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Alterations of type II classical cadherin, cadherin-10 (CDH10), is associated with pancreatic ductal adenocarcinomas

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Abstract

Pancreatic ductal adenocarcinoma (PDAC), either sporadic or familial, has a dismal prognosis and finding candidate genes involved in development of the cancer is crucial for the patient care. First, we identified two patients with germline alterations in or adjacent to *CDH10* by chromosome studies and sequencing analyses in 41 familial pancreatic cancer (FPC) cases. One patient had a balanced translocation between chromosome 5 and 20. The breakpoint on chromosome band 5p14.2 was ~810 Kb upstream of *CDH10*, while that on chromosome arm 20p was in the pericentromeric region which might result in inactivation of one copy of the gene leading to reduced expression of *CDH10*. This interpretation was supported by loss of heterozygosity (LOH) seen in this region as determined by short tandem repeat analyses. Another patient had a single nucleotide variant in exon 12 (p.Arg688Gln) of *CDH10*. This amino acid was conserved among vertebrates and the mutation was predicted to have a pathogenic effect on the protein by several prediction algorithms. Next, we analyzed LOH status in the *CDH10* region in sporadic PDAC and at least 24% of tumors had evidence of LOH. Immunohistochemical stains with CDH10 antibody showed a different staining pattern between normal pancreatic ducts and PDAC. Taken together, our data supports the notion that *CDH10* is involved in sporadic pancreatic carcinogenesis, and might have a role in rare cases of FPC. Further functional studies are needed to elucidate the tumor suppressive role of *CDH10* in pancreatic carcinogenesis.

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1 | INTRODUCTION

Pancreatic cancer has a dismal prognosis. The most common type of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC), which is generally sporadic in origin and accounts for >90% of pancreatic cancers. Up to 10% of pancreatic cancer patients are designated familial pancreatic cancer (FPC),[1] which is defined as a kindred with at least two first-degree relatives affected by pancreatic cancers.[2]

Large-scale genome-wide screening, for example, next generation sequencing and SNP array analysis, provides an extensive and unbiased way to search for susceptibility genes in cancers. Using these approaches, genomic regions showing copy number variations and mutations are often found in PDAC.[3–5] Jones et al.[4] showed that point mutations contributed the most to the genetic alterations in PDAC by analyzing sequences of ~23,000 transcripts in 24 pancreatic cancers using Sanger sequencing. These mutations may change expression of genes involving in core signaling pathway and regulatory processes, such as DNA damage control (*TP53*), KRAS signaling (*KRAS*), and homophilic cell adhesion (*CDH* gene family). Further whole-exome or whole-genome sequencing studies help identify more genomic aberrations in PDAC, which can be further categorized into four different subtypes—stable, locally rearranged, scattered, and unstable—with potential clinical utility.[3,5]

Although relatively rare, several genes associated with FPC such as *BRCA2*, *BRCA1*, *STK11*, *CDKN2A*, *PALB2*, *ATM,* and mismatch repair genes[6–9] have been identified. Conducting whole-genome sequencing of 638 patients with FPC and exome sequencing of 39 FPC tumor tissues, a recent study further identified more genes carrying private heterozygous premature truncations or deleterious mutations in FPC.[8] However, variants identified in the FPC kindreds are highly heterogeneous—more than 60% of the genes identified only appear in one single FPC kindred. As the risk of developing pancreatic cancer is significantly higher—4.6 to 32 fold—in members of FPC kindreds,[2,10] it remains an important task to understand the genetic underpinning of FPC.

Either familial or sporadic, finding candidate genes that are involved in PDAC tumorigenesis should contribute to the development of early detection biomarkers, offer the opportunities of preventive medicine.

In this study, we first performed chromosome studies in 41 FPC cases followed by direct sequencing of cadherin-10 (*CDH10*) gene, a lesser-known gene that encodes a type II classical cadherin,[11] in all cases. We further carried out the loss of heterozygosity (LOH) analysis of *CDH10* in 50 sporadic PDAC tissues. Our results found alterations of *CDH10* genes and LOH of *CDH10* regions in PDAC tissues, suggesting that *CDH10* may be involved in pancreatic carcinogenesis.

2 | MATERIALS AND METHODS

2.1 | Materials

This study was approved by Johns Hopkins University Institutional Review Board. For FPC cases, DNA from lymphoblastoid cell lines created from individuals in the National Familial

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Pancreas Tumor Registry (NFPTR; www.nfptr.org) was used. Individuals studied had pancreatic cancer documented by review of the pathology report with the first degree member of the family also having pancreatic cancer (Supporting Information Table S1). Individuals ($n = 39$) or other family members ($n = 2$) had been tested for mutations in *BRCA2* and *PALB2* and they were negative (Supporting Information Table S1).

For sporadic pancreatic carcinoma cases, formalin-fixed, paraffin-embedded (FFPE) tissue from 28 individuals at Johns Hopkins Hospital and snap-frozen fresh tissue from 22 individuals at Thomas Jefferson University were used. Pancreas cancer cell lines including Hs766T, PL45, MIA PaCa-2, Capan-1, and BxPC-3 were used in this study.

Anonymized samples of bone marrow or peripheral blood from individuals serving as bone marrow transplantation donors were used as controls for sequencing.

