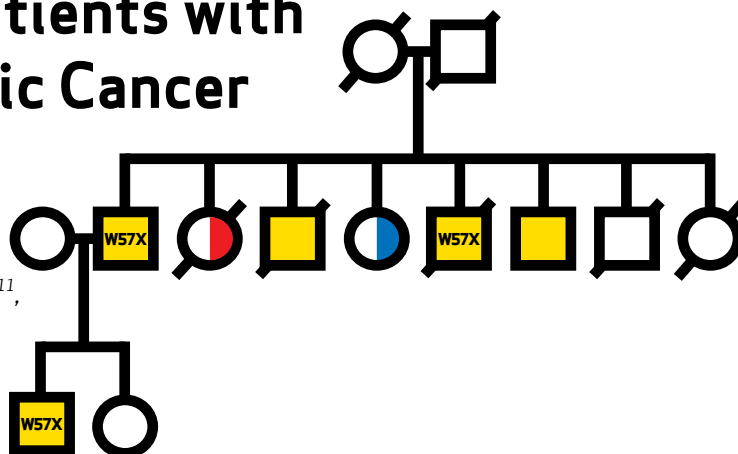


# ATM Mutations in Patients with Hereditary Pancreatic Cancer

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## ABSTRACT

Pancreatic cancers are the fourth most-common cause of cancer-related deaths in the Western world, with >200,000 cases reported in 2010. Although up to 10% of these cases occur in familial patterns, the hereditary basis for predisposition in the vast majority of affected families is unknown. We used next-generation sequencing, including whole-genome and whole-exome analyses, and identified heterozygous, constitutional, ataxia telangiectasia mutated (*ATM*) gene mutations in 2 kindreds with familial pancreatic cancer. Mutations segregated with disease in both kindreds and tumor analysis demonstrated LOH of the wild-type allele. By using sequence analysis of an additional 166 familial pancreatic cancer probands, we identified 4 additional patients with deleterious mutations in the *ATM* gene, whereas we identified no deleterious mutations in 190 spouse controls ( $P = 0.046$ ). When we considered only the mostly severely affected families with 3 or more pancreatic cancer cases, 4 deleterious mutations were found in 87 families ( $P = 0.009$ ). Our results indicate that inherited *ATM* mutations play an important role in familial pancreatic cancer predisposition.

**SIGNIFICANCE:** The genes responsible for the majority of cases of familial pancreatic ductal adenocarcinoma are unknown. We here identify *ATM* as a predisposition gene for pancreatic ductal adenocarcinoma. Our results have important implications for the management of patients in affected families and illustrate the power of genome-wide sequencing to identify the basis of familial cancer syndromes. *Cancer Discovery*; 2(1): 41–6. ©2011 AACR.

## INTRODUCTION

Pancreatic ductal adenocarcinomas carry a dismal prognosis, with fewer than 5% of patients surviving 5 years (1). This disease is particularly tragic when several members of

a single family are affected, a situation that occurs in 5% to 10% of cases of pancreatic ductal adenocarcinomas diagnosed in the United States (2). The genes responsible for 85% to 90% of familial pancreatic cancer kindreds, defined

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as those with at least 2 affected first-degree relatives, are unknown. In the remaining families, germline mutations of genes, including *BRCA2*, *PALB2*, *CDKN2A*, *STK11*, and possibly *BRCA1*, have been identified (2).

Unbiased, genome-wide sequencing can be used to identify familial pancreatic genes, as demonstrated by the finding that *PALB2* is a pancreatic cancer susceptibility gene (3). To identify additional familial pancreatic cancer genes, we evaluated the whole-genome sequences of 16 subjects from 6 families and the whole-exome sequences of an additional 22 subjects from 10 families. All families included at least 3 members with pancreatic ductal adenocarcinoma, and DNA was available from at least 2 such members. Families had enrolled into one of the familial pancreatic cancer registries participating in the Pancreatic Cancer Genetic Epidemiology Consortium (National Familial Pancreas Tumor Registry at Johns Hopkins, Mayo Clinic, Dana-Farber Cancer Institute, MD Anderson Cancer Center, Ontario Pancreas Cancer Study, and Karmanos Cancer Center). In this brief report, we present data from 2 families (FPC-A and FPC-B) that harbored inactivating mutations of a putative pancreatic cancer susceptibility gene.

