TITLE: Assessing the Spectrum of Germline Variation in Fanconi Anemia Genes among Patients with Head and Neck Carcinoma before Age 50

Running Title: Germline Variants in FA Genes- HN Cancer

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Abbreviations: FA, Fanconi anemia; HNSCC, head and neck squamous cell carcinoma.

Precis: Patients with Fanconi's Anemia (FA) are at increased risk of head and neck squamous cell carcinoma (HNSCC), but the prevalence of undiagnosed FA patients with HNSCC is unknown. Targeted germline sequencing of 417 patients with HNSCC identified several known FA variant mutations and several novel FA mutations; although no patients with HNSCC were found to have clinical signs of FA, FA mutations in HNSCC may explain a potential mechanism for tumorigenesis.

ABSTRACT

Background: Patients with Fanconi anemia (FA) have increased risk for head and neck squamous cell carcinoma (HNSCC). We sought to determine the prevalence of undiagnosed FA and FA carriers in patients with HNSCC and an age cutoff for FA genetic screening.

Methods: Germline DNA from 417 HNSCC patients under age 50 was screened for sequence variants by targeted next-gen sequencing of the entire length of 16 FA genes.

Results: The sequence revealed 194 FA gene variants in 185 patients (44%). The variant spectrum was comprised of 183 nonsynonymous point mutations, nine indels, one large deletion, and one synonymous variant predicted to effect splicing. 108 patients (26%) had at least one rare variant predicted to be damaging, and 57 (14%) had at least one rare variant predicted to be damaging and previously reported. Fifteen patients carried two rare variants, or an X-linked variant, in an FA gene. Overall, we did not identify an age cutoff for FA screening among young HNSCC patients, as there were no significant differences in mutation rates when patients were stratified by age, tumor site, ethnicity, smoking status, or human papillomavirus status. However, we observed an increased burden, or mutation load, of FA gene variants in FANCD2, FANCE, and FANCL in our HNSCC patient cohort relative to the 1000 Genomes population.

Conclusions: FA germline functional variants offer a novel area of study in HNSCC Tumorigenesis. FANCE and FANCL, components of the core complex, are known to be responsible for the recruitment and ubiquitination, respectively, of FANCD2, a critical step in the FA DNA repair pathway. The increased mutation load of FANCD2, FANCE, and FANCL variants in our younger HNSCC patient cohort indicates the importance of the FA pathway in HNSCC.

Keywords: Fanconi Anemia, Recessive inherited disorders, Head and Neck Cancers, Squamous Cell Carcinoma, Germline Variations

INTRODUCTION

Fanconi anemia (FA) is a rare, predominantly recessive inherited disorder with an incidence of 1 in 130,000 births and a carrier rate estimated to be 0.6% (1,2). Genetically, FA is a heterogeneous disease with 21 causative genes known so far, as five new genes were added within the past year (3,4). Phenotypically, FA is associated with congenital defects (short stature, renal defects, café au lait spots, microphthalmia, hearing difficulties and abnormal thumb or radii) and progressive bone marrow failure. In addition to the congenital anomalies, and the inevitable bone marrow failure, FA patients are at increased risk of acute myelogenous leukemia and head and neck squamous cell carcinoma (HNSCC) (5,6). However, 30% of patients fail to display the FA congenital defects (7,8), and up to 25% present with solid or hematological malignancies as the first sign of the condition (9). In many such instances, FA diagnosis is prompted after encountering severe toxicities upon initiating chemotherapy or radiation therapy to treat the malignancy (10). The pathogenesis is based on the fact that the normal function of FA genes is related to DNA repair and genome stability; bi-allelic mutations of these genes confer a 0.7-2% annual risk of cancer (5,9,11), and some, including *FANCD1* (*BRCA2*) and *FANCN* (*PALB2*), are closely associated with solid organ malignancies (12-14)

Head and neck squamous cell carcinoma is traditionally related to tobacco and alcohol consumption and recently associated with human papillomavirus; the risk is a 10 to 15-fold increase compared to those without these exposures (15,16). However, FA confers a 500-700-fold increased risk of HNSCC compared to the normal population (9,17,18). The median age of HNSCC onset in FA patients is 33 years compared to 60 years in traditional HNSCC patients; thus FA patients develop HNSCC at a significantly younger age than sporadic HNSCC (19).

Younger HNSCC patients have reduced DNA repair capacity but the prevalence of FA mutations in HNSCC patients remains unknown (20). Given 30% of patients with FA mutations do not display the congenital stigmata of FA, we sought to determine: 1) the prevalence of undiagnosed FA among HNSCC patients younger than 50 years, 2) an age cutoff for FA

screening among younger HNSCC patients, and 3) the prevalence of FA carriers (heterozygote germline mutations) among younger HNSCC patients.

METHODS

DNA extraction

DNA was isolated from blood using the Puregene kit and DNeasy blood and tissue DNA extraction kit (Qiagen), and subjected to phenol/chloroform extraction and ethanol precipitation.

Samples for sequencing

Six hundred forty-seven HNSCC patients younger than 50 years were enrolled in a prospective molecular epidemiologic study of newly diagnosed HNSCC that included completion of a prospective standardized epidemiologic questionnaire and blood draw. Patients with cancer (including known FA) prior to HNSCC diagnosis were excluded. The Institutional Review Board of the University of Texas MD Anderson Cancer Center approved this study, and all patients gave informed consent.

Of the 617 patients initially enrolled for the study (Supplemental Figure 1), DNA was available from 468 patients. DNA from 417/468 patients was of sufficient quality and quantity required for the targeted capturing and sequencing approach. Thus, DNA from 417 patients was sequenced. There is no difference between the sequenced group (417 patients) and the non-sequenced group (230 patients) in terms of age, gender, ethnicity, smoking, and alcohol drinking (Supplemental Table 1). There was a higher proportion of Larynx/Hypopharynx among those excluded and a higher proportion of Oral cavity in those included.

Targeted Next-Gen Sequencing

The genomic regions representing the entire length of 16 FA genes were targeted for capturing and sequencing (Supplemental Table 2). The exceptions were the exon 1 regions of *FANCA*, *FANCB*, and *FANCE*, which were not covered by the sequencing approach. Excessive repeat sequences prevented the successful design of probes for exon 1 of FANCA. Probes were

designed for the *FANCB* and *FANCE* exon 1 regions but did not yield product. The targeted Truseq (Illumina) capturing design, capture, and sequencing were done as previously described (21).

Variant calling and filtering

Novoalign (http://www.novocraft.com/products/novoalign/) was used for sequence alignment. The MPG ("Most Probably Genotype") genotype caller (https://research.nhgri.nih.gov/software/bam2mpg/; NHGRI Genome Technology Branch) was used to call variants, and SnpEff (22) was used to filter the high quality functional variants based on the following criteria: quality score ≥ 20, read depth ≥10, nonsense, missense, indel, splicing (+/- 2bp). The extracted functional variants were subsequently annotated and filtered using a population-specific maximum frequency, where applicable, of .5% in each of the 1000 Genomes (2,504 individuals), the NHLBI-ESP6500 (6,503 individuals), and the ExAC-nonTCGA (60,706 individuals) variant databases. The allele frequency threshold of .5% is derived from the FA carrier frequency of 1:181(2). All coordinates are in accord with human genome build 19.

Predicted damaging variants

To predict the functional consequence of a variant, we compared the results from five prediction algorithms: Sift (http://sift.jcvi.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2), MutationTaster (http://www.mutationtaster.org/), CADD (http://cadd.gs.washington.edu), and GERP++ (http://mendel.stanford.edu/SidowLab/downloads/gerp/). We used a minimum threshold of 20 for Phred-scaled CADD scores, representing the 1% most damaging variants in the genome, and a minimum threshold of 2 for GERP++ RS scores. GERP++ was included to highlight constrained sites. Annovar (23) was used to annotate the variants with the results from all five prediction algorithms. For consideration as "Damaging", at least 4 of the 5 algorithms had to meet their specified threshold. "Benign" variants did not meet the threshold in at least 4 of the 5 algorithms. Variants lacking consensus between at least 4 algorithms were labeled as "Indeterminate" (Figure 1, Supplemental Table 3)

Previously reported variants

The following databases were used to identify previously reported variants: The Leiden Open Variation Database (LOVD) for Fanconi anemia (http://www.rockefeller.edu/fanconi/) ClinVar NCBI database (http://www.ncbi.nlm.nih.gov/clinvar/), and BIC (Breast Cancer Information Core).

Synonymous variants

Synonymous variants with quality score ≥ 20 and a read depth ≥ 10 were analyzed using SILVA-v1.1.1 (24). Variants determined to be "potentially pathogenic" by SILVA were further analyzed by NetGene2 (25) to predict splicing effects.

SNP array analysis

The patient DNA samples were run on the Illumina HumanExome Bead Chip, containing ~250,000 SNPs. The data were processed using GenomeStudio (Illumina, Inc.), and CNVs were detected using cnvPartition v3.2 (Illumina, Inc.) and Nexus v7.5 (BioDiscovery, Inc.).

Statistical analysis of mutation load

The Mann-Whitney-WIlcoxon non-parametric statistical test was used to evaluate the burden or mutation load of FA gene variants in the HNSCC patient cohort compared to the 1000 Genomes dataset. Given the HNSCC cohort was comprised predominantly of patients with Caucasian ethnicity (Table 1A), the statistical test was performed using data from the 356 Caucasian HNSCC patients and the 503 EUR 1000 Genomes individuals to create a more homogenous comparison group. We obtained the nonsynonymous and indel variant alleles from each set and implemented the test in R (26).

Low frequency variants

LoFreq (27) was implemented to call low frequency variants occurring between 5 – 40 percent with a genotype quality > 500.

RESULTS

Of the 417 patients with DNA available for sequencing, 88 (21%) were younger than 40 (a traditionally accepted definition of "young" for a patient with HNSCC), 108 (26%) were 40-44, and 221 (53%) were 45-49 years of age. Tumor site was oral cavity in 149 (36%), oropharynx in 230 (55%) and larynx in 38 (9%). The cohort was comprised of four different ethnic populations, 356 (85%) are Caucasian, 40 (10%) are Hispanic, 14 (3%) are Asian, and 7 (2%) are African American (Table 1A).

No patient in the cohort had a known diagnosis of FA, and there were very few with any potential signs of a FA phenotype. Classic phenotypes of FA were then evaluated in the cohort. Seventeen patients (4%) had a first-degree relative with a hematologic malignancy, 25 (6%) had short stature (<fifth percentile), and eight (2%) had macrocytic anemia and/or leukopenia, and among the patients who received chemotherapy, there were no grade IV toxic effects.

Germline DNA targeted capturing and next-gen sequencing of 16 FA genes revealed 11,968 initial variants. The targeted region for capturing and sequencing included the entire gene. The post sequence coverage of high quality sequence, particularly for the entire coding region of 56,120 bp, was 100% for all genes except for a total of 287 bp from the exon 1 of *FANCA*, *FANCB* and *FANCE*, with a depth of coverage around 240 reads at each base (Supplemental Table 2) (21).

Among the 11,968 initial called variants, there were 137 synonymous variants and 358 functional variants (nonsense, missense, indel, or splicing) (Figure 1). Using a population-specific variant frequency threshold of ≤ .5% in each of 1000 Genomes, NHLBI-ESP6500, and ExAC databases, the subset of functional variants was further reduced to 192 (183 SNVs and 9 indels). Functional prediction algorithms (SIFT, PolyPhen, MutationTaster, CADD, and GERP++) analyzed the 183 rare SNVs to identify which may be likely to induce deleterious functional consequences and which are likely benign. Assignment of a definitive prediction to a particular variant was dependent on at least four of the five algorithms reaching a consensus. If

a consensus was not met between at least 4 algorithms, the prediction was classified as indeterminate, and labeled as such. 80 rare SNVs were predicted to be damaging and 64 were predicted to be benign, while 39 were indeterminate. The 9 indels were presumed to be deleterious by the nature of the variant. Splicing prediction algorithms (SILVA and NetGene2) analyzed the 137 synonymous variants and identified 1 variant as likely pathogenic by creating a new donor site.

The 193 resulting variants (184 SNVs and 9 indels) were compared to variants that had been previously reported to the LOVD Fanconi anemia disease database, BIC (Breast Cancer Information Core), and/or listed in ClinVar. Forty-four percent (85/193) of variants had been previously reported in at least one of the three databases, but only 5/85 were listed, specifically, as pathogenic, while the rest were listed as either benign or uncertain significance. Variants in *BRCA2* (36) and *PALB2* (9) comprised 52% (45/85) of the reported variants. In addition to the 85 reported variants, our cohort carried 71 variants that were unreported but present at a frequency below .5% in the public databases, and 38 completely novel variants.

In addition to high throughput sequencing of the 16 FA genes, genotype data was collected by SNP array, from Illumina's HumanExome BeadChip, for all 417 HNSCC patients. Copy number analysis revealed a ~154kb heterozygous deletion of *SLX4*, spanning the entire gene, in a HNSCC patient. This patient also carried a *SLX4* missense mutation that was predicted to be damaging (Table 2).

We also implemented LoFreq to call low frequency variants occurring between 5%-40% with a quality score greater than 500 and a minor allele frequency less than .5% in public databases. It is known that revertant mosaicism in Fanconi anemia may result in the loss of a variant, or a variant being present to a lower extent, and in such cases cancer may precede the diagnosis of FA (28). However, our search for low frequency variants did not yield any potential candidates.

Of the 417 patients, 185 (44%) carried at least one rare variant (Table 1B). The 185 patients carried 194 variations (192 rare variants, a large deletion, and a synonymous variant that was predicted to affect splicing). A rare variant was observed in 42%-55% of patients from Caucasian, Hispanic, and African American ancestry, whereas 79% (11/14) of Asian patients carried a variant. A rare variant was seen in a similar proportion of patients irrespective of tumor site, Larynx (42%), Oral Cavity (44%) or Oropharynx (45%), or age group, < 40 (40%), 40-44 (49%), or 45-49 (44%) years (Table 1B,1D). The proportion of patients carrying rare predicted damaging variants was also similar irrespective of tumor site, 25%-28%, or age group, 24%-28% (Table 1C-D).

Fifteen patients (4%) had either two rare FA variants in the same FA gene or an X-linked variant of FA (Table 2). Among these 15 patients, the median age was 45 years (only 2 were <40 years), 10 (67%) had oropharyngeal primary tumors, 8 (47%) were never smokers, 2 (13%) had a first-degree relative with a hematologic malignancy, 5 (33%) had short stature (<fifth percentile), and 3 (20%) had macrocytic anemia and/or leukopenia. Relative to the entire cohort, there were no differences in age, sex, smoking status, tumor site, or human papillomavirus status between patients carrying two rare variants in the same FA gene or an X-linked FA variant or not. Of the 15 patients with two rare variants, or an X-linked variant, six carried variants that had been previously documented in either the FA mutation database, ClinVar, or BIC.

