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Mutations of *HNRNPA0* and *WIF1* predispose members of a large family to multiple cancers

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Abstract

We studied a large family that presented a strong familial susceptibility to multiple early onset cancers including prostate, breast, colon, and several other uncommon cancers. Through targeted gene, linkage, and whole genome sequencing analyses, we show that the presence of a variant in the regulatory region of *HNRNPA0* associated with elevated cancer incidence in this family (Hazard ratio = 7.20, $p = 0.0004$). Whole genome sequencing identified a second rare protein changing mutation of *WIFI* that interacted with the *HNRNPA0* variant resulting in extremely high risk for cancer in carriers of mutations in both genes ($p = 1.98 \times 10^{-13}$). Analysis of downstream targets of the mutations in these two genes showed that the *HNRNPA0* mutation affected expression patterns in the PI3 kinase and ERK/MAPK signaling pathways, while the *WIFI* variant influenced expression of genes that play a role in NAD biosynthesis. This is a first report of variation in *HNRNPA0* influencing common cancers or of a striking interaction between rare variants coexisting in an extended pedigree and jointly affecting cancer risk.

Keywords

Whole genome sequencing; Expression analysis; Linkage analysis; Complex disease; Colon cancer; Prostate cancer

Introduction

While extensive family studies have shown that almost all cancers are influenced by genetic factors, rather little of the overall genetic cancer burden has to date been assigned to specific genetic factors. It has been hypothesized that much of the missing heritability explaining common diseases like cancer results from an inadequate assessment of interactions that may jointly substantially affect risk. In this study, we studied a large family presenting initially through a proband with very early onset breast cancer. Subsequently, a second relative in the family developed an extremely early onset colorectal cancer. Further contact with the family showed that a large number of relatives had developed cancers, with many common cancers occurring at an early age. Analysis of mutations in all known cancer predisposing loci failed to yield any relevant findings. We therefore undertook a comprehensive analysis of the family, including linkage analysis using dense single nucleotide polymorphism analysis, whole genome sequencing of key family members and expression analysis to characterize findings associated with specific mutations that were identified. Here we report two novel mutations in heterogeneous nuclear ribonucleoprotein A0 (*HNRNPA0*) and WNT Inhibitory Factor 1 (*WIFI*) genes in this family, which are associated with the elevated incidence of cancers in this family.

Materials and methods

Patients

Families were accrued through collaboration among the University of Texas MD Anderson Cancer Center, the Baylor University Medical Center and the University of Texas Southwestern Medical Center. Participants included family members with cancer; first-degree relatives without cancer and more distant relatives used to reconstruct any deceased or missing family members as well as spouses of family members. All participants were enrolled on Institutional Review Board-approved protocols at all institutions and were asked to provide blood for genetic analysis and expression profiling after signing informed consent forms. Participants also completed a health and diet questionnaire and medical history information was requested from all individuals.

DNA extraction

Blood from each participant was collected with a Vacutainer tube containing ethylenediamine tetraacetic acid (EDTA) (Becton Dickinson Vacutainer System, Rutherford, NJ, USA) and DNA was isolated with Qiagen Kits (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions.

Expression analyses

Total RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA). Affymetrix GeneChips were used for expression analysis. Target labeling was performed according to the manufacturer's standard protocol (Affymetrix Inc., Santa Clara, CA, USA). Biotinylated cRNA targets were purified and subsequently hybridized to Affymetrix HG-U133A and U133B GeneChips (>44,000 probe sets). Arrays were scanned using an Affymetrix confocal laser scanner. Microarray Suite, Version 5.0 (MAS 5.0; Affymetrix) software was used to assess fluorescent hybridization signals, to normalize signals, and to evaluate signal detection calls. Normalization of signal values per chip was achieved using the MAS 5.0 global method of scaling to the target intensity value of 500 per GeneChip.

Sanger sequencing and mutation analysis

Sanger sequencing method was used to analyze mutations in known cancer predisposing loci (targeted gene) and novel mutations we identified through linkage analysis and whole genome sequencing. Mutation analysis of known cancer predisposing genes, *PTEN*, *BRCA1*, *BRCA2*, *CHEK2*, *p53*, *APC*, *hMLH1*, *hMSH2*, *hMSH6*, *TGF β R1*, *MYH*, *ARLTS*, *DNMT3B*, *DNMT3A*, *DNMT1* and *CCND1* were performed. In the linkage analysis phase, the whole candidate gene *HNRNPA0* was sequenced for DNA samples from six cancer carriers and six healthy marry-ins and then the identified novel mutations in this gene were further sequenced for the rest of the family members. For the novel mutation identified *WIFI* genes through whole genome sequencing analysis, we performed the mutation analysis on all available family members.