## RESULTS

Whole-genome sequencing was applied to 3 members of FPC-A and whole-exome sequencing was applied to 3 members of FPC-B. These efforts produced an average of 113.0 and 7.1 gigabases per sample, resulting in 34-fold and 97-fold distinct coverage of the genome and exome, respectively (Table 1). On average, whole-genome sequencing identified 6,132,376 variants per person, of which 61.6% (3,778,674 variants) matched those deposited in single-nucleotide polymorphism (SNP) databases. Similarly, exome sequencing resulted in an average of 35,440 variants per individual, of which 55.3% (19,581 variants) matched those deposited in SNP databases (Table 1).

Sequence data were first evaluated for known predisposition genes (*BRCA2*, *PALB2*, *CDKN2A*, *BRCA1*, *TP53*, *STK11*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *PRSS1*, and *PRSS2*). No mutations in these genes were observed. We hypothesized

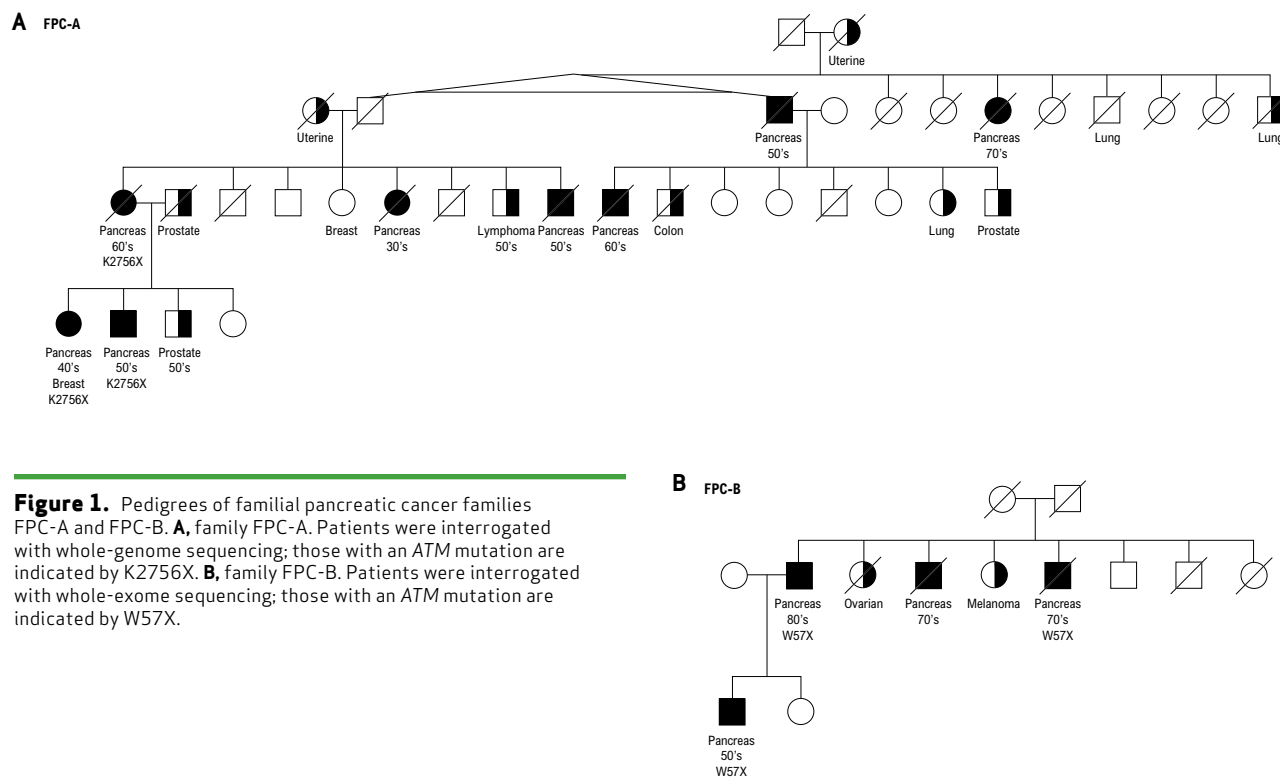
that any causative variant would be rare in the general population and therefore heterozygous in an affected individual. In addition, a causative variant would be inactivating and shared among all affected members of a family. We therefore filtered the variants according to the following criteria: (i) variants present in SNP databases were excluded from further analysis; (ii) the fraction of distinct sequence reads containing a variant had to be between 25% and 75% of the total number of distinct sequence reads of the corresponding base (i.e., it had to be heterozygous); (iii) the variant had to be present in all affected members of an affected kindred; and (iv) the variant had to be inactivating, that is, predicted to produce a nonsense mutation, frameshift insertion or deletion, or splice-site alteration (IVS -1 or -2; IVS +1 or +2).

