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Original Article

Identification of candidate genes in a family with cancer overload by whole-exome sequencing

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ABSTRACT

Background. Approximately 120 out of every 1 million children in the world develop cancer each year. In Turkey, 2500-3000 children are diagnosed with new cancer each year. The causes of childhood cancer have been studied for many years. It is known that many cancers in children, as in adults, cause uncontrolled cell growth, and develop as a result of mutations in genes that cause cancer.

Methods. The investigation of family history within this context in the study, a total of 13 individuals consisting of all children and adults in the family were examined using the whole-exome sequencing (WES) with the individuals who were diagnosed with cancer in the family, who were detected to have different cancer profiles, and defined as high risk and to determine the gene or genes through which the disease has developed.

Results. At the end of the study, a total of 30 variants with a pathogenic record in the family were identified. A total of 10 pathogenic variants belonging to 8 different genes from these variants have been associated with various cancer risks.

Conclusions. A significant scientific contribution has been made to the mechanism of disease formation by studying a family with a high cancer burden and by finding the genes associated with the disease. In addition, by the results obtained, family members with cancer predisposition were selected after a risk analysis conducted in this family, and the necessary examinations and scans were recommended to provide an early diagnostic advantage.

Key words: cancer, gene mutation, candidate genes, whole exome sequencing.

Cancer is a disease known to be caused by the accumulation of different genetic changes in a cell over the years. These changes lead to abnormal cell proliferation and clonal expansion, which can eventually invade other tissues. In most cases, genetic changes that promote tumor formation occur in somatic cells and do not involve germline mutations.[1]

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A large number of genes that cause tumor development have been identified and classified into 3 different categories: tumor suppressor genes, proto-oncogenes, and genes involved in genome stability. Tumor-suppressor genes control cell proliferation, inhibit the progression of the cell cycle, or induce apoptosis. Generally, a single functional copy of the gene is sufficient for the development of cancer. Inactivation of both alleles allows uncontrolled proliferation and thus contributes to the development of tumors. On the contrary, proto-oncogenes promote cell proliferation and contribute to tumor progression when they are permanently activated as a result of mutations. In such a

case, mutations in a single allele are sufficient for uncontrolled reproduction. Genes involved in DNA stability do not play a direct role in the regulation of cell proliferation, however, the dysfunction in these genes contributes to an increased number of mutations and consequently, to an increased likelihood of tumor development.[2]

Childhood cancers were detected in 11.5 per 100,000 children in 1975, and at a rate of 14.8 in 2004.[3] This means that about 150 out of every 1 million children worldwide will develop cancer before the age of 20 years.[4] The incidence rates of childhood cancers are highest in children aged 0-4 years.[5] The causes of childhood cancer have been studied for many years. Although the genetic basis of childhood cancer is currently not fully elucidated, the immune system and exposure to environmental factors are suggested to have a significant contribution. The hereditary syndromes caused by the high-penetration germline DNA mutations[6,7], chromosomal aneuploidy[8] or epigenetic disorders[9] are known to account for 5-10% of childhood cancers.[10] It is believed that many cancers that occur in children, as in adults, are suggested to develop as a result of uncontrolled cell growth and ultimately due to the mutations in the genes that lead to cancer. In adults, these gene mutations reflect the cumulative effects of aging and long-term exposure to cancer-causing substances. However, it is difficult to identify the potential environmental causes of childhood cancer.

Overall, a hereditary predisposition to cancer is suspected in cases such as families with 2 or higher number of relatives with the same cancer type on the same side of the family, with multiple primary cancer individuals, the emergence of different types, presence of the genetically associated cancers (such as breast and ovarian cancer or colon, and uterine cancer), in increased bilateral or multifocal prevalence in contrast to unilateral involvement, and with the emergence of malignant changes in the same individual or the family.[11]

The application of new gene sequencing techniques in children with cancer allows us to expand our view of the molecular basis of childhood tumors. Unlike conventional molecular techniques, high-productive techniques such as large sequencing or next-generation sequencing (NGS) can sequence millions of DNA fragments, at ever-decreasing costs and in less time. They can also detect different types of genomic changes with a single test.[12]

In a recent study, 1120 cancer patients aged 0-19 years were screened with next-generation sequencing, and germline mutations that cause a predisposition to cancer, which were detected as 1% in the control group, were observed in 8.5% of pediatric patients.[13] Pediatric cancer patients having germline mutations at a higher rate than the general population which raises some questions: Is developing routine genetic screening of newborns to identify patients at risk ethical and cost-effective? Is it possible to track all these patients? Can they benefit from early diagnosis? The answer to all these questions can be found by studying the genes that affect the formation of diseases in families with a high cancer burden at the entire genome level and clinically evaluating the obtained results.

Genetic features are known to be the most important cause of cancer formation, especially in childhood tumors. The transmission of the disease, which is genetically inherited, to the next generation is also inevitable. In addition, the risk of developing a secondary tumor is also significantly increased. Therefore, the early diagnosis of childhood cancers which are known to have high genetic inheritance, and demonstrating whether they showed genetic structure are highly important for the patient's own life, the quality of life, and for the next generation.