Thirty-nine patients carried a rare variant in two or more different FA genes (Supplemental Table 4). When comparing age at presentation of patients with and without multiple variants, there was no difference in age, sex, smoking, alcohol use, or HPV status.

The 194 germline variations were comprised of 176 missense, 9 indels, 6 nonsense (including one stoploss), 2 splicing (including one synonymous), and 1 large deletion (Figure 2,

Supplemental Table 3). These variants amounted to 255 occurrences throughout the HNSCC cohort (Table 3). *BRCA2*, *FANCP*, *FANCM*, *FANCA,* and *FANCI* were the most common genes to carry rare variants (21% (54/255), 14% (36/255), 11% (27/255), 9% (22/255), and 8% (20/255), respectively). Twenty-six percent of patients (108/417) carried 91 FA rare variants predicted to be damaging, where *BRCA2* had the highest proportion of occurrences at 10% (25/255), and *SLX4, FANCI, FANCM*, *FANCQ*, each accounted for 5% (12-14/255). Fourteen percent of patients (57/417) had rare variants predicted to be damaging and previously reported. *BRCA2* with 10% (25/255), and *SLX4* and *FANCQ* each with 3% (7-8/255) are the top three most prevalent carriers. Detailed characterization of each of the 194 rare variants is presented in Supplemental Table 3.

Table 4 presents the incidence of rare variants, by FA gene, among the HNSCC cohort, segregated by tumor site, ethnicity, and age group. There were no significant differences in mutation rates when patients were stratified by age, tumor site, ethnicity, smoking status, or human papillomavirus status.

Finally, we looked for an increased burden or mutation load of FA gene variants in our cohort in comparison to the 1000 Genomes dataset using the Mann-Whitney-Wilcoxon nonparametric test. Because our cohort is comprised, predominantly, of patients with Caucasian ethnicity, the statistical comparison was between the 356 Caucasian HNSCC patients and the 503 individuals of European ancestry in the 1000 Genomes dataset. This comparison revealed that *FANCD2*, *FANCE*, and *FANCL* had a significantly increased burden in our cohort (Table 5). At the same time, the mutation burden for *BRCA2*, *FANCG*, and *FANCQ* was significantly reduced in our cohort.

DISCUSSION

Though HNSCC is highly associated with FA, the prevalence of FA germline variants in younger HNSCC populations has not been explored. While the primary purpose of this study

was to define an age cutoff among patients with HNSCC to undergo genetic screening for FA, there was no correlation of younger age with FA germline variants. In a previously reported tumor genomic analysis of HNSCC in low-risk patients (nonsmokers <45 years old) and traditional high-risk patients (smokers >45 years old), age was not a marker of genome instability: rates of gene-specific mutations and copy-number alterations were similar in oral tongue cancers in low-risk patients and oral tongue cancers in traditional high-risk patients (29). Similarly, we did not see a difference in prevalence of FA germline mutations between patients younger than 40, 40-44, and 45-50 years of age or between smokers and nonsmokers.

Our patient cohort consisted of four different ethnicities, so it was important to use an approach that involved population-specific frequencies to ensure that low frequency variants were properly characterized. 185 patients carried 194 total variations, including 183 rare nonsynonymous variants, a synonymous variant predicted to affect splicing, 9 indels, and a large deletion (Figure 1). 108 patients carried at least one variant predicted to be damaging. We identified 38 novel FA germline variants, including a synonymous variant and a large deletion, not present in control populations or previously documented in disease-associated databases, 23 (61%) of which were predicted to be damaging (Figure 1, Supplemental Table 3). There were 15 patients carrying two rare alleles in the same FA gene, which included three that were known to have anemia and/or leukopenia and two presenting with short stature. FA germline variants, and genome instability, may play a broader role in HNSCC susceptibility regardless of age or traditional risk factors.

Mutations in the FA core complex (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FANCM, and the recently identified FANCT) affect ubiquitination of the FANCD2/FANCI complex, a critical step in the FA pathway that repairs DNA damage and maintains genomic stability (5,30). FANCL is a member of the FA core complex with E3 ligase enzymatic activity for FANCD2 mono-ubiquitination (31). FANCE recruits FANCD2 to the FA core complex for ubiquitination, and subsequent DNA repair (32). Interestingly, FANCD2, FANCL, and FANCE

homologs form a subset of the FA genes present in *Ciona intestinalis*, thought to be the closest invertebrate relative of vertebrates (33,34), and thus appear to be an evolutionarily conserved part of the FA pathway. It is likely that mutations affecting *FANCD2*, *FANCE*, or *FANCL* modify cancer susceptibility through altered DNA repair and genomic instability. In the current study, we observed an increased burden, or mutation load, of variants in *FANCD2*, *FANCL,* and *FANCE* in HNSCC patients compared to population-level estimates (Table 5). One would expect that FA patients with mutations in *FANCD2, FANCL,* and *FANCE* may be predisposed to developing HNSCC. However, it is not practical to evaluate this from clinical experience of FA patients, as *FANCL*, *FANCE,* and *FANCD2* patients represent only 0.4%, 1% and 4% of all FA patients (4), respectively, and may often succumb to other maladies, not surviving long enough to develop HNSCC. Recently, missense mutations in the FANCD2/FANCI complex have been associated with colorectal carcinoma (35). The FA pathway, FANCD2 in particular, is implicated in facilitating replication through common fragile sites (36), a critical process in maintaining genomic stability. Interestingly, a study reporting results from sequencing 190 patients with esophageal squamous cell carcinoma (ESCC) for germ line variants in 12 FA genes identified heterozygous indel variants, one each, in *FANCD2*, *FANCE*, and *FANCL* in three patients, each with a strong family history of ESCC (37). ESCC and squamous cell carcinoma of the anogenital tract, in addition to HNSCC, form the cancer spectrum displayed in FA patients (17). Germline mutations in *FANCD2*, *FANCE,* and *FANCL* may provide a novel area of study for HNSCC susceptibility and tumorigenesis.

In conclusion, our analysis of FA gene germline variations in 417 HNSCC patients under the age of 50 identified 15 patients carrying two variants in an FA gene, five of whom presented with a FA-associated phenotype, but we did not identify a specific age cut-off for FA screening. We identified 44% (185/417) of the patients in our cohort as heterozygous carriers of a rare FA gene variant, and variants in 60% of these patients (108/185) were predicted to be damaging. In addition, the increased mutation burden of FANCE, FANCL and FANCD2, key players in the

activation of the DNA repair pathway, may indicate the importance of the FA pathway in HNSCC tumorigenesis.

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FIGURE LEGENDS

Figure 1. Schematics of FA gene variant analysis in HNSCC patients. Data from SNP array were analyzed for copy number variations. SNV discovery was performed from targeted sequencing of 16 FA genes. Synonymous variants were analyzed for splicing effects. Indels, nonsynonymous, and splicing variants were screened for quality and filtered using population-specific frequencies, where applicable. The resulting rare variants were screened for presence in the FA mutation database (FAmutDB) and ClinVar, and analyzed by functional prediction algorithms to determine potential pathogenicity.

^ Unreported variants were present at a frequency below .5% in the public databases but not reported to the FA mutation database, ClinVar, or BIC.

* Novel variants were not present at any frequency in the public databases used for filtering.

Figure 2. The 194 rare variants, by mutation type, observed in the 16 FA genes from 185/417 HNSCC patients

The plot shows the number and type of rare variants observed in each FA gene along with the number of rare variants that were predicted to be damaging. ^includes one stoploss; *includes one synonymous variant

Table 1: Distribution of patients by age, sex, ethnicity, and primary tumor site, as well as by prevalence of FA gene variants.

A. 417 HNSCC Patients, segregated by cancer site, age group, and ethnicity

	Oral Cavity (n=149)			Oropharynx (n=230)			Larynx (n=38)			
	<40	40-44	45-49	<40	40-44	45-49	<40	40-44	45-49	Total
Caucasian	40	32	45	23	54	129	3	9	21	356
African American	0	0	2	0	1	1	1	0	2	7
Hispanic	8	4	8	7	5	7	0	0	1	40
Asian	3	4	3	1	0	2	0	0	1	14
Total	51	40	58	31	60	139	4	9	25	417

B. 185 HNSCC Patients Carrying a Rare FA Gene Variant

	Oral Cavity (n=66)			Oropharynx (n=103)			Larynx (n=16)				
	<40	40-44	45-49	<40	40-44	45-49	<40	40-44	45-49	Total	%
Caucasian	16	11	22	4	29	55	2	4	6	149	42
African American	0	0	0	0	0	0	1	0	2	3	43
Hispanic	5	3	2	4	3	5	0	0	0	22	55
Asian	1	3	3	1	0	2	0	0	1	11	79
Total	22	17	27	9	32	62	3	4	9	185	
Percent (%)	45			42			44				

C. 108 HNSCC Patients Carrying a Rare Predicted Damaging FA Gene Variant

	Oral Cavity (n=41)			Oropharynx (n=57)			Larynx (n=10)				
	<40	40-44	45-49	<40	40-44	45-49	<40	40-44	45-49	Total	%
Caucasian	10	7	13	1	15	32	2	1	4	85	24
African American	0	0	0	0	0	0	0	0	2	2	29
Hispanic	4	3	0	3	3	2	0	0	0	15	38
Asian	1	2	1	0	0	1	0	0	1	6	43
Total	15	12	14	4	18	35	2	1	7	108	
Percent (%)	28			25			26				

D. Percentage of HNSCC Patients Carrying Rare variants, and Rare Predicted Damaging variants, in FA Genes, segregated by age group.

AGE GROUP	PATIENT TOTAL	PATIENTS CARRYING A RARE VARIANT (%)	PATIENTS CARRYING A PREDICTED DAMAGING RARE VARIANT
< 40	86	34 (40)	21 (24)
40-44	109	53 (49)	31 (28)
45-49	222	98 (44)	56 (25)
TOTAL	417	185 (44)	108 (26)

Table 2. Patients carrying two variants in one Fanconi anemia (FA) gene or an X-linked variant of FA

									Mutation 1				Mutation 2			
Patient	Eth[¥]	Sex	Age (yrs)	Cancer site*	FA phenotypes[†]	Smoking[¥]	HPV[¥]	Gene	cDNA	protein	In FA db**	Functional Prediction[††]	cDNA	protein	In FA db**	Functional Prediction[††]
A5421[β]	C	**M**	46	OP	None	*Never*	*Positive*	*BRCA2*	c.1792A>G	p.T598A	Yes	Benign	c.1804G>A	p.G602R	Yes	Benign
A5809	C	F	45	OP	None	*MD*	*Positive*	*BRCA2*	c.8573A>G	p.Q2858R	Yes	Damaging	c.1151C>T	p.S384F	Yes	Damaging
A1105	C	M	44	OP	None	*Never*	*MD*	*ERCC4*	c.1336G>T	p.A446S	--	Indeterminate	c.1347C>A	p.V449V[^]	--	Damaging
A4798	C	M	42	OP	None	*Current*	*Positive*	*FANCA*	c.1046C>T	p.A349V	--	Indeterminate	c.2390C>T	p.A797V	--	Benign
A4675	C	M	43	OP	A	*Never*	*Positive*	*FANCB*[§]	c.30C>A	p.N10K	--	Benign	--	--	--	--
A2766	H	M	31	OC	None	*Never*	*MD*	*FANCI*	c.868G>A	p.V290M	--	Benign	c.1114G>A	p.V372I	--	Indeterminate
A3494	H	M	47	OP	A	*Never*	*MD*	*FANCI*	c.3493delG	p.D1105fs	Yes	Damaging	c.3946G>A	p.G1316R	--	Benign
A4164	C	M	49	OC	None	*Never*	*MD*	*FANCI*	c.1461T>A	p.Y487X	Yes	Damaging	c.362T>C	p.L121P	--	Damaging
A4741	C	F	33	OC	None	*Former*	*MD*	*FANCM*	c.5117A>C	p.N1706T	--	Benign	c.3827C>T	p.S1276L	--	Benign
A2217	C	M	46	OP	L & A	*Current*	*MD*	*SLX4*	c.3739G>A	p.E1247K	--	Damaging	c.833G>A	p.R278Q	--	Benign
A2281	C	M	43	OP	None	*Former*	*MD*	*SLX4*	c.2182G>A	p.A728T	--	Damaging	c.5281C>T	p.R1761C	--	Damaging
A3094	C	F	43	OC	Ht	*Current*	*Positive*	*SLX4*	c.4264C>G	p.P1422A	--	Indeterminate	c.2364G>C	p.Q788H	--	Benign
A4325	C	F	49	OP	None	*Never*	*MD*	*SLX4*	c.3368C>A	p.S1123Y	Yes	Damaging	Large deletion[‡]	--	--	Damaging
A5423	H	M	47	OP	None	*Former*	*Positive*	*SLX4*	c.4261A>T	p.I1421F	Yes	Indeterminate	c.2290C>G	p.P764A	--	Benign
A2674	AA	F	49	Lar	Ht	*Current*	*MD*	*ERCC4*	c.109C>T	p.R37C	--	Damaging	c.109C>T	p.R37C	--	Damaging

[¥] Ethnicity (C = Caucasian; AA = African American; H = Hispanic); * OP = oropharynx; Lar = larynx; OC = oral cavity; † L = leukopenia; A = anemia; Ht = short stature; ¥ MD = Missing Data

‡ *SLX4* deletion removes the entire gene (deletion coordinates: chr16_3586230-3740926)

[β] carries a third mutation in BRCA2, also predicted to be benign (c.125A>G; p.Y42C)

[§] *FANCB* is X-linked

** Previously reported in the Leiden Open Variation Database for Fanconi anemia (http://www.rockefeller.edu/fanconi/) and/or ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/)

†† Functional prediction consensus result from SIFT, PolyPhen, MutationTaster, CADD, and GERP++; synonymous variants analyzed by SILVA and NetGene2; nonsense mutations, indels, and the large deletion presumed to be damaging by nature of variant.