2.2 | Copy number and SNP array analysis of T(5;20) somatic cell hybrids

Genomic DNAs for SNP array analysis were isolated from two t(5;20) human/mouse somatic cell hybrid clones (human der(5)- and der(20)-retaining clones). Copy number variation was evaluated using Illumina HumanHap 550 arrays. SNP genotyping was performed at the SNP Center of The Johns Hopkins University Genetic Resources Facility. Samples were processed and analyzed according to the Infinium II Assay protocol (Illumina, San Diego, CA) and the processed BeadChips were imaged on an Illumina BeadArray reader. The signal intensity (log *R* ratio) and allelic composition (B allele frequency) of human der(5) and der(20) chromosomes were analyzed by direct observation of the scan data using BeadStudio v.3.0.27. The minimal size of detected aberrant findings was calculated from the base position of the proximal and distal aberrant SNPs based on the UCSC Genome Browser, Human Dec. 2013 assembly (hg38). It is estimated that <3% of human SNP probes on Illumina array are potentially conserved in rodent. Therefore, the human DNA signals can be clearly separated from those of mouse, and balanced translocation breakpoints can be mapped with high resolution using this method.

2.3 | Genomic DNA and total nucleic acid isolation

Genomic DNAs were isolated from peripheral blood, bone marrow, somatic cell hybrid clones or transformed lymphoblast cells with the Gentra PureGene DNA isolation kit or QIAGEN QIAamp Midi kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total nucleic acid was isolated from the FFPE tumor samples using the Agencourt FormaPure kit (Agencourt Bioscience Corp., Beverly, MA) according to the manufacturer's protocol. Isolated DNA and total nucleic acid were quantified by Nanodrop and examined for size by agarose gel electrophoresis.

2.4 | Polymerase chain reaction (PCR) and direct sequencing of CDH10

PCR reactions using primers for *CDH10* gene (Supporting Information Table S2) were carried out in a reaction containing 1× PCR buffer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 0.25 μM each of forward and reverse primers, 1.25 units Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA), and 20 ng of DNA in a 20-μL reaction volume. PCR amplification was performed using the ABI9700 and touchdown thermal cycling conditions as follows: 94°C

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for 2 min; 3 cycles of 94°C for 30 s, 64°C for 30 s, 72°C for 30 s; 3 cycles of 94°C for 30 s, 61°C for 30 s, 72°C for 30 s; 3 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s; 35 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min.

PCR products were purified using QiaQuick reagents (Qiagen, Valencia, CA) or ExoSAP-IT (USB Corp., Cleveland, OH) and were cycle sequenced using Big Dye v3.1 reagents (Applied Biosystems, Foster City, CA) and the standard M13F or M13R sequencing primers according to the manufacturer's protocol. Sequencing products were purified with CleanSEQ Sequencing Purification System (Agencourt Bioscience Corp., Beverly, MA), and automated sequencing was performed by capillary electrophoresis (CE) on an ABI3700 (Applied Biosystems, Foster City, CA). Sequences were aligned and examined using Sequencher software (Gene Codes, Ann Arbor, MI).

2.5 | Short tandem repeat (STR) analysis

The following six STRs around *CDH10* on chromosome 5 were analyzed: *D5S2845* (5p14.3), *D5S1473* (5p14.2), *D5S813* (5p14.2), *D5S648* (5p14.1), *D5S814* (5p14.1), *D5S419* (5p14.1) (Supporting Information Table S3). No STRs are described within *CDH10* itself. Reactions were individually prepared and thermalcycled according to the PCR protocol described above. After amplification, 2 μL of each PCR product was mixed with 8 μL of deionized formamide/GeneScan 500 [ROX] size standard (Applied Biosystems, Foster City, CA). Samples were denatured at 95°C for 2 min and placed on ice for at least 1 minbefore analyzing on the ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). CE data from the tumor samples and from nontumor control samples were analyzed to identify alleles at each locus and determine the allelic ratios.

2.6 | CDH10 immunohistochemistry (IHC)

Unconjugated Rabbit Anti-Human CDH10, C-Terminus polyclonal antibody was obtained from Abgent, San Diego CA(Cat.# AP1482b). Chromogenic IHC labeling for CDH10 was performed as follows: 5 μm tissue sections of FFPE tissues on charged slides were deparaffinized and rehydrated by sequential 10 min room temperature incubations in xylene, 100% ethanol, 95% ethanol, 70% ethanol, and distilled water. A 60-s immersion in distilled water containing 1% Tween-20 detergent (Sigma-Aldrich, St. Louis, MO, Cat. # P-7949) was followed by heat-induced antigen retrieval. The slides were immersed in an EDTA target retrieval buffer (Invitrogen, Carlsbad, CA, #00–5500) and steamed in a vegetable steamer (Black and Decker Handy Steamer Plus, Black and Decker, Towson, MD) for 45 min. Endogenous peroxidase activity was blocked by 10 min treatment with peroxidase blocking reagent (Dako, Carpinteria, CA, Cat. # S2001). The primary antibody was applied at a dilution of 1:50 diluted in antibody dilution buffer (ChemMate Cat. # ADB250) and incubated 14 h at 4°C. The primary antibody was detected using the Power Vision Plus HRP-polymer detection system (Leica Cat. # PV6119) per manufacturer's instructions. All washing steps utilized Tris Buffered Saline with Tween (TBST; Sigma-Aldrich, Cat. # T-9039). DAB chromogen (Sigma-Aldrich, Cat. # D4293) was applied to develop the secondary detection reagent. Slides were then counter stained with Mayer's hematoxylin (Dako Cat. # S3309), dehydrated and cover slips were mounted.

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2.7 | Databases

Single Nucleotide Polymorphism Database (dbSNP, https://www.ncbi.nlm.nih.gov/projects/SNP/) build 147, 1000 Genomes May 2013 release (http://browser.1000genomes.org/), and the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/) were used to identify the minor allele frequency of *CDH10*'s missense mutations. Expression level of *CDH10* in various tissue types was obtained from the Unigene's expressed sequence tag (EST) database, and the Genotype-Tissue Expression project (GTEx Portal, http://www.gtexportal.org/home/).