When family history is examined, it is very difficult to determine which gene or genes cause the disease in individuals diagnosed with cancer in families which were identified as having many and different types of cancer,

and defined as high-risk. Therefore, panel gene testing applications are needed that allow the investigation of multiple genes in the study of genes that may be the cause of disease in these families. Our goal in the project is to identify candidate genes that may affect the incidence of cancer in a family with individuals that were diagnosed with different cancers and are considered as high-risk. For this purpose, a total of 13 individuals diagnosed with cancer in the family, and who were considered at high risk were examined using the whole-exome sequencing, and high-risk genes were detected in this family.

Material and Methods

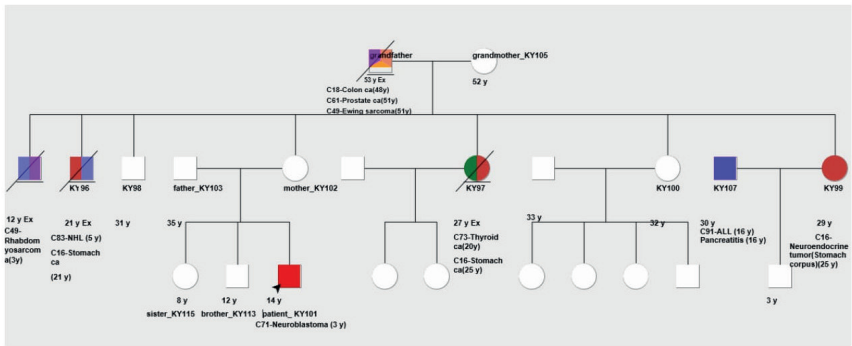
The whole-exome sequencing (WES) analyses were performed from the peripheral blood samples of 13 individuals who were selected after genetic counseling from patients with childhood cancer diagnoses with high-risk cancer profiles, and from the family members who presented to Istanbul University Institute of Oncology, Department of Clinical Oncology, Pediatric Hematology-Oncology Unit. Patient consent was obtained from all the patients included in the study that they accepted the test, and this study was approved by the Ethics Committee of İstanbul University.The data of the pediatric patient and the family members is shown in Table I. The disease codes and descriptions of the family was shown in Figure 1.

After receiving the approvals for the project, about 10 ml of peripheral blood was taken into the EDTA tube, and lymphocyte cells were isolated using the Fikol (Sigma-Aldrich, Germany) method.[14] DNA was isolated from lymphocytes obtained from blood samples using the QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's instructions.[15] First, the genomic DNA was measured with a Qubit fluorimeter (Life Technologies), and then the genomic DNA concentration was adjusted to be 10 ng/μL using a 10 mM pH of 8.5 Tris-HCl. Fluorometric measurement was repeated, and the same buffer solution was readjusted to obtain the concentration as 5 ng/μL, and 50 ng was made ready for use in sequencing. The obtained genomic DNA samples were stored in the -80 device or nitrogen tanks until the whole-exome sequencing process was performed.

Table I. The clinical features of the proband, and other family members.

Family ID	Patient no.	Gender	Cases	Last Status	Consanguinity
F1	KY96	Male	NHL (5y) + Stomach ca (21y)	Ex (21y)	Maternal-Uncle
F1	KY97	Female	Tyroid ca (20y) +Stomach ca (25y)	Ex (27y)	Maternal-Aunt
F1	KY98	Male	Healthy	Alive	Maternal-Uncle
F1	KY99	Female	Neuroendocrine tumor (Stomach corpus) (25y)	Alive	Maternal-Aunt
F1	KY100	Female	Healthy	Alive	Maternal-Aunt
F1	KY101	Male	Neuroblastoma (3y)	Alive	Proband
F1	KY102	Female	Healthy	Alive	Mother
F1	KY103		Healthy	Alive	Father
F1	KY105	Female	Healthy	Alive	Maternal-Grandmother
F1	KY107	Male	ALL (16y) + Pancreatitis (16y)	Alive	Brother in law (Husband of KY99)
F1	KY108	Male	Healthy	Alive	Maternal-Cousin (Child of KY99)
F1	KY113	Male	Healthy	Alive	Brother
F1	KY115	Female	Healthy	Alive	Sister

NHL: non-hodgkin lymhoma, ALL: acute lymphoblastic leukemia.



Disease Codes and Descriptions

C16- Stomach malignant neoplasm* Stomach ca/Neuroendocrine tumor (Stomach Corpus)

C18- Colon malignant neoplasm* Colon ca

C49- Other malignant neoplasm of connective tissue and soft tissue* Rhabdomyosarcoma/Ewing sarcoma

C61- Prostate malignant neoplasm* Prostate ca

C71- Brain malignant neoplasm* Neuroblastoma

C73- Malignant neoplasm of thyroid gland* Thyroid ca

C83- Non-Hodgkin lymphoma(NHL)

C91- Acute Lymphoblastic Leukemia(ALL)

Fig. 1. The pedigree of the F1 family.