Linkage analysis

57 DNA samples including 14 from family members with cancer, 30 from first-degree relatives without cancer and 13 from non-first degree relatives were subjected to linkage analysis. Genotyping was performed on the Illumina Hu-manHap300v2_A chip for 317,498 single nucleotide polymorphic (SNP) markers. We used Merlin [1] and SIMWALK2 [9] programs to perform linkage analysis on this family. Whereas Merlin can effectively deal with markers that are in strong linkage disequilibrium, we had to divide the pedigree into four parts to overcome its limitation on pedigree sizes. We also duplicated a few individuals to assure that we can trace common haplotypes among affected people. We used SIMWALK2 to analyze specific regions of the strongest signals we observed by Merlin program to better delineate the regions of linkage. With SIMWALK2 we analyzed data from the entire family jointly, but we trimmed the markers at a 0.5 cM density to reduce false positive results caused by linkage disequilibrium among the markers. We used a model with a 0.5 % penetrance in non-carriers and 5 % penetrance in carriers. This parametric analysis gives most weight to the affected individuals and becomes essentially model free (since the unaffected do not contribute much to the analysis, and the affected contribute equally to evidence for linkage). Results of multi-point analysis are presented in terms of the LOD score.

Whole genome sequencing (WGS)

Eight DNA samples including four from cancer affected, one unaffected blood relative, and three healthy marry-ins were subject to whole genome sequencing. Samples were sequenced using the Illumina HiSeq2000 platform with an average depth of coverage of ~47. The sequences were aligned and the variants were called using the Illumina CASAVA pipelines and re-called using the GATK best practice [2]. Variants called from both pipelines are analyzed but candidate variants that are called from only one of the pipelines were examined against aligned reads from which variants are called. We excluded variants with phred-scaled quality score of 20 or less. We also excluded variants from the 1,000 genomes projects to focus on novel variants that exist only in cancer patients in this family. The variants were annotated and analyzed using Variant Tools [8].

Results

Description of the family

The family was initially ascertained because of very early onset breast cancer occurring in the proband at age 26 (person 001 in Fig. 1). Subsequently colon cancer arose as 3 × 3 cm lobulated rectal adenocarcinoma in an 11-year old girl (individual 144). Subsequent colonoscopies and endoscopies identified 22 0.2–0.3 cm sessile polyps throughout the colon, with no evidence of other lesions in the ileum, duodenum, stomach, or esophagus. Upon pathological review, these polyps were all found to have similar morphology being sessile with only minimal loss of crypt orientation. A few crypts were distended by mucin. This child was treated aggressively with local radiation and continuous infusion of 250 mg/m² per day 5-fluorouracil, a total colectomy with endorectal pull-through and six 5-day courses at 4- to 5-week intervals of 20 mg/m² intravenous leucovorin and 425 mg/m² 5-fluorouracil. The patient was without disease for a little over 2 years following treatment but eventually

developed metastases and died at age 16. The pedigree structure, shown in Fig. 1 shows the presence of five prostate cancers (including one occurring at age 47), a melanoma at age 25, and several other cancers, further presented in Table 1.

Targeted mutation analysis

Germline DNA from individual 001 who had early onset breast cancer was submitted for analysis of *PTEN*, *BRCA1*, *BRCA2*, *CHEK2* and *p53* analysis, a germline sample from individual 003 was submitted for analysis of *APC*, *hMLH1*, *hMSH2*, *hMSH6*, *TGF β R1*, and *MYH* and a germline DNA sample from individual 152 was submitted for p16 analysis. No causal mutations were identified in any of these genes. Two sequence variants of Trp149Stop and Cys148Arg in *ARLTS* were noted in individual 003. Within the *ARLTS* gene family members more often carried the Trp149Stop polymorphism than spouses ($\chi^2_1=5.14$, $p=0.02$), but these variants were not consistently transmitted among family members. Targeted sequencing of *DNMT3B*, *DNMT3A*, *DNMT1* and *CCND1* in multiple family members failed to show an association of any polymorphisms in these genes with familial cancer risk.