When these filters were used, the 18,503,448 variants initially identified in the sequenced members of families FPC-A and FPC-B were narrowed to 156. Of these 156 variants, the most interesting were in *ATM* because FPC-A and FPC-B family members harbored different heterozygous nonsense variants of this gene (c.8266A>AT; p.K2756X and c.170G>GA; p.W57X). The pedigrees of these families are shown in Figure 1. Both of these variants have been reported previously in patients with ataxia-telangiectasia, an autosomal-recessive condition associated with an increased risk of multiple cancer types (4, 5).

To estimate the prevalence of deleterious *ATM* mutations in patients with familial pancreatic cancer and control subjects, we conventionally sequenced the entire coding region of the *ATM* gene in an additional 166 familial pancreatic cancer patients and 190 spouse controls (Supplementary Table S1). Patients with pancreatic cancer and control subjects were recruited as part of the National Familial Pancreas Tumor Registry. Of the 166 patients with familial pancreatic cancer, 71 were from kindreds with 3 or more pancreatic cancers. Chromatograms for all variants in *ATM* were visually inspected. Variants present in the Human Gene Mutation Database (6) were then manually curated and pathogenic alterations previously seen in ataxia-telangiectasia patients were confirmed with Sanger sequencing in a second, independent PCR amplification. Four variants (c.3214G>GT; p.E1072X, c.6095G>GA;

**Table 1. Summary data for familial pancreatic cancer families with inactivating *ATM* variants**

Type	Family	Individual	Read length	Bases mapped to genome	Bases mapped to exome	Exome bases with $\geq 10$ reads	Average raw coverage	Effective coverage	Total number of variants	Variants in SNP database
Whole-genome sequencing	FPC-A	1	100	115,388,854,300	1,294,030,427	34,681,602	35	95.1%	6,024,866	3,758,900
		2	100	110,742,763,000	1,219,892,546	34,328,889	33	94.7%	6,181,369	3,793,150
		3	100	112,771,643,500	1,271,909,140	34,656,841	34	95.1%	6,190,892	3,783,971
Whole-exome sequencing	FPC-B	1	75	8,110,266,675	4,118,378,295	36,458,593	109	97.9%	35,674	19,573
		2	75	7,597,985,850	4,076,171,309	36,560,365	108	98.0%	35,724	19,605
		3	75	5,649,542,100	2,815,928,672	36,067,752	74	97.6%	34,923	19,554



**Figure 1.** Pedigrees of familial pancreatic cancer families FPC-A and FPC-B. **A**, family FPC-A. Patients were interrogated with whole-genome sequencing; those with an *ATM* mutation are indicated by K2756X. **B**, family FPC-B. Patients were interrogated with whole-exome sequencing; those with an *ATM* mutation are indicated by W57X.