^ synonymous variant predicted to effect splicing

Table 3. Variant counts, for each FA gene, and number of occurrences present in our HNSCC cohort, characterized as Rare, Predicted damaging, and Predicted damaging & reported

	Rare (185 patients)		Predicted damaging (108 patients)		Predicted damaging & reported (57 patients)	
Gene	**variants**	**# occurrences**	**variants**	**# occurrences**	**variants**	**# occurrences**
FANCA	20	22	7	9	2	2
FANCB	1	1	0	0	0	0
FANCC	6	8	4	5	3	4
FANCD1/BRCA2	37	54	15	25	15	25
FANCD2	11	11	6	6	2	2
FANCE	6	6	5	5	1	1
FANCF	2	2	2	2	0	0
FANCG	6	6	1	1	0	0
FANCI	17	20	11	14	2	2
FANCJ/BRIP1	8	13	4	5	3	4
FANCL	8	13	3	8	0	0
FANCM	20	27	12	13	0	0
FANCN/PALB2	12	15	3	3	2	2
FANCO/RAD51C	3	4	2	3	2	3
FANCP/SLX4	25	36	9	14	3	8
FANCQ/ERCC4	12	17	7	12	3	7
	194	255	91	125	38	60

Rare – exists at a frequency <.5% in 1000Genomes, NHLBI-ESP6500, or ExAC

Predicted damaging – prediction algorithim (criteria); SIFT (D), Polyphen (D or P), MutationTaster (D or A), CADD (Phred-scaled score > 20), GERP++ (RS score > 2). If 4/5 of the required criteria were met, the variant was considered to be “predicted damaging”.

Predicted damaging & reported – The above criteria were met and the variant was present in either the FA mutation database and/or ClinVar.

Table 4. Distribution of rare FA gene variants among HNSCC patients, segregated by cancer site, age of onset, and ethnicity

	Cancer Site	Oral Cavity											Oropharynx										Larynx								
	No. of patients	149											230										38								
Patient Totals	Age Group	<40			40-44			45-49					<40			40-44			45-49					<40		40-44		45-49				
	No. of patients	51			40			58					31			60			139					4		9		25				
	Ethnicity	A	H	C	A	H	C	A	H	C	Aa		A	H	C	H	C	Aa	A	H	C	Aa		Aa	C	C	A	Aa	C	H		
	No. of patients	3	8	40	4	4	32	3	8	45	2	Total	1	7	23	5	54	1	2	7	129	1	Total	1	3	9	1	2	21	1	Total	All
	No. of patients with rare variants	1	5	16	3	3	11	3	2	22	0	66	1	4	4	3	29	0	2	5	55	0	103	1	2	4	1	2	6	0	16	185
FA gene rare variants	*BRCA2/FANCD1*		2	6	2		2	1		6		19		2	1		10		1		18		32			1	1		1		3	54
	FANCJ/BRIP1		1	1			2	1		1		6					2				3		5				1	1			2	13
	FANCQ/ERCC4			1						2		3	1				4				3		8		1	1		2	2		6	17
	FANCA		1	1	1		2			2		7				1	4		1		8		14			1					1	22
	FANCB																1						1									1
	FANCC		1				1			2		4				1	2						3			1					1	8
	FANCD2		1				1	1		2		5				1					4		5			1					1	11
	FANCE			2								2		1			1				1		3					1			1	6
	FANCF				1							1									1		1									2
	FANCG	1	1					1				3					2				1		3									6
	FANCI		2	1		1				3		7			1	1	1			3	5		11						2		2	20
	FANCL	1	1	1			2					5		1	1	1	2			1	2		8									13
	FANCM		1	6			2		1	6		16					2				7		9				1	1			2	27
	FANCN/PALB2					2			1	1		4					1			1	5		7	1					3		4	15
	FANCO/RAD51C	1		1						1		3									1		1									4
	FANCP/SLX4	1	1	2			4	1		3		12			1		6			2	12		21		1	1			1		3	36
Variant Totals	Ethnicity	4	12	22	4	3	16	5	2	29	0		1	4	4	5	38	0	2	7	71	0		1	2	6	3	5	9	0		255
	Age Group	38			23			36					9			43			80					3		6		17				
	Cancer Site	97											132											26								
	Total Occurrences	255																														

Table 5. Analysis of FA gene mutation burden in HNSCC patients

FA Gene	Adjusted p-value	HNSCC direction
FANCA	0.93744	--
FANCB	2.8672	--
FANCC	15.3552	--
FANCD1 [BRCA2]	**3.52E-15**	↓
FANCD2	**3.52E-15**	↑
FANCE	**0.0081088**	↑
FANCF	1.55936	--
FANCG	**3.52E-15**	↓
FANCI	11.1664	--
FANCJ [BRIP1]	2.5776	--
FANCL	**3.52E-15**	↑
FANCM	8.1728	--
FANCN [PALB2]	6.2352	--
FANCO [RAD51C]	14.776	--
FANCP [SLX4]	1.7248	--
FANCQ [ERCC4 or *XPF]*	**3.52E-15**	↓

Figure 1

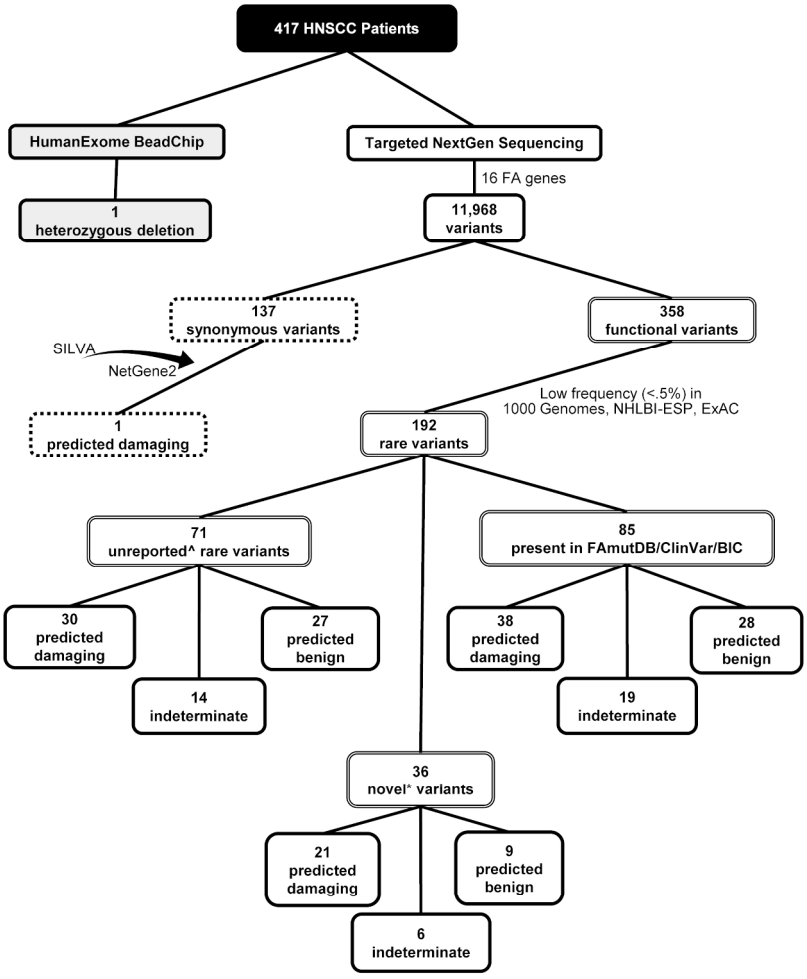

Figure 1. Schematics of FA gene variant analysis in HNSCC patients.

190x254mm (300 x 300 DPI)

Figure 2

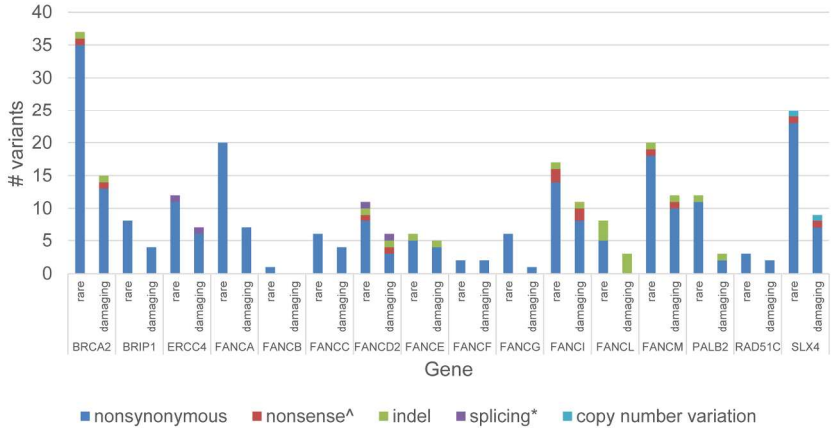

Figure 2. ^nonsense group includes stoploss; *splicing group includes a synonymous variant

190x254mm (300 x 300 DPI)

Supplemental Table 1. Comparison between samples included in the study and samples excluded

	Cases selected (n=417)		Cases unselected (n=230)		
Variables	**n**	**(%)**	**n**	**(%)**	**p-value**
Age, mean (SD)	43.1 (6.1)		42.5 (6.6)		0.514
Age, grouped					0.566
<45	195	(46.8)	113	(49.1)	
45-49	222	(53.2)	117	(50.9)	
Sex					1.0
Male	306	(73.4)	169	(73.5)	
Female	111	(26.6)	61	(26.5)	
Race					1.0
Non-Hispanic whites	356	(85.4)	197	(85.7)	
Others	61	(14.6)	33	(14.3)	
Site					<0.001
Oral cavity	149	(35.7)	61	(26.5)	
Oropharynx*	230	(55.2)	127	(55.2)	
Larynx/hypopharynx^	38	(9.1)	39	(17.0)	
SYN	0	(0)	3	(1.3)	
Smoking					0.067
Current	136	(34.5)	83	(38.8)	
Former	68	(17.3)	48	(22.4)	
Never	190	(48.2)	83	(38.8)	
Alcohol drinking					0.284
Current	194	(49.1)	112	(52.3)	
Former	57	(14.4)	37	(17.3)	
Never	144	(36.5)	65	(30.4)	

*The oropharynx group includes five patients with upper neck lymph node metastasis with unidentified primary site

^The larynx group includes three patients with hypopharyngeal cancer

"SYN" = patients with synchronous primaries at more than one site

Supplemental Table 2: High quality sequence coverage of FA gene regions for HNSCC samples

			Coverage (enti			
Name	RefSeq Gene ID	UCSC Transcript ID	chr	start	end	TargetSize (bp)
FANCA	NM_000135	uc002fou.1	chr16	89803958	89888065	84107
FANCB	NM_001018113	uc004cwg.1	chrX	14861528	14896184	34656
FANCC	NM_000136	uc004avh.3	chr9	97861335	98084991	223656
FANCD1/BRCA2	NM_000059	uc001uub.1	chr13	32884616	32973809	89193
FANCD2	NM_001018115	uc003bux.1	chr3	10063112	10143614	80502
FANCE	NM_021922	uc003oko.1	chr6	35415137	35434881	19744
FANCF	NM_022725	uc001mql.1	chr11	22644078	22652387	8309
FANCG	NM_004629	uc003zwb.1	chr9	35073834	35085013	11179
FANCI	NM_001113378	uc010bnp.1	chr15	89782193	89860362	78169
FANCJ/BRIP1	NM_032043	uc002izk.2	chr17	59756546	59945920	189374
FANCL	NM_018062.3	uc002rzw.4	chr2	58386377	58473515	87138
FANCM	NM_020937.2	uc001wwd.4	chr14	45600135	45670093	69958
FANCN/PALB2	NM_024675.3	uc002dlx.1	chr16	23614482	23657678	43196
FANCO/RAD51C	NM_058216.1	uc002iwu.3	chr17	56764962	56811692	46730
FANCP/SLX4	NM_032444.2	uc002cvp.2	chr16	3631183	3666585	35402
FANCQ/ERCC4	NM_005236	uc002dce.2	chr16	14009014	14046205	37191

Coverage refers to high quality sequence score for the targeted region, MPG score >=10.

• Coverage of CDS for the FANCA, FANCB and FANCB of 98%, 99% and 93% resepctively is exclusively

ire gene)			Coverage (c		
Mean Coverage %	Min Coverage %	Max Coverage %	CDS (bp)	Minimum (bp)	Maximum (bp)
96.22	92.86	97.83	4373	4294	4294
95.67	93.67	97.56	2580	2418	2580
94.33	93.00	95.19	1827	1827	1827
94.35	93.21	95.24	10257	10257	10257
93.03	90.59	94.71	4491	4483	4490
98.50	95.91	99.50	1611	1362	1516
95.89	93.73	99.76	1125	1124	1125
98.31	93.83	99.99	1869	1868	1869
96.76	95.03	97.86	3987	3987	3987
96.35	95.13	97.47	3750	3750	3750
90.13	89.03	90.87	1143	1142	1143
98.66	97.20	99.79	6155	6154	6155
93.37	90.28	95.62	3561	3561	3561
93.64	90.85	96.37	1135	1134	1135
94.92	92.71	96.19	5686	5569	5686
99.27	97.56	100.00	2570	2517	2570

to the reduced coverage of exon 1 in these genes

coding region)	
Mean (bp)	Mean % Coverage (bp)•
4294	98
2547	99
1827	100
10257	100
4490	100
1439	93
1125	100
1869	100
3987	100
3750	100
1143	100
6155	100
3561	100
1135	100
5685	100
2569	100

Supplemental Table 3: Characteristics of rare variants from sequence analysis of 16 FA genes in 417 H

185/417 HNSCC patients carry 194 rare FA gene variants
Including 1 large deletion from SNP array & 1 synonymous variant

80 variants are predicted to be damaging by prediction algorithms (at least 4 out of 5 agree - SIFT, Poly
11 variants are predicted to be damaging due to the nature of the variant

65 variants are predicted to be benign by prediction algorithms (at least 4 out of 5 agree - SIFT, Polyphe

39 variants have inconsistent predictions (at least 4 out of 5 do not agree - SIFT, Polyphen, MutationTa

56 variants are present in the FA Mutation database (FAMutdb)
19 of these are predicted to be benign
27 are predicted to be damaging
10 have inconsistent predictions

SIFT	Damaging if "D"
Polyphen	Damaging if "P" or "D"
MutationTaster	Damaging if "A" or "D"
CADD	Damaging if >= 20 (top 1% most damaging variants in the genome)
GERP++	Conserved position if > 2 (RS score threshold of 2 provides high sensitivity while s
nature of variant	Damaging if variant is of the type: stopgain, indel, large deletion, synonymous pr

"reported in FAmutDB/ClinVar/BIC" -- variant is present in at least one of these databases
"unreproted rare variant" -- variant is not present in FAmutDB, ClinVar, or BIC, but is observed in the 1(
"novel" -- variant has not been reported to FAmutDB, ClinVar, or BIC, nor is it observed in the 1000 Ger