Linkage analysis

We performed linkage analysis to identify genetic causes for disease by tracing the coinheritance of genetic markers with disease in families. Results from Merlin showed strongest signals on chromosome 2 (Fig. 2a) and 5 (Fig. 2b). We then used SIMWALK2 for a parametric linkage analysis by using the entire pedigree structure to narrow down the regions of linkage. This analysis supported evidence for linkage to chromosome 5 but denies much evidence for linkage on chromosome 2 giving a maximum location score of 0.562 when 20 markers around the most significant region were studied. Merlin analysis using a parametric model also showed strong evidence for a locus on chromosome 5q (Fig. 2c, d). This locus was inherited much more often than expected by chance with cancer in this family and so suggested that genes in this region influence risk for cancer in this family. We used SIMWALK2 to identify a haplotype that was commonly shared among affected individuals.

Meanwhile, we integrated linkage analysis results with the result from Illumina expression microarray of several family members, in which *MOCS2* ($p=0.0026$) and *HNRNPA0* ($p=0.0027$) were found to have the most significant changes in expression levels comparing individuals who have a shared haplotype associated with cancer risk compared with individuals not carrying a risk haplotype, therefore suggesting *MOCS2* or *HNRNPA0* as in the region of linkage between inherited susceptibility to cancers and genetic markers on chromosome 5. No variants were identified in *MOCS2* that associated with cancer risk in this family and this gene does not appear to have relevance to cancer susceptibility. The *HNRNPA0* gene is involved in RNA processing and localizes to the nucleus. Although its role in cancer development is unknown, it belongs to a broader class of proteins governing cell growth. We, therefore, sequenced the entire *HNRNPA0* gene from six cancer carriers and six healthy marry-in individuals using Sanger sequencing. This sequencing approach identified three novel mutations in this gene [M1: C deletion at position 137091457, M2: G to C mutation at position 137089865, and M3: G to A mutation at position 137090925]. M1

and M2 locate at the 3' and 5' UTR of the *HNRNPA0* gene respectively. Both M1 and M2 locate within the regulatory element of the gene. Predictions from a transcriptional factor search tool (<http://www.cbrc.jp/research/db/TFSEARCH.html>) suggested that M1 and M2 have an effect on transcriptional factor binding sites, and therefore influence its gene expression.

Next, we sequenced those three mutations on all available family members (including cancer carrier, blood relative and marry-ins) and found that the M1 and M2 mutations were only observed in cancer carriers and blood relative but not in marry-ins. M3 mutations existed in some marrying-in individuals. To further test whether these mutations are family specific, rather than common mutations in cancer patients, we randomly selected 31 DNA samples from patients with Lynch Syndrome to serve as convenient controls for this gene. None of these three mutations were observed for Lynch syndrome patients who are predisposed to development of cancer (data not shown).

Interestingly, we observed one participant (cancer carrier of family member; ID 001) carries a homozygous mutation of M1, which is a deletion at position 137091457, while her mother (sample ID 003) is a heterozygous mutation carrier. We did not have a sample from her father and could not deduce his genotype from the other relatives in the family. To evaluate whether this second hit at this position is a single point mutation or a large fragment deletion, we searched the published SNPs of this gene in NCBI SNPs database. There are a total of 8 SNPs and all of them locate at the 3' UTR of this gene. We reviewed the sequencing data on the whole gene, but no SNP variations were observed in this particular participant. Failing to observe any variation could reflect either chance homozygosity for all the SNPs or the presence of a deletion on one strand so that the remaining genotyping all appears to result in homozygous genotype results.

Whole genome sequencing analysis—To confirm observed variants on chromosome 5 and uncover other cancer-associated factors which might have been overlooked previously, we sequenced complete genomes of 8 samples, including four cancer carriers, one blood relative, and three marry-ins as controls. The cancer carriers include a patient (sample ID 001) with early onset breast cancer (age 26), her mother (sample ID 003) with breast (age 50) and colon cancer (age 65), an affected relative (sample ID 107) with early onset prostate cancer (age 47) and another affected relative (sample ID 072) with prostate cancer (age 65) who had several offspring affected with cancers. Since we did not have DNA for the girl who died of CRC at age of 16 (sample ID 144), we included her mother (sample ID 134, blood relative), her father (marry-in, sample ID 140), and her grandmother (marry-in, sample ID 132) for whole genome sequencing. In addition, we sequenced another marry-in (sample ID 113). The next generation sequencing data confirmed the M1–M3 mutations observed in *HNRNPA0* by Sanger sequencing analysis. The result ruled out the possibility of a large deletion on one strand of *HNRNPA0* in the participant who showed homozygous mutation of M1 and also filtered out the M3 because it was present in some marrying-in individuals.