p.R2032K, IVS41-1G>GT; and c.3801delG; Table 2) previously observed in patients with ataxia-telangiectasia (7–10) were identified in the 166 patients with pancreatic cancer, whereas none were identified in the controls. The prevalence of *ATM* mutations was significantly greater in patients with familial pancreatic cancer compared with control subjects (4/166 vs. 0/190; the Fisher exact test,  $P = 0.046$ ). This association was even stronger in the most severe pancreatic cancer families, containing 3 affected members, where 4.6% (4/87 vs. 0/190; the Fisher exact test,  $P = 0.009$ ) of families carried deleterious *ATM* mutations. There was no significant difference in the youngest age at which a family member was diagnosed with pancreatic cancer among *ATM* carrier families versus the 162 pancreatic cancer families without *ATM* mutations.

Pancreatic tumor tissue with adequate DNA was available from 1 of the 10 pancreatic cancer patients with a germline *ATM* mutation (IV-1 in family FPC-A; Fig. 1). The entire coding region of *ATM* was sequenced by the use of microdissected tumor DNA. This patient was heterozygous for a nonsense variant (c.8266A>AT, p.K2756X) in DNA from peripheral blood cells. Analysis of the pancreatic tumor from this patient demonstrated LOH at the *ATM* locus with retention of the mutant, nonsense allele (Fig. 2). Losses of the *ATM* locus are uncommon in patients with sporadic pancreatic ductal adenocarcinoma (11), suggesting that the *ATM* loss in this patient was driven by a classic 2-hit model for tumor suppressor genes (12).

## DISCUSSION

The *ATM* protein is a serine/threonine kinase involved in DNA double-strand break repair (13, 14). Ataxia-telangiectasia is caused by the inheritance of biallelic deleterious mutations in the *ATM* gene (13) and occurs in 1/40,000 to 1/300,000 live births. The reported carrier frequency of deleterious *ATM* variants in the population is 0.5% to 1% (14, 15). Ataxia-telangiectasia is characterized by progressive cerebellar ataxia, oculomotor apraxia, telangiectasias of the conjunctiva and skin, immunodeficiency, sensitivity to ionizing radiation, and an increased rate of malignancies, particularly lymphoma and leukemia (14–16).

Women who carry pathogenic *ATM* mutations have an increased risk of breast cancer (15–17). However, the role played by *ATM* mutations in a patient's susceptibility to breast cancer has proven controversial (18–21). There are no other previous reports of *ATM* mutations in the germline of patients with pancreatic ductal adenocarcinoma. Similarly, no somatic mutations of *ATM* have been reported in pancreatic ductal adenocarcinoma. Pancreatic neuroendocrine tumors have little in common with pancreatic ductal adenocarcinoma, and occasionally harbor a somatic mutation of *ATM* (22). However, these mutations may be passengers because there is no genetic or biochemical evidence indicating that these mutations inactivate the gene product.

In our series of 166 familial pancreatic cancer probands, 2.4% (4/166) carried deleterious *ATM* mutations. When we considered only families with more than 3 affected members,

**Table 2. Summary of heterozygous deleterious *ATM* variants found in patients with pancreatic cancer**

Variant	Pancreatic cancer type	Nucleotide (genomic) <sup>a</sup>	Nucleotide (cDNA) <sup>b</sup>	Amino acid (protein) <sup>c</sup>	Type	Number of affected individuals sequenced
1	Familial <sup>d</sup>	g.chr11:107711896A>T	c.8266A>T	p.K2756X	Nonsense	3
2	Familial <sup>e</sup>	g.chr11:107603810G>A	c.170G>A	p.W57X	Nonsense	3
3	Familial	g.chr11:107648719G>T	c.3214G>T	p.E1072X	Nonsense	1
4	Familial	g.chr11:107691848G>A	c.6095G>A	p.R2032K	Missense	1
5	Familial	g.chr11:107693309G>T	IVS41-1G>T	sp	Splice site	1
6	Familial	g.chr11:107660218delG	c.3801delG	fs	INDEL	1

<sup>a</sup>Genomic positions are coordinates in the March 2006, hg1836.1 UCSC release of the human genome. Genomic coordinates and sequences of mutations are on the coding strand. All changes are heterozygous. g., genomic sequence; c., cDNA sequence; p., protein sequence; del, deletion.