					Variant Info	
chr	start	end	Column1	ref	alt	gene
2	58386933	58386933	2:58386933-58386933	-	AATT	FANCL
2	58387285	58387286	2:58387285-58387286	CT	-	FANCL
2	58388673	58388673	2:58388673-58388673	-	ATTT	FANCL
2	58456974	58456974	2:58456974-58456974	C	T	FANCL
3	10085255	10085255	3:10085255-10085255	-	TGGA	FANCD2
3	10106085	10106085	3:10106085-10106085	G	T	FANCD2
3	10122823	10122823	3:10122823-10122823	C	T	FANCD2
6	35424011	35424013	6:35424011-35424013	GGA	-	FANCE
6	35427507	35427507	6:35427507-35427507	T	C	FANCE
9	35074140	35074140	9:35074140-35074140	A	G	FANCG
9	35075959	35075959	9:35075959-35075959	C	G	FANCG
9	97873915	97873915	9:97873915-97873915	A	G	FANCC
9	98011571	98011571	9:98011571-98011571	C	T	FANCC
13	32911967	32911967	13:32911967-32911967	T	A	BRCA2
14	45605328	45605328	14:45605328-45605328	C	T	FANCM

4	45669125	45669127	14:45669125-45669127	GAG	-	FANCM
5	89835919	89835919	15:89835919-89835919	G	T	FANCI
5	89838241	89838241	15:89838241-89838241	A	G	FANCI
5	89859688	89859688	15:89859688-89859688	T	A	FANCI
6	3586230	3740926	16:3586230-3740926	--	--	SLX4
6	3639105	3639105	16:3639105-3639105	C	T	SLX4
6	3639265	3639265	16:3639265-3639265	C	A	SLX4
6	3639375	3639375	16:3639375-3639375	G	C	SLX4
6	3639723	3639723	16:3639723-3639723	G	A	SLX4
6	3639900	3639900	16:3639900-3639900	C	T	SLX4
6	3641275	3641275	16:3641275-3641275	C	G	SLX4
6	3641304	3641304	16:3641304-3641304	C	G	SLX4
6	14028111	14028111	16:14028111-14028111	A	C	ERCC4
6	14028150	14028150	16:14028150-14028150	G	C	ERCC4
6	14029125	14029125	16:14029125-14029125	G	T	ERCC4
6	14029136	14029136	16:14029136-14029136	C	A	ERCC4
6	23641406	23641406	16:23641406-23641406	T	C	PALB2
6	23646577	23646578	16:23646577-23646578	CT	-	PALB2
6	89807245	89807245	16:89807245-89807245	C	A	FANCA
6	89831326	89831326	16:89831326-89831326	C	T	FANCA
6	89849271	89849271	16:89849271-89849271	G	T	FANCA
6	89857890	89857890	16:89857890-89857890	A	G	FANCA
7	59878645	59878645	17:59878645-59878645	T	C	BRIP1
	10115047	10115047	3:10115047-10115047	G	A	FANCD2
	35426215	35426215	6:35426215-35426215	C	T	FANCE
5	89824480	89824480	15:89824480-89824480	T	A	FANCI
5	89849381	89849381	15:89849381-89849381	G	-	FANCI
6	3639378	3639378	16:3639378-3639378	T	A	SLX4
6	3640271	3640271	16:3640271-3640271	G	T	SLX4
6	3640461	3640461	16:3640461-3640461	G	A	SLX4
6	3641280	3641280	16:3641280-3641280	C	T	SLX4
6	3647691	3647691	16:3647691-3647691	T	C	SLX4
6	3656645	3656645	16:3656645-3656645	A	G	SLX4
	58386913	58386913	2:58386913-58386913	C	G	FANCL
	58392901	58392901	2:58392901-58392901	C	G	FANCL
	58456962	58456962	2:58456962-58456962	C	G	FANCL
	10070369	10070369	3:10070369-10070369	T	C	FANCD2
	10107551	10107551	3:10107551-10107551	G	C	FANCD2
	10107587	10107587	3:10107587-10107587	A	G	FANCD2
	10132062	10132062	3:10132062-10132062	C	T	FANCD2
	35423672	35423672	6:35423672-35423672	C	T	FANCE
	35427531	35427531	6:35427531-35427531	T	C	FANCE
	35075077	35075077	9:35075077-35075077	C	T	FANCG
	35077006	35077006	9:35077006-35077006	G	T	FANCG
1	22646497	22646497	11:22646497-22646497	T	C	FANCF
1	22646921	22646921	11:22646921-22646921	G	C	FANCF
4	45605397	45605397	14:45605397-45605397	G	A	FANCM

4	45605405	45605405	14:45605405-45605405	G	C	FANCM
4	45605503	45605503	14:45605503-45605503	C	T	FANCM
4	45605551	45605551	14:45605551-45605551	A	G	FANCM
4	45605730	45605730	14:45605730-45605730	G	A	FANCM
4	45606286	45606286	14:45606286-45606286	T	C	FANCM
4	45618149	45618149	14:45618149-45618149	T	C	FANCM
4	45623204	45623204	14:45623204-45623204	G	C	FANCM
4	45628478	45628478	14:45628478-45628478	C	G	FANCM
4	45633740	45633740	14:45633740-45633740	T	C	FANCM
4	45644816	45644816	14:45644816-45644816	A	C	FANCM
4	45645661	45645661	14:45645661-45645661	G	T	FANCM
4	45645784	45645784	14:45645784-45645784	C	T	FANCM
4	45645892	45645892	14:45645892-45645892	T	C	FANCM
4	45658326	45658326	14:45658326-45658326	C	T	FANCM
4	45658342	45658342	14:45658342-45658342	A	C	FANCM
4	45665612	45665612	14:45665612-45665612	C	T	FANCM
4	45669105	45669105	14:45669105-45669105	T	C	FANCM
5	89804889	89804889	15:89804889-89804889	T	C	FANCI
5	89811698	89811698	15:89811698-89811698	T	C	FANCI
5	89811742	89811742	15:89811742-89811742	G	A	FANCI
5	89819943	89819943	15:89819943-89819943	G	A	FANCI
5	89824418	89824418	15:89824418-89824418	G	A	FANCI
5	89825056	89825056	15:89825056-89825056	A	G	FANCI
5	89826381	89826381	15:89826381-89826381	G	A	FANCI
5	89843162	89843162	15:89843162-89843162	A	G	FANCI
5	89848573	89848573	15:89848573-89848573	A	T	FANCI
5	89849316	89849316	15:89849316-89849316	C	T	FANCI
5	89859649	89859649	15:89859649-89859649	G	A	FANCI
6	3633211	3633211	16:3633211-3633211	C	A	SLX4
6	3639991	3639991	16:3639991-3639991	C	A	SLX4
6	3642722	3642722	16:3642722-3642722	C	G	SLX4
6	3642737	3642737	16:3642737-3642737	G	C	SLX4
6	3642845	3642845	16:3642845-3642845	C	T	SLX4
6	3644515	3644515	16:3644515-3644515	C	T	SLX4
6	3647490	3647490	16:3647490-3647490	G	A	SLX4
6	3651147	3651147	16:3651147-3651147	C	A	SLX4
6	3652236	3652236	16:3652236-3652236	C	T	SLX4
6	3656698	3656698	16:3656698-3656698	C	A	SLX4
6	14014131	14014131	16:14014131-14014131	C	T	ERCC4
6	14029437	14029437	16:14029437-14029437	C	T	ERCC4
6	14041578	14041578	16:14041578-14041578	G	A	ERCC4
6	14042100	14042100	16:14042100-14042100	G	A	ERCC4
6	23637718	23637718	16:23637718-23637718	T	C	PALB2
6	89811442	89811442	16:89811442-89811442	C	G	FANCA
6	89816220	89816220	16:89816220-89816220	G	A	FANCA
6	89818612	89818612	16:89818612-89818612	G	C	FANCA
6	89836359	89836359	16:89836359-89836359	G	A	FANCA

16	89842161	89842161	16:89842161-89842161	T	A	FANCA
16	89842176	89842176	16:89842176-89842176	C	G	FANCA
16	89858916	89858916	16:89858916-89858916	G	A	FANCA
16	89865628	89865628	16:89865628-89865628	G	A	FANCA
16	89871709	89871709	16:89871709-89871709	C	T	FANCA
16	89877149	89877149	16:89877149-89877149	C	T	FANCA
16	89877377	89877377	16:89877377-89877377	G	A	FANCA
16	89877386	89877386	16:89877386-89877386	G	C	FANCA
17	56780607	56780607	17:56780607-56780607	A	G	RAD51C
X	14883603	14883603	X:14883603-14883603	G	T	FANCB
2	58390589	58390589	2:58390589-58390589	A	C	FANCL
9	98011497	98011497	9:98011497-98011497	G	A	FANCC
13	32953550	32953550	13:32953550-32953550	G	A	BRCA2
13	32914815	32914815	13:32914815-32914815	G	A	BRCA2
13	32893369	32893369	13:32893369-32893369	G	C	BRCA2
17	59885856	59885856	17:59885856-59885856	T	C	BRIP1
13	32953549	32953549	13:32953549-32953549	G	T	BRCA2
13	32968861	32968861	13:32968861-32968861	T	C	BRCA2
16	23646942	23646942	16:23646942-23646942	T	C	PALB2
13	32911295	32911295	13:32911295-32911295	G	A	BRCA2
13	32907401	32907401	13:32907401-32907401	G	C	BRCA2
13	32900252	32900252	13:32900252-32900252	A	G	BRCA2
15	89835982	89835982	15:89835982-89835982	C	A	FANCI
16	3632567	3632567	16:3632567-3632567	G	A	SLX4
17	59924572	59924572	17:59924572-59924572	G	A	BRIP1
13	32907419	32907419	13:32907419-32907419	G	A	BRCA2
13	32953604	32953604	13:32953604-32953604	G	A	BRCA2
13	32906766	32906766	13:32906766-32906766	C	T	BRCA2
13	32937521	32937521	13:32937521-32937521	G	A	BRCA2
17	59924505	59924505	17:59924505-59924505	A	G	BRIP1
13	32914809	32914809	13:32914809-32914809	T	C	BRCA2
13	32972771	32972771	13:32972771-32972771	C	T	BRCA2
13	32929047	32929047	13:32929047-32929047	G	C	BRCA2
9	98009786	98009786	9:98009786-98009786	C	T	FANCC
3	10074646	10074646	3:10074646-10074646	G	C	FANCD2
3	10103845	10103845	3:10103845-10103845	C	T	FANCD2
3	10122797	10122797	3:10122797-10122797	G	A	FANCD2
6	35426122	35426122	6:35426122-35426122	G	C	FANCE
9	35075738	35075738	9:35075738-35075738	G	T	FANCG
9	35079445	35079445	9:35079445-35079445	T	C	FANCG
16	14014038	14014038	16:14014038-14014038	C	T	ERCC4
16	14029516	14029516	16:14029516-14029516	G	C	ERCC4
16	14041570	14041570	16:14041570-14041570	T	C	ERCC4
16	89805672	89805672	16:89805672-89805672	C	T	FANCA
16	89836978	89836978	16:89836978-89836978	G	A	FANCA
16	89877157	89877157	16:89877157-89877157	C	T	FANCA
13	32918724	32918724	13:32918724-32918724	A	G	BRCA2

3	32953616	32953616	13:32953616-32953616	C	T	BRCA2
7	59821830	59821830	17:59821830-59821830	C	A	BRIP1
3	32893386	32893386	13:32893386-32893386	A	G	BRCA2
3	32914328	32914328	13:32914328-32914328	T	C	BRCA2
3	32906571	32906571	13:32906571-32906571	A	C	BRCA2
3	32930598	32930598	13:32930598-32930598	T	C	BRCA2
3	32945172	32945172	13:32945172-32945172	A	C	BRCA2
3	32911754	32911754	13:32911754-32911754	C	T	BRCA2
3	32929042	32929042	13:32929042-32929042	C	G	BRCA2
6	23637715	23637715	16:23637715-23637715	G	A	PALB2
3	32906502	32906502	13:32906502-32906502	A	G	BRCA2
6	23619286	23619286	16:23619286-23619286	C	G	PALB2
6	23647211	23647211	16:23647211-23647211	T	C	PALB2
6	23652456	23652456	16:23652456-23652456	G	A	PALB2
3	32905152	32905153	13:32905152-32905153	GA	-	BRCA2
6	89813298	89813298	16:89813298-89813298	T	C	FANCA
	97864005	97864005	9:97864005-97864005	A	G	FANCC
3	32912735	32912735	13:32912735-32912735	G	T	BRCA2
6	14041848	14041848	16:14041848-14041848	C	T	ERCC4
	98009773	98009773	9:98009773-98009773	A	C	FANCC
6	23619288	23619288	16:23619288-23619288	C	T	PALB2
3	32911175	32911175	13:32911175-32911175	G	A	BRCA2
7	59926512	59926512	17:59926512-59926512	C	T	BRIP1
3	32911700	32911700	13:32911700-32911700	G	T	BRCA2
3	32907407	32907407	13:32907407-32907407	A	G	BRCA2
3	32893271	32893271	13:32893271-32893271	A	G	BRCA2
7	56787304	56787304	17:56787304-56787304	G	A	RAD51C
3	32913271	32913271	13:32913271-32913271	A	C	BRCA2
7	59870998	59870998	17:59870998-59870998	T	C	BRIP1
3	32911818	32911818	13:32911818-32911818	C	T	BRCA2
6	23641186	23641186	16:23641186-23641186	C	G	PALB2
6	23646872	23646872	16:23646872-23646872	A	T	PALB2
6	23649272	23649272	16:23649272-23649272	C	T	PALB2
7	56774172	56774172	17:56774172-56774172	G	A	RAD51C
7	59761303	59761303	17:59761303-59761303	C	T	BRIP1
3	32954018	32954018	13:32954018-32954018	G	A	BRCA2
3	32945178	32945178	13:32945178-32945178	A	G	BRCA2
3	32937324	32937324	13:32937324-32937324	C	A	BRCA2

HNSCC patients

phen, MutationTaster, CADD, GERP++)

en, MutationTaster, CADD, GERP++)

ster, CADD, GERP++)

still strongly enriching for truly constrained sites)
edicted to induce splicing,