Whole exome sequencing is a DNA sequencing strategy that allows the investigation of base matches in genomic coding regions and other interested regions. Since the encoded part of the genome covers only 1-2% of the entire genome, this approach is preferred because it is a more cost-effective strategy for detecting DNA changes that can change protein function compared to the whole genome sequence. Although the research community reveals and identifies the functional effects of sequence changes in non-coding regions of the genome, WES is a test that provides valuable information for both exploratory research and precision medicine applications.[16] The whole-exome sequencing process was performed on the GenoXome_MGISeq G-400 Platform. According to the panel protocol, DNA was fragmented after DNA quality determination was made. Later, adapters were connected to this fragment with the ligation process to the DNA. For the sequencing quality to be at the optimum level, non-specific DNA was removed by purification. Then, to obtain the amplified DNA, the marked library was replicated by a 10-cycle PCR process. The enriched library was loaded into the "Flow Cell" before being put on the device, and the "Flow Cell" loaded with the sample was placed in the HiSeq device for the sequencing process.

The raw data in the BCL format obtained from the device was primarily converted to the VCF file format. The resulting raw data were examined using the computer programs such as VarAFT (https://varaft.eu/) and VariED (http://varied.cgm.ntu.edu.tw/) in accordance with the filter and quality control scores, and then genomic changes that are seen to exist in gene regions were evaluated. The Variant Effect Predictor (VEP) is a central source for the annotation of transcript results. VEP also uses the databases such as NCBI Reference Sequence Database (RefSeq) and algorithms such as Polymorphism Phenotyping (PolyPhen) and SIFT. Information

about the known disease relationship was obtained from the Catalog of Somatic Mutations in Cancer (COSMIC), from the ClinVar database and Online Human Mendelian Inheritance (OMIM) catalog. The resources such as dbSNP, Ensembl 1000 Genome Project and the Exome Variant Server provide information about the occurrence and frequencies of variants within a population. As a result, the descriptions of the variants indicated in all relevant databases and algorithms were obtained. Various filtering options were also used to determine the relationships of the annotation-treated variants with the phenotype. In particular, variants with a pathogenic record of the Clinvar were examined in detail. Variants that have not been previously reported in the literature or databases were defined as candidate (novel) variants. The variables obtained in the study were evaluated considering those with a Q30 quality score above 80%. According to the emerging pathogenic variants, a detailed clinical report was prepared for each person and was given accompanied by genetic counseling.

Furthermore, Copy number variations (CNVs) were evaluated with the CNVkit tool using WES data. We used WES data of 13 individuals, who have high-risk cancer load in the family. The Likely pathogenic/Pathogenic deletion/duplication were included in the study whereas Uncertain significance/Benign deletion/duplication were excluded. The reference Genome HG38 were used to analyze the CNV data.

Results and Discussion

At the end of the study, a total of 30 variants with a pathogenic record in the family were identified. A total of 10 pathogenic variants belonging to 8 different genes from these variants have been associated with various cancer risks. These pathogenic gene mutations, and individuals detected with these gene mutations, and the records of the clinical database of these mutations are shown in Table II.

Table II. The pathogenic gene mutations detected in F1 family and ClinVar records.

Family ID	Cases	Mutation	ClinVar_Associated Diseases
F1	KY96, KY97, KY98, KY99	FCN3 (NM_003665), Exon5, HET, c.349delC, p. (Leu117Serfs*65)	Immunodeficiency_due_to_ficolin_3_deficiency
F1	KY96, KY97, KY98, KY99, KY100, KY102, KY107, KY108	KLKB1 (NM_000892), Exon5, HET, c.428G>A, p. (Ser143Asn)	Prekallikrein_deficiency
F1	KY96, KY97, KY98, KY100, KY101, KY102, KY105, KY113, KY115	C7: NM_000587: exon12:c.C1561A: p.R521S	Complement_component_7_deficiency|C7_and_C6_deficiency, combined_subtotal
F1	KY96, KY97, KY98, KY100, KY101, KY102, KY103, KY113	IRGM (NM_001145805), Exon2, HET, c.313C>T, p. (Leu105Leu)	Inflammatory_bowel_disease_19
F1	KY96, KY97, KY98, KY99, KY100, KY102, KY105, KY107, KY113, KY115	PRSS1 (NM_002769), Exon2, HET, c.86A>T, p. (Asn29Ile)	Hereditary_pancreatitis|not_provided
F1	KY99, KY101, KY102, KY103, KY107, KY108, KY115	PRSS1 (NM_002769), Exon2, HET, c.161A>G, p. (Asn54Ser)	Hereditary_pancreatitis
F1	KY96, KY102, KY107, KY108, KY115	MBL2 (NM_000242), Exon1, HET, c.161G>A, p. (Gly54Asp)	Mannose-binding_protein_deficiency
F1	KY98, KY99, KY105	MBL2 (NM_000242), Exon1, HET, c.154C>T, p. (Arg52Cys)	Mannose-binding_protein_deficiency
F1	KY96, KY97, KY99, KY100, KY105, KY108	PTPRJ (NM_001098503), Exon5, HET, c.827A>C, p. (Gln276Pro)	Carcinoma_of_colon
F1	KY101, KY103, KY107, KY108	FGFR4 (NM_001354984), Exon9, HET, c.1162G>A, p. (Gly388Arg)	Cancer_progression_and_tumor_cell_motility