<sup>b</sup>Mutations in non-coding sequences are annotated by intron number preceded by "IVS"; with positive numbers starting from the G of the GT splice donor site and negative numbers starting from the G of the AG splice acceptor site.

<sup>c</sup>fs, frameshift mutation; sp, splice site mutation.

<sup>d</sup>Family FPC-A.

<sup>e</sup>Family FPC-B.

we found that 4.6% (4/87) carried deleterious *ATM* mutations. The evidence that these mutations are functional is unambiguous because we defined deleterious mutations in the most rigorous way possible: only those mutations that are known to cause ataxia-telangiectasia when inherited were considered deleterious. Thus, these mutations cause a recessive disease phenotype in humans under natural conditions. We identified other germline mutations of *ATM* in the families we studied but could not conclude that they were functionally inactive because they had not been demonstrated to occur in the germline of patients with ataxia-telangiectasia. Future functional studies of these additional mutations may reveal an even greater role for *ATM* in the susceptibility of pancreatic cancer.

The identification of deleterious *ATM* mutations in probands has substantial implications for risk assessment and surveillance in other family members. Moreover, because *ATM* is a key participant in DNA repair, it is possible that new therapeutics based on synthetic lethal interactions can be developed to treat these patients with pancreatic ductal adenocarcinoma, as has been accomplished for patients with *BRCA* gene mutations (23, 24).

## METHODS

### Study Participants

This study was reviewed and approved by the Institutional Review Board of the Johns Hopkins Medical Institutions, and informed consent was obtained from all study participants. Study participants were enrolled into one of the familial pancreatic cancer registries participating in the Pancreatic Cancer Genetic Epidemiology Consortium (National Familial Pancreas Tumor Registry at Johns Hopkins, Mayo Clinic, Dana-Farber Cancer Institute, MD Anderson Cancer Center, Ontario Pancreas Cancer Study, and Karmanos Cancer Center). The Pancreatic Cancer Genetic Epidemiology Consortium and National Familial Pancreas Tumor Registry have been previously described (25, 26).

### Preparation of Genomic DNA

Genomic DNA from familial pancreatic cancer cases was extracted from peripheral blood lymphocytes (PBL) or Epstein-Barr virus transformed PBLs via use of the QIAamp DNA mini kit (catalog number 51304; QIAGEN, Valencia, CA) according to the manufacturer's protocol. Similarly, genomic DNA from control individuals was extracted from PBLs with the QIAamp DNA mini kit (catalog number 51304; QIAGEN).

### Preparation of Genomic DNA Libraries and Whole-Genome Sequencing

A total of 5 to 10 µg of genomic DNA per sample were sequenced with the Illumina GAIIX Genome Analyzer via use of the Illumina Whole (Illumina, San Diego, CA) Genome Fast-Track Sequencing Service to yield 200 (2 × 100) base pairs from the final library fragments. Sequencing reads were analyzed and aligned to human genome hg18 with the Eland algorithm in CASAVA 1.7 software (Illumina). The Database of Single Nucleotide Polymorphisms (National Center for Biotechnology Information) was used in the analysis of whole-genome sequencing data (27).