In summary, including variants called from both the Illumina CASAVA and GATK pipelines but excluding genotypes with quality score lower than 20, we observed a total of

10,669,728 genetic variants (8,123,823 SNPs and 1,970,559 INDELs) from the eight individuals sequenced, with on average 4.77 and 4.47 million variants called by GATK and CASAVA pipelines, respectively. Of these, 68,558 variants exist in all cancer cases and blood relative but are not in the controls. In order to identify novel high-penetrant mutations in this family, we excluded 65,337 variants that were present in the 1,000 genomes project and 53,435 variants from seven controls sequenced for other projects. 2,235 variants remained, among which 28 locate in exon regions of ref seq genes.

We carefully examined these 28 variants against the aligned reads (Table 2). Among those variants, 11 are in mononucleotide or short-tandem repeat sequences and were not consistently called by two pipelines, 4 are in segmental duplication regions with one variant exhibiting extremely high depth of coverage, 2 variants are synonymous and are unlikely to be functional, 1 is observed in the Exome Sequencing project, 5 of them are not in the conserved regions of the human genome, and 2 variants have function estimates but are considered benign by SIFT, PolyPhen2, MutationAssessor, and eXtasy. After removing all these variants, only three variants (*HNRNPA0*, *WIF1* and *NPLOC4*) left on the list. Although Nuclear protein localization protein 4 homolog (*NPLOC4*, *NPL4*) is recognized as a critical component of the endoplasmic reticulum-associated degradation (ERAD) pathway (1, 2) and is suspected to be cancer related.

Since the variant in *NPLOC4* locates at the 3' UTR, we used microRNA prediction tool (available at <http://www.targetscan.org>) to analyze whether this variant falls into a miRNA target site, which might influence expression of the gene. However the prediction shows that this variant doesn't fall into any miRNA target site suggesting that it does not impact microRNA binding. Therefore, we excluded *NPLOC4* from further analysis.

The variant on *WIF1* is reported to be potentially damaging. *WIF1* is a negative regulator of WNT and hypermethylation of *WIF1* is commonly observed in many cancers including breast, prostate, bladder, and aerodigestive cancers. This Cys294Phe mutation is predicted to have a strong impact on the structure of the protein but that this position is not evolutionarily conserved. Therefore, we carried out the Sanger sequencing to test Cys294Phe mutation in *WIF1* on every family members. Again these two mutations were only observed in either cancer-carrier or blood-relatives but not in the marry-ins.

The association of the mutation in *HNRNPA0* and *WIF1* and cancer risk

Next, we performed survival analysis evaluate the association between the mutations we identified in *HNRNPA0* M2 and *WIF1* with the cancer risk in this family, either individually or jointly. Both the M2 mutation in *HNRNPA0* and mutation in *WIF1* are significant in both single-variant log-rank test (p values 0.0001 and 0.0113, respectively) and cox regression test (p values 0.0004 and 0.0062). When analyzing these two variants jointly, the M2 mutation becomes less significant (with p value 0.0874), but effect of *WIF1* and the interaction between M2 and *WIF1* are significant (<0.0001) (Table 3). The Kaplan–Meier survival curves (Fig. 3) show significant differences between family members with and without these mutations individually and jointly, with p values 0.0001 (Fig. 3a), and 0.0066 (Fig. 3b), and 0.0012 (Fig. 3c). All these analyses suggest that the presence of these two

novel mutations in *HNRNPA0* and *WIF1* are strongly associated with the elevated cancer risk in this family.