In our study, **FCN3 (NM_003665), Exon5, HET, c. 349delC, p. (Leu117Serfs*65)** variant were detected in individuals with the codes KY96, KY97, KY98, and KY99. This variant is registered in the ClinVar database as: RCV000005603.6 (Uncertain significance*- Immunodeficiency due to ficolin 3 deficiency). In the dbSNP database, it is registered as rs532781899 (MAF/MinorAlleleCount:-=0.019/2- Clinical significance: CLIN_pathogenic). This variation creates a frameshift starting from the Leu106 codon. The new reading frame creates a STOP Codon at position 65. Ficolin-3 (FCN3), is a circulating model recognition molecule of the lectin pathway that participates in host immune responses against cancer.[17] Ficolin-3 is mainly synthesized in the liver and lung and is known to participate in both systemic and local natural immune responses.[18] Recent studies suggested that the FCN3 gene may be an indicator of ovarian and prostate cancer.[19,20] Also, FCN3 was reported to have a distinctive potential biological marker role in leukemia aggressiveness.[21] H-ficolin(FCN3) serum concentration in pediatric cancer patients is shown to be effective in susceptibility to fever and neutropenia.[22] FCN3 has an important role in the innate immune response to infections, and there is convincing evidence that its deficiency is associated with a predisposition to infections and autoimmunity.[23] Considering family members with a high burden of cancer, detection of **FCN3 (NM_003665), Exon5, HET, c.349delC, p.(Leu117Serfs*65)** variant in FCN3 gene in four siblings, and three of which have been diagnosed with many different types of cancer were striking. In addition, according to the segregation assessment, this variant was not found in the mother. Their father was diagnosed with colon ca+prostate ca+ewing's sarcoma and died at the age of 55 years. Therefore, genetic testing could not be performed on the father, however, considering the intensive clinical condition of the father, it is assumed that this variant was most likely transferred from the father. The reason why this variant has not been detected in a patient with the code KY101 diagnosed with cancer in the family is that his mother and father do not carry this variant. The evaluation of the results found in the light of clinical data showed that the **HET, c. 349delC, p. (Leu117Serfs*65)** variant detected in the FCN3 gene was suggested to have been associated with cancer aggressiveness.

As a result of the study, individuals with the codes KY96, KY97, KY98, KY99, KY100, KY102, KY107, and KY108 were found to have the **KLKB1 (NM_000892), Exon5, HET, c. 428G>A, p. (Ser143Asn)** variant. In the Clinvar database was indicated as RCV000012817.26 (conflicting interpretations of pathogenicity- Prekallikrein deficiency), and in the dbSNP database as rs3733402 (MAF/MinorAlleleCount: G=0.395/783- Clinical significance: CLIN_ pathogenic). KLKB1 secretes bradykinin by cutting the plasma kallikrein enzyme, the Lys-Arg and Arg-Ser bonds in the kininogen and has functions related to blood clotting, fibrinolysis, hemostasis, and inflammatory response.[24] In various databanks, **KLKB1 (NM_000892), Exon5, HET, c.428G>A, p. (Ser143Asn)** variant was reported to be associated with Prekallikrein deficiency.[25] In the investigation of the association of the KLKB1 gene with cancer, it was reported that its expression increases when treated with the demethylating agent 5-azacytidine in lung cancer cells, and therefore it can act as a tumor suppressor gene.[26] PSA, which is used as a biomarker to detect prostate cancer and reduce cancer deaths, is a protein product copied from the KLKB1 gene and is secreted by the epithelial cells of the prostate.[27] Considering the cancer history of the family, although four of the eight individuals with whom the variant was detected were diagnosed with cancer, none of them had lung or prostate cancers. Additional studies are required in the wider patient and control groups for clearly understanding the clinical effect of **KLKB1 (NM_000892), Exon5, HET, c.428G>A, p. (Ser143Asn)** variant.

Within the scope of the study, people with the codes KY96, KY97, KY98, KY100, KY101, KY102, KY105, KY113, and KY115 were detected to have the variant **C7 (NM_000587),**