### Preparation of Genomic DNA Libraries and Whole-Exome Sequencing

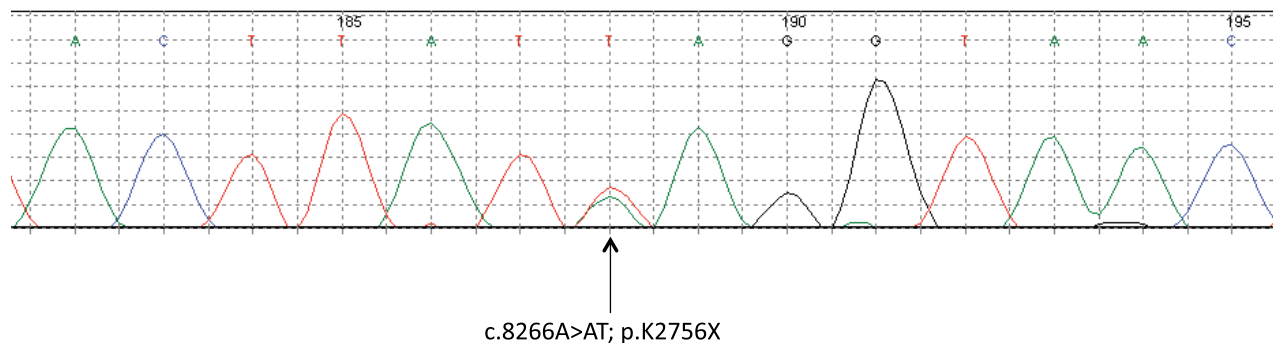
Genomic DNA libraries were prepared with the use of 1.5 to 3 µg of genomic DNA and human exome capture was performed following a modified protocol from Agilent's SureSelect Paired-End Version 2.0 Human Exome Kit (Agilent, Santa Clara, CA) as previously described (28).

Captured DNA libraries were sequenced with the Illumina GAIIX Genome Analyzer, yielding 150 (2 × 75) base pairs from the final library fragments. Sequencing reads were analyzed and aligned to human genome hg18 with the Eland algorithm in CASAVA 1.7 software (Illumina). The Database of Single Nucleotide Polymorphisms was used in the analysis of whole-exome sequencing data (27).

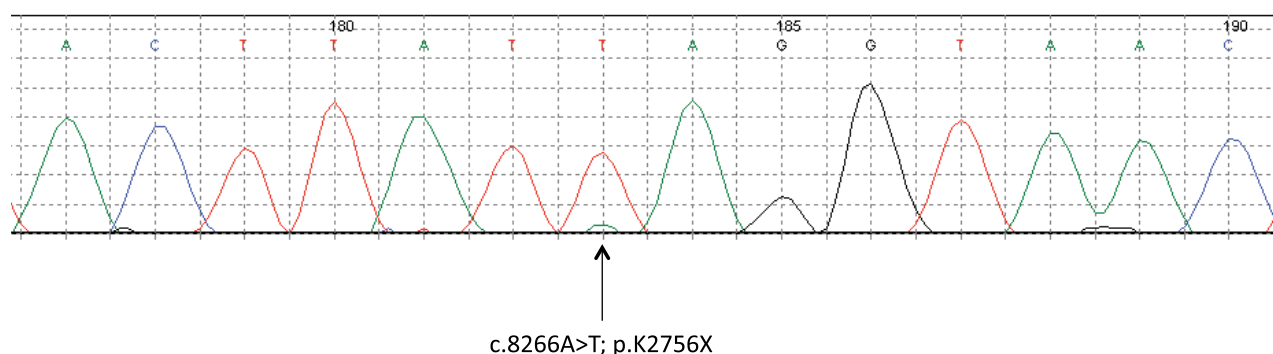
### Evaluation of *ATM* in Additional Pancreatic Cancers, Controls, and Tumor Samples

The coding region of the *ATM* gene was sequenced in 166 familial pancreatic cancer patients and 190 spouses who served as controls.

## A Peripheral blood lymphocytes



## B Tumor



**Figure 2.** Chromatograms from IV-1 of family FPC-A. **A**, PBLs of IV-1 demonstrating heterozygosity for the *ATM* inactivating mutant c.8266A>T; p.K2756X. **B**, laser capture microdissected pancreatic tumor of IV-1 showing LOH with retention of the *ATM* inactivating mutant c.8266A>T; p.K2756X.