3 | RESULTS

3.1 | Germline alterations of CDH10 identified in FPC

Through routine karyotyping of lymphoblastoid cell lines of NFPTR enrollees, we identified one individual with a typical pancreatic adenocarcinoma showing a balanced constitutional translocation t(5;20)(p14; p11.1) in all metaphases (Figure 1A). Metaphase fluorescent in-situ hybridization (FISH) using bacterial artificial chromosomes (BACs) obtained from the BACPAC Resource Center (Children's Hospital Oakland, Oakland, CA) were used to further define the breakpoint. Several primer sets (Figure 1D) were designed in the regions covered by the BAC clone that straddled the breakpoint on chromosome 5 (Figure 1B). We further conducted Illumina HumanHap 550 array analysis of the human-mouse somatic cell hybrid clones containing only the derivative chromosomes to identify the translocation breakpoint on chromosome 5 (Figure 1C).

Interestingly, the breakpoint is at 810 kb upstream of (*CDH10*), which is the nearest gene without any microdeletions or duplications in the putative breakpoint regions (Figure 1D). Subsequent sequencing analysis identified no germline *CDH10* mutation in this individual. We further analyzed the genomic stability of the patient's tumor using 6 STR markers on chromosome band 5p14.2. The results demonstrated a region of LOH covering *CDH10* (Figure 3, Familial t(5;20) case), which is commonly seen in cancers. This indicated that *CDH10* might be associated with FPC. However, limited tumor tissue was available from a biopsy of this patient's tumor and no additional materials were available for *CDH10* expression analysis by RT-PCR or IHC.

We further extended our studies by performing karyotyping and all-exon sequencing of germline *CDH10* in 41 FPC cases. *CDH10* germline sequence changes were also analyzed in 106 deidentified normal bone marrow donors. *CDH10* polymorphisms were checked against public variant databases (dbSNP build 147, 1000 Genomes May 2013 release) before reporting as a possible novel variant.

Among 41 patients, we observed a patient who demonstrated a germline G > A transition base substitution at coding DNA position 2063 resulting in an amino acid arginine (R) to glutamine (Q) substitution at amino acid codon 688 (p.R688Q) (Figure 2A). This alteration occurs in a highly conserved cadherin cytoplasmic domain in exon 12. The amino acid is conserved among vertebrates (Figure 2B). To be noted, this patient had two first degree relatives who had developed pancreatic cancer. Tumor tissue was not available from this individual for analysis. In contrast, no nonsynonymous changes in exon 12 were found

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among the 106 control samples or in public databases (dbSNP build 147, 1000 Genomes May 2013 release; see more detail in discussion). Although one synonymous alteration in coding sequence of exon 12 that had not been previously described was found in two control individuals, this variant was less likely to cause any deleterious effect on *CDH10* function (DNA position 2019 C > T). These results further suggest that alteration of *CDH10* gene may be related to small portion of FPC cases.

To investigate whether the amino acid change at the position 688 affects the protein function, we performed predictions of the pathogenic effect of this variant using MutationTaster2,[12] PolyPhen-2,[13] and SIFT.[14] As expected, all three algorithms suggested pathogenic effect of this variant with disease causing probabilities 1.00 (MutationTaster2), 0.982 (PolyPhen-2), and 100% (SIFT).

3.2 | Somatic alterations in *CDH10* gene region

We further examined if somatic alteration in *CDH10* gene region is present in sporadic PDAC, and analyzed LOH status using six microsatellite markers surrounding *CDH10*. DNA isolated from 28 microdissected FFPE PDACs and 22 grossly dissected fresh frozen PDACs were analyzed to identify somatic alterations. For LOH analysis, we included one sample of familial tumor for comparisons. One tumor (1/51, 2%) demonstrated an A > T transition at 2176, which resulted in amino acid threonine (T) to serine (S) substitution at codon 726 (p.T726S; mutation id: COSM84892). We subsequently confirmed that this mutation was identified in the tumor cell line from the same individual.[4] Additional mutations were not identified.

Next, we examined LOH status of a total of 51 samples including 50 sporadic tumors and one familial tumor, which was included for comparisons. Twelve cases (24%) including 11 FFPE samples and 1 fresh PDAC sample demonstrated LOH at one or more of the markers most proximal to *CDH10*, that is, *D5S813* or *D5S648* (Figure 3 and Table 1). The remaining 38 sporadic cases (76%) demonstrated no definitive evidence of LOH at a locus adjacent to *CDH10*, whereas the familial tumor from the patient with a constitutional t(5;20) demonstrated LOH at a locus adjacent to *CDH10*. Among the 38 cases without evidence of LOH in the two loci, 7 of them showed LOH in at least one of the 4 loci analyzed (14%; Locus *D5S2845*, *D5S1473*, *D5S814,* and *D5S419*).

We noted that STR marker *D5S648*, the closest upstream marker to *CDH10*, was only informative in 13 out of the 50 tumors tested (26%; Figure 3). The heterozygosity scores for this STR marker were shown to be similar to *D5S813*, which are 0.74 and 0.75, respectively. However, STR *D5S813* was informative (heterozygous) in 64% of the tumors (32/50).

In addition, DNA microdissected from FFPE specimens demonstrated a significantly higher frequency of LOH compared to DNA isolated from fresh tumor specimens (11/28, 39.3% vs. 1/22, 4.5%, respectively). This raises the possibility that some of the DNA isolated from fresh frozen tumors may have contained a low percentage of tumor cells, which made it inadequate for LOH detection.