Exon12, HET, c.1561C>A, p. (Arg521Ser). In the ClinVar database it was registered as RCV000788233. 1 (Pathogenic-Complement component 7 deficiency) and in the dbSNP database it is registered as rs121964920 (MAF/MinorAlleleCount: A=0.001/0- Clinical significance: CLIN_pathogenic). It is associated with complement C7 deficiency in various databases.[28,29] The complement system connects innate immunity to adaptive immunity. The complement system is an important component of the inflammatory response and is involved in various stages of inflammation, tumorigenesis, and cancer progression.[30] Activation of the complement regulates the adaptive immune response and is involved in the regulation of the T cell response in tumors.[31,32] Complement deficiency disrupts both B and T cell responses.[31] The inflammation that stimulates the tumor has an important role in carcinogenesis and cancer progression.[33] Various studies reveal that complement system activation is an important component of tumor-stimulating inflammation.[32,34] When the distribution of variants in the family was evaluated, this variant was found in five of the six siblings. Two of these siblings were diagnosed with cancer. This variant was detected to be transferred from the healthy mother. Also, it has been described in a patient diagnosed with cancer from the third generation. This variant was detected to be transmitted from the mother to both the sick child and to his two healthy siblings. When the variant was evaluated considering the clinical data, the variant could not be directly associated with the pathogenesis of the disease. There is a need for additional studies for understanding the association of **C7 (NM_000587), Exon12, HET, c.1561C>A, p. (Arg521Ser)** variant, and clinical findings.

Within the scope of the study, individuals with the codes KY96, KY97, KY98, KY100, KY101, KY102, KY103, and KY113 were detected to have the **IRGM (NM_001145805), Exon2, HET, c. 313C>T, p. (Leu105Leu)** variant. In the Clinvar database it is regşstered as RCV000023694.2 (Pathogenic- 19 inflammatory bowel disease (Crohn's disease (CD)), in the dbSNP database it is registered as rs10065172 (MAF/MinorAlleleCount: T=0.304/462- Clinical significance: CLIN_pathogenic). Crohn's disease (CD) is a common form of chronic inflammatory bowel disease. The genetic evidence associated with the **IRGM (NM_001145805), Exon2, HET, c. 313C>T, p. (Leu105Leu)** indicates that there are defects in the early immune response in the pathogenesis of CD, in particular in the innate immune pathways and the processing of intracellular bacteria. With the conducted IRGM expression analysis, it was noted that it shows transcription in many tissues, including the colon, small intestine, and peripheral blood leukocytes.[35] Only the immunity-related guanosine triphosphatase family M (IRGM) gene out of three IRG genes located in the human genome (IRGC, IRGQ, and IRGM) encodes a functional IRG.[36] Immunity-associated guanosine triphosphatase has critical importance in defense against pathogens by regulating the progression of autophagy.[37] Autophagy is an "autodigestive" process that plays an important role in the enabling of intracellular components for terminal degradation and recycling.[38] This process has been associated with various aspects of innate and adaptive immunity and has a role in many autoimmune diseases such as the abnormalities in the autophagy pathway, rheumatoid arthritis, systemic lupus erythematosus (SLE).[39] IRGM plays an important role in autophagy. IRGM genetic polymorphisms were confirmed to have been associated with many types of inflammatory, and autoimmune diseases. Also, **IRGM (NM_001145805), Exon2, HET, c. 313C>T, p. (Leu105Leu)** variant is known to be associated with autoimmune thyroid disease (AITD).[40] Recent research has suggested that autophagy may play a critical role in tumorigenesis. The GTPase family M (IRGM) associated with immunity, is a human protein that has been highlighted for its contribution to autophagy on inflammation, and infections. Studies have shown that IRGM plays a role in the development of several cancers. One study reported that the genetic polymorphisms in

IRGM were associated with a predisposition to stomach cancer.[41] In another study, a positive correlation was reported between IRGM upregulation, and IRGM levels in stomach cancer tissues and cancer stages, and the IRGM gene might have a role in the pathogenesis of stomach cancer and may reflect the progression of the disease.[42] The evaluation of the clinical data of the family including all this information, the detection of (KY96, KY97) IRGM gene mutation in 2 siblings who have been diagnosed with stomach cancer out of 3 siblings was noteworthy. However, the fact that this gene mutation, which was also detected in a patient with the code KY101, was seen in both his healthy mother and his healthy father raises doubts about the pathogenicity of this variant. Additional studies should be conducted to understand the level of contribution of **IRGM (NM_001145805), Exon2, HET, c. 313C>T, p. (Leu105Leu)** variant to the process of cancer formation.

PRSS1 (NM_002769), Exon2, HET, c. 86A>T, p. (Asn29Ile) variant was detected in individuals with the codes KY96, KY97, KY98, KY99, KY100, KY102, KY105, KY107, KY113, and KY115; and **PRSS1 (NM_002769), Exon2, HET, c. 161A>G, p. (Asn54Ser)** variant was detected in individuals with the codes KY99, KY101, KY102, KY103, KY107, KY108, and KY115. In the ClinVar database **PRSS1 (NM_002769), Exon2, HET, c.86A>T, p.(Asn29Ile)** variant is registered as RCV000763166.1 (Pathogenic* - hereditary pancreatitis), in dbSNP database it is registered as rs111033566 (dbSNP entry validated Clinical significance: CLIN_pathogenic). Hereditary pancreatitis (HP) is a genetic disease in which the risk of pancreatitis and pancreatic cancer can be passed from generation to generation in a family. The most commonly associated gene with HP is the PRSS1 gene. The mutations in the PRSS1 gene create an increased risk of pancreatitis and pancreatic cancer in the individual.[43] The assessment of the family history showed that it is worth noting that **PRSS1 (NM_002769), Exon2, HET, c. 86A>T, p. (Asn29Ile)** variant was found in all 6 siblings. Three of these individuals had various cancer diagnoses, however, there were no pancreatic cancer diagnoses. Perhaps because two of the three siblings diagnosed with cancer had died at an early age, pancreatic cancer had not yet been detected by that date. The fact that this variant, which is also found in other siblings, was transferred from their 53-year-old healthy mother has raised doubts about the pathogenicity of this variant. Further studies are required on this topic. **PRSS1 (NM_002769), Exon2, HET, c.161A>G, p. (Asn54Ser)** variant was detected only in patients with the codes KY99 and KY101 who were diagnosed with cancer. It is noteworthy that this variant was detected both in the mother and father of the patient with the code KY101. Therefore, it raises doubts about its pathogenicity property. Further studies are needed on this issue.