The association of *HNRNPA0* and *WIF1* mutations with gene expression

Next we analyzed the expression data categorized by sample genotype of *HNRNPA0* mutation 2 and *WIF1* identified by Sanger sequencing. There were 1,640 genes whose expression levels varied significantly ($p < 0.05$) according to *WIF1* genotypes and 4,303 genes with significant variation by genotype for *HNRNPA0* ($p < 0.05$) (Supplemental Table 1). To characterize the effects that these variants have on pathways that may influence cancer risk, we used Ingenuity pathway analysis to organize the gene relationships. As showed in Table 4, the top canonical pathways for *HNRNPA0* variant were PI3K/AKT signaling ($p = 6.19 \times 10^{-12}$), ERK/MAPK signaling ($p = 9.0 \times 10^{-11}$), NRF2 mediated oxidative stress response ($p = 6.11 \times 10^{-10}$), B cell receptor signaling ($p = 1.71 \times 10^{-9}$) and ceramide signaling ($p = 3.0 \times 10^{-9}$). As showed in Table 5, the top canonical pathways for *WIF1* variant were much less significant, including NAD biosynthesis III ($p = 3.68 \times 10^{-3}$), hepatic fibrosis/hepatic stellate cell activation ($p = 1.78 \times 10^{-2}$), Proline degradation ($p = 2.07 \times 10^{-2}$), nucleotide excision repair pathway ($p = 2.80 \times 10^{-2}$) and retinoic biosynthesis I ($p = 3.07 \times 10^{-2}$). Despite the name, the *WIF1* variant did not influence any of the WNT expression levels that are thought to be regulated by WIF1, suggesting that the particular variant of *WIF1* in this family did not affect the WIF1 inhibitory function of WNT. The WIF1 protein contains an N-terminal WIF domain and five EGF-like domains. We used NCBI domain search tool and found out that Cys294Phe locates at one of the EGF-like domain other than N-terminal WIF domain. This predication supports our observation of gene expression analysis and pathway analysis.

Discussion

The present work links the two novel mutations in the *HNRNPA0* and *WIF1* genes to the excess cancers in this family. Gene expression study of blood samples showed that the *HNRNPA0* and *WIF1* mutations influence specific cellular signaling pathways related to cancer growth. In addition, we observed a striking interaction between rare variants in *HNRNPA0* and *WIF1* jointly affect cancer risk in an extended pedigree.

HNRNPA0 is a member of the hnRNP A/B family of related RNA binding proteins that bind pre-mRNA and are involved in the processing, metabolism, and transport of nuclear pre-mRNA transcripts [7]. *HNRNPA0* is phosphorylated at Ser84 by MAPKAPK-2 in response to LPS treatment in mouse macrophage cells, which might play a key role in stimulating translation of the TNF-pre-mRNA tr [7]. The novel mutation M2 we identified locates at the 5' UTR of the gene and is predicted to have an effect on transcriptional factor binding sites and therefore influence its expression. On the other hand, *WIF1* is a secreted protein that binds to Wnt proteins and inhibits their activity [3]. It contains an N-terminal WIF domain and five EGF-like repeats [4]. It has been reported that *WIF1* expression is downregulated in many types of cancers [5, 6, 10, 11].

The region on chromosome 5 with a significant linkage signal is relatively wide and encompasses over 120 genes. Besides three mutations in *HNRNPA0*, we also identified a

variant in *SQSTM1* on chromosome 5 but outside the linkage region, which demonstrates a significant association with cancer risk in this family (p value 0.0005), but further sequencing analyses showed that this variant was present in some controls individuals and it was therefore removed from further analyses. Whole genome sequencing also identified a rare mutation in *WIF1* that is associated with cancer risk in this family, though not as strongly as the *HNRNPA0* variant. Joint analysis of *WIF1* and *HNRNPA0* showed a highly significant interaction among carriers of both mutations, but the comparison of the log likelihoods for models with or without the interaction showed only a very modest improvement in the fit of the model with *WIF1* and the interaction ($\chi^2_2 = 1.04, p = 0.57$), suggesting that the *HNRNPA0* variant may be sufficient to explain increased risk for cancer in this family. Further analysis to explore the impact that the *HNRNPA0* versus *WIF1* variants have on expression of genes showed that the *HNRNPA0* variant influenced well known pathways to cancer development such as PI3K/AKT and MAPK signaling, while the relevance of the *WIF1* variant to cancer pathways was much less clear. Despite the much stronger prior biological evidence that *WIF1* variation could explain risk for cancer in this family and annotation that showed an effect of the *WIF1* variant on gene function, results for *HNRNPA0* were much more compelling than for *WIF1*. Results therefore implicate *HNRNPA0* as playing the dominant role in cancer risk for this family. Further studies to explore the effects of *WIF1* or *HNRNPA0* on cancer risk will be of value in this family. Given striking interactions that we observed for risk among individuals carrying both variants, further study of this combination could present an opportunity for predictive assessment of risk, but the results are not yet sufficient for clinical action because they have not been validated in a CLIA laboratory and have only been detected in this single family. During the course of the study of this family, two additional cancers arose, both of which carried the rare variants of *HNRNPA0* and *WIF1* further strengthening the case for these variations influencing risk. Variations in *HNRNPA0* might also further be studied for relevance to early onset cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Pedigree of the high-risk family