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Because of this concern, we analyzed DNA from each tumor specimen for oncogene *KRAS* codons 12 and 13 mutations as evidence of tumor content. DNA isolated from FFPE has a significantly higher mutant allele percentage compared to DNA isolated from fresh tissue [39.68 ± 4.20% and 21.12 ± 3.26% (mean ± SEM), respectively, $P = .026$; Table 1). In general, a tumor percentage >30% is needed to identify LOH. Although a low or negative *KRAS* result is not definitive for a low percentage of tumor DNA present in the sample, our results suggest that some of our DNA specimens may have had an inadequate percentage of tumor cells to detect LOH (or mutations) and actual frequency of LOH in this region may be higher.

3.3 | Localization of CDH10 protein in PDAC

Lastly, we examined CDH10 protein expression in PDACs in order to determine whether CDH10 expression and distribution is altered. Immunohistochemical stains (IHC) with anti-CDH10 were performed on 31 sporadic PDACs on a tissue microarray with two normal pancreatic tissues as a control. The polyclonal antibody we used targets the C-terminal region of CDH10 corresponding to the cytoplasmic domain region. The two normal pancreas tissue samples demonstrated a staining pattern consistent with localization of the protein to gap/tight junctions (Figure 4A,B). In contrast, very weak cytoplasmic staining was observed with CDH10 in PDAC specimens (Figure 4C,D). Expression of CDH10 in PDAC specimens was greatly reduced compared to that in normal pancreas. IHC was also attempted with another polyclonal antibody to CDH10 (Sigma HPA010651) but this antibody failed to stain normal controls. These results suggest that decreased CDH10 expression in PDAC possibly correlates with LOH in *CDH10* gene region.

4 | DISCUSSION

Abnormal cadherin expression has been associated with a large spectrum of disease, including metastatic cancer (Berx and van Roy, 2009). Members of the cadherin superfamily are increasingly shown to have a defining role in cancer. Its best-known member, E-cadherin, has been shown to suppress invasion and metastasis, and germline mutations in this gene causes an autosomal dominant predisposition to diffuse gastric cancer and early onset breast cancer.[15,16] *CDH10* was found to be one of a number of genetically altered cadherins involved in the homophilic cell adhesion pathway in pancreatic cancer, where it was considered a driver mutation.[4] We hypothesized that *CDH10* may play a role in the development of pancreatic cancer. Here we identified two novel germline alterations in individuals with FPC, who were negative for mutations in other known pancreatic cancer risk/causative genes (*BRCA2* and *PALB2*). Of note, one of two cases had variant of uncertain significance (VUS) in *BRCA1* and *FANCA* genes (data not shown), and significance of these alterations in pathogenesis is not known. Roberts et al.[8] showed that more than half of the germline alterations in FPC were only found in one kindred, indicating that most FPC-associated variants are very heterogeneous and only contribute to a small number of cases. It is known that whole genome sequencing approach is more technically challenging to identify structural variation. In addition, discovering structural variants especially in primary tumors is difficult due to the artifact created by contamination of normal stromal cells and lymphocytes.

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CDH10 has been proposed to be one of the driver mutation genes in sporadic PDACs.[4] Additionally, cadherins are important in cell-cell adhesion, and are known to function in cell recognition, coordinated cell movement, and inducing and maintaining both structural and functional cell and tissue polarity. Abnormal expression of cadherins, such as the most well-known member E-cadherin, often results in increased tumor cell invasion, which ultimately leads to metastasis of tumors.[17] *CDH10* is a lesser known gene that encodes a type II classical cadherin, which is defined based on the lack of a HAV cell adhesion recognition sequence specific to type I cadherins.[11] It is predominantly expressed in brain and is putatively involved in synaptic adhesions and in axon outgrowth and guidance.[18,19] Recent studies further suggest that somatic mutations in *CDH10* are associated with colorectal cancer, gastric cancer, and lung cancer.[20–22] Therefore, it is of interest to clarify the association between genetic alterations in *CDH10* and pancreatic cancer.

In our study, germline alterations in or nearby *CDH10* were observed in 2 out of 41 FPC patients using a combination of classical chromosome analysis and direct sequencing in order to precisely capture the multifaceted genetic alterations. First, we identified a patient with FPC who has a balanced translocation between chromosome 5 and 20 by analyzing karyotypes of participants in National Familial Pancreas Tumor Registry (NFPTR). We narrowed the breakpoints of the constitutional t(5;20)(p14.2p11.1) using FISH with BAC and long-range PCR probes, somatic cell hybrids and SNP array to a region 810 Kb from *CDH10* on chromosome 5 and in the pericentromeric region of chromosome 20. We postulate that *CDH10* expression has been affected by proximity to the pericentromeric region of chromosome 20. Genomic rearrangements that put euchromatic genes near the heterochromatin of a centromere often result in gene inactivation due to the proximity of heterochromatin.[23] This effect, known as position effect variegation, has been recognized since 1930, although direct demonstration of this effect in humans has been limited, possibly due to the difficulty of cloning breakpoints that involve heterochromatin. There are identified examples in humans of cis-acting elements that mediate their effect on gene regulation over large genomic distances. Individuals with campomelic dysplasia without a mutation in the coding region of *SOX9*, a gene known to cause the syndrome, have been found; several have translocations with breakpoints up to 932 kb upstream of the gene.[24] Other examples include a noncoding sequence ~10 kb downstream of the promoter of *RET* in Hirschsprung disease, and a SNP within a conserved noncoding sequence 10 kb upstream from the promoter of *IRF6* in Van der Woude syndrome (reviewed in Ref. 25).

