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

Barbara Anna Bokor M.D.

PhD Thesis

Szeged

2026

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

1. Table of contents

2. 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.

3. List of abbreviations

ABCA1: ATP binding cassette subfamily A member 1

ACD: ACD shelterin complex subunit and telomerase recruitment factor

ACMG: American College of Medical Genetics and Genomics

ADAMTSL3: ADAMTS like 3

AP: acute pancreatitis

ASIP: agouti signaling protein

ATM: ATM serine/threonine kinase

ATP8B1: ATPase phospholipid transporting 8B1

BAP1: BRCA1 associated protein 1

BRCA1: breast cancer 1 gene

BRCA2: breast cancer 2 gene

CASP8: caspase 8

CCND1: cyclin D1

CDK4: cyclin dependent kinase 4

CDKAL1: CDK5 regulatory subunit associated protein 1 like 1

CDKN2A: cyclin dependent kinase inhibitor 2A

CEL: carboxyl ester lipase

CEL-MODY: carboxyl ester lipase – mature onset diabetes of the young

CEL-HYB1: carboxyl ester lipase hybride 1

CFTR: cystic fibrosis transmembrane conductance regulator

CLDN2: claudin 2

CP: chronic pancreatitis

CPA1: procarboxypeptidase A1

CTRC: chymotrypsinogen C

CUBN: cubilin

CYP1B1: cytochrome P450 family 1 subfamily B member 1

DDIT3/CHOP: DNA damage-inducible transcript 3/ C/EBP homologous protein

DIP2C: disco interacting protein 2 homolog C

EGFL6: EGF like domain multiple 6

EPHA3: EPH receptor A3

EPHB6: EPH receptor B6

ER: endoplasmic reticulum

EVE: evolutionary model of variant effect

FAMMM: familial atypical multiple mole melanome syndrome

FANCI: Fanconi anemia, complementation group I gene

FBXW7: F-box and WD repeat domain containing 7

FLNB: filamin B

FTO: fat mass- and obesity-associated gene

GNAS: Guanine Nucleotide binding protein, Alpha Stimulating activity polypeptide

MACF1: microtubule actin crosslinking factor 1

MC1R: melanocortin 1 receptor

min: minute

MITF: melanocyte inducing transcription factor

MLL3=KMT2C: lysine methyltransferase 2C

n.a.: not applicable

NGS: next generation sequencing

OBSCN: obscurin

OCA2: oculocutaneous albinism type 2

PRSS1: serine protease 1, human cationic trypsinogen

PALB2: partner and localizer of BRCA2

PARP1: poly(ADP-ribose) polymerase 1

PCR: polymerase chain reaction

PKHD1: PKHD1 ciliary IPT domain containing fibrocystin/polyductin

PLA2G6: phospholipase A2 group VI

PM2: pathogenic moderate

POLE: DNA polymerase epsilon, catalytic subunit

POT1: protection of telomeres 1

PVS1: pathogenic very strong

RAD54B: RAD54 homolog B

sec: secundum

SETDB1: set domain protein bifurcated 1

SLC45A2: solute carrier family 45 member 2

SPINK1: serine protease inhibitor Kazal type 1, pancreatic secretory trypsin inhibitor

SPTAN1: spectrin alpha, non-erythrocytic 1

SYNE1: spectrin repeat containing nuclear envelope protein 1

TECTA: tectorin alpha

TERF2IP: TERF2 interacting protein

TERT: telomerase reverse transcriptase

TRPV6: transient receptor potential cation channel subfamily V member 6

TYR: tyrosinase

VUS: variant of unknown significance

WBRT: whole brain radiation therapy

WES: whole exom sequencing

WGS: whole genom sequencing

WRN: WRN RecQ like helicase

ZNF668: zinc finger protein 668

4. Introduction

4.1. Genetic testing and counseling in complex diseases

In recent decades, there has been rapid evolvement in genetic testing methods, resulting not only in a wider spectrum of genetic testing modalities, but also an exponential increase in the amount of genetic information available. The development of high-throughput testing methods like whole-exom sequencing (WES), whole-genom sequencing (WGS) and different array-based methods have made it possible to gain invaluable genetic information throgh programs like the Human Genome Project, the 1000 Genomes Project and a great number of genome-wide association studies (GWAS) completed in recent years [1-3]. With the growing genetic knowledge at our disposal, we gained a deeper insight not only into the genetic background of rare Mendelian diseases, but also the genetic factors contributing to the development of complex, polygenic diseases [1-5].

Increasing evidence uncovered in the last decades suggest that most common, complex diseases have an underlying genetic background [3-6]. The development of these complex diseases can be attributed to the presence of a high-risk, rare pathogenic variant in 1-10% of cases, causing familial forms with a high-penetrance Mendelian inheritance pattern [4]. However, in the vast majority of cases, complex diseases are caused by the presence of several low-impact risk variants, which together form a polygenic risk score and result in varying degrees of individual disease risk, which can be modified by environmental and lifestyle factors due to the multifactorial nature of these diseases [4-7]. The development of complex, polygenic diseases can usually not be attributed to one single underlying pathomechanism, but rather the combined effect of impairment in multiple pathways caused by the accumulation of recurrent low-risk variants [7].

In the clinical practice, genetic testing and counseling traditionally aimed to identify the underlying rare, high-impact genetic variants causing rare genetic diseases with a Mendelian inheritance. However, the genetic information about complex diseases gained through technological advances in recent years led to the need of a paradigm-shift in patient care towards the introduction of personalized medicine [3]. While the genetic testing and counseling of patients with common complex diseases is not part of the routine patient care yet, it is very important to gain as much information as possible about the genetic background of these diseases, and create a framework for genetic counseling of complex diseases, as personal risk,

risk of family members, recommendations about prevention, screening and therapy all need special considerations in case of patients with polygenic, multifactorial diseases [3-7].

To better understand the genetic background and genotype-phenotype correlations of complex diseases, our research work presented in this thesis centered around two conditions, which are both considered primarily complex diseases: melanoma malignum and chronic pancreatitis [8-13]. While the genetic background of these diseases is considered primarily multifactorial and polygenic, both of these diseases have monogenic forms, that can be inherited with a Mendelian inheritance in an autosomal dominant manner.

4.2. 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 [8-10]. Regarding its frequency, it is one of the most commonly diagnosed malignant tumors worldwide. According to the data of the American Cancer Society, the overall lifetime risk of developing melanoma malignum is approximately 3% in Caucasian people. Approximately 330.000 new cases of melanoma malignum were diagnosed worldwide in 2022, leading to the death of 60.000 people [9].

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 [8-10]. The germline, rare, 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*) 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 (*TYR, 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 [8].

To note, some melanoma susceptibility genes are not only associated with the development of melanoma, but also with other malignant tumors [8, 9, 14]. The high penetrance melanoma predisposing gene *CDKN2A* is associated with melanoma-pancreatic cancer syndrome (OMIM 606719) and melanoma and neural system tumor syndrome (OMIM 155755). The low penetrance melanoma predisposing gene *ATM* is associated with breast cancer susceptibility (OMIM 114480).

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 [8]. 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 [15].

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. This prompted us to broaden our analysis, selecting a panel of 19 genes 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: *ABCA1*, *ADAMTSL3*, *ATP8B1*, *CUBN*, *DIP2C*, *EGFL6*, *EPHA3*, *EPHB6*, *FBXW7*, *FLNB*, *GNAS*, *MACF1*, *MLL3*, *OBSCN*, *PKHD1*, *SPTAN1*, *SYNE1*, *TECTA*, and *ZNF668* [16].

4.3. 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 [11]. 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 [11-13]. To our current knowledge, genetic factors may act as causative genetic variants in the *PRSS1* (serine protease 1, human cationic trypsinogen, OMIM 276000) gene, while variants in the *CFTR* (cystic fibrosis transmembrane conductance regulator, OMIM 602421), *SPINK1* (serine protease inhibitor Kazal type 1, pancreatic secretory trypsin inhibitor, OMIM 167790), *CTRC* (chymotrypsinogen C, OMIM 601405), *CLDN2* (claudin 2, OMIM 300520), *CPA1* (procarboxypeptidase A1, OMIM 114850), *TRPV6* (transient receptor potential cation channel subfamily V member 6, MIM 606680) genes, *CEL*-MODY variants and the *CEL-HYB1*

haplotype in the *CEL* (carboxyl ester lipase, MIM 114840) gene can cause susceptibility to pancreatitis [11-13, 17].

The *PRSS1* gene (7q34; NM_002769.5) encodes human cationic trypsinogen, the zymogen form of the pancreatic digestive enzyme trypsin. Whitcomb et al. identified *PRSS1* as a susceptibility gene for hereditary pancreatitis in 1996 [17], while also found the most common disease-causing variant c.365G>A, p.R122H. Most disease-causing mutations in the *PRSS1* gene lead to increased autoactivation or decreased degradation of trypsinogen, causing increased intrapancreatic trypsin activity [18, 19] (Figure 1). 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 [12, 13, 17, 18].

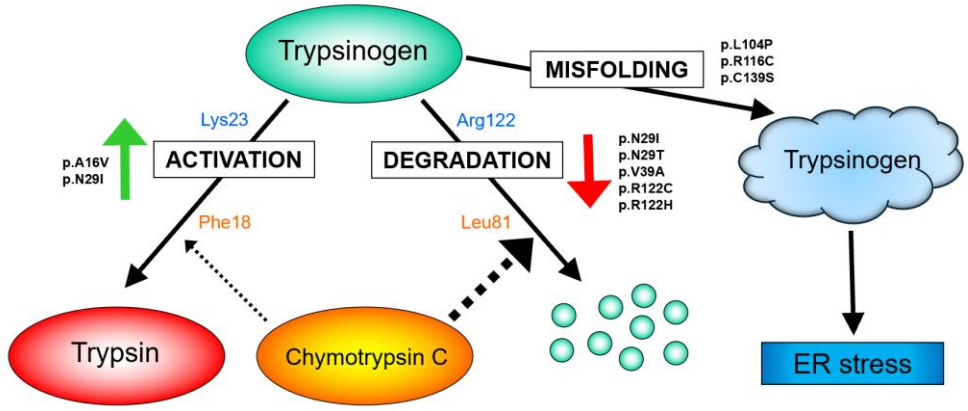

Figure 1. Molecular pathomechanisms of hereditary pancreatitis caused by pathogenic *PRSS1* variants. Pathogenic variants in the *PRSS1* gene can lead to the development of pancreatitis through the trypsinogen-dependent pathway, or the more recently discovered misfolding-dependent pathway. Pathogenic *PRSS1* variants can lead to the increased autoactivation, or the decreased degradation of trypsinogen. Pathogenic *CTRC* mutations may also predispose to the development of pancreatitis via the trypsin-dependent pathway, as chymotripsin C plays an important role in regulating trypsinogen autoactivation. Some pathogenic *PRSS1* mutations do not cause pancreatitis through the trypsinogen-dependent pathway, but their presence leads to the misfolding of the defective enzyme, intracellular retention of the misfolded protein, and consequently to ER stress, that results in the development of CP.

Modified figure based on the publication: Németh BC, Sahin-Tóth M: Human cationic trypsinogen (PRSS1) variants and chronic pancreatitis. 2014, Am J Physiol Gastrointest Liver Physiol. 306: G466–G473. [18]

The *CTRC* gene (1p36.21; NM_007272.3) encodes the serine protease chymotrypsin C, which, based on functional studies, plays an important role in regulating trypsinogen activation [20, 21], acting as a protective mechanism for intrapancreatic trypsin activation. Loss-of-function

mutations in the *CTRC* gene lead to diminished degradation of trypsinogen [20, 21], therefore leading to increased intrapancreatic trypsin activation, which can predispose to the development of pancreatitis by the trypsin-dependent pathway (Figure 1). The common *CTRC* c.180C>T, p.G60G variant, which we identified in some of the family members in the investigated Hungarian family, is a known predisposing factor for chronic pancreatitis [22, 23], which confers a 2-fold risk for the development of CP in heterozygous form, and a 5-fold risk in homozygous form [22].

In the last decade misfolding-causing *PRSS1* variants that result in chronic pancreatitis independently from trypsinogen activation were published [18, 24-29]. Kereszturi et al. were first to discover, that some rare pathogenic variants in the *PRSS1* gene do not stimulate autoactivation or diminish degradation of trypsinogen, but cause misfolding of the defective trypsinogen molecule, leading to increased ER stress [24] (Figure 1). The first identified misfolding-causing *PRSS1* variants were the p.R116C and p.C139S variants [24], since then many other variants leading to pancreatitis through the misfolding-dependent pathway were identified, including the p.D100H, p.C139F, p.K92N, p.S124F, p.G208A variants reported by Schnúr et al. [25], the p.S127C variant [26] or most recently, the novel p.A61V variant [28]. In case of the *PRSS1* c.311C>T, p.L104P variant, it was Balázs et al. who first described ER stress as the pathomechanism behind the variant's disease-causing role, proving the pathogenic role of the p.L104P variant [29].

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 [30-32].