Fig. 2. Linkage analysis of the family. **a, b** LOD and Zmean scores on chromosomes 2 (**a**) and chromosomes 5 (**b**) using a non-parametric model estimated by Merlin. **c** Parametric linkage analysis using **SIMWALK2**. **d** Parametric analysis using Merlin

Fig. 3. Kaplan–Meier survival of family members with and without *HNRNPA0* and *WIF1* mutations. **a** With *HNRNPA0* mutations only, **b** with *WIF1* mutations only, **c** with both *HNRNPA0* and *WIF1* mutations

Table 1

Characteristics of affected family member and mutations

Case ID	Sex	Age	Age Dx	Cancer type	<i>HNRNP40</i> (M2 mutation)	<i>WIFI</i> (Cys294Phe)
001	F	71	26 31	Breast adenocarcinoma Breast adenocarcinoma	G/G->G/C	G/G->G/T
003	F	91	50 65	Colon adenocarcinoma Breast adenocarcinoma	G/G->G/C	G/G->G/T
004	M	68	68	Tongue squamous cell carcinoma	G/G->G/C	G/G->G/T
032	F	90	75	Breast adenocarcinoma	WT	WT
052	M	37	21	Cervix intraepithelial severe dysplasia	WT	WT
056	M	58	54 54 54	Skin squamous cell carcinoma Liver metastatic adenocarcinoma Bladder adenocarcinoma	WT	WT
072	M	74	65	Prostate adenocarcinoma	G/G->G/C	G/G->G/T
079	M	84	67	Prostate adenocarcinoma	G/G->G/C	G/G->G/T
080	M	87	76 77	Prostate adenocarcinoma Bone marrow malignant Lymphoma	G/G->G/C	G/G->G/T
104	M	60	60	Bone marrow thymic large B-cell Lymphoma	G/G->G/C	G/G->G/T
107	M	55	47	Prostate adenocarcinoma	G/G->G/C	G/G->G/T
144	F	16	11	Colon polyp, adenocarcinoma	Unknown	Unknown
152	M	34	25 25	Melanoma Skin basal cell carcinoma	G/G->G/C	WT
64	M	63	53	Colon polyp, adenoma	G/G->G/C	WT
191	M	61	52	Thyroid papillary adenocarcinoma	WT	WT
194	F	68	56	Breast adenocarcinoma	G/G->G/C	G/G->G/T

Sex: F female, M male; Age current age, Age Dx Age at diagnosis

Table 2

Variants that appear only in cases and blood relatives, in exon regions of ref seq genes, and are not in 1,000 genomes project

