Mutations in Known and Novel Cancer Susceptibility Genes in Young Patients With Pancreatic Cancer

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Abstract

**Background:** Pancreatic cancer is the fourth most common cause of mortality due to cancer, globally. It has a poor prognosis and is usually diagnosed at later stages when tumor resection is not possible. Heritability for pancreatic cancer is relatively high and clinically significant.

**Methods:** A group of 24 pancreatic cancer patients with young age at onset, from a referral hospital in Tehran University of Medical Sciences were screened for mutations in 710 cancer relevant genes using next generation sequencing technology.

**Results:** Two patients had pathogenic mutations in known pancreatic cancer susceptibility genes, **BRCA1/2**. Two other patients also had potentially pathogenic mutations in 2 novel candidate genes including **PARP4** and **EXO1**.

**Conclusion:** **BRCA1/2** genes are the most commonly mutated pancreatic cancer susceptibility genes that should be considered in all pancreatic cancer cases with young age at onset or a family history of cancer. **PARP4** and **EXO1** also are potential candidate genes for susceptibility to pancreatic cancer. Identifying the hereditary cases of pancreatic cancer will help to offer more targeted treatments to the patients and also to prevent cancer in family members who might be a mutation carrier.

**Keywords:** **BRCA1, BRCA2**, Hereditary, Pancreatic cancer, Susceptibility genes


**Introduction**

Pancreatic cancer (OMIM accession number: 260350) ranks the fourth most common cause of fatalities due to cancer worldwide. Pancreatic cancer holds one of the worst prognoses among different cancer types. Five-year survival rate for pancreatic cancer differs between 3% to 29% at different stages. According to the World Health Organization (WHO), incidence of pancreatic cancer was estimated to be 337,872 new cases in 2012 in both men and women globally. The average lifetime risk for pancreatic cancer in both men and women is 1.5%.1

Previously, family-based studies have shown how familial clustering of cancer and intergenerational propagation of mutations may increase the risk of disease in some families compared to the general population.1 Lifetime risk for pancreatic cancer is 1.1%, and risk for a monozygotic twin and a dizygotic twin is 4.3% and 3.7% respectively, if one of the twins is affected.2 These estimates suggest that heritability for pancreatic cancer is relatively high and clinically significant. Cancer heritability is defined as the proportion of variance in cancer risk due to genetic differences.

In the last few decades, a number of genes were reported to be associated with pancreatic cancer including **BRCA1**, **BRCA2**, **PALB2**, **MLH1**, **MSH2**, **MSH6**, **PMS2**, **EPCAM**, **APC**, **STK11/LKB1**, **CDKN2A**, **P53**, **SPINK1**, **CFTR**, and **ATM**.2,3 Highly penetrant germline mutations in any of these genes could enhance the risk of pancreatic cancer development.1 These variations are transmitted through an autosomal dominance model and can cause familial clustering.1 Known pancreatic cancer-associated genes are estimated to account for only 20% of familial pancreatic cancers.2 There are still many familial cases of pancreatic cancer that could not be explained by mutations in known genes, and probably more pancreatic cancer susceptibility genes yet to be discovered.
In this study, we sequenced 24 patients with early onset pancreatic cancer on a panel of 710 genes including known cancer susceptibility genes and genes that could potentially be involved in cancer pathogenesis. We evaluated these genes in search for known, and also novel pancreatic cancer susceptibility genes. We found 2 variations in BRCA1/2 (OMIM accession number: 113705, 600185), the well-known pancreatic cancer susceptibility genes, in addition to 2 truncating mutations in PARP4 (OMIM accession number: 607519), and EXO1 (OMIM accession number: 606063) genes.