The second germline alteration, a missense variant, has previously been reported as somatic mutation in lung adenocarcinoma.[26,27] This missense variant (p.R688Q; mutation id: COSM738261) occurs in the predicted cadherin cytoplasmic superfamily region. Therefore, while it is difficult to predict exactly what effect this missense variant would exert on the function of CDH10, comparison between the conserved amino acids of the existing cadherin cytoplasmic domains in various cadherin genes shows that R688 is among the highly conserved amino acid sequences of this domain. This suggests that the amino acid substitution may be significant. Although further functional analysis of CDH10 is required to elucidate this, all three predictions algorithms suggested the variant likely has a pathogenic effect on the protein. Additionally, the allele frequency of this missense variant is reported to be 0.00000826 in ExAC database version 0.3.1 (http://exac.broadinstitute.org/

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variant/5–24488076-C-T) suggesting that it may be a rare pathogenic variant. However, a recent whole genome sequencing study by Roberts et al. on 638 FPC cases and 967 controls identified only one premature truncating variant in *CDH10*. Hence, *CDH10* alterations we identified may be an uncommon cause of FPC.

Next, we studied a possible role of somatic alterations of *CDH10* in sporadic pancreatic tumors. We identified one mutation and a significant number of tumors with LOH in the region. Our analysis may underestimate LOH for several reasons. First, there may not have been enough tumor cell percentage in the specimens analyzed to accurately identify LOH. Second, the marker closest to *CDH10* (*D5S648*) had a much lower heterozygosity than expected. Since normal tissue from these tumors was not available for comparison, it is possible that we have underestimated the frequency of LOH at the *D5S648* locus. This speculation is supported by the result of LOH status of PDAC cell lines: 4 out of the 5 cell lines showed noninformative in locus *D5S648* (Supporting Information Figure S1). Since cell lines are pure populations of tumor cells, there is high frequency of "noninformative" allele, which could be a result of LOH. Third, FFPE tissues have degraded DNA, and 3 of six markers (*D5S1473, D5S813,* and *D5S419*) had relatively long allele lengths (>200 bp), causing variations in the ratios we observed in our normals and therefore a relatively wide range of ratios consistent with no loss. Nevertheless, our data indicate that *CDH10* alteration is seen in both familial and sporadic pancreatic cancer patients, which is consistent with recent observations showing FPC undergoes a similar somatic molecular pathogenesis as sporadic PDAC.[1]

Unigene's EST profiles show that *CDH10* is highly expressed in brain (23/1,092,688 total ESTs), while lower expression has been detected in pancreas (2/213,440 total ESTs). GTEx (http://www.gtexportal.org/home/), which contains gene expression data from microarray and RNA-Seq platforms, shows that *CDH10* has the highest expression in brain, particularly cerebellar hemisphere. Medium Reads Per Kilobase of transcript per Million mapped reads of the tissue is around 15, while it is very low or not detectable in pancreases. Our data indicate that CDH10 protein expression is altered in PDAC. CDH10 expression was previously shown in normal human prostate luminal epithelial cells but was absent in prostate cancer.[28] The authors developed their own antisera to CDH10, and concluded that expression of CDH10 was involved in a specific role of secretory cell terminal differentiation. Using commercially available antisera, we preliminarily demonstrated a difference in expression and localization in normal pancreas as compared with normal prostate.

Mature cadherin proteins are composed of a large N-terminal extracellular domain, a single membrane-spanning domain, and a small, highly conserved C-terminal cytoplasmic domain. The extracellular domain consists of five subdomains, each containing a cadherin motif, and appears to determine the specificity of the protein's homophilic cell adhesion activity. Relatively little is known about CDH10. It was first discovered in 1999[11] and was found to be expressed in brain, where it was shown to be involved in blood-brain barrier "synaptic adhesions, axon outgrowth and guidance."[19] *CDH10* spans 157.7kb on chromosome 5 (chr5: 24,487,209–24,645,087, hg38), and produces two transcript variants. It is a Type II

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(atypical) cadherin, that is, it lacks a HAV cell adhesion recognition sequence specific to type I cadherins.

Previous literature suggests that sequences near *CDH10* might be involved in synaptic adhesion, axon outgrowth and guidance and genetic variations nearby *CDH10* are associated autism.[29,30] In addition, Biankin et al.[3] found aberrations in axon guidance pathway genes in pancreatic cancer genomes. Furthermore, mutations in *CDH10* were identified in colorectal cancer and lung cancer recently.[20–22] These findings are in line with our studies that variations in *CDH10* coding or nearby genomic regions may play roles in pancreatic cancer.