Chr	Pos	Ref	Alt	Gene	Mean GQ	Mutation type	Mutation impact	Mutation class
1	118420020	-	T	GDAP2	91.5	-	-	-
1	152191884	G	A	HRNR	101.3	-	-	-
1	159023386	G	A	IFI16	111.6	NON_SYNONYMOUS_CODING	MODERATE	MISSENSE
1	160398161	G	A	VANGL2	113.0	UTR_3_PRIME	MODIFIER	-
1	174980243	AAAAAAAA	-	CACYBP	88.2	-	-	-
1	180772617	C	T	XPR1	112.1	NON_SYNONYMOUS_CODING	MODERATE	MISSENSE
11	70281359	G	T	CTTN	99.0	-	-	-
12	65449852	C	A	WIFI	116.1	NON_SYNONYMOUS_CODING	MODERATE	MISSENSE
16	11929051	T	C	RSL1D1	107.6	-	-	-
16	11966239	-	A	GSPT1	96.6	-	-	-
16	17197814	G	A	XYLT1	121.1	UTR_3_PRIME	MODIFIER	-
16	24267639	-	CA	CACNG3	190.0	-	-	-
16	33961995	C	T	LINC00273	100.6	-	-	-
17	78938525	G	A	RPTOR	99.3	UTR_3_PRIME	MODIFIER	-
17	79525590	C	G	NPLOC4	115.6	UTR_3_PRIME	MODIFIER	-
17	79687655	C	T	SLC25A10	103.0	UTR_3_PRIME	MODIFIER	-
18	109348	G	A	ROCKIP1	95.6	-	-	-
19	4523091	-	T	PLIN5	63.0	-	-	-
19	34843754	CCCCACCCACAGC	-	KIAA0355	189.1	CODON_DELETION	MODERATE	-
19	39399199	G	A	NFKBIB	87.4	-	-	-
22	21637039	C	G	POM121L8P	95.8	-	-	-
3	12581722	T	C	C3orf83	110.5	SYNONYMOUS_CODING	LOW	SILENT
3	12598526	-	CGGCGTGCGC	MKRN2	263.5	-	-	-
4	1945715	A	T	WHSC1	108.3	DOWNSTREAM	MODIFIER	-
4	4865498	AA	-	MSX1	88.6	-	-	-
4	88537204	C	T	DSPP	90.0	SYNONYMOUS_CODING	LOW	SILENT
5	137089865	C	G	HNRNPA0	99.0	UTR_5_PRIME	MODIFIER	-
8	42878531	TCCT	-	HOOK3	109.5	-	-	-

Mutations of *HNRNPA0* and *WIFI* predispose members of a large family to multiple cancers

Univariate analysis of the impact of HNRNPA0 [Mutation 2 (M2)] and WIF1 on the survival of cancer patients, assuming a Cox proportional hazards model

Table 3

Effect	DF	Parameter estimate	Standard error	Chi square	p value	Hazard ratio	-2 log L
<i>Model with HNRNPA0 Mutation 2 (M2) or WIF1 Cys294Phe</i>							
<i>HNRNPA0</i>							
G/C = 1	1	1.97481	0.56227	12.3354	0.0004	7.205	85.737
WT = 0							
<i>WIF1</i>							
G/T = 1	1	1.34752	0.49253	7.4851	0.0062	3.848	92.445
WT = 0							
<i>Model with HNRNPA0 Mutation 2 (M2) and WIF1 Cys294Phe</i>							
<i>HNRNPA0</i>							
G/C = 1	1	1.90097	0.66603	8.1464	0.0043	6.692	85.713
WT = 0							
<i>WIF1</i>							
G/T = 1	1	0.10722	0.56786	0.0356	0.8502	1.113	
WT = 0							
<i>Model with HNRNPA0 Mutation 2 (M2), WIF1 Cys294Phe and their interaction</i>							
<i>HNRNPA0</i>							
G/C = 1	1	1.55145	0.90774	2.9211	0.0874	7.306	84.700
WT = 0							
<i>WIF1</i>							
G/T = 1	1	-14.74948	0.30867	2,283,3232	<10 ⁻³⁰⁰	0.	
WT = 0							
<i>HNRNPA0 × WIF1</i>							
1	1	15.13919	0.91213	275,4833	1.98 × 10 ⁻¹³	0.61 × 10 ⁶	

Table 4

Top canonical pathways of HNRNPA0

Name	<i>p</i> value	Ratio of affected genes to pathway members
PI3K/AKT signaling	6.19×10^{-12}	45/152 (0.296)
ERK/MAPK signaling	9×10^{-11}	56/211 (0.265)
NRF2-mediated oxidative stress response	6.11×10^{-10}	52/195 (0.267)
B cell receptor signaling	1.71×10^{-09}	48/175 (0.274)
Ceramide signaling	3×10^{-09}	31/91 (0.341)

Table 5

Top canonical pathways of WIF1

Name	<i>p</i> value	Ratio of affected genes to pathway members
NAD biosynthesis III	3.68×10^{-03}	3/10 (0.3)
Hepatic fibrosis/hepatic stellate cell Activation	1.78×10^{-02}	10/155 (0.065)
Proline degradation	2.07×10^{-02}	2/7 (0.286)
Nucleotide excision repair pathway	2.8×10^{-02}	4/36 (0.111)
Retinoate biosynthesis	3.07×10^{-02}	4/37 (0.108)