PCR amplification and Sanger sequencing were performed following protocols described previously (29) with the primers listed in Supplementary Table S2.

### Laser Capture Microdissection and DNA Extraction of Pancreatic Tumor Samples

To identify somatic mutations and copy number changes in patients with germline *ATM* mutations, primary pancreatic cancer cells ( $\sim 1 \times 10^5$ ) were meticulously microdissected from frozen sections with the PALM micro-laser system (Carl Zeiss Microimaging Inc.; North America, Thornwood, NY) as previously described (29). Genomic DNA was then extracted with the QIAamp DNA Micro Kit (QIAGEN).

### Statistics

Two-tailed *P*-values were calculated using the Fisher exact test. A *P*-value < 0.05 was considered significant.

### Disclosure of Potential Conflicts of Interest

Under a licensing agreement between Myriad Genetics, Inc., and the Johns Hopkins University, K.W. Kinzler, V.E. Velculescu, B. Vogelstein, A.P. Klein, J.R. Eshleman, R.H. Hruban, and M. Goggins are entitled to a share of royalty received by the University on sales of products related to *PALB2*. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict-of-interest policies. Under agreements between the Johns Hopkins University, Inostics, PGDx, and QIAGEN, N. Papadopolous, B. Vogelstein,

K.W. Kinzler, and V.E. Velculescu are entitled to a share of the royalties received by the University on sales of products related to genes and technologies described in this manuscript. N. Papadopolous, B. Vogelstein, K.W. Kinzler, and V.E. Velculescu are co-founders of Inostics and Personal Genome Diagnostics and are members of their Scientific Advisory Boards. They own stock in Inostics and PGDx, which is subject to certain restrictions under Johns Hopkins University policy. The terms of these arrangements are managed by the Johns Hopkins University in accordance with its conflict-of-interest policies.

### Authors' Contributions

N.J. Roberts, V.E. Velculescu, M. Goggins, B. Vogelstein, N. Papadopolous, L. Kopelovich, R.H. Hruban, K.W. Kinzler, and A.P. Klein designed the study. J. Yu, G.M. Peterson, M.L. Bondy, S. Gallinger, A.G. Schwartz, S. Syngal, M.L. Cote, J. Axilbund, R. Schulick, S.Z. Ali, J.R. Eshleman, M. Goggins, B. Vogelstein, R.H. Hruban, and A.P. Klein collected and analyzed the familial pancreatic cancer samples. N.J. Roberts, Y. Jiao, N. Papadopolous, and K.W. Kinzler performed genomic sequencing. N.J. Roberts, M. Goggins, B. Vogelstein, N. Papadopolous, R.H. Hruban, K.W. Kinzler, and A.P. Klein analyzed the genetic data. N.J. Roberts, B. Vogelstein, K.W. Kinzler, and A.P. Klein wrote draft manuscripts. All authors contributed to the final version of the paper.