In summary, we have found germline alterations in and adjacent to *CDH10* in 2 of 41 individuals with FPC suggesting *CDH10* genomic alterations may play an as yet undefined role in predisposition of selected individuals for development of pancreatic cancer. The finding of LOH at the region most proximal to *CDH10* gene in at least 24% of sporadic pancreas cancer confirms and extends the report of Jones et al.[4] Our data supports the notion that *CDH10* is involved in sporadic pancreatic carcinogenesis, and might have some roles in rare cases of FPC. Further evaluation of the function of *CDH10* in epithelial neoplasms is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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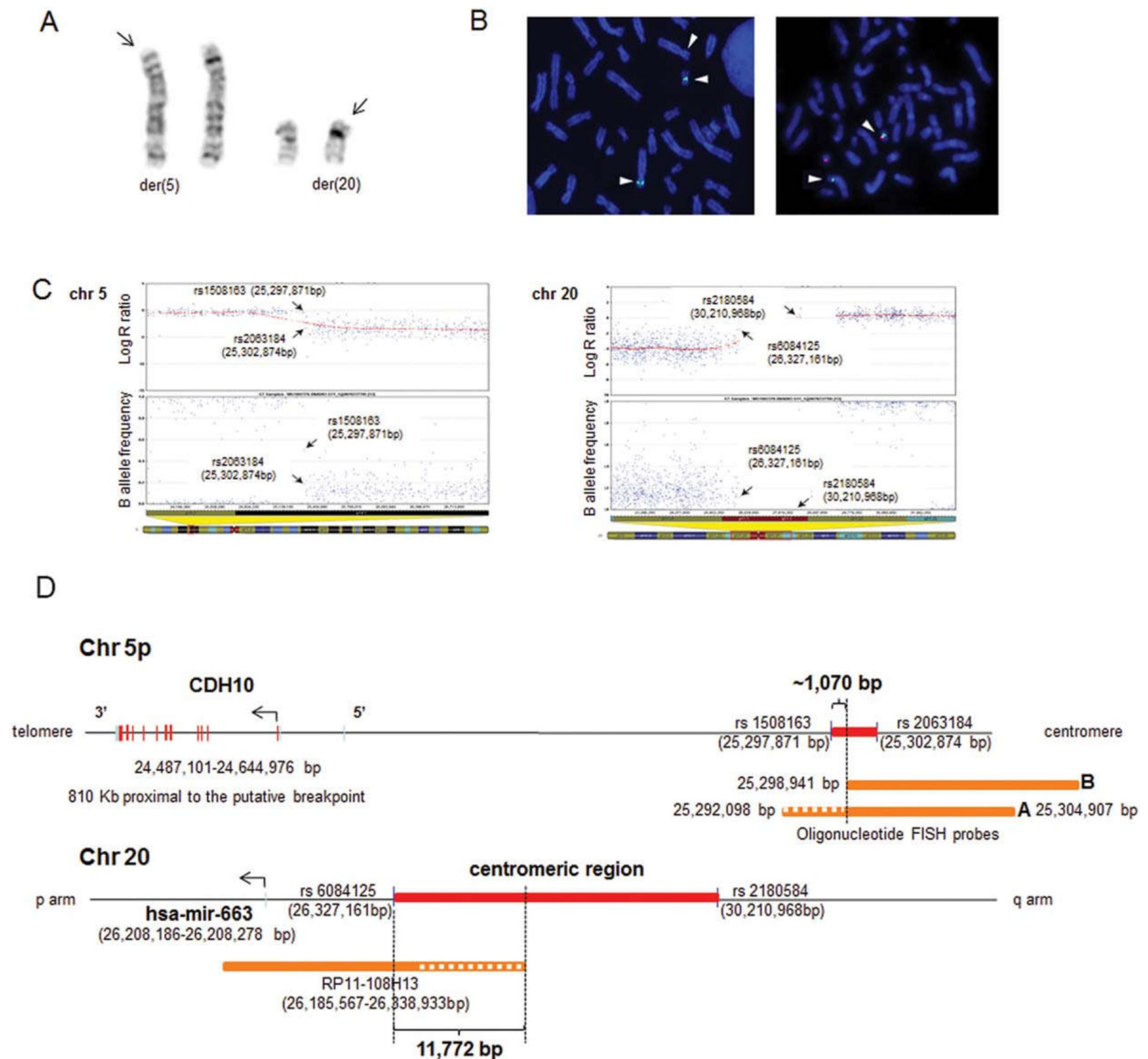

FIGURE 1.

Identification of the translocation breakpoint in a FPC patient with t(5;20)(p14;p11.1). **(A)** The G-banded chromosomes 5 and 20 from a lymphoblastoid cell line of the FPC patient showing an apparently balanced translocation t(5;20)(p14;p11.1) (black arrows). der(5), derivative chromosome 5; der(20), derivative chromosome 20. **(B)** FISH analysis of the translocation breakpoint. Left panel: Oligonucleotide probes PCR-amplified from RP11–184E9 (chromosome band 5p14) shows green split signals (white arrows) between chromosome arms 5p and 20p. Right panel: BAC FISH analysis showing the split signals (white arrows) of RP11–108H13 (chromosome band 20q11.1; green) on 20p and 5p, while those of RP11–348i14 (chromosome band 20q11.1; orange) are intact. **(C)** Illumina HumanHap550 genotyping beadchip analysis of the human-mouse somatic cell hybrids containing a copy of der(20). Log R ratio and B allele frequency plots demonstrating the translocation breakpoints on chromosomes 5 (left panel) and 20 (right panel). The breakpoint flanking SNPs are also shown (black arrows). Both der(5) and der(20)-containing somatic hybrids were analyzed with SNP array, but this figure only shows der(20) cell

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results. **(D)** A schema illustrating the translocation breakpoint regions on chromosomes 5 and 20 identified using a combination of FISH, somatic cell hybrid coupled with SNP array analysis. The nearest genes to the putative breakpoints on chromosome bands 5p14 and 20p11.1 are *CDH10* and *hsa-miR-663*, respectively. Genomic locations shown are based on the UCSC genome browser (hg38).