000 Genomes, NHLBI-ESP, and/or ExAC
nomes, NHLBI-ESP, or ExAC

type	occurrence	note	protein	cDNA (hgvs)	SIFT
frameshift insertion	6		p.P370fs	c.1094_109	fs
frameshift deletion	1		p.Q355fs	c.1049_105	fs
frameshift insertion	1		p.Q340fs	c.1003_100	fs
nonsynonymous SNV	1		p.S64N	c.191G>A	T
frameshift insertion	1		p.T359fs	c.1077_107	fs
nonsynonymous_SNV	1		p.V665L	c.1993G>T	T
stopgain	1		p.Q1006X	c.3016C>T	.
nonframeshift deleti	1		p.246_246del	c.736_738d	nfs
nonsynonymous SNV	1		p.L429P	c.1286T>C	D
nonsynonymous SNV	1		p.F612L	c.1834T>C	T
nonsynonymous SNV	1		p.R381S	c.1143G>C	T
nonsynonymous SNV	1		p.C387R	c.1159T>C	T
nonsynonymous SNV	1		p.M1I	c.3G>A	D
nonsynonymous SNV	1		p.C1159S	c.3475T>A	T
nonsynonymous SNV	1		p.P32S	c.94C>T	D

nonframeshift deleti	1		p.2021_2021d	c.6061_606	nfs
nonsynonymous SNV	1		p.D665Y	c.1993G>T	D
nonsynonymous SNV	1		p.Q851R	c.2552A>G	T
stoploss	1		p.X1329K	c.3985T>A	.
large deletion	1	deletion spans e	--	--	ld
nonsynonymous SNV	1		p.D1512N	c.4534G>A	D
nonsynonymous SNV	1		p.M1458I	c.4374G>T	T
nonsynonymous SNV	1		p.P1422A	c.4264C>G	T
stopgain	1		p.Q1306X	c.3916C>T	.
nonsynonymous SNV	1		p.E1247K	c.3739G>A	D
nonsynonymous SNV	1		p.Q788H	c.2364G>C	T
nonsynonymous SNV	1		p.V779L	c.2335G>C	T
nonsynonymous SNV	1		p.K389Q	c.1165A>C	T
nonsynonymous SNV	1		p.G402R	c.1204G>C	T
nonsynonymous SNV	1		p.A446S	c.1336G>T	T
synonymous	1	predicted to affe	p.V449V	c.1347C>A	syn
nonsynonymous SNV	1		p.Q690R	c.2069A>G	T
frameshift deletion	1		p.Q430fs	c.1289_129	fs
nonsynonymous SNV	1		p.L1265F	c.3795G>T	D
nonsynonymous SNV	1		p.R917Q	c.2750G>A	T
nonsynonymous SNV	1		p.T541N	c.1622C>A	T
nonsynonymous SNV	1		p.M427T	c.1280T>C	D
nonsynonymous SNV	1		p.N370S	c.1109A>G	T
splicing	1		--	c.2715+1G>	.
nonsynonymous SNV	1		p.R371W	c.1111C>T	D
stopgain	1		p.Y487X	c.1461T>A	.
frameshift deletion	1		p.D1105fs	c.3493delG	fs
nonsynonymous SNV	3		p.I1421F	c.4261A>T	D
nonsynonymous SNV	4		p.S1123Y	c.3368C>A	D
nonsynonymous SNV	2		p.R1060W	c.3178C>T	D
nonsynonymous SNV	2		p.E787K	c.2359G>A	T
nonsynonymous SNV	2		p.K458E	c.1372A>G	D
nonsynonymous SNV	2		p.V197A	c.590T>C	T
nonsynonymous SNV	1		p.G377A	c.1115G>C	T
nonsynonymous SNV	1		p.E222Q	c.649G>C	T
nonsynonymous SNV	1		p.R68P	c.203G>C	T
nonsynonymous SNV	1		p.S10P	c.28T>C	T
nonsynonymous SNV	1		p.C758S	c.2273G>C	D
nonsynonymous SNV	1		p.K770R	c.2309A>G	T
nonsynonymous SNV	1		p.S1257L	c.3770C>T	T
nonsynonymous SNV	1		p.L133F	c.397C>T	D
nonsynonymous SNV	1		p.M437T	c.1310T>C	D
nonsynonymous SNV	1		p.A495T	c.1483G>A	T
nonsynonymous SNV	1		p.Q247K	c.739C>A	T
nonsynonymous SNV	1		p.Y287C	c.860A>G	D
nonsynonymous SNV	1		p.L146V	c.436C>G	D
nonsynonymous SNV	1		p.D55N	c.163G>A	D

nonsynonymous SNV	5		p.L57F	c.171G>C	T
nonsynonymous SNV	1		p.P90L	c.269C>T	D
nonsynonymous SNV	1		p.N106S	c.317A>G	D
nonsynonymous SNV	1		p.A166T	c.496G>A	T
nonsynonymous SNV	1		p.S175P	c.523T>C	T
nonsynonymous SNV	1		p.I290T	c.869T>C	D
nonsynonymous SNV	1		p.G378R	c.1132G>C	D
nonsynonymous SNV	2		p.L526V	c.1576C>G	D
nonsynonymous SNV	2		p.I587T	c.1760T>C	D
nonsynonymous SNV	1		p.K953N	c.2859A>C	D
nonsynonymous SNV	1		p.G1235V	c.3704G>T	D
nonsynonymous SNV	1		p.S1276L	c.3827C>T	T
nonsynonymous SNV	1		p.L1312P	c.3935T>C	D
stopgain	2		p.Q1701X	c.5101C>T	.
nonsynonymous SNV	1		p.N1706T	c.5117A>C	T
nonsynonymous SNV	1		p.R1860C	c.5578C>T	D
nonsynonymous SNV	1		p.V2014A	c.6041T>C	D
nonsynonymous SNV	1		p.L121P	c.362T>C	D
nonsynonymous SNV	2		p.I275T	c.824T>C	D
nonsynonymous_SNV	1		p.V290M	c.868G>A	T
nonsynonymous_SNV	1		p.V372I	c.1114G>A	T
nonsynonymous SNV	1		p.V467I	c.1399G>A	T
nonsynonymous SNV	3		p.M525V	c.1573A>G	D
nonsynonymous SNV	1		p.R533Q	c.1598G>A	D
nonsynonymous SNV	1		p.Y923C	c.2768A>G	D
nonsynonymous SNV	1		p.D1063V	c.3188A>T	D
nonsynonymous SNV	1		p.T1143I	c.3428C>T	T
nonsynonymous SNV	1		p.G1316R	c.3946G>A	T
nonsynonymous SNV	1		p.R1680S	c.5040G>T	T
nonsynonymous SNV	1		p.Q1216H	c.3648G>T	T
nonsynonymous SNV	2		p.E769Q	c.2305G>C	T
nonsynonymous SNV	1		p.P764A	c.2290C>G	T
nonsynonymous SNV	1		p.A728T	c.2182G>A	D
nonsynonymous SNV	1		p.G700E	c.2099G>A	D
nonsynonymous SNV	1		p.R525C	c.1573C>T	T
nonsynonymous SNV	1		p.Q332H	c.996G>T	D
nonsynonymous SNV	1		p.R278Q	c.833G>A	T
nonsynonymous SNV	1		p.E179D	c.537G>T	D
nonsynonymous SNV	2	homozygous	p.R37C	c.109C>T	D
nonsynonymous SNV	1		p.P550S	c.1648C>T	T
nonsynonymous SNV	1		p.V709M	c.2125G>A	D
nonsynonymous SNV	1		p.E883K	c.2647G>A	T
nonsynonymous SNV	1		p.N863D	c.2587A>G	T
nonsynonymous SNV	1		p.R1184P	c.3551G>C	D
nonsynonymous SNV	1		p.R1053C	c.3157C>T	T
nonsynonymous SNV	1		p.H1000Q	c.3000C>G	T
nonsynonymous SNV	1		p.A797V	c.2390C>T	T

nonsynonymous SNV	1	p.E630V	c.1889A>T	D
nonsynonymous SNV	3	p.C625S	c.1874G>C	D
nonsynonymous SNV	1	p.A349V	c.1046C>T	T
nonsynonymous SNV	1	p.A280V	c.839C>T	D
nonsynonymous SNV	1	p.V230I	c.688G>A	T
nonsynonymous SNV	1	p.R163H	c.488G>A	D
nonsynonymous SNV	1	p.A129V	c.386C>T	T
nonsynonymous SNV	1	p.T126R	c.377C>G	T
nonsynonymous SNV	1	p.I208V	c.622A>G	T
nonsynonymous SNV	1	p.N10K	c.30C>A	T
nonsynonymous SNV	1	p.F257C	c.755T>G	T
nonsynonymous SNV	1	p.S26F	c.77C>T	D
nonsynonymous_SN	6	p.A2951T	c.8851G>A	D
nonsynonymous SNV	1	p.R2108H	c.6323G>A	T
nonsynonymous SNV	2	p.A75P	c.223G>C	D
nonsynonymous SNV	5	p.K297R	c.890A>G	T
nonsynonymous SNV	1	p.K2950N	c.8850G>T	D
nonsynonymous SNV	1	p.Y3098H	c.9292T>C	T
nonsynonymous_SN	1	p.I309V	c.925A>G	T
nonsynonymous SNV	1	p.D935N	c.2803G>A	D
nonsynonymous SNV	2	p.D596H	c.1786G>C	D
nonsynonymous SNV	1	p.Q147R	c.440A>G	T
nonsynonymous_SN	1	p.Q686K	c.2056C>A	T
nonsynonymous_SN	2	p.R1761C	c.5281C>T	T
nonsynonymous SNV	2	p.R173C	c.517C>T	D
nonsynonymous SNV	1	p.G602R	c.1804G>A	T
nonsynonymous SNV	1	p.V2969M	c.8905G>A	D
nonsynonymous SNV	1	p.S384F	c.1151C>T	D
nonsynonymous SNV	2	p.V2728I	c.8182G>A	T
nonsynonymous SNV	1	p.L195P	c.584T>C	T
nonsynonymous SNV	1	p.L2106P	c.6317T>C	T
nonsynonymous SNV	1	p.T3374I	c.10121C>T	D
nonsynonymous SNV	2	p.G2353R	c.7057G>C	D
nonsynonymous SNV	2	p.V60I	c.178G>A	T
nonsynonymous_SN	1	p.Q65H	c.195G>C	D
nonsynonymous SNV	1	p.P593S	c.1777C>T	T
nonsynonymous SNV	1	p.R997Q	c.2990G>A	T
nonsynonymous SNV	1	p.G340R	c.1018G>C	T
nonsynonymous SNV	1	p.P386H	c.1157C>A	T
nonsynonymous SNV	1	p.Q26R	c.77A>G	T
nonsynonymous SNV	1	p.P6S	c.16C>T	T
nonsynonymous SNV	1	p.R576T	c.1727G>C	T
nonsynonymous SNV	4	p.I706T	c.2117T>C	D
nonsynonymous SNV	1	p.A1346T	c.4036G>A	T
nonsynonymous SNV	1	p.P739L	c.2216C>T	D
nonsynonymous SNV	1	p.M160I	c.480G>A	T
nonsynonymous SNV	1	p.N2291D	c.6871A>G	T

nonsynonymous SNV	2	p.R2973C	c.8917C>T	D
nonsynonymous SNV	1	p.Q740H	c.2220G>T	D
nonsynonymous SNV	1	p.I80M	c.240A>G	T
nonsynonymous SNV	1	p.S1946P	c.5836T>C	T
nonsynonymous SNV	1	p.N319T	c.956A>C	D
nonsynonymous_SNV	4	p.I2490T	c.7469T>C	T
nonsynonymous SNV	1	p.E2856A	c.8567A>C	T
nonsynonymous SNV	1	p.P1088S	c.3262C>T	T
nonsynonymous SNV	1	p.A2351G	c.7052C>G	D
nonsynonymous SNV	4	p.P864S	c.2590C>T	T
nonsynonymous SNV	1	p.Y296C	c.887A>G	T
nonsynonymous SNV	1	p.E1083D	c.3249G>C	D
nonsynonymous SNV	1	p.D219G	c.656A>G	T
nonsynonymous SNV	1	p.P8L	c.23C>T	D
frameshift deletion	1	p.E260fs	c.778_779d	fs
nonsynonymous SNV	1	p.R1117G	c.3349A>G	D
nonsynonymous SNV	2	p.L554P	c.1661T>C	D
stopgain	1	p.E1415X	c.4243G>T	.
nonsynonymous SNV	2	p.R799W	c.2395C>T	D
nonsynonymous SNV	1	p.F64C	c.191T>G	D
nonsynonymous SNV	1	p.E1083K	c.3247G>A	D
nonsynonymous SNV	1	p.A895T	c.2683G>A	T
nonsynonymous SNV	1	p.R162Q	c.485G>A	D
nonsynonymous SNV	1	p.A1070S	c.3208G>T	T
nonsynonymous SNV	3	p.T598A	c.1792A>G	T
nonsynonymous_SNV	2	p.Y42C	c.125A>G	T
nonsynonymous SNV	2	p.G264S	c.790G>A	D
nonsynonymous SNV	1	p.E1593D	c.4779A>C	T
nonsynonymous SNV	1	p.H478R	c.1433A>G	T
nonsynonymous SNV	2	p.A1109V	c.3326C>T	D
nonsynonymous SNV	1	p.L763F	c.2289G>C	D
nonsynonymous SNV	1	p.L332H	c.995T>A	D
nonsynonymous SNV	1	p.R37H	c.110G>A	D
nonsynonymous SNV	1	p.A175T	c.523G>A	D
nonsynonymous SNV	1	p.R1035H	c.3104G>A	D
nonsynonymous SNV	1	p.A3029T	c.9085G>A	D
nonsynonymous SNV	1	p.Q2858R	c.8573A>G	D
nonsynonymous SNV	1	p.T2662K	c.7985C>A	D

Prediction Results			
Polyphen2	lutationTast	CADD	GERP++
fs	fs	fs	
fs	fs	fs	
fs	fs	fs	
B	N	0.126	-4.57
fs	fs	fs	
D	D	24.5	5.58
.	A	38	4.91
nfs	nfs	nfs	
D	D	25.7	5.41
B	N	11.97	4.25
B	D	19.27	5.33
B	N	0.015	-2.75
D	D	29.7	5.13
B	N	0.001	-3.49
P	N	23.5	2.76

nfs	nfs	nfs	
D	D	28.7	5.97
D	D	20.7	4.71
.	N	10.09	-4.08
ld	ld	ld	
D	D	28.3	5.67
B	N	0.04	-11.3
P	N	0.074	3.78
.	A	35	2.43
D	N	23.9	4.7
D	N	12.73	-3.65
B	N	19.52	-0.651
B	D	9.398	5.91
B	D	25.7	5.91
B	D	19.08	5.78
syn	syn	syn	
B	N	0.002	-3.38
fs	fs	fs	
D	D	27	1.87
B	N	5.401	0.027
P	N	4.896	3.22
P	D	21.6	5.43
D	D	23.6	5.13
.	D	25.4	5.83
D	D	35	3.48
.	A	38	4.83
fs	fs	fs	
P	N	13.21	-4.05
P	N	25.4	6.07
B	N	14.1	2.83
P	N	22.7	1.33
D	N	27.1	4.22
B	N	0.024	-3.52
B	D	7.847	3.35
B	D	23.9	4.85
B	D	22.4	2.99
D	N	21.7	1.47
D	D	27.8	5.68
B	N	10.59	-2.5
B	N	14.18	2.58
D	N	25.7	5.37
P	N	23.6	5.41
B	N	8.56	1.25
P	D	23.8	5.12
D	D	28.3	4.23
D	D	25	5.2
D	D	31	4.99