5. Aims

The aim of our study was to better understand the genotype-phenotype associations of two complex diseases, melanoma malignum and chronic pancreatitis, and draw conclusions regarding the genetic investigations and genetic counseling in case of these complex, multifactorial diseases. The genetic background of both melanoma malignum and chronic pancreatitis are complex [8-13], with the uniqe trait that both melanoma and CP can be inherited in an autosomal dominant, as well as a polygenic, multifactorial manner.

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 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 correlate with the clinical findings of the patients including the advanced stage of melanoma, poor prognosis, bad survival and resistance to therapies.

For those patients who were negative on the melanoma-specific panel, we expanded our investigation to include a broader spectrum of cancer-associated genes. 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, in a previous communication, our study group published the data of a Hungarian family, with multiple family members carrying the *PRSS1* p.L104P variant, most of whom developed chronic pancreatitis by adulthood [30]. In our actual longitudinal study, we expanded the genetic testing of family members, and followed the clinical manifestations of misfolding-induced hereditary pancreatitis in the same family to observe and better understand the disease course.

6. Patients and methods

6.1. Melanoma malignum

6.1.1. Patients and samples

In our studies regarding familial melanoma malignum, 17 unrelated Hungarian 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 dermato-histological examinations (Table 1). 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.

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 for genetic testing 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 [14]. 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 (FAMMM). Three patients (Patient 2, 9, and 16) had a positive family history of malignant melanoma, three patients (Patient 5, 6 and 15) were diagnosed with two or more primary melanomas, and 12 patients (Patient 1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 16) had multiple dysplastic nevi removed. Tumors other than melanoma were diagnosed in six patients: five patients (Patient 1, 8, 10, 11 and 12) had an additional tumor (prostate cancer, basal cell carcinoma, thyroid cancer, adenomatous polyp of the transverse colon flexurae lienalis et sigmoidal with dysplasia grade 3, and lung adenocarcinoma), whereas one patient (Patient 6) had two primary cancers other than melanoma (adenocarcinoma of the large intestine and basal cell carcinoma infiltrating the skin). One patient (Patient 6) had lymphatic metastases at the time of diagnosis, and one patient (Patient 15) 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, cholecyst, and bladder, with lymphadenomegalia colli l.s. et hilii l.d.).

Patients	Sex	Age of onset	Number of primary MM	Number of dysplastic naevi	Other cancers	Family history
1	female	58	1	>5	none	negative
2	male	76	1	>3	prostate caner	positive
3	female	82	1	>10	none	negative
4	male	55	1	>3	none	negative
5	female	26	2	>10	none	negative
6	female	51	3	>10	adenocarcinoma invasivum intestini crassi, flexura lieanlis; carcinoma basocellulare	negative
7	female	44	0	>10	none	negative
8	female	57	0	>10	carcinoma basocellulare 2x	negative
9	female	42	1	<3	none	positive
10	female	42	1	>5	thyroid carcinoma	negative
11	male	51	1	>100	2 colon polyps with grade 3 dysplasia	negative
12	male	50	1	>10	lung adenocarcinoma, prostate hypertrophy	negative
13	male	42	1	>10	none	negative
14	female	43	1	>5	none	negative
15	male	31	2	>5	multiplex metastases	negative
16	female	53	0	>5	none	positive
17	male	40	1	>10	none	negative

Table 1. Summary of clinical characteristics in our cohort of Hungarian melanoma malignum patients.

Each patient diagnosed with melanoma malignum underwent the excision of the melanotic lesion, three patients received immunotherapy (Patient 6, 12 and 15), one patient received combined targeted molecular therapy (Patient 15), while none of the patients required traditional chemotherapy. Patient 15 underwent palliative radiotherapy of the brain metastases (whole brain radiation therapy, WBRT) (Table 2.).

The studies were approved by the Hungarian National Public Health Centre, the Institutional Ethics Committee of the University of Szeged (58523-4/2017/EKU) and were conducted according to the Helsinki guidelines. Our research was supported by the EFOP-3.6.1-16-2016-00008 grant and by the GINOP-2.3.2-15-2016-00039 grant.

Patient Nr.	Age of onset	Nr. 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	2	**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

Table 3. Clinical characteristics and administered therapies in the Hungarian melanoma malignum cohort with increased risk.

6.1.2. 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 human reference genome using the Burrows-Wheeler Aligner (BWA). Duplicates were marked using the Picard software package. 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 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. 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)* based on a review by Read, Wadt, and Hayward (2016) [8]. Secondary findings were screened and reported based on the current guidelines of the American College of Medical Genetics and Genomics (ACMG SF v3.2) [33].

In our second study, we interpreted the sequencing results using the Franklin Genoox website, creating and using a virtual panel that included 6 genes (*BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B*) influencing melanoma prognosis and survival [15].

In our third study of the Hungarian melanoma cohort, we interpreted the sequencing results using the Franklin Genoox website, creating and using a virtual cancer 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*) [16].

VarSome and Franklin bioinformatic platforms (https://franklin.genoox.com) were used and variants were interpreted based on the ACMG guidelines. The candidate variants were confirmed by bidirectional capillary Sanger sequencing in all three of our studies.

6.2. Hereditary chronic pancreatitis

6.2.1. Patients and samples

Follow-up investigations of family members with known hereditary pancreatitis from our previous communication [30] were carried out and further family members were recruited. Clinical data were obtained. Before sampling, patients received pretest genetic counseling from a clinical geneticist. If requested by the patient, the results of the genetic testing carried out for

research purposes were given to the patient or in case of minors (age <18 years), the legal representative, by a clinical geneticist within the framework of genetic counseling, as required by Hungarian law [34].

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.

6.2.2. Nomenclature

Nucleotide numbering started from the first nucleotide (denoted as c.+1) of the ATG translation initiation codon of the *PRSS1* (genomic reference: NC_000007.14, Homo sapiens chromosome 7, GRCh38.p14 primary assembly) and *CTRC* reference sequences (genomic reference: NC_000001.11, Homo sapiens chromosome 1, GRCh38.p14 primary assembly). Amino acids were numbered starting with the initiator methionine of the primary translation product.

6.2.3. PCR and DNA sequencing

All exons of the *PRSS1, SPINK1, CTRC* and *CPA1* genes and exons 4, 10 and 11 of the *CFTR* gene were sequenced in patients IV/2, III/2 and IV/4. Exons 2 and 3 of the *PRSS1*, exon 3 of SPINK1, exons 2,3 and 7 of *CTRC*, exons 7,8 and 10 of *CPA1* and exons 4 and 11 of *CFTR* genes were sequenced in patients V/4, V/6 and VI/1. In all unaffected family members *PRSS1* exon 3 was sequenced. In the unaffected children of the index patient's cousin (Figure 8., IV/4) and the unaffected daughter of patient V/4 *CTRC* exons 2 and 3 were also analyzed. Target sequences were amplified by conventional PCR. Polymerase chain reaction (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. The reaction started with a 15 min initial heat activation at 95 °C followed by 40 PCR cycles (20-30 sec of denaturation at 95 °C, 20-30 sec of annealing at 57-62 °C and 30-60 sec extension at 72 °C) and was completed by a final extension step for 5 min at 72 °C. PCR products were verified by 1.5 % agarose gel electrophoresis. The PCR amplicons (5 µL) were treated with 1 µL FastAP Thermosensitive Alkaline Phosphatase and 0.5 µL Exonuclease I (Thermo Fisher Scientific) for 15 min at 37 °C, and the reaction was stopped by heating the samples to 85 °C for 15 min. PCR amplicons were sequenced by the Sanger method.

6.2.4. Fecal elastase measurement

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

7. Results

7.1. Melanoma malignum

7.1.1. Germline melanoma predisposing variants

Using a 30-gene melanoma panel, melanoma predisposing germline genetic variants were identified in 10 (58.82%) patients of the 17-member Hungarian cohort (Table 3.).

We identified rare germline heterozygous variants (n=11; Table 3.), 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 a medium penetrance melanoma predisposing gene, the melanocortin 1 receptor gene (*MC1R*), and two nonsense variants in low penetrance genes: the p.Q218Ter in caspase 8 gene (*CASP8*) and the p.Q40Ter variant in fat mass- and obesity-associated gene (*FTO*) were detected.

The novel p.Y143C (c.428A>G) variant of the *MC1R* (16q24.3; NM_002386.4) is a missense variant in exon 1 causing a tyrosine to cysteine amino acid change in the 143th position of the protein. Based on the ACMG classification guideline, this variant is classified as likely pathogenic, considering the extremely low frequency of the variant in gnomAD population databases (PM2). Since it is a missense variant, most computational prediction tools (BayesDel, SIFT, DANN, MT) support a deletorious effect on the gene (PP2), while one prediction tool reports an uncertain effect (FATHMM).

The novel p.Q218Ter (c.652C>T) variant of the *CASP8* (2q33.1; NM_033358.4) is a nonsense variant in exon 7 resulting in the formation of a premature termination codon after the 218th amino acid of the polypeptide. 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 it has an extremely low frequency in the gnomAD database. However, most *in silico* prediction tools suggest a benign effect (BayesDel noAF, BayesDel addAF, EIGEN, FATHMM, DANN), and one prediction tool reports an uncertain effect (MutationTaster).

Patient No.	Gene	Variant	Classification	Frequency	Novelty	Penetrance
1	*ATM*	c.8734A>G p.Arg2912Gly	VUS with uncertain significance	rare	recurrent	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
2	*TYR*	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
3	*OCA2*	c.2433G>T p.Arg811Ser	Leaning pathogenic VUS	rare	recurrent	low
4	*CDKN2A*	c.343G>T p.Val115Leu	Leaning pathogenic VUS	rare	recurrent	high
	SETDB1	c.1744A>G p.Thr582Ala	Leaning pathogenic VUS	rare	novel	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
5	*TYR*	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
6	*TYR*	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
7	*FTO*	c.118C>T p.Gln40Ter	Likely pathogenic	rare	novel	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
8	*SETDB1*	c.3226C>T p.Arg1076Cys	Leaning pathogenic VUS	rare	recurrent	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
9	*CASP8*	c.652C>T p.Gln218Ter	Likely pathogenic	rare	novel	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
10						
11	*TYR*	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
12	*OCA2*	c.1817C>T p.Thr606Ile	VUS with uncertain significance	rare	novel	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
13	*TYR*	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
14						
15	*PARP1*	c.523G>A p.Glu175Lys	VUS with uncertain significance	rare	novel	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
16	*MC1R*	c.428A>G p.Tyr143Cys	Likely pathogenic	rare	novel	medium
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low
17	*TYR*	c.325G>A p.Gly109Arg	Pathogenic	rare	recurrent	low
	TYR	c.1205G>A p.Arg402Gln	VUS/risk factor	high	recurrent	low

Table 3. Summary of the identified germline variants in the 17-member cohort of Hungarian familial melanoma patients. All the variants were detected in heterozygous form.

The p.Q40Ter (c.118C>T) variant of the *FTO* gene (16q12.2; NM_001080432.3) is a nonsense variant in exon 2 resulting in the formation of a premature termination codon after the 40th

amino acid of the amino acid chain. This change may either cause a severely impaired protein function, or lead to mRNA-mediated decay of the truncated protein. Based on the ACMG classification guideline, this variant is classified as likely pathogenic, considering its extremely low frequency in gnomAD population databases (PM2), and the fact that the variant is a null variant, and loss-of-function is a known mechanism of disease in the *FTO* gene (PVS1). The predictions of *in silico* computational tools are conflicting: some support a pathogenic effect (BayesDel addAF, BayesDel noAF, EIGEN), while others support a benign effect (FATHMM, LRT), and some tools report an uncertain result (Dann, Mutationtaster).

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 as follows: the p.T582A missense variant is a leaning pathogenic VUS in the set domain protein bifurcated 1 (*SETDB1*) gene. The p.E175L VUS is located in the poly(adp-ribose) polymerase 1 (*PARP1*) gene, and the p.T606I VUS was detected in the oculocutaneous albinism type 2 (*OCA2*) gene.

Among the identified recurrent rare germline heterozygous variants (n=5), a pathogenic one was identified in low penetrance melanoma predisposing gene, the tyrosinase (*TYR*) gene. Three of the five recurrent variants are classified as leaning pathogenic VUS, one is located in a high penetrance gene, the cyclin-dependent kinase inhibitor 2a (*CDKN2A*) gene, and two in low penetrance genes, *OCA2* and *SETDB1*. One rare recurrent germline VUS was detected in the ataxia-telangiectasia mutated gene (*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 (Table 3.). 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 [35]. After performing the segregartion analysis, we did not detect the CA albinism risk haplotype in any of our melanoma patients.

In one melanoma patient, we identified the *BRCA1* (17q21.31 NM_007294.4):c.181T>G p.C61G pathogenic variant as a secondary finding, which is a known disease-causing variant for hereditary breast and ovarian cancer.

7.1.2. Germline variants influencing melanoma prognosis

We identified further mutations using a 6-gene panel in four of the 17 patients (23,5%). None of them overlaps with the variants in the *BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B* genes reported by Amaral et al. (2020), who were the first to investigate this panel of genes in connection with melanoma progression, survival and therapy resistance [15]. However, we identified three novel variants in the Fanconi anemia, complementation group I gene (*FANCI*) in 3 patients (Patient 9, 15 and 16), and one novel variant in the RAD54 Homolog B gene (*RAD54B*) in one melanoma patient (Patient 14) (Figure 2.).