MBL2 (NM_000242), Exon1, HET, c.161G>A, p. (Gly54Asp) variant was detected in individuals with the codes KY96, KY98, KY99, KY102, KY107, KY108, and KY115, however **MBL2 (NM_000242), Exon1, HET, c.154C>T, p. (Arg52Cys)** variant has been detected in the individuals with the code KY105. **MBL2 (NM_000242), Exon1, HET, c.161G>A, p.(Gly54Asp)** variant in Clinvar database is registered as RCV000015424.24 (Pathogenic - Mannose-binding protein deficiency), and as rs1800450 (MAF/MinorAlleleCount: T=0.122/75 - Clinical significance: CLIN_pathogenic) in dbSNP database. **MBL2 (NM_000242), Exon1, HET, c.154C>T, p. (Arg52Cys)** variant in Clinvar database is registered as RCV000015426.28 (Pathogenic*-, mannose-binding protein deficiency), and in the dbSNP database as rs5030737 (MAF/MinorAlleleCount: A=0.027/4- Clinical significance: CLIN_pathogenic). Mannose-binding lectin deficiency (MBL) is a condition that affects the immune system. People with this condition have a deficiency in the levels of an immune system protein called mannose-binding lectin in their blood. It is not clear whether this deficiency predisposes the affected individuals to recurrent infections. Individuals with the deficiency of mannose-

binding lectin can develop infections of the upper respiratory tract and other body systems. People with this condition can also get more serious infections, such as pneumonia and meningitis. Depending on the type of infection, the frequency and severity of the symptoms caused by infections vary. Infants and young children with mannose-binding lectin deficiency appear to be more susceptible to infections than the affected adults, however, adults may also develop recurrent infections.[44] In addition, affected people who have undergone chemotherapy or are taking drugs that suppress the immune system are especially prone to infections. Increased susceptibility to sepsis and chemotherapy-related infections has been shown in people with MBL deficiency.[45,46] Mannose-binding lectin is a gene that is a key activator in the lectin complement pathway. The complement pathway has recently been found to play a role in oncogenesis.[47] Genetic polymorphisms in the MBL gene have been associated with risk for several cancers including breast cancer[48], stomach cancer[49,50], colon cancer[51] and cervical cancer.[52] In these studies, MBL2 polymorphisms were reported to have been resulted in lower serum levels and were associated with a high risk of cancer. On the contrary, in a study on lung cancer, it has been reported that low serum MBL levels due to MBL2 gene polymorphisms created positive effects on survival.[53] No association was reported on MBL2 gene and colon cancer survival in another study.[54] The evaluation of the distribution of **MBL2 (NM_000242), Exon1, HET, c.161G>A, p. (Gly54Asp)** variant among the family members showed that this variant was found in 2 out of 6 siblings. However, only one of these 2 siblings was diagnosed with cancer. Therefore, additional studies are required for the pathogenic effect of the **MBL2 (NM_000242), Exon1, HET, c.161G>A, p. (Gly54Asp)** variant. This variant has not been found in the mothers of individuals (KY105). Their father, on the other hand, was diagnosed with three different cancers (colon ca, prostate ca and Ewing's sarcoma and died at the age of 55 years. Therefore, genetic testing could not be performed on the father. However, considering the intensive clinical condition of the father, it is assumed that this variant was most likely transferred from the father. **MBL2 (NM_000242), Exon1, HET, c.154C>T, p. (Arg52Cys)** variant was found in the mothers of the siblings with the codes KY98 and KY99, and KY105. The assessment of the cancer history in the family showed that the detection of this variant in the healthy mother raises doubts about its pathogenic effect. In the HGMD data bank **MBL2 (NM_000242), Exon1, HET, c.154C>T, p. (Arg52Cys)** variant is registered as DFP (Disease-associated polymorphism with supporting functional evidence). Further studies are needed to understand the relationship between the **MBL2 (NM_000242), Exon1, HET, c.154C>T, p. (Arg52Cys)** variant and cancer risk. However, given the effects of the MBL gene on the oncogenesis process, clinical follow-up especially against some cancers such as breast cancer, stomach cancer, and colon cancer of people carrying variants is recommended.