Materials and Methods

Study Subjects

The methods for cases and controls recruitment were extensively explained before, and are briefly described here.7 Cases were selected from patients who referred to a university affiliated hospital (Shariati hospital) in Tehran, Iran for endoscopic ultrasonography (EUS), from January 2011 to December 2014. All cases had pathology proven pancreatic adenocarcinoma. Informed consent was obtained from each patient included in the study. A structured valid and reliable questionnaire was used for data collection by a few trained interviewers.8 Twenty-four of the cases younger than 50 years were used for this candidate genes assessment.

Laboratory Method

The germline DNA extracted from white blood cells at the time of cancer diagnosis, was sequenced on the Cancer Relevant Genes (CRG) panel by using Agilent Sure Select chemistry (Agilent Technologies Inc., Santa Clara, CA) for capturing the sequence regions of interest. The CRG panel targets 10,165 coding exons (plus 10 bp from introns in each side of an exon) of 710 known cancer susceptibility genes, cancer driver genes as reported in Cancer Genes Census (http://cancer.sanger.ac.uk/census), or genes that could potentially contribute to cancer if mutated. The genes on the CRG panel are implicated with cell processes known to be of significance in cancer development, such as genome maintenance, cell cycle control mechanisms, and cellular differentiation signaling. Briefly, the germline DNA samples were sheared (200–400 bp) and illumina adaptors were added to their ends. For each sample, the DNA fragments were barcoded by tagging a specific oligonucleotide to all DNA fragments of that sample. The regions of interest from each sample DNA library were captured from the rest of the genome by hybridizing them with biotinylated RNA strand probes which were complementary to the DNA sequence of the regions of interest. The hybridized DNAs with biotinylated probes bound to streptavidin-coated magnetic beads and were separated from the rest of the genomic DNA in a magnetic field. Every 12 samples were pooled and used for paired-end sequencing for 600 cycles (generating 300 bp reads) on an Illumina MiSeq while using a V3 sequencing cartridge (Illumina Inc., San Diego CA). All pathogenic variants identified using next-generation sequencing were confirmed by using Sanger sequencing.

Data Analysis

The Burrows-Wheeler Aligner1 aligned the sequence reads to the reference human. Picard (http://broadinstitute.github.io/picard/; accessed August 2, 2016) converted SAM files to BAM format, and organized BAM files. GATK2 removed reads that went unmapped, were aligned to more than one human genome region, or were duplicates. The HaplotypeCaller module of GATK identified SNVs and indels. Variants were considered if they had at least 20x depth of coverage and the alternate allele ratio was present in at least 25% of the reads. Variants on CRG panel were narrowed down in multiple steps. We focused on truncating variants and also checked all missense variants to see if there are any previously known pathogenic mutations among them. The types of mutations considered as truncating includes nonsense mutations, frame shift insertion/deletion, splice site consensus mutations, and initial codon mutations.

Results

To search for mutations in known and novel pancreatic cancer susceptibility genes, we undertook sequencing of 24 early onset pancreatic cancer patients with a 50:50 male to female distribution. The average age of the probands was 43.9 years (range: 29-49 years). Detailed characteristics of the cases are shown in Table 1.

The data on the CRG panel were evaluated for all known pancreatic cancer susceptibility genes. As a result, 2 out of 24 probands were found to harbor each unique mutation in BRCA1 (c.301+1G>A), and BRCA2 (c.260_261delCT). These mutations are presented in Table 2. Subsequently, we checked the mutations in other genes of the CRG panel that could potentially increase the risk of cancer if they have been mutated. We detected 2 splicing mutations in 2 genes that could be potentially pathogenic in 2 other patients each harboring one: EXO1 (c.2212-1G>C), and PARP4 (c.3667-1G>A). These variations are presented in Table 3.