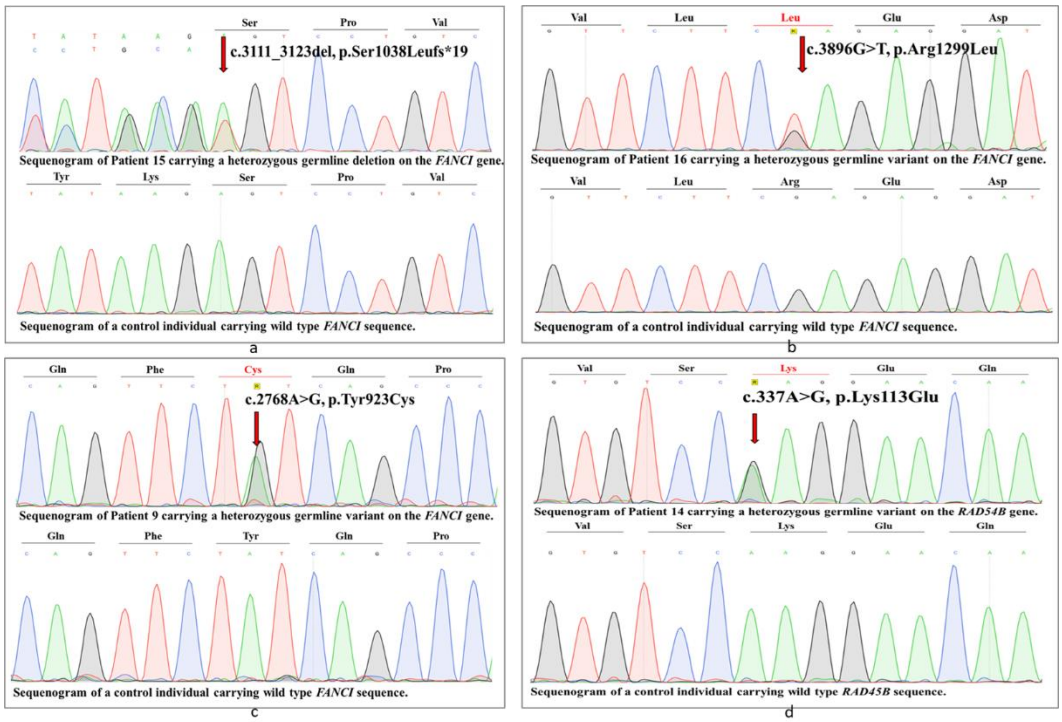

Figure 2. Sequenograms of the novel *FANCI* and *RAD54B* variants identified in the Hungarian melanoma cohort (n=17). (a) The novel likely pathogenic *FANCI* variant c.3111_3123del, p.S1038LfsTer19 is carried by Patient 15. (b) Among the novel missense VUS *FANCI* variants, the c.2768A>G, p.Y923C is present in Patient 9, (c) the c.3896G>T, p.R1299L in Patient 16. (d) The novel likely pathogenic *RAD54B* variant is detected in Patient 14.

The novel c.3111_3123del, p.S1038LfsTer19 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 2a.). 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 it has an extremely low frequency in the gnomAD database (PM2).

The novel c.2768A>G, p.Y923C 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 2b.). 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 gnomAD population databases (PM2). EVE (evolutionary model of variant effect; https://evemodel.org/) 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).

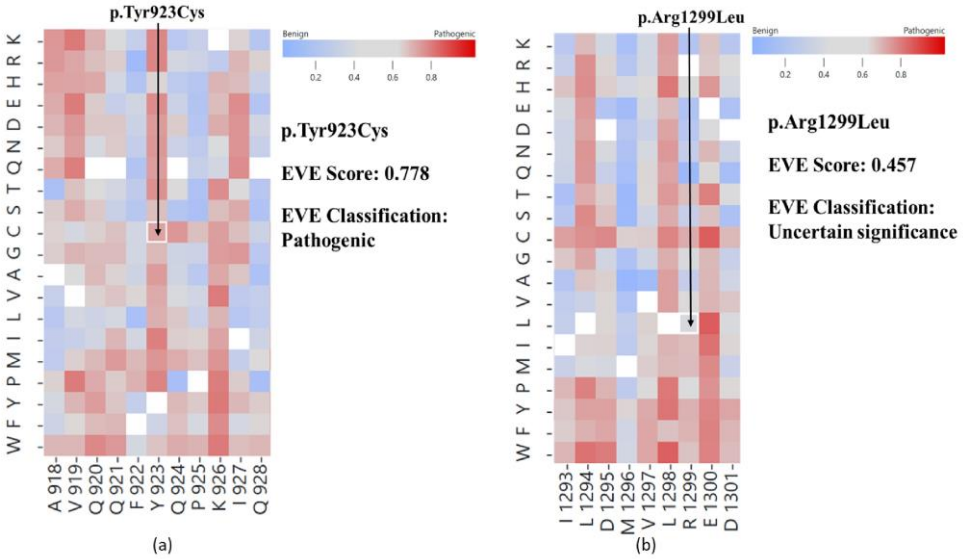

Figure 3. Heatmaps of the evolutionary model of variant effect (EVE) scores of the missense variants of the FANCI protein. (a) Concerning the high EVE score (0.778) of the p.Y923C missense variant it is classified as pathogenic according to the EVE classification. (b) Based on the medium EVE score (0.457) of the p.R1299L missense variant, it is classified as uncertain significance according to the EVE classification (https://evemodel.org/).

The c.3896G>T, p.R1299L 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 in the 1299th position of the protein (Figure 2c.). 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 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 only published previously in one paper as a candidate for ovarian cancer susceptibility [36].

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&) (Figure 4.).

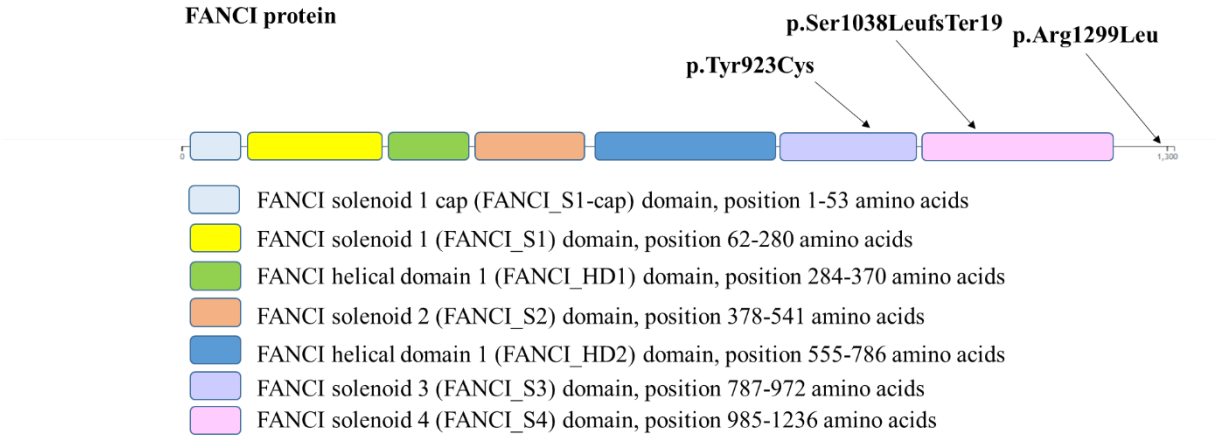

Figure 4. The position of the identified variants on the FANCI protein. The p.Y923C variant is located within the FANCI solenoid 3 (FANCI_S3) functional domain on the protein. The other two identified *FANCI* variants do not affect any known FANCI domain (https://www.rcsb.org/sequence/3s51).

The novel c.337A>G, p.K113E 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 2d.). 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 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).

Amaral et al. (2020) identified an association among the reported variants of the *BRCA2, POLE, WRN, FANCI, PALB2* and *RAD54B* genes and the patients' clinical outcomes as well as therapy resistance [15]. Therefore, in case of the four variants (three in the *FANCI* gene and one in the

RAD54B gene) identified by this study, we also analyzed the clinical characteristics of the patients harboring 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.) [37]. The staging examinations showed no signs of metastases, consequently only the excision of the melanoma was performed, without any additional treatment. After the removal of melanoma malignum, the patient attended follow-up examinations yearly, which did not show any signs of late metastases, relapse or second primary melanomas in the following years. In March of 2023 a neck, thorax, abdominal and pelvic CT scan was performed of the asymptomatic patient, where the 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. The contrast cranial MRI confirmed the presence of multiplex brain metastases. The tissue biopsy taken from the liver and consequent histopathology showed a liver metastasis of melanoma malignum. 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, genetic examination was performed from 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. In the meantime, dabrafenib + trametinib targeted molecular therapy was administered for three months between April and July 2023, leading to 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 the immunotherapy. The treatment of the patient was once again switched to dabrafenib + trametinib combined molecular targeted therapy, but the therapy could not control further progression of the disease, which lead to the exitus of the patient in March 2024.

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 (Table 1.).

In the case of Patient 9, we identified another novel *FANCI* variant, the c.2768A>G, p.Y923C variant. 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, without adjuvant therapy. The patient had a positive family history of melanoma malignum (aunt on the father's side). Patient 9 is under regular dermatological care, her condition is unchanged.

We also identified the c.3896G>T, p.R1299L variant in the *FANCI* gene in a 53 years 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.

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 the role of these variants in melanoma disease progression and therapy response.

Additionally, we identified a VUS variant in the *RAD54B* gene in a 43 years old female patient (Patient 14), who had a stage pT4 melanoma malignum at the time of diagnosis, without lymphatic involvement or other metastases. 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* c.337A>G, p.K113E variant regarding the unfavourable outcome, progression of melanoma malignum, or resistance to immunotherapy. However, the fact that the patient carries a VUS variant in a gene (*RAD54B*) that had been implicated in modifying disease progression alerts us that she needs a careful follow-up.

7.1.3. Germline variants of other tumor predisposing genes in melanoma patients

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 (III./4., Figure 5.).

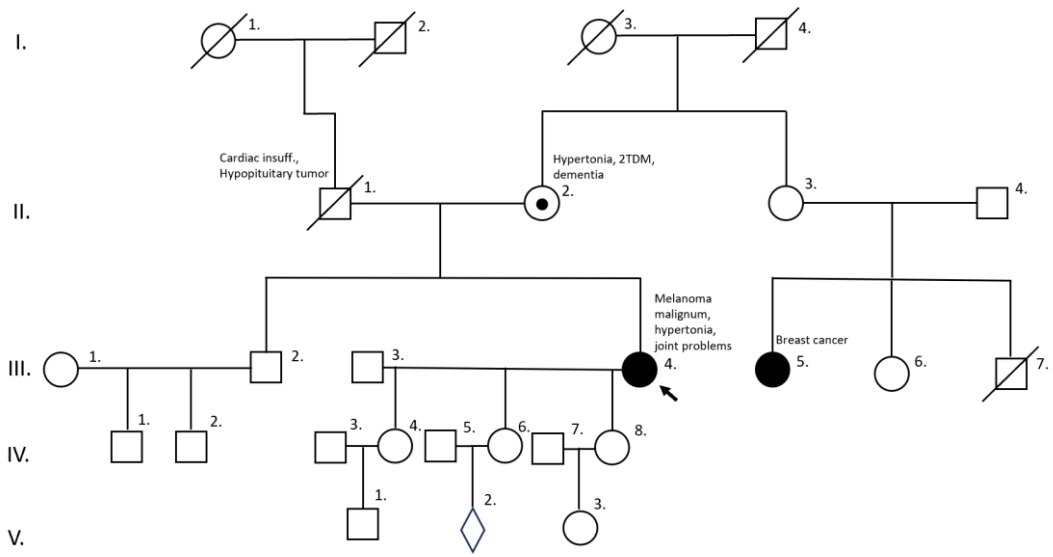

Figure 5. Pedigree of the Hungarian melanoma patient carrying the novel germline *OBSCN* variant. The proband (arrow) was diagnosed with a primary cutaneous melanoma and found to carry the heterozygous frameshift variant c.21322_21323insCTGG, p.G7108AfsTer10. The patient's mother also carries the same variant but has no cancer history to date. The father was not available for testing (deceased). The family member affected by breast cancer declined genetic testing. Filled symbols indicate individuals affected by cancer, open symbols indicate unaffected individuals, empty symbol with a black dot at the center indicates a symptomless carrier and diagonal lines indicate deceased family members.