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## REFERENCES

- Howlander N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, et al. SEER Cancer Statistics Review, 1975–2008. National Cancer Institute, 2011. Available from: [http://seer.cancer.gov/csr/1975\\_2008/](http://seer.cancer.gov/csr/1975_2008/).
- Shi C, Hruban RH, Klein AP. Familial pancreatic cancer. *Arch Pathol Lab Med* 2009;133:365–74.
- Jones S, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 2009;324:217.
- Li A, Swift M. Mutations at the ataxia-telangiectasia locus and clinical phenotypes of A-T patients. *Am J Med Genet* 2000;92:170–7.
- Telatar M, Wang Z, Udar N, Liang T, Bernatowska-Matuszkiewicz E, Lavin M, et al. Ataxia-telangiectasia: mutations in ATM cDNA detected by protein-truncation screening. *Am J Hum Genet* 1996;59:40–4.
- Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, et al. The Human Gene Mutation Database: 2008 update. *Genome Med* 2009;1:13.
- Gilad S, Khosravi R, Harnik R, Ziv Y, Shkedy D, Galanty Y, et al. Identification of ATM mutations using extended RT-PCR and restriction endonuclease fingerprinting, and elucidation of the repertoire of A-T mutations in Israel. *Hum Mutat* 1998;11:69–75.
- McConville CM, Stankovic T, Byrd PJ, McGuire GM, Yao QY, Lennox GG, et al. Mutations associated with variant phenotypes in ataxia-telangiectasia. *Am J Hum Genet* 1996;59:320–30.
- Telatar M, Teraoka S, Wang Z, Chun HH, Liang T, Castellvi-Bel S, et al. Ataxia-telangiectasia: identification and detection of founder-effect mutations in the ATM gene in ethnic populations. *Am J Hum Genet* 1998;62:86–97.
- Teraoka SN, Telatar M, Becker-Catania S, Liang T, Onengut S, Tolun A, et al. Splicing defects in the ataxia-telangiectasia gene, ATM: underlying mutations and consequences. *Am J Hum Genet* 1999;64:1617–31.
- Iacobuzio-Donahue CA, van der Heijden MS, Baumgartner MR, Troup WJ, Romm JM, Doheny K, et al. Large-scale allelotyping of pancreaticobiliary carcinoma provides quantitative estimates of genome-wide allelic loss. *Cancer Res* 2004;64:871–5.
- Berger AH, Knudson AG, Pandolfi PP. A continuum model for tumour suppression. *Nature* 2011;476:163–9.
- Lavin MF. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nat Rev Mol Cell Biol* 2008;9:759–69.
- Taylor AM, Byrd PJ. Molecular pathology of ataxia telangiectasia. *J Clin Pathol* 2005;58:1009–15.
- Swift M, Sholman L, Perry M, Chase C. Malignant neoplasms in the families of patients with ataxia-telangiectasia. *Cancer Res* 1976;36:209–15.
- Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. *N Engl J Med* 1987;316:1289–94.
- Geoffroy-Perez B, Janin N, Ossian K, Lauge A, Croquette MF, Griscelli C, et al. Cancer risk in heterozygotes for ataxia-telangiectasia. *Int J Cancer* 2001;93:288–93.
- Athma P, Rappaport R, Swift M. Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. *Cancer Genet Cytogenet* 1996;92:130–4.
- FitzGerald MG, Bean JM, Hegde SR, Unsal H, MacDonald DJ, Harkin DP, et al. Heterozygous ATM mutations do not contribute to early onset of breast cancer. *Nat Genet* 1997;15:307–10.
- Swift M, Su Y. Link between breast cancer and ATM gene is strong. *BMJ* 1999;318:400.
- Prokopcova J, Kleibl Z, Banwell CM, Pohlreich P. The role of ATM in breast cancer development. *Breast Cancer Res Treat* 2007;104:121–8.
- Corbo V, Beghelli S, Bersani S, Antonello D, Talamini G, Brunelli M, et al. Pancreatic endocrine tumours: mutational and immunohistochemical survey of protein kinases reveals alterations in targetable kinases in cancer cell lines and rare primaries. *Ann Oncol*. 2011 Mar 29. [Epub ahead of print]
- Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005;434:917–21.
- Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123–34.
- Petersen GM, de Andrade M, Goggins M, Hruban RH, Bondy M, Korczak JF, et al. Pancreatic cancer genetic epidemiology consortium. *Cancer Epidemiol Biomarkers Prev* 2006;15:704–10.
- Brune KA, Lau B, Palmisano E, Canto M, Goggins MG, Hruban RH, et al. Importance of age of onset in pancreatic cancer kindreds. *J Natl Cancer Inst* 2010;102:119–26.
- Database of Single Nucleotide Polymorphisms (dbSNP) Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. Available from: <http://www.ncbi.nlm.nih.gov/SNP/>.
- Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, et al. DAXX/ATRAX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* 2011;331:1199–203.
- Sjblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006;314:268–74.