B	D	17.99	1.07
P	D	27.9	5.65
D	D	25.3	5.65
D	D	29.2	5.52
B	N	9.26	-1.38
B	D	22.7	5.55
D	D	29	4.88
B	D	17.71	3.42
B	N	23.1	5.94
D	N	22.8	1.19
D	D	27.6	5.61
B	N	1.058	2.24
B	N	3.258	-7.41
.	A	35	-2.53
B	N	0.003	-0.275
D	D	35	5.27
D	D	26.8	5.93
D	D	23.2	5.27
P	D	23.1	3.54
B	N	9.427	-0.231
P	N	13.8	3.97
B	D	10.24	-0.076
P	D	23.1	4.67
D	D	35	6.03
B	N	15.55	-0.064
D	D	27.2	5.92
D	D	23.4	5.76
B	N	5.046	0.541
B	N	17.66	-0.564
B	N	1.206	-2.5
B	N	18.62	4.64
B	N	0.003	-1.6
D	N	25.9	4.65
D	D	27	4.35
B	N	15.35	-1.55
B	N	21.8	1.16
B	N	0.095	-5.38
B	N	9.08	0.712
D	D	35	5.13
D	D	32	5.33
D	D	32	5.49
B	N	0.615	-10.5
B	N	22.9	2.5
P	N	15.18	-1.96
B	N	3.673	-4.92
B	N	1.19	3.01
B	N	10.84	-9.26

B	N	19.26	3.93
D	D	21.4	5.03
P	N	23.2	3.48
D	N	23.3	3.74
B	N	0.626	-1.86
D	D	26.2	4.01
B	N	12.03	-3.76
B	N	0.694	-10.1
P	D	16.25	5.77
B	N	0.002	-11.6
B	D	13.07	1.24
D	D	26.9	4.22
D	D	29.5	5.6
B	N	13.2	2.76
D	D	23.8	5.59
P	N	10.41	5.29
D	D	24	1.76
B	N	0.006	1.6
B	N	0.001	-12.2
B	N	8.869	-0.456
D	N	24.9	3.38
B	N	0.001	-1.65
B	N	18.29	4.88
P	D	21.1	2.21
D	D	27.6	5.26
B	N	18.77	3.64
P	D	24.3	0.674
D	N	23.4	3.52
B	N	0.002	-7.77
B	D	4.462	-0.011
B	N	0.939	-5.6
B	N	23.2	1.53
D	N	27.9	2.97
B	N	0.014	-5.4
D	N	23.2	3.37
B	N	0.004	2.2
B	N	23.6	2.82
D	N	23.1	3.6
B	N	0.147	1.37
D	D	16.64	4.96
B	N	6.133	1.88
P	D	24.1	5.33
D	D	26.9	5.49
P	N	6.518	-1.73
D	D	25.6	4.9
P	N	10.37	1.67
P	N	23.5	2.43

D	D	33	4.61
D	D	25.7	2.09
P	N	23	1.6
B	N	0.002	-9.18
P	N	10.84	1.15
B	N	8.41	-5.83
D	D	28	5.28
B	N	1.114	2.73
P	N	23.1	2.03
P	N	18.44	2.82
B	N	0.005	-2.14
D	N	23.1	3.8
B	N	0.008	-5.08
B	D	26.8	1.14
fs	fs	fs	
D	D	22.5	5.09
D	A	25.7	4.76
.	A	35	4.19
D	A	35	6.16
D	D	26.6	4.32
B	N	23.7	3.05
B	N	0.057	2.16
D	D	34	5.85
B	N	0.001	-2.47
B	N	1.104	-2.76
B	D	0.506	0.279
B	D	24.9	6
B	N	17.41	-1.14
D	N	10.99	5.24
D	D	31	5.75
D	N	17.95	4
B	N	13.49	3.3
D	D	27.9	4.75
D	D	28.7	4.67
B	N	23.2	4.66
D	D	28.4	4.81
D	D	28.2	5.28
B	N	25.3	4.93

Prediction Consensus	atabase Presenc	FAMutdb	ClinVar	linVar_significanc
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Benign	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Benign	novel	--	--	--
Indeterminate	novel	--	--	--
Benign	novel	--	--	--
Damaging	novel	--	--	--
Benign	novel	--	--	--
Damaging	novel	--	--	--

Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Benign	novel	--	--	--
Indeterminate	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Benign	novel	--	--	--
Benign	novel	--	--	--
Indeterminate	novel	--	--	--
Indeterminate	novel	--	--	--
Indeterminate	novel	--	--	--
Damaging	novel	--	--	--
Benign	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Benign	novel	--	--	--
Indeterminate	novel	--	--	--
Damaging	novel	--	--	--
Damaging	novel	--	--	--
Damaging	present in FAmu	Yes	--	--
Damaging	present in FAmu	Yes	--	--
Damaging	present in FAmu	Yes	--	--
Damaging	present in FAmu	Yes	--	--
Indeterminate	present in FAmu	Yes	--	--
Damaging	present in FAmu	Yes	--	--
Indeterminate	present in FAmu	Yes	--	--
Indeterminate	present in FAmu	Yes	--	--
Damaging	present in FAmu	Yes	--	--
Benign	present in FAmu	Yes	--	--
Indeterminate	unreported rare	--	--	--
Indeterminate	unreported rare	--	--	--
Indeterminate	unreported rare	--	--	--
Indeterminate	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Benign	unreported rare	--	--	--
Benign	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Benign	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Damaging	unreported rare	--	--	--

Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Indeterminate	unreported rare --	--	--
Indeterminate	unreported rare --	--	--
Indeterminate	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Indeterminate	unreported rare --	--	--
Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Indeterminate	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Damaging	unreported rare --	--	--
Benign	unreported rare --	--	--
Indeterminate	unreported rare --	--	--
Indeterminate	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--
Benign	unreported rare --	--	--

Indeterminate	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Indeterminate	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Benign	unreported rare	--	--	--
Damaging	unreported rare	--	--	--
Benign	unreported rare	--	--	--
Benign	unreported rare	--	--	--
Indeterminate	unreported rare	--	--	--
Benign	unreported rare	--	--	--
Benign	present in FAmu	--	Yes	Benign
Damaging	present in FAmu	Yes	Yes	Benign|Benign|B
Damaging	present in FAmu	Yes	Yes	Benign|Benign|B
Benign	present in FAmu	Yes	Yes	Benign|Benign|B
Damaging	present in FAmu	Yes	Yes	Benign|Benign|U
Indeterminate	present in FAmu	--	Yes	Benign|Likely ber
Damaging	present in FAmu	Yes	Yes	Benign|Likely ber
Benign	present in FAmu	Yes	Yes	Benign|Likely ber
Benign	present in FAmu	Yes	Yes	Benign|not provi
Benign	present in FAmu	Yes	Yes	Benign|other|oth
Damaging	present in FAmu	Yes	Yes	Benign|other|oth
Benign	present in FAmu	Yes	Yes	Benign|other|Un
Benign	present in FAmu	--	Yes	Likely benign
Damaging	present in FAmu	--	Yes	Likely benign|Ber
Damaging	present in FAmu	--	Yes	Likely benign|Ber
Benign	present in FAmu	Yes	Yes	Likely benign|Ber
Damaging	present in FAmu	Yes	Yes	Likely benign|Ber
Damaging	present in FAmu	Yes	Yes	Likely benign|Ber
Benign	present in FAmu	Yes	Yes	Likely benign|Ber
Benign	present in FAmu	--	Yes	Likely benign|not
Benign	present in FAmu	Yes	Yes	Likely benign|oth
Indeterminate	present in FAmu	Yes	Yes	Likely benign|oth
Damaging	present in FAmu	Yes	Yes	Likely benign|oth
Benign	present in FAmu	Yes	Yes	Likely benign|Un
Damaging	present in FAmu	--	Yes	not provided
Benign	present in FAmu	--	Yes	not provided
Indeterminate	present in FAmu	--	Yes	not provided
Indeterminate	present in FAmu	Yes	Yes	not provided
Benign	present in FAmu	--	Yes	not provided
Indeterminate	present in FAmu	--	Yes	not provided
Benign	present in FAmu	--	Yes	not provided
Damaging	present in FAmu	--	Yes	not provided
Damaging	present in FAmu	--	Yes	not provided
Benign	present in FAmu	Yes	Yes	not provided
Damaging	present in FAmu	--	Yes	not provided
Benign	present in FAmu	--	Yes	not provided
Indeterminate	present in FAmu	--	Yes	not provided|Un

Damaging	present in FAmu	Yes	Yes	not provided|Un
Damaging	present in FAmu	--	Yes	not provided|Un
Indeterminate	present in FAmu	--	Yes	not provided|Un
Benign	present in FAmu	Yes	Yes	not provided|Un
Indeterminate	present in FAmu	Yes	Yes	other|Benign|Be
Benign	present in FAmu	Yes	Yes	other|Benign|ot
Damaging	present in FAmu	Yes	Yes	other|Likely beni
Benign	present in FAmu	Yes	Yes	other|Likely beni
Damaging	present in FAmu	Yes	Yes	other|Likely beni
Indeterminate	present in FAmu	Yes	Yes	other|Likely beni
Benign	present in FAmu	Yes	Yes	other|not provid
Damaging	present in FAmu	Yes	Yes	other|Uncertain
Benign	present in FAmu	Yes	Yes	other|Uncertain
Indeterminate	present in FAmu	Yes	Yes	other|Uncertain
Damaging	present in FAmu	Yes	Yes	Pathogenic
Damaging	present in FAmu	Yes	Yes	Pathogenic
Damaging	present in FAmu	Yes	Yes	Pathogenic|not p
Damaging	present in FAmu	--	Yes	Pathogenic|not p
Damaging	present in FAmu	Yes	Yes	Pathogenic|not p
Damaging	present in FAmu	--	Yes	Uncertain signific
Indeterminate	present in FAmu	--	Yes	Uncertain signific
Benign	present in FAmu	--	Yes	Uncertain signific
Damaging	present in FAmu	--	Yes	Uncertain signific
Benign	present in FAmu	--	Yes	Uncertain signific
Benign	present in FAmu	Yes	Yes	Uncertain signific
Benign	present in FAmu	Yes	Yes	Uncertain signific
Damaging	present in FAmu	Yes	Yes	Uncertain signific
Benign	present in FAmu	Yes	Yes	Uncertain signific
Indeterminate	present in FAmu	--	Yes	Uncertain signific
Damaging	present in FAmu	--	Yes	Uncertain signific
Indeterminate	present in FAmu	Yes	Yes	Uncertain signific
Indeterminate	present in FAmu	Yes	Yes	Uncertain signific
Damaging	present in FAmu	Yes	Yes	Uncertain signific
Damaging	present in FAmu	Yes	Yes	Uncertain signific
Indeterminate	present in FAmu	--	Yes	Uncertain signific
Damaging	present in FAmu	Yes	Yes	Uncertain signific
Damaging	present in FAmu	Yes	Yes	Uncertain signific
Indeterminate	present in FAmu	--	Yes	Uncertain signific

				NHLBI-ESP6500		
			# Individuals	6503	2203	4300
linVar_DB	BIC	BIC_significance	ARC - BRCA2 (Ta	esp6500_all	esp6500_aa	
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--	--	--	0	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0.0002	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0.0006	0
--	--	--	0.0008	0
--	--	--	0.0007	0.0005
--	--	--	0.0012	0.0009
--	--	--	0.0002	0
--	--	--	0.0021	0.0002
--	--	--	0.0006	0.0018
--	--	--	0.000077	0.0002
--	--	--	0.0009	0.0005
--	--	--	0.0002	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0	0
--	--	--	0.0008	0.0002
--	--	--	0.0002	0
--	--	--	0.0014	0.0041
--	--	--	0	0
--	--	--	0	0
--	--	--	0.0002	0

--	--	--	0.0018	0.0005
--	--	--	0.0006	0.0005
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0.000077	0
--	--	--	0.0009	0.0005
--	--	--	0.0002	0.0002
--	--	--	0.0017	0.0005
--	--	--	0.000077	0
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0.0004	0
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0.000077	0
--	--	--	0.000077	0
--	--	--	0.0005	0.0002
--	--	--	0.0172	0.0505
--	--	--	0.0175	0.0516
--	--	--	0.000077	0.0002
--	--	--	0.0024	0.0007
--	--	--	0.000077	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0	0
--	--	--	0.0002	0
--	--	--	0.0015	0.0005
--	--	--	0.0021	0.0061
--	--	--	0	0
--	--	--	0.000077	0.0002
--	--	--	0.000077	0.0002
--	--	--	0.000077	0
--	--	--	0.000077	0
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0.000077	0
--	--	--	0.000077	0
--	--	--	0.0002	0
--	--	--	0	0
--	--	--	0.000077	0
--	--	--	0.0002	0
--	--	--	0	0
--	--	--	0.0003	0.0005

--	--	--	--	0	0
--	--	--	--	0.0023	0.0007
--	--	--	--	0.0002	0.0002
--	--	--	--	0	0
--	--	--	--	0.0002	0
--	--	--	--	0.0002	0.0002
--	--	--	--	0	0
--	--	--	--	0	0
--	--	--	--	0	0
--	--	--	--	0	0
Fanconi_a	--	--	--	0.000077	0.0002
not_speci	--	--	--	0.005	0.0014
not_provi	Yes	Class 1 - Not Pat	--	0.0044	0.0018
Breast-ov	Yes	unknown	1 - Not pathoger	0.0033	0.0068
Hereditary	Yes	unknown	1 - Not pathoger	0.0003	0
Hereditary	--	--	--	0.0013	0.0007
Familial_c	Yes	unknown	1 - Not pathoger	0.0005	0
Breast-ov	Yes	unknown	1 - Not pathoger	0	0
Familial_c	--	--	--	0.0097	0.0287
Hereditary	Yes	not clinically imp	--	0.0007	0.0002
Hereditary	Yes	not clinically imp	--	0.0004	0.0002
Hereditary	Yes	unknown	--	0	0
Fanconi_a	--	--	--	0.0046	0.0136
not_speci	--	--	--	0.0082	0.0239
not_speci	--	--	--	0.0036	0.0011
Familial_c	Yes	unknown	1 - Not pathoger	0	0
Familial_c	Yes	unknown	1 - Not pathoger	0.0002	0.0002
not_provi	Yes	not clinically imp	1 - Not pathoger	0.0011	0.0002
not_provi	Yes	not clinically imp	--	0.0033	0.0009
Hereditary	--	--	--	0.0014	0.0005
Familial_c	Yes	unknown	--	0.0002	0
Familial_c	Yes	unknown	--	0.0012	0.0036
Familial_c	Yes	unknown	--	0.000077	0
Hereditary	--	--	--	0.001	0.0002
not_speci	--	--	--	0.0071	0.0207
not_speci	--	--	--	0.0007	0
not_speci	--	--	--	0	0
not_speci	--	--	--	0.0005	0.0002
not_speci	--	--	--	0	0
not_speci	--	--	--	0	0
not_speci	--	--	--	0.0009	0
not_speci	--	--	--	0.0008	0.0005
not_speci	--	--	--	0.002	0.0007
not_speci	--	--	--	0.0005	0.0014
not_speci	--	--	--	0.0031	0.0075
not_speci	--	--	--	0.0003	0
Familial_c	Yes	unknown	--	0	0