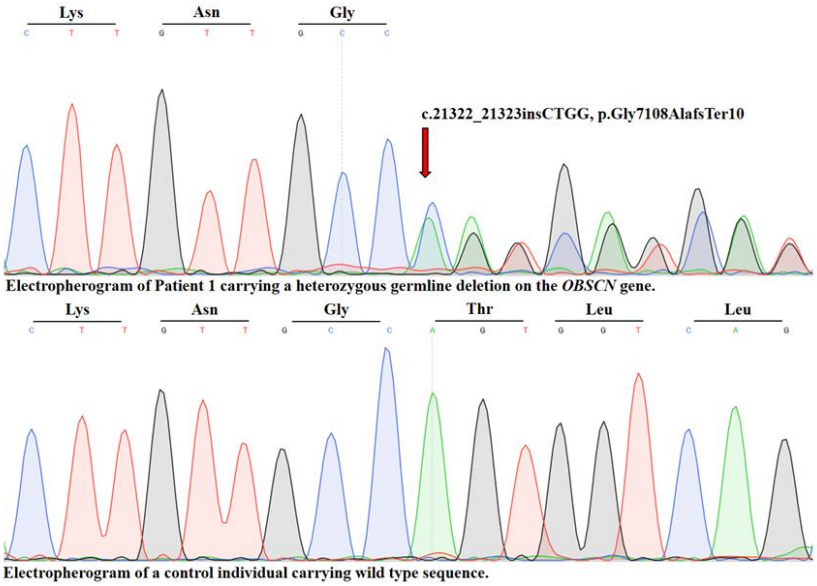

Figure 6. Sanger sequencing confirmation of the novel *OBSCN* variant. Bidirectional capillary sequencing verified the heterozygous frameshift insertion (c.21322_21323insCTGG, p.G7108AfsTer10) in exon 89 of the *OBSCN* gene (NM_001386125.1). The electropherogram demonstrates the site of insertion (arrow), confirming the presence of the variant in the proband.

The detected variant is described as c.21322_21323insCTGG, p.G7108AfsTer10 (NM_001386125.1) (Figure 6.). This frameshift insertion occurs in exon 89, introducing a premature stop codon after amino acid position 7118 and leading to a truncated protein (Figure 7.)

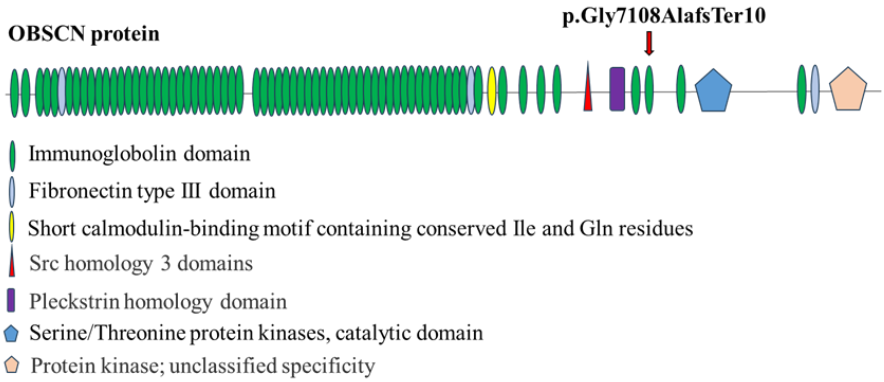

Figure 7. Schematic representation of the OBSCN protein domains and the location of the novel variant. The OBSCN protein comprises multiple structural and signaling domains, including immunoglobulin-like repeats, fibronectin type III domains, RhoGEF, and kinase domains. The novel c.21322_21323insCTGG, 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).

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 (II./2., Figure 5.) 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 5.). One third-degree relative, a cousin on the mother's side (III./5., Figure 5.), was affected by breast cancer, however, this family member declined genetic testing.

According to the ACMG guidelines, the variant was classified as likely pathogenic, based on the following criteria. The variant is a null variant (frameshift/nonsense) in a gene where loss of function is a known disease mechanism (PSV1), and the variant is absent, or extremely rare in large population databases such as gnomAD, suggesting it is not a common benign polymorphism (PM2). *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.

7.2. Hereditary chronic pancreatitis

Previously, our group published a Hungarian family with hereditary pancreatitis carrying the *PRSS1* p.L104P mutation as a causative factor with ~43% penetrance and later age of onset [30]. In the actual follow-up study we longitudinally observed the natural course and clinical characteristics of hereditary pancreatitis in the same family that we followed for 9 years (from 2016 until 2025).

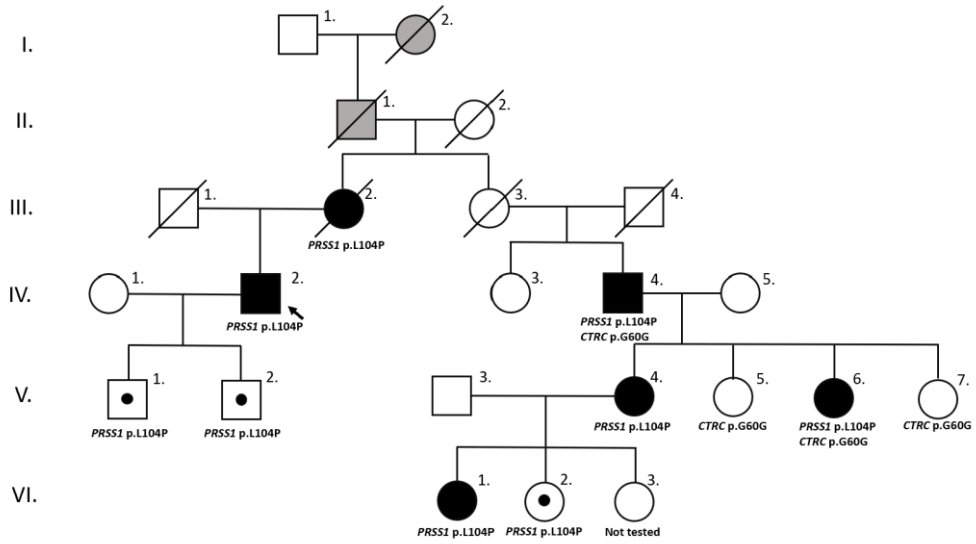

Figure 8. Pedigree of the family with the *PRSS1* p.L104P variant. The empty circles represent the healthy females and the squares represent the healthy males on the pedigree, the black circles represent females diagnosed with chronic pancreatitis, the black squares represent males diagnosed with chronic 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.

Initially, we carried out the genetic testing of the index patient, a 51-year-old male (Figure 8., IV/2.) with diagnosed chronic pancreatitis and a positive family history in our previous

communication [30]. The patient was diagnosed with chronic pancreatitis at the age of 38, and he had multiple episodes of acute pancreatitis (AP). The patient reported chronic diarrhoea, but otherwise no abdominal symptoms. The imaging tests showed a dilated Wirsungian duct, pancreatolithiasis, calcification and atrophy of the pancreas. Since our original report in 2017 the patient developed one episode of AP in 2023, caused by wirsungolithiasis, for which the patient consequently underwent pancreatic stenting. The exocrine function of the pancreas showed a medium functional impairment (Lundh). In the follow-up period the fecal elastase test carried out showed severe exocrine dysfunction (Table 4). The patient also developed endocrine dysfunction of the pancreas, he was diagnosed with Type 3c diabetes mellitus at age 37. Considering other etiologies of CP, the patient reported previous smoking but no regular or significant alcohol consumption, and there were no signs of hyperlipidaemia in the laboratory findings, however, the patient did have a cholecystectomy (Table 5). During the follow-up period, no data supported the presence of pancreatic cancer.

After investigating the above mentioned exons of the *PRSS1, CFTR, CTRC, CPA1* and *SPINK1* genes, we identified the *PRSS1* p.L104P variant in the patient [30], which is a known pathogenic variant for hereditary pancreatitis and can be considered the genetic cause for the patient developing chronic pancreatitis. We identified no further susceptibility variants in the genes tested.

We carried out the family testing of both symptomatic and asymptomatic family members, taking into account the possible differences in the age-of-onset in patients harboring pathogenic *PRSS1* variants and the incomplete penetrance of these variants. The genetic testing of the index patient (Figure 8., IV./2.), and family members III./2., IV./4., V./4., V./5., V./6., V./7. and V./1., V./2. (Figure 8.) were carried out during our initial investigations [30], while genetic testing of family members VI./1. and VI./2. (Figure 8.) were carried out during our current longitudinal study.

We identified the *PRSS1* p.L104P variant in the index patient's two sons (Figure 8., V./1.and V./2.), at the time of the sampling 8 and 6 years old, respectively. The boys showed no characteristic symptoms of acute or chronic pancreatitis and have not had any documented episodes of acute pancreatitis. However, during the follow-up perriod, the younger boy (Figure 8., V./2.) showed slightly reduced growth rate, with his height on the 10th percentile continuously since 2017. The fecal elastase test of both boys carried out at ages 15 and 13 during the follow-up period showed normal exocrine function (Table 4). Ultrasound imaging of the pancreas carried out during the follow-up period showed no morphological changes of

the pancreas or any other signs of acute or chronic pancreatitis in either boy, and laboratory tests also did not show any significant changes (Table 4 and 5).

Place on pedigree	**Age at the time of sampling for fecal elastase test**	**Age at diagnosis of CP**	**Exocrine pancreatic function (based on fecal elastase test)**	**Endocrine pancreatic function**	**Age at diagnosis of diabetes mellitus**
IV./2.	59	36	**severe dysfunction**	**Type 3c diabetes mellitus**	37
V./1.	15	asymptomatic	normal function	normal function	n.a.
V./2.	13	asymptomatic	normal function	normal function	n.a.
IV./4.	55	18	**severe dysfunction**	**Type 3c diabetes mellitus**	46
IV./5.	55	asymptomatic	normal function	normal function	n.a.
V./4.	32	25	**severe dysfunction**	normal function	n.a.
V./5.	29	asymptomatic	normal function	normal function	n.a.
V./6.	26	19	**severe dysfunction**	normal function	n.a.
VI./1.	4	5	normal function (before diagnosis of CP)	normal function	n.a.
VI./2.	2	asymptomatic	normal function	normal function	n.a.

Table 4. Exocrine and endocrine pancreatic function of symptomatic and asymptomatic family members. The severity of the exocrine dysfunction was determined based on the results of the ScheBo ® Pancreatic Elastase 1 Stool Test (normal exocrine function: 200-500 µg/g, moderate exocrine pancreatic dysfunction: 100-199 µg/g, severe exocrine pancreatic dysfunction: <100 µg/g). Endocrine pancreatic dysfunction was determined based on the laboratory results of the patients (HbA1C) or the documented diagnosis of diabetes mellitus.

Place on pedigree	IV/2	III/2	IV/4.	V./4.	V./6.	VI./1
Identified pathogenic variant	*PRSS1* p.L104P heterozygous variant	*PRSS1* p.L104P heterozygous variant	*PRSS1* p.L104P heterozygous variant	*PRSS1* p.L104P heterozygous variant	*PRSS1* p.L104P heterozygous variant	*PRSS1* p.L104P heterozygous variant
Identified susceptibility variant	-	*CFTR* c.1210-12T(5) heterozygous variant	*CTRC* p.G60G heterozygous variant	-	*CTRC* p.G60G heterozygous variant	-
Date of birth	1966.	1944.	1970.	1993.	1999.	2020.
Age of onset	24	32	18	25	19	4
Age at first acute episode	24	32	18	25	19	4
Year of first acute episode	1990.	1976.	1988.	2018.	2018.	2025.
Age at diagnosis of chronic pancreatitis	38	58	18	25	20	5
Year of diagnosis of chronic pancreatitis	2005.	2002.	1988.	2018.	2019.	2025.
Current diagnosis	CP	CP	CP	CP	CP	CP
Nr. Of acute episodes	multiple	2	multiple	multiple	3	3
Smoking (pack/year)	no; previously yes (pack year unknown)	no; previously 10 cigarette/day (20 pack year)	no; previously yes (10 pack year)	no	no	no
Alcohol consumption	not significant	not significant	not significant	not significant	not significant	not significant
Cholecystolithiasis	no	yes	no	no	no	no
Dilation of the Wirsung duct	yes	yes	yes	yes	yes	yes
Calcification	yes	yes	yes	yes	yes	yes
Atrophy	yes	yes	yes	no	yes	no
Pancreas cyst	no	no	no	no	no	no
Pseudocyst	no	no	no	no	yes	yes
WOPN	no	no	no	no	no	no
Wirsungolithiasis	yes	yes	yes	no	yes	yes
Surgeries	laser hemorrhoid-ectomy, appendectomy, tonsillectomy, cholecystectomy	hysterectomy and adnexectomy, cholecystectomy, strumectomy	none	none	none	none
Chronic pain	no	yes	no	no	?	no
Chronic diarrhoea	yes	yes	yes	no	?	no
Pancreas carcinoma	no	no	no	no	no	no
Comorbidities	myeloma multiplex, coeliakia, duodenum aphta, acute hepatitis A	duodenum and gastric ulcer, myoma, struma, gastro-oesophageal reflux, acute hepatitis C, hypercholes-terinaemia	none	none	none	none

Table 5. The clinical characteristics of family members diagnosed with chronic pancreatitis carrying the *PRSS1* p.L104P variant.

We identified the *PRSS1* p.L104P variant in the index patient's mother (Figure 8., III./2.) during our initial investigation [30], who was also diagnosed with chronic pancreatitis at the age of 58, and preivously had 2 episodes of acute pancreatitis, beginning at the age of 32. The patient reported chronic pain and chronic diarrhoea (Table 5). The imaging tests showed atrophy and calcification of the pancreas, significant dilation in the Wirsungian duct with pancreatic stones, and stricture of the common biliary duct (which required the implantation of three stents). The patient did not develop diabetes mellitus. Considering other etiologies of pancreatitis, the patient reported previous smoking for 40 years (20 pack year), and occasional alcohol consumption previously. The patient also had cholecystolithiasis, and a resulting cholecystectomy at 56. During the follow-up period this patient passed away due to a cause unrelated to pancreatitis, diffuse gastric cancer. No novel acute episode or pancreatic cancer was diagnosed in the follow-up period.