Discussion

In an effort to identify known and novel mutations associated with pancreatic cancer, we conducted sequencing of 710 cancer relevant genes in 24 early onset pancreatic cancer patients. In this panel, 2 mutations in known pancreatic cancer susceptibility genes BRCA1,
Table 1. Characteristics of 24 Pancreatic Cancer Patients Screened in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Age</th>
<th>Gender</th>
<th>Education</th>
<th>Smoke</th>
<th>Opium</th>
<th>Alcohol</th>
<th>Family History</th>
<th>Tumor Characteristics</th>
<th>Stage</th>
<th>Survival (Months)</th>
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<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
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<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
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<td>IIB</td>
<td>6</td>
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<td>(-)</td>
<td>(+)</td>
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<td>(-)</td>
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<td>(+)</td>
<td>(+)</td>
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<td>IIA</td>
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<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>Tail, 36 mm, T4-N0-M0</td>
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<td>(-)</td>
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<td>IV</td>
<td>5.3</td>
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<td>F</td>
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<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
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<td>(-)</td>
<td>(-)</td>
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<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
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<td>(-)</td>
<td>(+)</td>
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<td>Head, 45 mm, T4-N1-M1</td>
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<td>(-)</td>
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<td>(-)</td>
<td>(-)</td>
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<td>Body, 35 mm, T3-N1-M1</td>
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<tr>
<td>131195</td>
<td>48</td>
<td>M</td>
<td>High school</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>Head, 35 mm, T3-N1-M0</td>
<td>IIB</td>
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<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>Second-degree relative, Unknown origin, 19 years old</td>
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<td>IV</td>
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<tr>
<td>131256</td>
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<td>Head, 51 mm, T2-N1-M1</td>
<td>IV</td>
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and BRCA2 were identified in 2 out of 24 probands (8.3%). According to previous studies, BRCA1/2 are the most commonly mutated pancreatic cancer susceptibility genes.10–12 Mutations in BRCA1/2 genes happen in 3%–5% of the pancreatic cancer patients. The results of our study confirm the findings in previous studies on the importance of BRCA1 and BRCA2 in pancreatic cancer. The higher mutation frequency rate among our patients is explained by the fact that the study was restricted to patients with early onset pancreatic cancer, therefore the chance of pancreatic cancer being hereditary is expected to be higher. The carrier of the BRCA2 mutation was diagnosed with pancreatic cancer at age 48 and her sister had a history of breast cancer as well. The BRCA1 mutation carrier was diagnosed at age 40 which is considered a young age for pancreatic cancer onset; however, he did not have any family history of breast or ovarian cancer.

Subsequently, we identified 2 rare mutations in 2 other patients in candidate cancer susceptibility genes, EXO1 and PARP4. None of these 2 mutation carriers had any family history of cancer, however, both of them were diagnosed with pancreatic cancer at very young ages (29 and 42 for EXO1 and PARP4, respectively). The frequency of truncating mutations among non-TCGA patients in the ExAC database is one out of 200 and 232 for EXO1 and PARP4 respectively, vs. 1/24 in our series. ExAC is an aggregate of exome sequencing data from a variety of large-scale sequencing projects, and although it is not a perfect control set for our patients, it gives a sense of expected frequencies of truncating mutations in these genes among general population.

PARP4 is a member of the poly (ADP-ribose) polymerase (PARP) protein family.12 The PARP proteins are responsible for a variety of cell sustaining events such as genome maintenance and regulation, and RNA interference.13,14 PARP4 contains BRCA1 carboxyterminal (BRCT) domain repeats, owing to that PARP4 is thought to have a role in genome repair.15–16 The BRCT domain in PARP4 binds to damage-sensing surveillance proteins signaling PARPs for DNA repair initiation.15 The impairment of DNA repair pathways by and large is pathogenic in nature, and is a well-known event as initiation of tumorigenesis. A previous study indicated that proliferation of PARP4-knocked down breast cancer cells was expedited suggesting a tumor suppression role for PARP4.16 Moreover, PARP4 has a specific N-terminal motif with the ability to bind and co-regulate estrogen receptors playing a role in breast cancer development.17 Interestingly, in addition to its role in genome maintenance, another role of PARP4 is in telomere replication regulation.18 Telomeres, base pair repeats at the ends of chromosomes, are vital for survival of cancer cells.19 Another member of the PARP family is Tankyrase (PARP5) which contains a similar N-terminal domain as PARP4, regulating telomere elongation.18 The highest Tankyrase activity has been seen in the pancreas.20 The accumulation of these facts indicates that Tankyrase has the ability to regulate telomere elongation and therefore tumorigenesis, and this action mostly takes place in the pancreas. The polypeptide enzyme Tankyrase is similar to PARP4 protein in this effect. Therefore, these lines of evidence support the findings of this study suggesting that PARP4 could be potentially a candidate pancreatic cancer susceptibility gene.