Familial_c	Yes	unknown	1 - Not pathoger	0.000077	0
not_speci	--		--	0.0006	0.0007
Familial_c	Yes	unknown	--	0	0
Familial_c	Yes	unknown	--	0	0
Breast-ova	Yes	unknown	1 - Not pathoger	0	0
Breast-ova	Yes	Class 1 - Not Pat	--	0.0016	0.0043
Breast-ova	Yes	not clinically imp	2 - Likely not pat	0.0015	0.0005
Breast-ova	Yes	unknown	--	0	0
Breast-ova	Yes	unknown	2 - Likely not pat	0	0
Familial_c	--		--	0.0024	0.0009
Breast-ova	Yes	unknown	--	0	0
Familial_c	--		--	0.000077	0
Familial_c	--		--	0.0002	0.0002
Familial_c	--		--	0.0007	0.002
Breast-ova	Yes	Class 5 - Pathoge	5 - Definitely pat	0	0
Fanconi_a	--		--	0.000077	0
Fanconi_a	--		--	0.000077	0
Breast-ova	--		5 - Definitely pat	0	0
XERODERI	--		--	0.0008	0.0002
Hereditary	--		--	0.000077	0
Familial_c	--		--	0	0
Hereditary	--		--	0	0
Hereditary	--		--	0	0
Familial_c	--		--	0	0
not_provi	Yes	not clinically imp	--	0.0015	0.0009
not_provi	Yes	unknown	1 - Not pathoger	0.0025	0.0009
Hereditary	--		--	0.0031	0.0023
not_provi	Yes	unknown	--	0	0
Hereditary	--		--	0	0
Familial_c	Yes	unknown	--	0	0
Familial_c	--		--	0	0
Hereditary	--		--	0.000077	0
Familial_c	--		--	0	0
Fanconi_a	--		--	0	0
Hereditary	--		--	0.000077	0
Familial_c	Yes	unknown	--	0.000077	0
Familial_c	Yes	unknown	2 - Likely not pat	0.000077	0
Breast-ova	--		--	0	0

1000Genomes			
2504	661	503	347
esp6500_ea	**1000g2015aug_all**	**1000g2015aug_afr**	**1000g2015aug_eur**
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0

0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
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0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0.0002	0.000199681	0	0.001
0	0	0	0
0	0	0	0
0	0	0	0
0.0009	0.000199681	0	0.001
0.0013	0.000199681	0	0.001
0.0008	0.000199681	0.0008	0
0.0013	0.000199681	0.0008	0
0.0003	0.000199681	0	0
0.003	0.000399361	0	0
0	0.00219649	0.0083	0
0	0	0	0
0.0012	0.000998403	0	0.003
0.0003	0	0	0
0	0.000199681	0	0.001
0	0.000199681	0	0.001
0.0001	0	0	0
0	0	0	0
0.001	0.000599042	0	0.003
0.0002	0	0	0
0	0.000199681	0	0
0	0	0	0
0	0	0	0
0.0003	0	0	0

0.0026	0.000399361	0	0.002
0.0007	0	0	0
0	0.000199681	0	0
0.0001	0	0	0
0	0	0	0
0.0001	0	0	0
0.0001	0	0	0
0.0012	0	0	0
0.0001	0	0	0
0.0023	0	0	0
0.0001	0	0	0
0	0	0	0
0.0001	0	0	0
0.0006	0.000798722	0	0.004
0	0	0	0
0.0001	0	0	0
0.0001	0.000199681	0	0.001
0.0001	0	0	0
0.0007	0.000399361	0	0.002
0.0001	0.0181709	0.0681	
0.0001	0.0185703	0.0696	
0	0.000199681	0	0
0.0033	0.000798722	0	0.001
0.0001	0	0	0
0	0.000399361	0	0.001
0	0	0	0
0	0	0	0
0.0001	0	0	0
0	0	0	0
0.0003	0	0	0
0.002	0.000399361	0	0.002
0	0.00199681	0.0076	0
0	0	0	0
0	0	0	0
0	0.000199681	0.0008	0
0.0001	0.000199681	0	0
0.0001	0.000399361	0	0
0	0	0	0
0.0001	0.000199681	0	0.001
0.0001	0	0	0
0.0001	0	0	0
0.0002	0.000599042	0	0.001
0	0	0	0
0.0001	0.000199681	0	0
0.0002	0	0	0
0	0	0	0
0.0002	0.000199681	0.0008	0

0	0.000199681	0	0
0.0031	0.00119808	0	0.005
0.0001	0	0	0
0	0	0	0
0.0003	0	0	0
0.0001	0	0	0
0	0.000199681	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0.00559105	0	0
0.0069	0.00259585	0.0008	0.0089
0.0057	0.00998403	0.0008	0.003
0.0015	0.00379393	0.0136	0.001
0.0005	0.000199681	0	0
0.0016	0.000399361	0	0.002
0.0008	0.00119808	0	0.004
0	0.000399361	0	0.002
	0.0115815	0.0303	
0.0009	0.000399361	0.0008	0.001
0.0005	0	0	0
0	0	0	0
	0.00658946	0.0234	
0.0001	0.00858626	0.031	
0.0049	0.00159744	0	0.007
0	0	0	0
0.0002	0.000599042	0.0023	0
0.0015	0	0	0
0.0045	0.000599042	0	0.002
0.0019	0.000798722	0	0.001
0.0002	0	0	0
0	0.000998403	0.0038	0
0.0001	0	0	0
0.0014	0	0	0
0.0001	0.00978435	0.0287	
0.001	0.00119808	0	0.002
0	0	0	0
0.0006	0.000199681	0	0.001
0	0	0	0
0	0.00519169	0	0
0.0014	0.000399361	0	0.002
0.001	0.000998403	0	0.003
0.0027	0	0	0
0.0001	0.0109824	0.0008	0
0.0008	0.00379393	0.0129	0.001
0.0005	0	0	0
0	0	0	0

0.0001	0	0	0
0.0006	0	0	0
0	0	0	0
0	0.000199681	0.0008	0
0	0	0	0
0.0002	0.0159744	0.0023	
0.002	0.000199681	0	0.001
0	0	0	0
0	0.000798722	0	0
0.0031	0.00119808	0	0.002
0	0	0	0
0.0001	0	0	0
0.0001	0.000199681	0	0
0	0	0	0
0	0	0	0
0.0001	0	0	0
0.0001	0	0	0
0	0	0	0
0.001	0	0	0
0.0001	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0.0017	0.000599042	0	0.003
0.0033	0.000798722		0.003
0.0035	0	0	0
0	0.000998403	0	0
0	0.00339457	0	0
0	0	0	0
0	0	0	0
0.0001	0	0	0
0	0.000599042	0	0
0	0	0	0
0.0001	0	0	0
0.0001	0	0	0
0.0001	0	0	0
0	0	0	0

	ExAC_nonTCGA frequencies		
504	60706	5203	5789
1000g2015aug_amr	1000g2015aug_eas	ExAC_nontcga_ALL	ExAC_nontcga_AFR
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0

0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0.0002	0.0001
0	0	0.00006591	0
0	0	0.00001883	0.0001
0	0	0	0
0	0	0.0006204	0.0002237
0	0	0.0003	0
0	0	0.0009	0.0003
0	0	0.0012	0.0008
0.0014	0	0.0006	0.0001
0.0029	0	0.0015	0.0003
0	0	0.0004	0.0042
0	0	0.00002825	0
0.0029	0	0.0004	0
0	0	0.0001	0
0	0	0.0004	0
0	0	0.00003775	0
0	0	0.00003965	0
0	0	0.00008548	0
0	0	0.0022	0.0002
0	0	0	0
0.0014	0	0.0003	0.0035
0	0	0.00007535	0
0	0	0.00001905	0
0	0	0.0004	0.0002

0	0	0.0015	0.0004
0	0	0.0002	0
0	0	0.00002825	0
0	0	0.000009466	0
0	0	0.0005	0
0	0	0.00005673	0
0	0	0.000009423	0
0	0	0.0009	0.0004
0	0	0.0001	0
0	0	0.001	0.0005
0	0	0.000009493	0
0	0	0.00006713	0
0	0	0.0001	0
0	0	0.0014	0.0001
0	0	0.00001887	0
0	0	0.00001883	0.0001
0	0	0.0000377	0
0	0	0.00001883	0
0	0	0.0005	0.0001
0.0014		0.005	0.0538
0.0014		0.005	0.0554
0	0.001	0.0002	0.0002
0.0029	0	0.002	0.0003
0	0	0.000009415	0
0	0	0.0001	0
0	0	0.00002825	0
0	0	0.00002825	0
0	0	0.00001256	0
0	0	0.0001	0
0	0	0.00008515	0
0	0	0.0009	0.0005
0	0	0.0007	0.0083
0	0	0.00002917	0
0	0	0.0001	0.0001
0	0	0.00003855	0.0002
0	0	0.0002	0
0	0	0.0007	0
0	0	0.0000754	0.0001
0	0	0.00001979	0
0	0	0.000009419	0
0	0	0.00001883	0
0	0	0.0006	0
0	0	0.00001883	0
0.0014	0	0.0002	0.0001
0	0	0.0002	0
0	0	0.0000471	0
0	0	0.0003	0.0007

0	0.001	0	0
0.0014	0	0.0026	0.0009
0	0	0.00002828	0.0001
0	0	0.00001887	0
0	0	0.0002	0
0	0	0	0
0	0	0.00002831	0
0	0	0.0003	0
0	0	0.000009428	0
0	0	0.00001352	0
0.0014	0	0.0039	0.0001
0.0014	0	0.0043	0.0009
0.036		0.0083	0.001
0	0	0.0012	0.009
0.0014	0	0.0002	0.0002
0	0	0.0011	0.0003
0.0029	0	0.0006	0.0001
0	0	0.0003	0.0001
0.0014	0.0169	0.0035	0.0297
0	0	0.0005	0.0001
0	0	0.0004	0.0002
0	0	0.0003	0
0.0029		0.0017	0.0173
0.0029		0	0.02555
0.0014	0	0.0025	0.0017
0	0	0.00002885	0
0	0	0.0004	0.0002
0	0	0.0007	0.0001
0.0014	0	0.0019	0.0009
0.0014	0	0.0016	0.0003
0	0	0.00006678	0
0	0	0.0004	0.0031
0	0	0.00004724	0
0	0	0.0009	0.0003
0.0058	0.003	0.0031	0.024
0.0043	0	0.0009	0.0001
0	0	0.0001	0
0	0	0.0002	0.0001
0	0	0.00001012	0
0	0	0.0032	0
0	0	0.0005	0
0.0029	0	0.0005	0.0001
0	0	0.0013	0.0001
0	0	0.0059	0.0011
0.0014	0	0.0016	0.0131
0	0	0.0002	0.0001
0	0	0	0

0	0	0.00000944	0
0	0	0.0004	0.0002
0	0	0	0
0	0	0.00002834	0.0001
0	0	0.0002	0
0.1066	0.003	0.0159	0.0023
0	0	0.0008	0.0006
0	0	0.000009702	0
0	0.004	0.0001	0
0.0058	0	0.0024	0.0004
0	0	0.00001899	0
0	0	0.0002	0
0.0014	0	0.0002	0
0	0	0.0002	0.0016
0	0	0	0
0	0	0	0
0	0	0.00000951	0
0	0	0	0
0	0	0.0004	0.0002
0	0	0.00001891	0
0	0	0.000009418	0
0	0	0	0
0	0	0.00001883	0
0	0	0	0
0	0	0.0024	0
0.0014		0.0016	0.0002
0	0	0.0018	0.0007
0	0	0.0005	0
0	0	0.0034	0.0001
0	0	0.00001939	0.0001
0	0	0.0000565	0
0	0	0.00004717	0
0	0.002	0.00002825	0
0	0	0.00004708	0
0	0	0.00002825	0
0	0	0.00004859	0
0	0	0.00001885	0
0	0	0	0

4327	33370	
ExAC_nontcga_AMR	:_nontcga_	:_nontcga_NFE
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0	0	0
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0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
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0	0	0
0	0	0
0.00008921	0	0.0003
0.0003	0	7.36E-05
0	0	1.84E-05
0	0	0
8.72E-05	0	0.00099
0.0002	0	0.0005
0	0	0.0016
0.0007	0	0.0019
0.0017	0	0.0008
0.001	0	0.0025
0.0003	0	0
0	0	5.52E-05
0.0000897	0	0.0007
0	0	0.0002
0.0026	0	0.0002
0.00008961	0	5.53E-05
0	0	7.74E-05
0.0008	0	0
0	0	0.0022
0	0	0
0.0004	0	0
0	0	0.0001
0	0	0
0	0	0.0008

0.0004	0	0.0024
0	0	0.0003
0	0	3.68E-05
0	0	1.85E-05
0.0045	0	0
0	0	0.0001
0	0	1.84E-05
0.0022	0	0.0011
0.0008	0	4.2E-05
0.0003	0	0.0017
0	0	1.85E-05
0	0	5.58E-05
0	0	7.37E-05
0.00008929	0	0.0016
0	0	3.69E-05
0	0	1.84E-05
0	0	7.37E-05
0	0	3.68E-05
0.00008916	0	0.0009
0.0025	0	0.0001
0.0025	0	0.0001
0.00008925	0.0001	0.0002
0.0008	0	0.0033
0	0	1.84E-05
0	0	0.0001
0	0	5.52E-05
0.0003	0	0
0.0001	0	0
0	0	1.84E-05
0	0	0.0002
0.0007	0	0.0013
0.0003	0	1.89E-05
0	0	0
0	0	0
0.00009189	0	1.87E-05
0	0	0.0003
0.0004	0	0.0001
0	0	0.0001
0	0	3.86E-05
0	0	1.84E-05
0	0	3.68E-05
0.0002	0	0.0002
0.0002	0	0
0.0002	0	0.0003
0.001	0	0.0001
0	0	9.2E-05
0	0	0.0005