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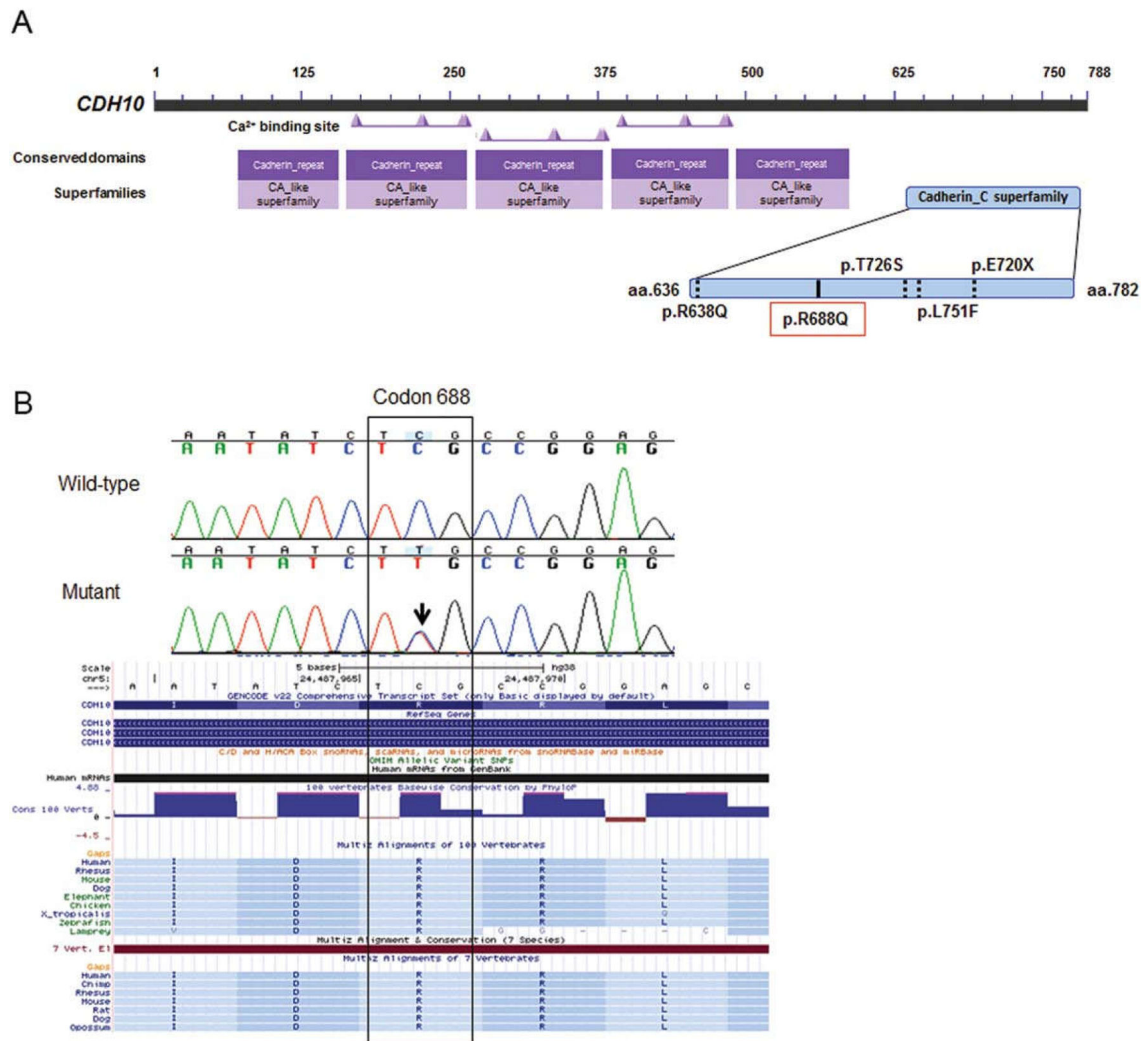

FIGURE 2.

CDH10 single nucleotide changes identified in the FPC and sporadic PDAC cases. (A) A diagram of CDH10 protein showing the locations of conserved domains and related domain superfamily as predicted by NCBI conserved domains. All previously reported *CDH10* somatic mutations (dotted line) and a novel germline variant (p.R688Q; solid line) identified in our study are clustered in exon 12 (Cadherin_C superfamily), which corresponds to the predicted cadherin cytoplasmic superfamily region. **(B)** Comparison between conserved amino acids of the existing cadherin cytoplasmic domains across vertebrate species. R688 is among the highly conserved amino acid component of this domain. Genomic locations shown are based on the UCSC genome browser (hg38).

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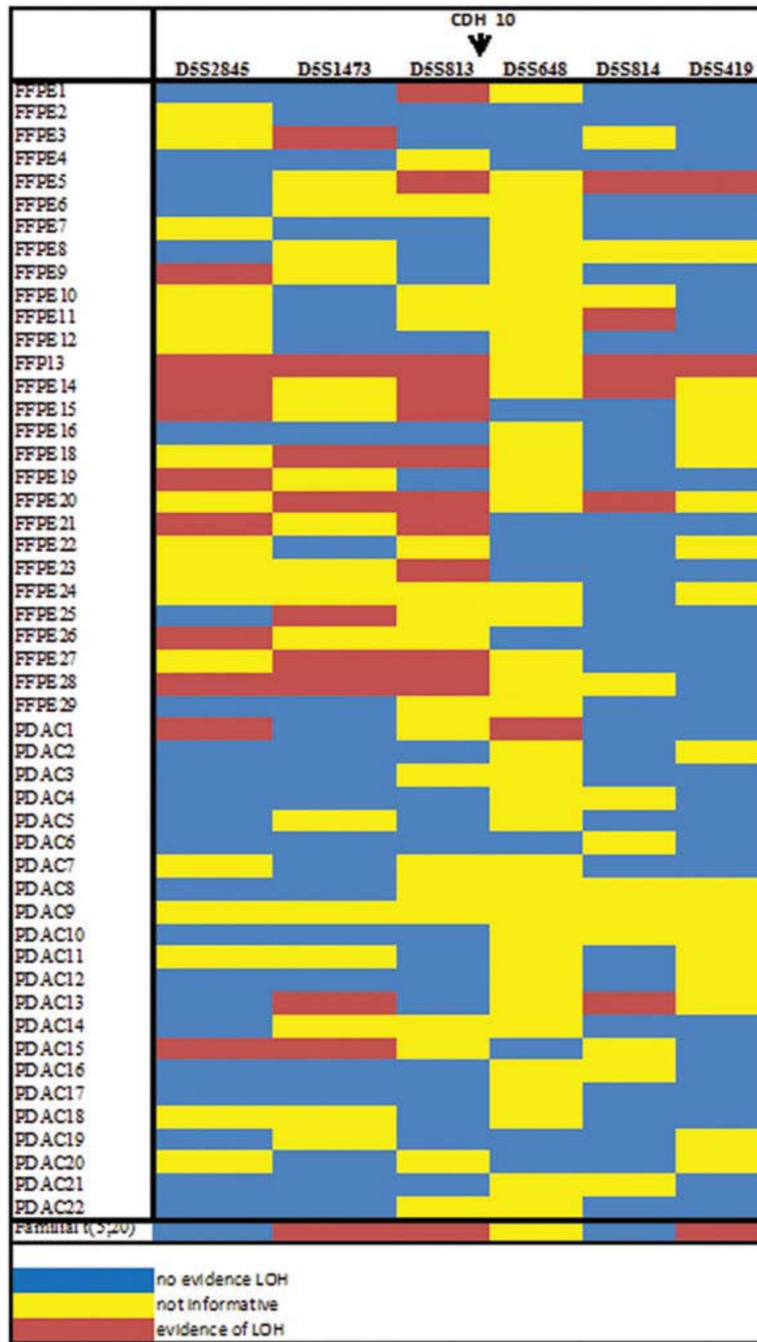