We also identified the pathogenic p.L104P variant in the index patient's first cousin (Figure 8., IV./4.) during our initial investigations [30], a 54-year-old male, who was also diagnosed with CP at the age of 18. We also identified the *CTRC* p.G60G mutation in the patient, which is a known genetic risk factor for pancreatitis and, in heterozygous form, confers a 2-fold risk of developing CP [22]. The patient first presented for gastroenterologic examinations in 1988 with upper gastrointestinal complaints of uncertain characteristics and periodic diarrhoea and vomiting. Following ultrasound imaging, calcifying chronic pancreatitis was diagnosed, which explained the patient's symptoms. The patient reported multiple episodes of AP, but no documentation on the acute episodes was provided. Imaging tests showed dilated main pancreatic duct, dilated common biliary duct and Wirsungolithiasis, for which ESWL was performed in the years following the first diagnosis (Table 5). The fecal elastase test carried out in our longitudinal study showed severe exocrine dysfunction, and considering the endocrine pancreatic function, the patient developed Type 3c diabetes mellitus by the age of 46 (Table 4). Considering other etiological factors of chronic pancreatitis, the patient did report previous smoking (10 pack year), but no sigificant alcohol consumption, and no other factors like cholelithiasis or hyperlipidaemia were present. During the follow-up period, no novel acute episode or pancreatic cancer development was observed in this patient.

We identified the p.L104P variant in two out of the four daughters of the index patient's symptomatic first cousin (Figure 8., V./4. and V./6.) [30]. At the time of the initial genetic testing, both daughters were asymptomatic [30], however, during the follow-up period of our

longitudinal study, the oldest daughter (Figure 8., V./4.) developed chronic pancreatitis by the age of 25, while the younger carrier sibling developed chronic pancreatitis at the age of 20.

The oldest sister (Figure 8., V./4.) did not have any diagnosed acute episodes before the diagnosis of chronic pancreatitis. Her medical examinations were initiated following epigastrial pain and vomiting, taking into account the family history and the previously identified pathogenic *PRSS1* p.L104P variant (Table 5). The imaging tests showed dilated pancreatic duct and calcifications in the pancreas, suggestive of the diagnosis of chronic pancreatitis, but there were no signs of pancreatic stones or cysts. The fecal elastase test carried out at the age of 32 years in our longitudinal study showed severe exocrine pancreatic dysfunction (Table 4). The patient reported no smoking or alcohol consumption, imaging tests did not show any signs of gallstones and there were no sings of hyperlipidaemia in the laboratory results. The patient did not develop diabetes mellitus to this date.

The younger sibling carrying the p.L104P variant (Figure 8., V./6.) and also the *CTRC* p.G60G variant developed her first episode of acute pancreatitis at the age of 19 years during the follow-up period of our longitudinal study, and had three documented acute episodes, before she was diagnosed with chronic pancreatitis at the age of 20 (Table 5). The imaging tests showed dilation of the pancreatic duct, calcification and atrophy of the pancreas, and multiple confluent pseudocysts, which later regressed to only one small pseudocyst in the head of the pancreas. The patient also needed stenting of the pancreatic duct, which was later removed. The fecal elastase test conducted during our longitudinal study at the age of 26 showed severe exocrine dysfunction of the pancreas (Table 4). Considering other etiologies of pancreatitis, the patient reported no smoking or alcohol consumption, the imaging tests did not show any signs of gallstones. The patient did not develop diabetes mellitus to this date.

The other two siblings (Figure 8., V./5. and V./7.) were both identified as carriers of the *CTRC* p.G60G variant during our initial study [30], but they remained asymptomatic during the follow-up period, neither of them developed either acute or chronic pancreatitis to this date.

As the age-of-onset of the symptoms can be quite variable, we also performed genetic testing of the two daughters of patient V./4. (Figure 8., VI./1. and VI./2.) during our longitudinal study, 4 and 2 year-old at the time of sampling, respectively. Patient V./4. (Figure 8.) was also pregnant with her third daughter (Figure 8., VI./3.), but considering the research purpose of our investigations and the risks of fetal genetic testing, we did not offer the testing of the fetus. At the time of the buccal swab sampling of the children, the mother reported her oldest daughter

(Figure 8., VI./1.) complaining of moderate to severe abdominal pain. After carrying out the genetic testing, we identified the pathogenic p.L104P variant in both of the children. The older daughter (Figure 8., VI./1.) developed three episodes of severe acute pancreatitis, presenting with abdominal pain, bloating and vomiting, starting a few months after the genetic investigation, at the age of 4 years (Table 5). The acute episodes required lengthy hospital stays, parenteral feeding and later nasogastric feeding. The imaging tests showed signs of acute pancreatitis, as well as two pancreatic pseudocysts and a large volumen of ascites and peripleural fluid. The patient was diagnosed with chronic pancreatitis shortly after the first acute episodes, at the age of 5 years, when MRI imaging and MRCP showed dilated pancreatic duct showing signs of calcification and a pancreas pseudocyst, the picture indicative of chronic pancreatitis. The fecal elastase test carried out before the first diagnosed acute episode showed normal exocrine function (Table 4).

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

8. Discussion

8.1. The importance of genetic testing in complex diseases

Studies aimed at the elucidation of the genetic background of complex diseases such as melanoma malignum and chronic pancreatitis are highly important for professionals to be able to determine the personal disease risk and necessary screening and therapeutic measures of patients. Identifying previously unknown risk variants can further refine the individual risk classification of these patients.

Determining the personal polygenic risk-score is most important in case of complex diseases, where there are preventive measures available to prevent or delay disease onset, or in diseases where effective screening options are available and an early detection and timely treatment can lead to significant health benefits. [6] In melanoma malignum, changing lifestyle factors – like limiting sun exposure, avoiding tanning beds or using sunscreen diligently – can lead to a decreased risk of melanoma development, and regular dermatological care and early detection of melanotic lesions can significantly improve disease outcome [38].

In case of complex diseases, where currently there are no effective preventive measures or no accurate early screening methods, like in the case of chronic pancreatitis, studies aimed at the deeper understanding of genotype-phenotype correlations can prove invaluable at determining early disease markers, that can help us develop accurate screening options. Furthermore, observing the natural disease course in sight of the genotype can help us define the possible disease progression based on the underlying pathomechanisms and develop new preventive and therapeutic measures [6].

8.2. Melanoma malignum

8.2.1. Germline predisposition of melanoma malignum

The landscape of germline genetic variants associated with melanoma susceptibility is becoming worldwide more and more elucidated. The results of our study further widens the spectrum of the identified germline predisposing variants adding the data of a 17-member Hungarian melanoma malignum cohort with increased risk.

The pathomechanisms 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, thus leading to an elevated risk for melanoma and other

tumor formation. 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) [8]. The results of our study seem to be in accordance with this, as most patients with low penetrance mutations had a very high number of naevi, fair hair and skin, and a general disposition prone to skin cancer formation.

While we identitfed predisposing 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, mainly high sun exposure (five patients), frequent sunburns in childhood and early adulthood (eight patients), and the frequent use of tanning salons (one patient).

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 p.Y143C was detected in the *MC1R*, medium penetrance melanoma predisposing gene. The likely pathogenic, nonsense variants p.Q218Ter and p.Q40Ter were present in *CASP8* and *FTO* low penetrance genes, respectively. 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 p.R2912G variant of the *ATM* gene was published in association with different types of cancers, for example breast and ovarian cancer [39], brain tumor, pancreatic cancer [40] and colorectal cancer [41]. The p.V115L variant of the *CDKN2A* gene was reported in Ewing sarcoma [42], laryngeal squamous cell carcinoma [43], cholangiocarcinoma and acute lymphoblastic leukaemia [44]. The p.R811S variant of the *OCA2* gene appeared in two publications in association with oculocutaneous albinism [45, 46]. The p.R1076C variant of the *SETDB1* gene was published in the literature in association with its role in intestinal epithelial homeostasis [47]. Finally, the p.G109R of the *TYR* was reported in association with oculocutan albinism [48, 49].

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, which in itself has only a putative and very mild association with melanoma risk.

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. We investigated, whether the reported risk *TYR* haplotype for albinism [35] is present in our melanoma cohort. We could not identify any patients in our cohort carrying the *TYR* risk haplotype, 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 the presence of pathogenic *BRCA1* or *BRCA2* mutations can lead to an increased risk of melanoma malignum, especially in males, as well as a number of more common *BRCA*-associated tumors [50, 51]. For this reason, in the instance of our patient, we considered this mutation as a pathogenic finding, rather than an actual secondary finding, and we would recommend considering the addition of the *BRCA1* and *BRCA2* genes to the list of melanoma-associated genes.

8.2.2. Germline variants influencing melanoma prognosis and survival

In our previous publication we have summarized the germline variants of melanoma-predisposing and melanoma susceptibility genes in our Hungarian melanoma cohort [37]. However, less attention is paid to the genetic testing of germline variants of genes influencing patients' survival outcomes or enhancing the design of new therapies [15]. Here, we investigated whether melanoma patients in this published cohort harbor any pathogenic or likely pathogenic germline variants in genes associated with unfavourable clinical outcomes [15].

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 [52]. The 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 [52-55]. Additionally, the germline variants in *BRCA2* have been found to increase the risk of melanoma and affect survival rates [56]. *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 [57-59]. Additionally, variants of *FANCI*, *PALB2*, and *RAD54B* genes are associated with altered survival outcomes in melanoma patients [52, 60, 61]. *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 [61, 62]. PALB2 protein partners with BRCA2 in homologous recombination, and mutations in the *PALB2* gene are similarly implicated in an increased melanoma risk and poorer survival [36].

FANCI has four distinct alpha solenoid segment domains (S1-S4). Regarding, the three novel *FANCI* gene 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* protein (Figure 4.). Our results correlate well with the previous findings, since the heterozygous germline deletion in exon 9 reported by Amaral et al. (2020) is also located within the solenoid 3 domain of the FANCI protein [15].

8.2.3. Additional tumor predisposing variants in melanoma patients

The *OBSCN* gene, located at 1q42.13, encodes obscurin, a very large cytoskeletal protein that belongs to the family of giant sarcomeric signaling proteins [63]. Obscurin contains multiple immunoglobulin-like (Ig-like) and fibronectin type III (FnIII) domains, in addition to signaling motifs such as RhoGEF and kinase domains [64]. It plays a fundamental role in cytoskeletal organization, cell adhesion, cell–cell recognition, and intracellular signaling pathways [65]. *OBSCN* is one of the largest genes in the human genome, and its extensive length inherently increases the probability of acquiring both germline and somatic mutations [66].

The identified frameshift *OBSCN* variant (p.G7108AfsTer10) is located within the Ig-like domain spanning residues 7077–7146 (Figure 7.). 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 the role of the loss of tumor suppressor function of the *OBSCN* gene across multiple cancer types [67-69]. 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 [67-69]. 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 predisposition 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 [16]. 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 so far remained 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 the contribution of *OBSCN* to melanoma susceptibility. To note, *OBSCN* expression is not prognostic in melanoma according to TCGA and Protein Atlas data, however, 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 compared to the previously reported somatic missense substitution. The germline occurrence raises the possibility that the *OBSCN* gene 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.

First, 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* gene variant that would otherwise remain undetected. 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* gene 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.

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 function loss. Definitive insights, however, will require *in vitro* studies assessing cytoskeletal organization and cell adhesion in melanoma cells expressing truncated OBSCN protein.

8.3. 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 chronic pancreatitis caused by the p.L104P variant in the *PRSS1* gene [30]. In accordance with this hypothesis, some of the family members, who were previously identified as asymptomatic carriers did develop chronic pancreatitis, and the new observed penetrance is ~67% that is higher compared to the ~43% penetrance previously reported in case of the *PRSS1* p.L104P variant in the same family [30].

Previously, we also hypothesized a late-onset caused by the p.L104P variant, however, during our investigations, a 4-year old girl (Figure 8., VI./1.) carrying only the pathogenic p.L104P variant also developed severe acute pancreatitis and was diagnosed with chronic pancreatitis shortly after the first acute episode at the age of 5. This development is in accordance with the observations of Enea et. al [70], who identified the p.L104P variant in two children, aged 10 and 13, who developed recurrent acute, and later chronic pancreatitis also at their young age. We also investigated the presence of the *CTRC* p.G60G variant present in the members of the Hungarian family, to determine, whether the early age-of-onset can be attributed to the disease-modifying effect of other pancreatitis susceptibility variants, but the young girl does not carry

the *CTRC* variant. These observations point to a very variable age-of-onset caused by the p.L104P variant, rather than a later one.

Considering the clinical picture of the patients, it is 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 clinical presentation of the disease also shows some characteristic features in the investigated family. While the imaging tests showed characteristic signs of chronic pancreatitis in each symptomatic family member – like calcification in the pancreas or the ductus Wirsungianus – the tests also detected pancreatic stones in the ductus Wirsungianus in almost each patient. The removal of the stones also prevented further attacks of acute pancreatitis in the affected family members. Based on these observations, we can hypothesize, that the *PRSS1* p.L104P variant causes calcifying chronic pancreatitis with pancreatic stone formation and dilation of the Wirsungian duct.

