associated with altered survival outcomes in melanoma patients. *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. PALB2 protein partners with BRCA2 in homologous recombination, and mutations in *PALB2* are similarly implicated in an increased melanoma risk and poorer survival.

FANCI has four distinct alpha solenoid segments (S1-S4). Regarding, the three novel *FANCI* variants identified by this study, the p.S1038LfsTer19 variant affects the solenoid 4 domain, and the p.Y923C variant is located within the solenoid 3 domain on the FANCI. Our results correlate well with the previous findings, since the heterozygous germline deletion in exon 9 reported by Amaral et al. (2020) also located within the solenoid 3 domain of the FANCI protein.

Other tumor predisposing variants in melanoma patients

The *OBSCN* gene encodes obscurin, a very large cytoskeletal protein that belongs to the family of giant sarcomeric signaling proteins. Obscurin contains multiple immunoglobulin-like (Ig-

like) and fibronectin type III (FnIII) domains, in addition to signaling motifs such as RhoGEF and kinase domains. It plays a fundamental role in cytoskeletal organization, cell adhesion, cell–cell recognition, and intracellular signaling pathways. *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.

The identified frameshift variant (p.G7108AfsTer10) is located within the Ig-like domain spanning residues 7077–7146. 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. 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.

Our finding 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, 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.

The identification of a novel germline *OBSCN* variant in a melanoma patient has several potential implications. First, this case supports the clinical relevance of using expanded multi-cancer panels in melanoma patients with negative standard results. Genetic counseling before and after the test is essential, and 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. Given the involvement of *OBSCN* in other cancers, carriers might benefit from comprehensive surveillance protocols.

Hungarian family with hereditary chronic pancreatitis

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 variant. In accordance with this hypothesis, some of the previously asymtomatic carriers did develop CP. The new observed penetrance is ~67%, that is higher, than the ~43% penetrance previously reported in the same family.

During our investigations, a young girl carrying only the p.L104P variant also developed CP at the age of five. This development is in contrast with our hypothesis, but in accordance with the observations of Enea et. al., who identified the p.L104P variant in two children, who developed CP also at a young age. These obesrvations point to a very variable age-of-onset caused by the p.L104P variant, rather than a later one.

It is also noteworthy, that most family members were diagnosed with CP 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 pancreatic 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 dilation in the ductus Wirsungianus in almost each symptomatic patient. Based on this observation, we can hypothesize, that the *PRSS1* p.L104P variant causes calcifying chronic pancreatitis with pancreatic stone formation and dilation of the Wirsungian duct.

CONCLUSIONS

Genetic testing and counseling should be implemented in the routine patient care of complex diseases in the future to achieve a more personalized approach in medicine. In case of complex diseases, high-throughput genetic testing methods should be used to find both rare, high-risk variants and common, low-risk variants contributing to personal disease risk, in order to identify high-risk patients based on their polygenic risk score, which in some cases can be comparable to a Mendelian risk.

With the use of high-throughput genetic testing methods, it is possible to examine the genetic profile of a patient in great detail, giving us the opportunity to not only identify disease-causing and predisposing variants, but also disease-modifying variants, causing individual differences in disease progression, survival and therapy response.

The implementation of these new approaches to genetic testing and the communication and interpretation of obtained genetic data through genetic counseling also needs special considerations in case of complex diseases. As risk-calculation is a highly important part of the genetic counseling process, we need to uncover the possible risk-variants as accurately as possible, as risk-calculation of complex diseases relies primarily on evidence of populational studies, and develop polygenic risk scores either specific to diseases or universally applicable for complex diseases that can help us calculate personal disease risk accurately.

The communication of this personal risk also needs special care from the genetic counselor's perspective, as the personal polygenic risk of a given disease can be quite variable, and for some patients, it can cause a significant psychological burden. During the communication, the counselor has to carefully explain the meaning and amount of a given risk, the advantages and disadvanteges of the methods used, the uncertainities of the observed and calculated risk that can arise from the possible incomplete knowledge about risk-variants and variants of unknown significance. The counselor also has to inform the patient about the possible health consequences of the complex disease, the screening and preventive options, potentially available therapies, and refer the patient to a specialist if needed. It is also very important to communicate the results in a way that the patient is able to understand and make informed decisions about their medical care and lifestyle choices.

Based on accumulating data about the genetic background of complex diseases available in the literature and presented in this thesis, a paradigm-shift and the development of a new, more personalized approach in the care of patients affected by complex diseases is needed, that should be based on identifying high-risk patients through genetic testing and developing new, universal guidelines for genetic counseling of patients with polygenic, multifactorial diseases.