FIGURE 3.

Summary of LOH analysis of *CDH10* gene region in sporadic PDACs. LOH analysis using 6 STR markers on chromosome 5p14.1–5p14.3 in 28 FFPE samples from Johns Hopkins Hospital and 22 fresh frozen tumor specimens from Thomas Jefferson University. Additionally, one FPC with t(5;20) was also analyzed. *CDH10* gene is located between *D5S813* and *D5S648* (indicated by arrow). Red = LOH, Blue = not compatible with LOH, NI = not informative.

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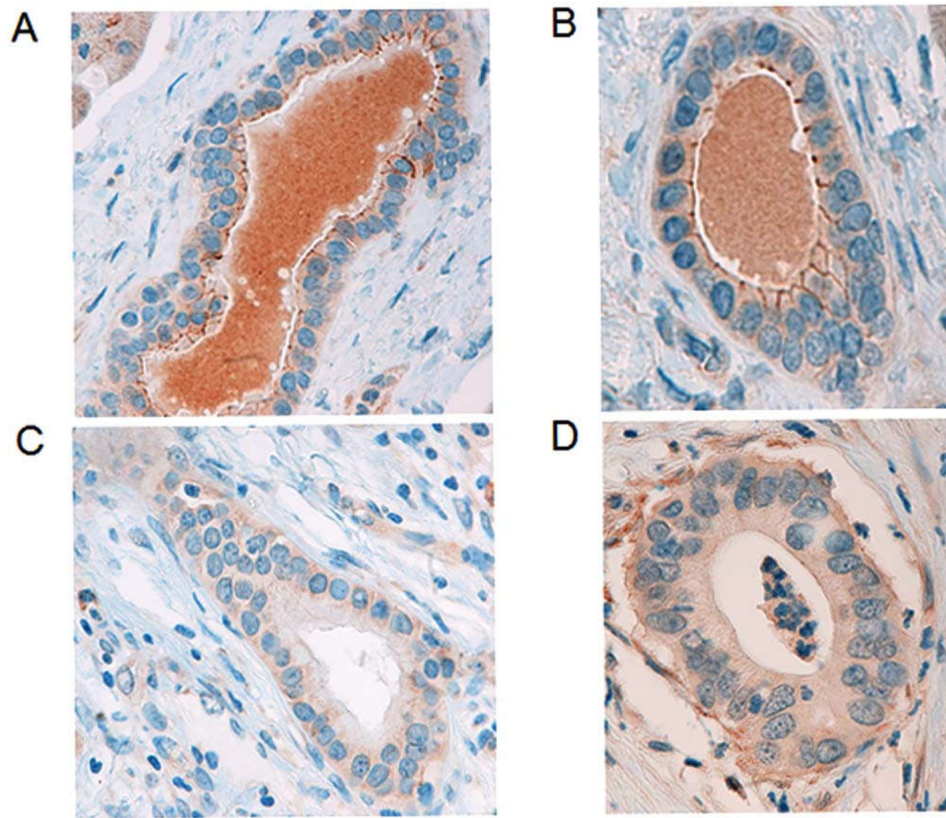

FIGURE 4.

Immunohistochemical stains with CDH10 antibody on normal pancreatic ducts (A and B) and PDACs (C and D). Images were taken at 200× (A and C) and 400× (B and D) original magnification.

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TABLE 1

Summary of LOH and *KRAS* mutation analysis in sporadic pancreatic cancer cases from formalin fixed paraffin embedded tissue (FFPE PDAC) and fresh tissue (Fresh PDAC)

	FFPE PDAC	**Fresh PDAC**	**Total**
Number of cases	28	22	50
LOH at loci adjacent to CDH10 (*D5S813* and/or *D5S648*)	11 (39.5%)	1 (4.5%)	12 (24%)
Suspicious for LOH	5 (10.7%)	2 (18.2%)	5 (10%)
KRAS mutation	24 (85.7%)	17 (77.3%)	41 (82%)
% of mutant allele (mean ± SEM)	39.68 ± 4.2	21.12 ± 3.3	32.2 ± 3.12

Suspicious for LOH: at least one loci showing LOH in another four loci if both *D5S813* and *D5S648* are not informative.