The p.L104P variant was first published by Teich et. al in a family with 3 unaffected family members carrying the variant [71], later by Chang et al. [31] and Sofia et al. [32] in two cases of patients with sporadic idiopathic chronic pancreatitis. Enea et al. reported two families affected by chronic pancreatitis, where they reported one of the affected patients having Wirsungian duct stenosis [70] and a family member of another affected family having a ruptured pancreatic cyst, but the clinical characteristics of affected patients were not discussed in more detail. Milani et al. presented a patient with chronic pancreatitis, where the imaging tests showed atrophy of the pancreas and torturous common bile duct [72], and they also reported additional affected family members, but no further specifications on the clinical characteristics of the chronic pancreatitis were described. Our study is the first to establish the connection between the *PRSS1* p.L104P variant and calcifying chronic pancreatitis with stone-formation based on our observations.

In the last decade, misfolding-causing variants were not only described in the *PRSS1* gene, but also in the *CPA1* [73-76], *CEL* [77-80] and *PNLIP* genes [81, 82]. The exact pathomechanism by which misfolding-causing genetic variants lead to the development of chronic pancreatitis is still unclear. Recently, knock-in mouse models mimicking human hereditary pancreatitis have been published, which develop misfolding-induced chronic pancreatitis due to the

presence of a single mutation in the *CPA1* (p.N256K) or the T7 (p.C140S) mouse trypsinogen or the *PNLIP* (p.T221M) genes or in the presence of *CEL-HYB1* hybrid allele carrier status [83-87]. These mouse models clearly demonstrated that the chronic pancreatitis-causing effect of mutations in genes expressed in the pancreas that cause protein misfolding result in chronic pancreatitis as well in artificially created mouse models, which confirms the clear role of protein misfolding in the development of the disease. Regarding the misfolding-induced pathogenesis of pancreatitis, recent cell culture experiments showed, that the degree of ER stress does not seem to directly correlate with the pathogenicity of misfolding *CPA1* variants [76], but showed correlation with the diminished secretion of the misfolded protein. Moreover, a later study with the *CPA1* p.N256K knock-in mouse model indicate that DDIT3/CHOP – a transcription factor that becomes upregulated as a result of ER stress – does not play any significant role in the development of misfolding-induced chronic pancreatitis [88].

The observations on the above mentioned mouse models undoubtedly clarify the chronic pancreatitis causing effect of misfolding-causing mutations. Description of misfolding-induced hereditary chronic pancreatitis in the Hungarian family carrying the *PRSS1* p.L104P mutation clearly confirms the role of misfolding in the pathogenesis of chronic pancreatitis. Clinical characterization and documentation of such a family helps to understand the natural disaese development. 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 for chronic pancreatitis, 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 activity of the trypsinogen.

It is also clear based on the clinical and diagnostic data available, that cellular and tissue-level changes leading to chronic pancreatitis begin long before the first symptoms occur.

Based on the autosomal dominant inheritance and incomplete penetrance of the p.L104P variant, in agreement with the earlier published practice guideline from 2020 [89], we recommend carrier testing of both symptomatic and asymptomatic family members, if this variant is identified in a patient with recurrent acute or chronic pancreatitis. Taking into account our observations about the girl with the age-of-onset at 5 years and the subclinical symptoms detected in the 13-year-old son of the index patient, and also considering the observations of Enea et al [70], carrier testing not only in adult family members, but also in children is recommended. 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, the

incomplete penetrance, and the psychological burden caused by identifying a pathogenic disease-causing variant in an asymptomatic family member.

9. 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.

10. Acknowledgements

I would like to express my deepest gratitude to my supervisors, Prof. Dr. Nikoletta Nagy and Dr. Balázs Németh, for their invaluable guidance, constant support, and encouragement throughout my researh journey. Their expertise, patience, academic precision and thoughtful insights have helped tremedously in shaping this doctoral thesis.

I am especially grateful to Prof. Dr. Márta Széll, for providing an inspiring scientific environment and for her support of my work. I would also like to thank Prof. Dr. Péter Hegyi, for fostering a collaborative and motivating research atmosphere.

I would also like to exptend my gratitude to all the co-authors of the publications forming the basis of this thesis, it has been a privilege to work alongside such dedicated and supportive professionals.

My sincere thanks go to all colleagues of the Medical Genetics Intitute of the University of Szeged and the HCEMM-SZTE Translational Pancreatology Research Group who contributed to the work underlying this dissertation. Their technical assistance and professional expertise were essential to the success of our research work.

Finally, I am deeply grateful to my family and loved ones for their unconditional love, patience, and encouragement. Their constant support gave me strength during challenging times and joy in moments of achievement. This thesis would not have been possible without them.

11. Bibliography/Reference/Appendix

[1] Collins FS, Morgan M, Patrinos A. The Human Genome Project: lessons from large-scale biology. Science. 2003 Apr 11;300(5617):286-90. doi: 10.1126/science.1084564. PMID: 12690187.

[2] 1000 Genomes Project Consortium; Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. Nature. 2015 Oct 1;526(7571):68-74. doi: 10.1038/nature15393. PMID: 26432245; PMCID: PMC4750478.

[3] Shelton CA, Whitcomb DC. Evolving Roles for Physicians and Genetic Counselors in Managing Complex Genetic Disorders. Clin Transl Gastroenterol. 2015 Nov 12;6(11):e124. doi: 10.1038/ctg.2015.46. PMID: 26561988; PMCID: PMC4817528.

[4] Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. Nat Rev Genet. 2018 Sep;19(9):581-590. doi: 10.1038/s41576-018-0018-x. PMID: 29789686.

[5] Bijanzadeh M. The recurrence risk of genetic complex diseases. J Res Med Sci. 2017 Mar 15;22:32. doi: 10.4103/1735-1995.202143. PMID: 28461818; PMCID: PMC5390543.

[6] Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, Natarajan P, Lander ES, Lubitz SA, Ellinor PT, Kathiresan S. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet. 2018 Sep;50(9):1219-1224. doi: 10.1038/s41588-018-0183-z. Epub 2018 Aug 13. PMID: 30104762; PMCID: PMC6128408.

[7] Lewis CM, Vassos E. Polygenic risk scores: from research tools to clinical instruments. Genome Med. 2020 May 18;12(1):44. doi: 10.1186/s13073-020-00742-5. PMID: 32423490; PMCID: PMC7236300.

[8] Read J, Wadt KAW, Hayward NK. Melanoma Genetics. J Med Genet. 2016;53:1–14.

[9] Soura E, Eliades PJ, Shannon K, Stratigos AJ, Tsao H. Hereditary melanoma: Update on syndromes and management: Genetics of familial atypical multiple mole melanoma syndrome. J Am Acad Dermatol. 2016 Mar;74(3):395-407; quiz 408-10. doi: 10.1016/j.jaad.2015.08.038. PMID: 26892650; PMCID: PMC4761105.

[10] Nan H, Kraft P, Hunter DJ, Han J. Genetic variants in pigmentation genes, pigmentary phenotypes, and risk of skin cancer in Caucasians. Int J Cancer. 2009 Aug 15;125(4):909-17. doi: 10.1002/ijc.24327. PMID: 19384953; PMCID: PMC2700213.

[11] Gardner TB, Adler DG, Forsmark CE, Sauer BG, Taylor JR, Whitcomb DC. ACG Clinical Guideline: Chronic Pancreatitis. Am J Gastroenterol. 2020 Mar;115(3):322-339. doi: 10.14309/ajg.0000000000000535. PMID: 32022720.

[12] Suzuki M, Minowa K, Isayama H, Shimizu T. Acute recurrent and chronic pancreatitis in children. Pediatr Int. 2021 Feb;63(2):137-149. doi: 10.1111/ped.14415. Epub 2021 Feb 18. PMID: 32745358.

[13] Ellison MA, Spagnolo DM, Shelton C, Orlova E, LaRusch J, Whitcomb DC, Haupt M. Complex Genetics in Pancreatitis: Insights Gained From a New Candidate Locus Panel. Pancreas. 2020 Aug;49(7):983-998. doi: 10.1097/MPA.0000000000001612. PMID: 32658084.

[14] Zocchi, L, Lontano A, Merli M, Dika E, Nagore E, Quaglino P, Puig S, Ribero S. Familial Melanoma and Susceptibility Genes: A Review of the Most Common Clinical and Dermoscopic Phenotypic Aspect, Associated Malignancies and Practical Tips for Management. J. Clin. Med. 2021;10:3760. https://doi.org/10.3390/jcm10163760

[15] Amaral, T.; Schulze, M.; Sinnberg, T.; Nieser, M.; Martus, P.; Battke, F.; Garbe, C.; Biskup, S.; Forschner A. Are Pathogenic Germline Variants in Metastatic Melanoma Associated with Resistance to Combined Immunotherapy? Cancers (Basel) 2020 12, 1101. doi: 10.3390/cancers12051101. PMID: 32354124; PMCID: PMC7281129.

[16] Balakrishnan, A.; Bleeker, F.E.; Lamba, S.; Rodolfo, M.; Daniotti, M.; Scarpa, A.; et al. Novel somatic and germline mutations in cancer candidate genes in glioblastoma, melanoma, and pancreatic carcinoma. Cancer Research 2007, 67(8), 3545–3550. https://doi.org/10.1158/0008-5472.CAN-07-0065.

[17] Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK Jr, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 14: 141–145, 1996.

[18] Németh BC, Sahin-Tóth M. Human cationic trypsinogen (PRSS1) variants and chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol. 2014 Mar;306(6):G466-73. doi: 10.1152/ajpgi.00419.2013. Epub 2014 Jan 23. PMID: 24458023; PMCID: PMC3949028.

[19] Hegyi E, Sahin-Tóth M. Genetic Risk in Chronic Pancreatitis: The Trypsin-Dependent Pathway. Dig Dis Sci. 2017 Jul;62(7):1692-1701. doi: 10.1007/s10620-017-4601-3. Epub 2017 May 23.

[20] Beer S, Zhou J, Szabó A, Keiles S, Chandak GR, Witt H, Sahin-Tóth M. Comprehensive functional analysis of chymotrypsin C (CTRC) variants reveals distinct loss-of-function mechanisms associated with pancreatitis risk. Gut. 2013 Nov;62(11):1616-24. doi: 10.1136/gutjnl-2012-303090. Epub 2012 Sep 1. PMID: 22942235; PMCID: PMC3660471.

[21] Szabó A, Sahin-Tóth M. Determinants of chymotrypsin C cleavage specificity in the calcium-binding loop of human cationic trypsinogen. FEBS J. 2012 Dec;279(23):4283-92. doi: 10.1111/febs.12018. Epub 2012 Oct 30. PMID: 23035638; PMCID: PMC3573857.

[22] Berke G, Beer S, Gede N, Takáts A, Szentesi A, Hegyi P, Rosendahl J, Sahin-Tóth M, Németh BC, Hegyi E. Risk of chronic pancreatitis in carriers of the c.180C>T (p.Gly60=) CTRC variant: case-control studies and meta-analysis. Pancreatology. 2023 Aug;23(5):481-490. doi: 10.1016/j.pan.2023.05.013. Epub 2023 May 29. PMID: 37321941; PMCID: PMC10586708.

[23] LaRusch J, Lozano-Leon A, Stello K, Moore A, Muddana V, O'Connell M, Diergaarde B, Yadav D, Whitcomb DC. The Common Chymotrypsinogen C (CTRC) Variant G60G (C.180T) Increases Risk of Chronic Pancreatitis But Not Recurrent Acute Pancreatitis in a North American Population. Clin Transl Gastroenterol. 2015 Jan 8;6(1):e68. doi: 10.1038/ctg.2014.13. PMID: 25569187; PMCID: PMC4418406.

[24] Kereszturi E, Szmola R, Kukor Z, Simon P, Weiss FU, Lerch MM, Sahin-Tóth M. Hereditary pancreatitis caused by mutation-induced misfolding of human cationic trypsinogen: a novel disease mechanism. Hum Mutat. 2009 Apr;30(4):575-82. doi: 10.1002/humu.20853. PMID: 19191323; PMCID: PMC2663013.

[25] Schnúr A, Beer S, Witt H, Hegyi P, Sahin-Tóth M. Functional effects of 13 rare PRSS1 variants presumed to cause chronic pancreatitis. Gut. 2014 Feb;63(2):337-43. doi: 10.1136/gutjnl-2012-304331. Epub 2013 Mar 1. PMID: 23455445; PMCID: PMC3681892.

[26] Thiel F, Reiser M, Weiss FU. A rare PRSS1 p.S127C mutation is associated with chronic pancreatitis and causes misfolding-induced ER-stress. Pancreatology. 2022 Dec;22(8):1112-1119. doi: 10.1016/j.pan.2022.10.005. Epub 2022 Nov 3. PMID: 36369231.

[27] Sahin-Tóth M. Genetic risk in chronic pancreatitis: the misfolding-dependent pathway. Curr Opin Gastroenterol. 2017 Sep;33(5):390-395. doi: 10.1097/MOG.0000000000000380.