PTPRJ (NM_001098503), Exon5, HET, c.827A>C, p. (Gln276Pro) variant has been found in individuals with the codes KY96, KY97, KY99, KY100, KY105, and KY108. In The Clinvar database it is registered as RCV000009227.4 (Pathogenic- Carcinoma of the colon) and as rs1566734 (MAF/MinorAlleleCount: C=0.190/181- Clinical significance: CLIN_ pathogenic) in dbSNP database. In the HGMD database, it has been associated with the risk of thyroid cancer. PTPRJ is a good candidate for the cancer predisposition gene. It has functional roles in tumor suppression, including inhibition of cell growth, migration, and angiogenesis.[55] PTPRJ also seems to play a role in human CRC as well.[56] PTPRJ is also a candidate tumor suppressor gene for other types of cancer. Loss of 11p11, the locus hosting the PTPRJ, is observed in 50% of lung cancers, 78% of breast cancers, and 38% of thyroid anaplastic carcinomas.[55,57,58] Considering the distribution of variants in the family in the light of this information, it is noteworthy that 4 out of 6 siblings have this

variant and three of them have been diagnosed with cancer regarding the pathogenic effect of the variant. In addition, the fact that one of the sick siblings had thyroid cancer increases the doubts. However, the fact that this variant has also been found in their healthy mother has led to the question of its pathogenic effect. Further studies are needed to clearly understand the effect of the **PTPRJ (NM_001098503), Exon5, HET, c.827A>C, p. (Gln276Pro)** variant on the risk of developing cancer.

GLUD2 (NM_012084), Exon1, HET, c.1492T>G, p.(SER4986) variant has been found in individuals with the codes KY96, KY98, KY99, KY100, KY105, KY107, and KY108. In the The Clinvar database is registered as: RCV000022827.24 (Pathogenic- as in Parkinson's disease), and as rs9697983 (MAF/MinorAlleleCount: G=0.033/4- Clinical significance: CLIN_pathogenic) in the dbSNP database. The age of the onset of Parkinson's disease (Gomes, #234), a common neurodegenerative disorder characterized by progressive loss of dopaminergic neurons and their termination in the basal ganglia is suggested to be associated with the **GLUD2 (NM_012084), Exon1, HET, c.1492T>G, p.(SER498Ala)** variant.[59] When evaluated from this point of view, it is recommended that people carrying this variant must undergo detailed examinations for Parkinson's disease.

FGFR4 (NM_001354984), Exon9, HET, c.1162G>A, p. (Gly388Arg) variant has been found in individuals with the codes KY101, KY103, KY107, and KY108. In the Clinvar database it is registered as RCV000017723.29 (Pathogenic Cancer progression and tumor cell motility), and in the dbSNP database as rs351855 (MAF/MinorAlleleCount: A=0.300/449- Clinical significance: CLIN_pathogenic). FGFR4 gene is a member of the fibroblast growth factor (FGF) family. Acts as a cell surface receptor for fibroblast growth factors, and plays a role in many mechanisms such as cell proliferation, differentiation, tissue repair, invasion, regulation of fat metabolism, bile acid biosynthesis, glucose uptake, vitamin D metabolism, and phosphate balance.[60] According to a research, cancer progression and tumor cell motility were associated with the 1162G>A (p.Gly388Arg) variant in the FGFR4 gene, and the increase in FGFR4 expression was associated with the development of breast and colon cancer. They also noted that it is statistically associated with lymph node metastasis and increased TNM stage, thereby indicating that it triggers the progression of cancer.[61] In another study it was emphasized that FGFR4 expression was associated with pancreatic cancers.[62] According to another research, this variant in the FGFR4 gene was effective in the onset and progression of prostate cancer.[63] Moreover, this variant in the FGFR4 gene was suggested to be used as a marker in Cushing's disease.[64] The c.G1162A:p.G388R variant in the FGFR4 gene which is also known for its oncogenic transformation activity was reported as a candidate gene for predicting the clinical development and assessing the stage of the disease in patients with advanced-stage retinoblastoma.[60,65] The evaluation in the light of this information shows that the detection of **FGFR4 (NM_001354984), Exon9, HET, c.1162G>A, p.(Gly388Arg)** variant only in the spouses of the siblings (KY107, and KY103), and in their children (KY108, and KY101) but not in the family members with high cancer burden suggest that additional studies are required for its pathogenic effect.