EXO1 encodes Exonuclease 1, a member of the RAD2 nuclease family involved in genome repair through interaction with DNA Mismatch Repair (MMR) complexes.21,22 Impairment of MMR repair system is pathogenic and results in accumulation of mutations in turn leading to formation of various types of tumors.23 Several studies on MMR pathway suggest that insufficiencies of this important system could lead to gastrointestinal, ovary and endometrial cancers, as well as pancreatic cancer.22,24–26 Furthermore, in addition to its role in maintaining DNA fidelity, dysfunctionality of MMR enzymes such as EXO1 could also prevent apoptosis in cancerous cells.26–27 A previous study suggests that deletion of EXO1 in mice and human cells lead to inactivation of apoptosis signaling pathway, generating gastric and colorectal cancers.28 Another study suggests that various SNPs in EXO1 induce susceptibility to lung cancer.29 A study suspected that pathogenic truncating
mutations in MMR genes such as EXO1 may be involved in development of cervical cancer.\textsuperscript{21} Interestingly, a previous study showed that variations in EXO1 could be involved in and exacerbate overall survival of pancreatic cancer patients.\textsuperscript{21} In addition, several studies suggest a role for EXO1 mutations in inducing hereditary nonpolyposis colorectal cancer (HNPPC) predisposition.\textsuperscript{29} Therefore, mostly due to the role this gene plays in controlling the MMR system, loss of function truncating mutations in EXO1 could be pathogenic in nature and could potentially contribute to tumorigenesis in pancreas.

One of the strengths of this study is the inclusion of well-characterized patients with early onset disease. These cases are more likely to be of a hereditary nature. Another notable strength is the large number of known and suspected cancer genes analyzed here. Limitations of the study may include the small sample size, lack of a specific control set, and no access to DNA samples from other family members for co-segregation analysis.

Most pancreatic tumors are highly aggressive and prone to metastasis by virtue of the constantly altering nature of the unstable genome in tumor cells.\textsuperscript{30} Surgical treatment is usually palliative and not always an option for some patients, since tumors are usually diagnosed at later stages.\textsuperscript{30} These features indicate the need for predictive and prognostic genetic markers, which may lead to better individualization of cancer therapy in addition to targeted treatment and prevention. BRCA1/2 genes are the most commonly mutated pancreatic cancer susceptibility genes which should be screened in all patients with young age at onset or family history of cancer, especially breast or ovarian cancers. This study identified the genes EXO1 and PARP4 as potential candidate susceptibility genes for pancreatic cancer. Further studies for confirming these findings are needed.

Authors’ Contribution
AP designed and directed the project. MRA contributed to the design, analysis of the results and to the writing of the manuscript. AM and SM contributed to sample preparation and processed the data. SA and ES performed the measurements and contributed to the interpretation of the results. PB and RM supervised the findings of this work. All authors discussed the results and commented on the manuscript.

Conflict of Interest Disclosures
The authors have no conflicts of interest.

Ethical Statement
The study protocol was approved by Institutional Review Board Digestive Disease Research Center, Tehran University of Medical Sciences (IRB number: IRB000001641, Federal wide Assurance number: FWA00015916) in December 2010, based on ethical guidelines of 1975 Declaration of Helsinki as reflected in a prior approval by institution’s human research committee.

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References
Hereditary Pancreatic Cancer