[28] Sándor M, Vitale DS, Nagy ZA, Ibrahim SY, Abu-El-Haija M, Lazou M, Vajda S, Sahin-Tóth M. Misfolding PRSS1 variant p.Ala61Val in a case of suspected intrauterine pancreatitis. Pancreatology. 2025 Feb;25(1):70-81. doi: 10.1016/j.pan.2024.12.013. Epub 2024 Dec 24. PMID: 39734120; PMCID: PMC12096409.

[29] Balázs A, Hegyi P, Sahin-Tóth M. Pathogenic cellular role of the p.L104P human cationic trypsinogen variant in chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol. 2016 Apr 1;310(7):G477-86. doi: 10.1152/ajpgi.00444.2015. Epub 2016 Jan 28. PMID: 26822915; PMCID: PMC4824176.

[30] Németh BC, Patai ÁV, Sahin-Tóth M, Hegyi P. Misfolding cationic trypsinogen variant p.L104P causes hereditary pancreatitis. Gut. 2017 Sep;66(9):1727-1728. doi: 10.1136/gutjnl-2016-313451. Epub 2016 Dec 23. PMID: 28011893; PMCID: PMC5481506.

[31] Chang YT, Wei SC, L PC, et al. Association and differential role of PRSS1 and SPINK1 mutation in earlyonset and late-onset idiopathic chronic pancreatitis in Chinese subjects. Gut 2009;58:885.

[32] Sofia VM, Da Sacco L, Surace C, Tomaiuolo AC, Genovese S, Grotta S, Gnazzo M, Petrocchi S, Ciocca L, Alghisi F, Montemitro E, Martemucci L, Elce A, Lucidi V, Castaldo G, Angioni A. Extensive molecular analysis suggested the strong genetic heterogeneity of idiopathic chronic pancreatitis. Mol Med 2016, 22:300-309

[33] Miller DT, Lee K, Abul-Husn NS, Amendola LM, Brothers K, Chung WK, Gollob MH, Gordon AS, Harrison SM, Hershberger RE, Klein TE, Richards CS, Stewart DR, Martin CL; ACMG Secondary Findings Working Group. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2023 Aug;25(8):100866. doi: 10.1016/j.gim.2023.100866. Epub 2023 Jun 22. PMID: 37347242; PMCID: PMC10524344.

[34] Act XXI Of 2008 on the protection of human genetic data, the regulation of human genetic studies and research, and the operational rules for biobanks.

[35] Michaud V, Lasseaux E, Green DJ, Gerrard DT, Plaisant C; UK Biobank Eye and Vision Consortium; Fitzgerald T, Birney E, Arveiler B, Black GC, Sergouniotis PI. The contribution of common regulatory and protein-coding TYR variants to the genetic architecture of albinism.

Nat Commun. 2022 Jul 8;13(1):3939. doi: 10.1038/s41467-022-31392-3. PMID: 35803923; PMCID: PMC9270319.

[36] Song, H.; Dicks, E.M.; Tyrer, J.; Intermaggio, M.; Chenevix-Trench, G.; Bowtell, D.D.; Traficante, N.; Group, A.; Brenton, J.; Goranova, T.; et al. Population-based targeted sequencing of 54 candidate genes identifies PALB2 as a susceptibility gene for high-grade serous ovarian cancer. J Med Genet 2021 58, 305-313. doi: 10.1136/jmedgenet-2019-106739. Epub 2020 Jun 16. PMID: 32546565; PMCID: PMC8086250.

[37] 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.

[38] Alaswad L, Galiatsatos P, Srivastava G. Lifestyle Medicine Strategies for Reducing Risk of Melanoma: Modifiable Factors and Clinical Implications. Am J Lifestyle Med. 2026 Feb 19:15598276261428425. doi: 10.1177/15598276261428425. Epub ahead of print. PMID: 41727279; PMCID: PMC12920161.

[39] Jarhelle E, Riise Stensland HMF, Hansen GÅM, Skarsfjord S, Jonsrud C, Ingebrigtsen M, Strømsvik N, Van Ghelue M. Identifying sequence variants contributing to hereditary breast and ovarian cancer in BRCA1 and BRCA2 negative breast and ovarian cancer patients. Sci Rep. 2019 Dec 27;9(1):19986. doi: 10.1038/s41598-019-55515-x. PMID: 31882575; PMCID: PMC6934654.

[40] Young EL, Thompson BA, Neklason DW, Firpo MA, Werner T, Bell R, Berger J, Fraser A, Gammon A, Koptiuch C, Kohlmann WK, Neumayer L, Goldgar DE, Mulvihill SJ, Cannon-Albright LA, Tavtigian SV. Pancreatic cancer as a sentinel for hereditary cancer predisposition. BMC Cancer. 2018 Jun 27;18(1):697. doi: 10.1186/s12885-018-4573-5. PMID: 29945567; PMCID: PMC6020441.

[41] Randon G, Fucà G, Rossini D, Raimondi A, Pagani F, Perrone F, Tamborini E, Busico A, Peverelli G, Morano F, Niger M, Antista M, Corallo S, Saggio S, Borelli B, Zucchelli G, Milione M, Pruneri G, Di Bartolomeo M, Falcone A, de Braud F, Cremolini C, Pietrantonio F. Prognostic impact of ATM mutations in patients with metastatic colorectal cancer. Sci Rep. 2019 Feb 27;9(1):2858. doi: 10.1038/s41598-019-39525-3. PMID: 30814645; PMCID: PMC6393680.

[42] Zhang N, Liu H, Yue G, Zhang Y, You J, Wang H. Molecular Heterogeneity of Ewing Sarcoma as Detected by Ion Torrent Sequencing. PLoS One. 2016 Apr 14;11(4):e0153546. doi: 10.1371/journal.pone.0153546. Erratum in: PLoS One. 2016 Oct 18;11(10):e0165251. PMID: 27077911; PMCID: PMC4831808.

[43] Todorova TA, Jordanov SH, Stancheva GS, Chalakov IJ, Melnicharov MB, Kunev KV, Mitev VI, Kaneva RP, Goranova TE. Mutational Status of CDKN2A and TP53 Genes in Laryngeal Squamous Cell Carcinoma. Pathol Oncol Res. 2015 Apr;21(2):413-21. doi: 10.1007/s12253-014-9836-0. Epub 2014 Aug 23. PMID: 25149524.

[44] Li C, Zhao X, He Y, Li Z, Qian J, Zhang L, Ye Q, Qiu F, Lian P, Qian M, Zhang H. The functional role of inherited CDKN2A variants in childhood acute lymphoblastic leukemia. Pharmacogenet Genomics. 2022 Feb 1;32(2):43-50. doi: 10.1097/FPC.0000000000000451. PMID: 34369425; PMCID: PMC8694244.

[45] Strauss KA, Gonzaga-Jauregui C, Brigatti KW, Williams KB, King AK, Van Hout C, Robinson DL, Young M, Praveen K, Heaps AD, Kuebler M, Baras A, Reid JG, Overton JD, Dewey FE, Jinks RN, Finnegan I, Mellis SJ, Shuldiner AR, Puffenberger EG. Genomic diagnostics within a medically underserved population: efficacy and implications. Genet Med. 2018 Jan;20(1):31-41. doi: 10.1038/gim.2017.76. Epub 2017 Jul 20. PMID: 28726809.

[46] Rooryck C, Morice-Picard F, Elçioglu NH, Lacombe D, Taieb A, Arveiler B. Molecular diagnosis of oculocutaneous albinism: new mutations in the OCA1-4 genes and practical aspects. Pigment Cell Melanoma Res. 2008 Oct;21(5):583-7. doi: 10.1111/j.1755-148X.2008.00496.x. PMID: 18821858.

[47] Južnić L, Peuker K, Strigli A, Brosch M, Herrmann A, Häsler R, Koch M, Matthiesen L, Zeissig Y, Löscher BS, Nuber A, Schotta G, Neumeister V, Chavakis T, Kurth T, Lesche M, Dahl A, von Mässenhausen A, Linkermann A, Schreiber S, Aden K, Rosenstiel PC, Franke A, Hampe J, Zeissig S. SETDB1 is required for intestinal epithelial differentiation and the prevention of intestinal inflammation. Gut. 2021 Mar;70(3):485-498. doi: 10.1136/gutjnl-2020-321339. Epub 2020 Jun 5. PMID: 32503845; PMCID: PMC7873423.

[48] Camand O, Marchant D, Boutboul S, Péquignot M, Odent S, Dollfus H, Sutherland J, Levin A, Menasche M, Marsac C, Dufier JL, Heon E, Abitbol M. Mutation analysis of the tyrosinase gene in oculocutaneous albinism. Hum Mutat. 2001 Apr;17(4):352. doi: 10.1002/humu.38. PMID: 11295837

[49] Lasseaux E, Plaisant C, Michaud V, Pennamen P, Trimouille A, Gaston L, Monfermé S, Lacombe D, Rooryck C, Morice-Picard F, Arveiler B. Molecular characterization of a series of 990 index patients with albinism. Pigment Cell Melanoma Res. 2018 Jul;31(4):466-474. doi: 10.1111/pcmr.12688. Epub 2018 Feb 14. PMID: 29345414.

[50] Ibrahim M, Yadav S, Ogunleye F, Zakalik D. Male BRCA mutation carriers: clinical characteristics and cancer spectrum. BMC Cancer. 2018 Feb 13;18(1):179. doi: 10.1186/s12885-018-4098-y. PMID: 29433453; PMCID: PMC5809938.

[51] Sun P, Li Y, Chao X, Li J, Luo R, Li M, He J. Clinical characteristics and prognostic implications of BRCA-associated tumors in males: a pan-tumor survey. BMC Cancer. 2020 Oct 14;20(1):994. doi: 10.1186/s12885-020-07481-1. PMID: 33054725; PMCID: PMC7556962.

[52] Liede, A.; Karlan, B.Y.; Narod, S.A. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. J Clin Oncol 2004 22, 735–742.

[53] Cruz, C.; Teule, A.; Caminal, J.M.; Blanco, I.; Piulats, J.M. Uveal melanoma and BRCA1/BRCA2 genes: a relationship that needs further investigation. J Clin Oncol 2011 29, e827–829.

[54] Mersch, J.; Jackson, M.A.; Park, M.; Nebgen, D.; Peterson, S.K.; Singletary, C. et al. Cancers associated with BRCA1 and BRCA2 mutations other than breast and ovarian. Cancer 2015 121, 269–275.

[55] Johansson, P.A.; Nathan, V.; Bourke, L.M.; Palmer, J.M.; Zhang, T.; Symmons, J.; Howlie, M., Patch, A.M.; Read, J.; Holland, E.A.; et al. Evaluation of the contribution of germline variants in BRCA1 and BRCA2 to uveal and cuta-neous melanoma. Melanoma Res 2019 29, 483-490. doi: 10.1097/CMR.0000000000000613. PMID: 31464824; PMCID: PMC6716616.

[56] Aoude, L.G.; Heitzer, E.; Johansson, P.; Gartside, M.; Wadt, K.; Pritchard, A.L.; Palmer, J.M.; Symmons, J.; Gerdes, A.M.; Montgomery, G.W.; et al. POLE mutations in families predisposed to cutaneous melanoma. Fam Cancer 2015 14, 621-628. doi: 10.1007/s10689-015-9826-8. PMID: 26251183.

[57] Stolarova, L.; Jelinkova, S.; Storchova, R.; Machackova, E.; Zemankova, P.; Vocka, M.; Kodet, O.; Kral, J.; Cerna, M.; Volkova, Z.; et al. Identification of Germline Mutations in Melanoma Patients with Early Onset, Double Primary Tumors, or Family Cancer History by

NGS Analysis of 217 Genes. Biomedicines 2020 8, 404. doi: 10.3390/biomedicines8100404. PMID: 33050356; PMCID: PMC7601281.

[58] Fidalgo, F.; Torrezan, G.T.; Sá, B.C.S.; Barros, B.D.F.; Moredo, L.F.; Valieris, R.; de Souza, S.J.; Duprat, J.P.; Krepischi, A.C.V.; Carraro, D.M. Family-based whole-exome sequencing identifies rare variants potentially related to cutaneous melanoma predisposition in Brazilian melanoma-prone families. PLoS One 2022 17, e0262419. doi: 10.1371/journal.pone.0262419. PMID: 35085295; PMCID: PMC8794197.

[59] Abdel-Rahman, M.H.; Sample, K.M.; Pilarski, R.; Walsh, T.; Grosel, T.; Kinnamon, D.; Boru, G.; Massengill, J.B.; Schoenfield, L.; Kelly, B.; et al. Whole Exome Sequencing Identifies Candidate Genes Associated with Hereditary Predisposition to Uveal Melanoma. Ophthalmology 2020 127, 668-678. doi: 10.1016/j.ophtha.2019.11.009. Epub 2019 Nov 18. PMID: 32081490; PMCID: PMC7183432.

[60] Vergani, E.; Frigerio, S.; Dugo, M.; Devecchi, A.; Feltrin, E.; De Cecco, L.; Vallacchi, V.; Cossa, M.; Di Guardo, L.; Manoukian, S.; et al. Genetic Variants and Somatic Alterations Associated with MITF-E318K Germline Mutation in Melanoma Patients. Genes (Basel) 2021 12, 1440. doi: 10.3390/genes12091440. PMID: 34573422; PMCID: PMC8469310.