Individuals with the code KY107 and KY108 were detected to have **CST3 (NM_000099), Exon1, HET, c.73G>A, p. (Ala25Thr)** variant. In the Clinvar database it was defined as RCV000005989.4 (conflicting interpretations of pathogenicity- age-related macular degeneration 11), as rs1064039 in dbsnp database (MAF/MinorAlleleCount: T=0.212/226- Clinical significance: CLIN_pathogenic). Age-related macular degeneration (AMD) and Alzheimer's disease (AD) are degenerative, multifactorial diseases that involve the age-related accumulation of extracellular deposits due to the irregularity of protein homeostasis. There is evidence that the **CST3 (NM_000099), Exon1, HET, c.73G>A, p. (Ala25Thr)** variant in CST3 (cysteine proteinase inhibitor cystatin C) gene

which was confirmed to have been associated with AD with meta-analysis, is associated with AMD.[66] Within the scope of the study, it is recommended that these people (KY107 and KY108) should be followed up clinically, as the **CST3 (NM_000099), Exon1, HET, c.73G>A, p. (Ala25Thr)** variant detected in KY107 and KY108 (The son of KY107) increases the risk of age-related macular degeneration (AMD) and Alzheimer's disease (AD

SAA1 (NM_000331), Exon3, HET, c.209C>T, p. (Ala70Val) variant has been found in individuals with the code KY96, KY97, KY98, KY99, KY100, KY101, KY102, KY103, KY105, and KY115. In the Clinvar database it is registered as RCV000019736.27 (Pathogenic- a variant of serum amyloid) and as rs1136743 in dbSNP database (dbSNP entry validated Clinical significance: CLIN_pathogenic). In the HGMD database, the SAA1 (NM_000331), Exon3, HET, c.209C>T, p. (Ala70Val) variant has been associated with familial Mediterranean fever (FMF).[67] When the family profile was examined, this variant was found in all 6 siblings and their mothers. Detailed clinical screening of these family members (KY96, KY97, KY98, KY99, KY100, KY101, KY102, KY103, KY105, and KY115) for FMF is recommended.

During the examination, people with the codes KY96, KY97, KY98, KY99, KY101, KY103, KY105, KY107, and KY115 were found to have the **TP53 (NM_000546), Exon4, HET, c.215C>G, p. (Pro72Arg)** variant. It is registered in the Clinvar database (December 2020) as RCV000211212.1 (drug response***- cyclophosphamide response-Efficacy), and in the dbsnp database as rs1042522 (MAF/MinorAlleleCount: G=0.457/1046 Clinical significance: CLIN_uncertain_significance, CLIN_benign, CLIN_drug_response). This change has been investigated by different researchers in different groups of diseases and has been associated with drug response and apoptosis. In a study conducted in gastric cancers, it was reported that people with advanced-stage gastric cancer treated with paclitaxel and cisplatin who carry this polymorphism were advantageous in terms of their response to chemotherapy and the longer time until progression.[68] A study conducted on patients with ovarian cancer reported that there was no effect of the corresponding polymorphism.[69] In a study conducted in breast cancer patients receiving neoadjuvant chemotherapy, it was emphasized that it is effective in responding to 5-FU and cyclofosfamide-based neoadjuvant chemotherapy.[70] As can be understood, **TP53 (NM_000546), Exon4, HET, c.215C>G, p. (Pro72Arg)** variant has advantages in response to apoptosis and chemotherapy. Therefore, drug selection should be made according to the identification of the **TP53 (NM_000546), Exon4, HET, c.215C>G, p. (Pro72Arg)** variant in these family members (KY96, KY97, KY98, KY99, KY101, KY103, KY105, KY107, KY115).

In addition to detection of the mutations, we also evaluated large deletions and duplications. After annotation by using the CNVkit tool, CNV results of 13 individuals were obtained. Although high number of deletions and duplications were detected in all individuals, only Likely pathogenic/Pathogenic variations were shown in Table III.

Both children and family members were informed in detail about the risks they may encounter later on. In addition, considering the candidate variants identified, various clinical follow-up recommendations are presented to minimize the risks. Their physicians were also

Table III. Evaluation of the Likely pathogenic/Pathogenic CNV results using WES data.

Patient no.	Chromosome	Type of variations	ACMG Classification of variations	Dosage-sensitive genes	Protein coding genes
KY96	chr15	Deletion	Likely pathogenic	*SPRED1*	*SPRED1*
KY98	chr11	Deletion	Likely pathogenic	*KMT2A*	*ATP5MG, KMT2A*
KY115	chrX	Deletion	Likely pathogenic	*DMD*	*DMD*

informed in detail about other tumors that may occur during the pediatric patient's long life process. Approaches such as radiation therapy for cancer treatment has additional risk of causing secondary tumors, especially in individuals known to have a genetic predisposition; therefore, it must be avoided as much as possible. In addition, since there is a risk of transmission to future generations in individuals with a genetic mutation, preimplantation genetic options for preventing a decrease or increase in the incidence of the disease were recommended to both patients and parents. In conclusion, the risk of developing cancer in people with the candidate genes was identified in the family with a high risk of cancer, and all follow-up plans have been formed in this direction. However, research with wider patient group and healthy controls will clarify in what direction pathogenic effects would the candidate variants create in the disease formation process.

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Ethical approval

This study was approved by the Ethics Committee of Istanbul University (25.02.2020, 341).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DAO, HY, RK; data collection: DAO, HY, RK; analysis and interpretation of results: DAO, RK, SBB, OSE, SBT, MH, SK, BC and HY; draft manuscript preparation: DAO, HY, RK. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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