[61] Ma, J.; Cai, X.; Kang, L.; Chen, S.; Liu, H. Identification of novel biomarkers and candidate small-molecule drugs in cutaneous melanoma by comprehensive gene microarrays analysis. J Cancer 2021 12, 1307-1317. doi: 10.7150/jca.49702. PMID: 33531976; PMCID: PMC7847648.

[62] Cai, Z.; Duan, Y.; Li, W.; Liu, Z.; Gong, Z.; Hong, S.; He, X.; Xuanyuan, X.; Chen, Y.; Bi, X.; et al. FANCI serve as a prognostic biomarker correlated with immune infiltrates in skin cutaneous melanoma. Front Immunol 2023 14, 1295831. doi: 10.3389/fimmu.2023.1295831. PMID: 38077326; PMCID: PMC10703153

[63] Young, P., Ehler, E., & Gautel, M. (2001). Obscurin, a giant sarcomeric rho guanine nucleotide exchange factor protein involved in sarcomere assembly. The Journal of Cell Biology, 154(1), 123-136. https://doi.org/10.1083/jcb.200102110.

[64] Kontrogianni-Konstantopoulos, A., Ackermann, M., Bowman, A., Yap, S., & Bloch, R. (2009). Muscle giants: molecular scaffolds in sarcomerogenesis. Physiological Reviews, 89(4), 1217-1267. https://doi.org/10.1152/physrev.00017.2009

[65] Ackermann MA, King B, Lieberman NAP, Bobbili PJ, Rudloff M, Berndsen CE, Wright NT, Hecker PA, Kontrogianni-Konstantopoulos A. Novel obscurins mediate cardiomyocyte adhesion and size via the PI3K/AKT/mTOR signaling pathway. J Mol Cell Cardiol. 2017 Oct;111:27-39. doi: 10.1016/j.yjmcc.2017.08.004. Epub 2017 Aug 4. PMID: 28826662; PMCID: PMC5694667.

[66] Mendiratta G, Ke E, Aziz M, Liarakos D, Tong M, Stites EC. Cancer gene mutation frequencies for the U.S. population. Nat Commun. 2021, 12(1), 5961. https://pubmed.ncbi.nlm.nih.gov/34645806/

[67] Guardia, T.; Eason, M.; Kontrogianni-Konstantopoulos, A. Obscurin: A multitasking giant in the fight against cancer. Biochimica et Biophysica Acta Reviews on Cancer 2021, 1876(1), 188567. https://doi.org/10.1016/j.bbcan.2021.188567.

[68] Sjöblom, T.; Jones, S.; Wood, L.D.; Parsons, D.W.; Lin, J.; Barber, T.D.; et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006, 314(5797), 268–274. https://doi.org/10.1126/science.1133427

[69] Babur, Ö.; Gönen, M.; Aksoy, B.A.; Schultz, N.; Ciriello, G.; Sander, C.; et al. Systematic identification of cancer driving sig-naling pathways based on mutual exclusivity of genomic alterations. Genome Biology 2015, 16(1), 45. https://genomebiology.biomedcentral.com/articles/10.1186/s13059-015-0612-6

[70] Enea A, Pizzol A, Pinon M, Cisarò F, Tandoi F, Arduino C, Calvo PL. Hereditary pancreatitis in Paediatrics: the causative role of p.Leu104Pro mutation of cationic trypsinogen gene also in young subjects. Gut. 2019 Apr;68(4):767-768. doi: 10.1136/gutjnl-2018-316443. Epub 2018 Apr 17. PMID: 29666173.

[71] Teich N, Bauer N, Mössner J, Keim V. Mutational screening of patients with nonalcoholic chronic pancreatitis: identification of further trypsinogen variants. Am J Gastroenterol. 2002 Feb;97(2):341-6. doi: 10.1111/j.1572-0241.2002.05467.x. PMID: 11866271.

[72] Milani I, Guarisco G, Chinucci M, Gaita C, Leonetti F, Capoccia D. The Challenge of Type 3c Diabetes: From Accurate Diagnosis to Effective Treatment. JCEM Case Rep. 2025 May 21;3(7):luaf109. doi: 10.1210/jcemcr/luaf109. PMID: 40401174; PMCID: PMC12093095.

[73] Witt H, Beer S, Rosendahl J, Chen JM, Chandak GR, Masamune A, Bence M, Szmola R, Oracz G, Macek M Jr, Bhatia E, Steigenberger S, Lasher D, Bühler F, Delaporte C, Tebbing J,

Ludwig M, Pilsak C, Saum K, Bugert P, Masson E, Paliwal S, Bhaskar S, Sobczynska-Tomaszewska A, Bak D, Balascak I, Choudhuri G, Nageshwar Reddy D, Rao GV, Thomas V, Kume K, Nakano E, Kakuta Y, Shimosegawa T, Durko L, Szabó A, Schnúr A, Hegyi P, Rakonczay Z Jr, Pfützer R, Schneider A, Groneberg DA, Braun M, Schmidt H, Witt U, Friess H, Algül H, Landt O, Schuelke M, Krüger R, Wiedenmann B, Schmidt F, Zimmer KP, Kovacs P, Stumvoll M, Blüher M, Müller T, Janecke A, Teich N, Grützmann R, Schulz HU, Mössner J, Keim V, Löhr M, Férec C, Sahin-Tóth M. Variants in CPA1 are strongly associated with early onset chronic pancreatitis. Nat Genet. 2013 Oct;45(10):1216-20. doi: 10.1038/ng.2730. Epub 2013 Aug 18. PMID: 23955596; PMCID: PMC3909499.

[74] Kujko AA, Berki DM, Oracz G, Wejnarska K, Antoniuk J, Wertheim-Tysarowska K, Kołodziejczyk E, Bal J, Sahin-Tóth M, Rygiel AM. A novel p.Ser282Pro CPA1 variant is associated with autosomal dominant hereditary pancreatitis. Gut. 2017 Sep;66(9):1728-1730. doi: 10.1136/gutjnl-2017-313816. Epub 2017 Mar 3. PMID: 28258133; PMCID: PMC5933945.

[75] Németh BC, Orekhova A, Zhang W, Nortman SA, Thompson T, Hegyi P, Abu-El-Haija M. Novel p.K374E variant of CPA1 causes misfolding-induced hereditary pancreatitis with autosomal dominant inheritance. Gut. 2020 Apr;69(4):790-792. doi: 10.1136/gutjnl-2019-318751. Epub 2019 Apr 20. PMID: 31005883; PMCID: PMC8596457.

[76] Sándor M, Sahin-Tóth M. Functional predictors of pathogenicity of missense CPA1 variants in chronic pancreatitis. Gut. 2024 Aug 8;73(9):1589-1590. doi: 10.1136/gutjnl-2023-331105. PMID: 37857479; PMCID: PMC11031613.

[77] Fjeld K, Weiss FU, Lasher D, Rosendahl J, Chen JM, Johansson BB, Kirsten H, Ruffert C, Masson E, Steine SJ, Bugert P, Cnop M, Grützmann R, Mayerle J, Mössner J, Ringdal M, Schulz HU, Sendler M, Simon P, Sztromwasser P, Torsvik J, Scholz M, Tjora E, Férec C, Witt H, Lerch MM, Njølstad PR, Johansson S, Molven A. A recombined allele of the lipase gene CEL and its pseudogene CELP confers susceptibility to chronic pancreatitis. Nat Genet. 2015 May;47(5):518-522. doi: 10.1038/ng.3249. Epub 2015 Mar 16. PMID: 25774637; PMCID: PMC5321495.

[78] Johansson BB, Fjeld K, El Jellas K, Gravdal A, Dalva M, Tjora E, Ræder H, Kulkarni RN, Johansson S, Njølstad PR, Molven A. The role of the carboxyl ester lipase (CEL) gene in pancreatic disease. Pancreatology. 2018 Jan;18(1):12-19. doi: 10.1016/j.pan.2017.12.001. Epub 2017 Dec 5. PMID: 29233499.

[79] Xiao X, Jones G, Sevilla WA, Stolz DB, Magee KE, Haughney M, Mukherjee A, Wang Y, Lowe ME. A Carboxyl Ester Lipase (CEL) Mutant Causes Chronic Pancreatitis by Forming Intracellular Aggregates That Activate Apoptosis. J Biol Chem. 2016 Oct 28;291(44):23224-23236. doi: 10.1074/jbc.M116.734384. Epub 2016 Sep 20. Erratum in: J Biol Chem. 2017 May 12;292(19):7744. doi: 10.1074/jbc.A116.734384. PMID: 27650499; PMCID: PMC5087739.

[80] Cassidy BM, Zino S, Fjeld K, Molven A, Lowe ME, Xiao X. Single nucleotide polymorphisms in CEL-HYB1 increase risk for chronic pancreatitis through proteotoxic misfolding. Hum Mutat. 2020 Nov;41(11):1967-1978. doi: 10.1002/humu.24105. Epub 2020 Sep 9. PMID: 32906201; PMCID: PMC7663422.

[81] Szabó A, Xiao X, Haughney M, Spector A, Sahin-Tóth M, Lowe ME. A novel mutation in PNLIP causes pancreatic triglyceride lipase deficiency through protein misfolding. Biochim Biophys Acta. 2015 Jul;1852(7):1372-9. doi: 10.1016/j.bbadis.2015.04.002. Epub 2015 Apr 7. PMID: 25862608; PMCID: PMC4645278.

[82] Toldi V, Kassay N, Szabó A. Missense PNLIP mutations impeding pancreatic lipase secretion cause protein misfolding and endoplasmic reticulum stress. Pancreatology. 2021 Oct;21(7):1317-1325. doi: 10.1016/j.pan.2021.07.008. Epub 2021 Aug 4. PMID: 34373204.

[83] Hegyi E, Sahin-Tóth M. Human CPA1 mutation causes digestive enzyme misfolding and chronic pancreatitis in mice. Gut. 2019 Feb;68(2):301-312. doi: 10.1136/gutjnl-2018-315994. Epub 2018 Jul 25.

[84] Sándor M, Németh BC, Demcsák A, Sahin-Tóth M. Chronic pancreatitis in T7C140S mice with misfolding cationic trypsinogen mutant. JCI Insight. 2025 Mar 11;10(8):e186516. doi: 10.1172/jci.insight.186516. eCollection 2025 Apr 22.

[85] Fjeld K, Gravdal A, Brekke RS, Alam J, Wilhelm SJ, El Jellas K, Pettersen HN, Lin J, Solheim MH, Steine SJ, Johansson BB, Njølstad PR, Verbeke CS, Xiao X, Lowe ME, Molven A. The genetic risk factor CEL-HYB1 causes proteotoxicity and chronic pancreatitis in mice. Pancreatology. 2022 Dec;22(8):1099-1111. doi: 10.1016/j.pan.2022.11.003. Epub 2022 Nov 9.

[86] Mao XT, Zou WB, Cao Y, Wang YC, Deng SJ, Cooper DN, Férec C, Li ZS, Chen JM, Liao Z. The CEL-HYB1 Hybrid Allele Promotes Digestive Enzyme Misfolding and Pancreatitis in Mice. Cell Mol Gastroenterol Hepatol. 2022;14(1):55-74. doi: 10.1016/j.jcmgh.2022.03.013. Epub 2022 Apr 7.

[87] Zhu G, Wilhelm SJ, George LG, Cassidy BM, Zino S, Luke CJ, Hanna M, Stone S, Phan N, Matiwala N, Ballentine SJ, Lowe ME, Xiao X. Preclinical mouse model of a misfolded PNLIP variant develops chronic pancreatitis. Gut. 2023 Jul;72(7):1340-1354. doi: 10.1136/gutjnl-2022-327960. Epub 2023 Jan 11.

[88] Németh BC, Demcsák A, Geisz A, Sahin-Tóth M. Misfolding-induced chronic pancreatitis in CPA1 N256K mutant mice is unaffected by global deletion of Ddit3/Chop. Sci Rep. 2022 Apr 15;12(1):6357. doi: 10.1038/s41598-022-09595-x.

[89] Hegyi P, Párniczky A, Lerch MM, Sheel ARG, Rebours V, Forsmark CE, Del Chiaro M, Rosendahl J, de-Madaria E, Szücs Á, Takaori K, Yadav D, Gheorghe C, Rakonczay Z Jr, Molero X, Inui K, Masamune A, Fernandez-Del Castillo C, Shimosegawa T, Neoptolemos JP, Whitcomb DC, Sahin-Tóth M; Working Group for the International (IAP – APA – JPS – EPC) Consensus Guidelines for Chronic Pancreatitis. International Consensus Guidelines for Risk Factors in Chronic Pancreatitis. Recommendations from the working group for the international consensus guidelines for chronic pancreatitis in collaboration with the International Association of Pancreatology, the American Pancreatic Association, the Japan Pancreas Society, and European Pancreatic Club. Pancreatology. 2020 Jun;20(4):579-585. doi: 10.1016/j.pan.2020.03.014. Epub 2020 Apr 8. PMID: 32376198.