0	0	0
0.002	0	0.0039
0	0	3.69E-05
0	0	0
0	0	0.0004
0	0	0
0	0	1.84E-05
0	0	0.0007
0	0	1.84E-05
0	0	2.69E-05
0.0003	0	0
0.0031	0	0.0065
0.0349	0	0.0044
0.0007	0	0.0008
0.00008921	0	0.0003
0.0003	0	0.0017
0.0019	0	0.0007
0.0011	0	0.0003
0.0012	0.0104	1.84E-05
0.0006	0	0.0008
0	0	0.0007
0	0.0034	0
0.0014	0	7.36E-05
0.0005197	0	4.54E-05
0.0004	0	0.0039
0	0	5.66E-05
0	0	0.0007
0.0005	0	0.001
0.0002	0	0.0033
0.001	0	0.002
0	0	0.0001
0.0009	0	1.84E-05
0.00008917	0	7.36E-05
0.0008	0	0.0013
0.0021	0.0032	0.0004
0.0009	0	0.0014
0.0004	0	5.52E-05
0.00008917	0	0.0003
0	0	1.98E-05
0	0	1.84E-05
0	0	0.001
0.001	0	0.0007
0.0017	0	0.0021
0.0002	0.0001	7.37E-05
0.0005	0	0.0007
0	0	0.0003
0	0	0

0	0	1.84E-05
0.0011	0	0.0005
0	0	0
0	0.0003	0
0.0013	0	7.42E-05
0.1421	0.0037	0.0002
0	0	0.0013
0	0	1.88E-05
0	0.0013	0
0.0027	0	0.004
0	0	3.71E-05
0.0017	0	3.68E-05
0.0003	0	0.0002
0.0003	0	0
0	0	0
0	0	0
0	0	1.85E-05
0	0	0
0	0.0003	0.0006
0	0	3.69E-05
0.00008925	0	0
0	0	0
0.00008917	0	1.84E-05
0	0	0
0.0002	0	0.0037
0.0007	0.0001	0.0023
0	0	0.0031
0	0	0
0.0004	0	2.74E-05
0	0	1.88E-05
0	0.0006	0
0	0	9.22E-05
0	0	1.84E-05
0	0	7.36E-05
0	0	5.52E-05
0	0	9.59E-05
0	0	3.68E-05
0	0	0

Supplemental Table 4. Patients with rare variants in two or more different FA g

patientID	# of variants	smoke (1=current; 2=former; 3-never)
A1917	2	1
A1917		
A1999	2	3
A1999		
A2083	3	1
A2083		
A2083		
A2154	2	1
A2154		
A2163	2	3
A2163		
A2236	2	3
A2236		
A2270	4	3
A2270		
A2270		
A2270		
A2322	2	3
A2322		
A2967	2	2
A2967		
A3023	4	2
A3023		
A3023		
A3023		
A3159	3	1
A3159		
A3159		
A3296	2	3
A3296		
A3421	2	1
A3421		
A3446	2	1
A3446		
A3623	3	3
A3623		
A3623		
A3630	2	3
A3630		
A3640	2	3
A3640		
A3769	2	3
A3769		

A4001	2	2
A4001		
A4025	2	3
A4025		
A4143	2	3
A4143		
A4303	2	2
A4303		
A4419	2	3
A4419		
A4603	2	3
A4603		
A4613	2	3
A4613		
A4636	2	3
A4636		
A4655	2	3
A4655		
A4676	2	3
A4676		
A4764	2	1
A4764		
A4830	2	3
A4830		
A4894	2	3
A4894		
A4932	2	2
A4932		
A5074	2	3
A5074		
A5086	2	2
A5086		
A5095	2	3
A5095		
A5098	3	3
A5098		
A5098		
A5159	2	1
A5159		
A5730	2	0
A5730		
A5823	3	2
A5823		
A5823		

genes

Patient details					
alcohol (1=current; 2=former; 3-never)	HPV Status (0 = N	cancerSite	ethnicity	age	
3	0	Oral Cavity	W	38	
1	--	Oropharynx	W	45	
1	--	Larynx	A	49	
1	--	Oropharynx	H	43	
3	--	Oropharynx	W	40	
3	0	Oral Cavity	W	42	
3	--	Oral Cavity	A	31	
3	--	Oropharynx	W	44	
1	0	Oropharynx	W	46	
3	0	Oral Cavity	H	36	
3	--	Larynx	W	48	
1	0	Oral Cavity	H	36	
3	--	Oral Cavity	W	47	
1	--	Oropharynx	W	49	
1	--	Oral Cavity	W	36	
1	--	Oral Cavity	W	49	
3	0	Oral Cavity	W	43	
2	1	Oropharynx	H	42	

3	--	Larynx	W	49
3	1	Oral Cavity	A	49
1	1	Oropharynx	W	47
3	--	Oral Cavity	A	42
1	--	Oropharynx	W	45
3	--	Oral Cavity	A	46
1	1	Oropharynx	W	45
1	1	Oral Cavity	W	32
1	0	Oral Cavity	W	49
3	--	Oral Cavity	H	35
1	--	Oropharynx	W	46
3	0	Oropharynx	W	48
3	1	Oropharynx	W	40
1	--	Oropharynx	W	47
3	1	Larynx	B	46
3	1	Oropharynx	W	49
3	0	Oral Cavity	W	49
2	--	Larynx	W	41
2	--	Oropharynx	W	48
0	--	Oral Cavity	W	34
1	--	Oral Cavity	W	41

		Varia						
ageGroup	sex	chr	start	end	ref	alt	gene	cDNA
40less	F	16	3642722	3642722	C	G	SLX4	c.2305G>C
		14	45605551	45605551	A	G	FANCM	c.317A>G
45-49	F	16	3639265	3639265	C	A	SLX4	c.4374G>T
		16	89842176	89842176	C	G	FANCA	c.1874G>C
45-49	M	13	32930598	32930598	T	C	BRCA2	c.7469T>C
		17	59924572	59924572	G	A	BRIP1	c.517C>T
		14	45658326	45658326	C	T	FANCM	c.5101C>T
40-44	M	16	89816220	89816220	G	A	FANCA	c.3157C>T
		2	58387285	58387286	CT	-	FANCL	c.1049_1050del
40-44	M	13	32929047	32929047	G	C	BRCA2	c.7057G>C
		15	89825056	89825056	A	G	FANCI	c.1573A>G
40-44	M	14	45605405	45605405	G	C	FANCM	c.171G>C
		16	89877157	89877157	C	T	FANCA	c.480G>A
40less	M	2	58390589	58390589	A	C	FANCL	c.755T>G
		9	35079445	35079445	T	C	FANCG	c.77A>G
		17	56780607	56780607	A	G	RAD51C	c.622A>G
		16	3644515	3644515	C	T	SLX4	c.2099G>A
40-44	M	16	3639991	3639991	C	A	SLX4	c.3648G>T
		13	32911175	32911175	G	A	BRCA2	c.2683G>A
45-49	F	13	32893271	32893271	A	G	BRCA2	c.125A>G
		16	23641406	23641406	T	C	PALB2	c.2069A>G
40less	F	16	3647490	3647490	G	A	SLX4	c.1573C>T
		17	59924572	59924572	G	A	BRIP1	c.517C>T
		3	10107551	10107551	G	C	FANCD2	c.2273G>C
		2	58386933	58386933	-	AATT	FANCL	c.1094_1095insAATT
45-49	M	16	23647211	23647211	T	C	PALB2	c.656A>G
		15	89811698	89811698	T	C	FANCI	c.824T>C
		16	3641280	3641280	C	T	SLX4	c.2359G>A
40less	F	9	98011571	98011571	C	T	FANCC	c.3G>A
		16	89865628	89865628	G	A	FANCA	c.839C>T
45-49	F	3	10070369	10070369	T	C	FANCD2	c.28T>C
		16	3639378	3639378	T	A	SLX4	c.4261A>T
45-49	M	2	58386933	58386933	-	AATT	FANCL	c.1094_1095insAATT
		15	89811698	89811698	T	C	FANCI	c.824T>C
40less	M	17	59926512	59926512	C	T	BRIP1	c.485G>A
		6	35426122	35426122	G	C	FANCE	c.1018G>C
		16	3647691	3647691	T	C	SLX4	c.1372A>G
45-49	M	15	89835982	89835982	C	A	FANCI	c.2056C>A
		9	97864005	97864005	A	G	FANCC	c.1661T>C
40-44	F	13	32953550	32953550	G	A	BRCA2	c.8851G>A
		17	59821830	59821830	C	A	BRIP1	c.2220G>T
40-44	M	3	10107587	10107587	A	G	FANCD2	c.2309A>G
		15	89849316	89849316	C	T	FANCI	c.3428C>T

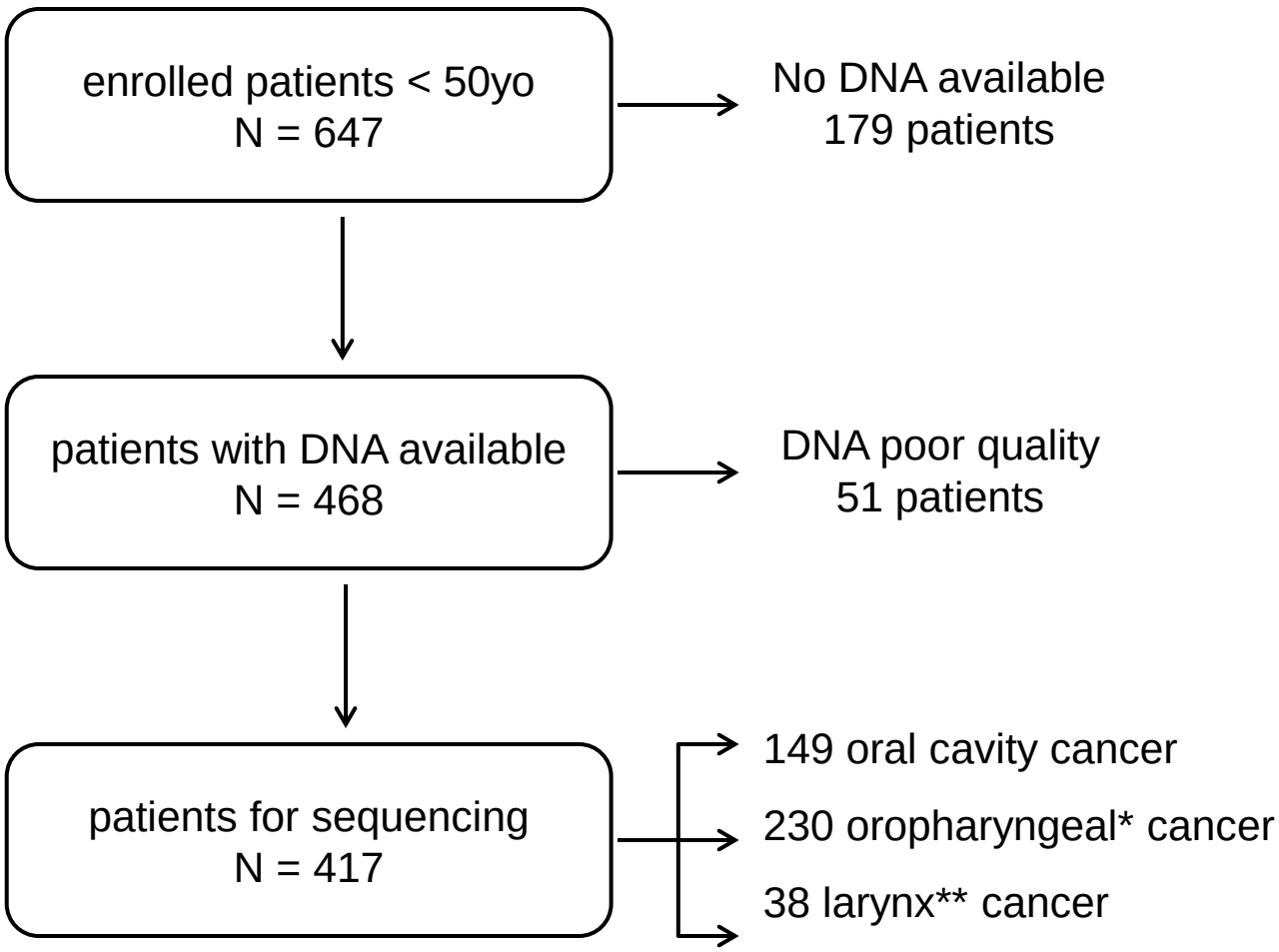
45-49	M	16	14041578	14041578	G	A	ERCC4	c.2125G>A
		15	89825056	89825056	A	G	FANCI	c.1573A>G
45-49	M	13	32930598	32930598	T	C	BRCA2	c.7469T>C
		3	10074646	10074646	G	C	FANCD2	c.195G>C
45-49	M	9	35075738	35075738	G	T	FANCG	c.1157C>A
		16	23646872	23646872	A	T	PALB2	c.995T>A
40-44	M	13	32913271	32913271	A	C	BRCA2	c.4779A>C
		11	22646921	22646921	G	C	FANCF	c.436C>G
45-49	M	14	45605405	45605405	G	C	FANCM	c.171G>C
		16	14029516	14029516	G	C	ERCC4	c.1727G>C
45-49	F	9	35074140	35074140	A	G	FANCG	c.1834T>C
		17	59870998	59870998	T	C	BRIP1	c.1433A>G
45-49	M	13	32954018	32954018	G	A	BRCA2	c.9085G>A
		16	3640461	3640461	G	A	SLX4	c.3178C>T
40less	M	16	89877386	89877386	G	C	FANCA	c.377C>G
		13	32945172	32945172	A	C	BRCA2	c.8567A>C
45-49	F	14	45605730	45605730	G	A	FANCM	c.496G>A
		16	3639378	3639378	T	A	SLX4	c.4261A>T
40less	F	9	35077006	35077006	G	T	FANCG	c.739C>A
		14	45628478	45628478	C	G	FANCM	c.1576C>G
45-49	M	14	45605405	45605405	G	C	FANCM	c.171G>C
		15	89825056	89825056	A	G	FANCI	c.1573A>G
45-49	M	13	32900252	32900252	A	G	BRCA2	c.440A>G
		16	23641186	23641186	C	G	PALB2	c.2289G>C
40-44	F	2	58386933	58386933	-	AATT	FANCL	c.1094_1095insAATT
		9	35075959	35075959	C	G	FANCG	c.1143G>C
45-49	M	14	45645661	45645661	G	T	FANCM	c.3704G>T
		17	59885856	59885856	T	C	BRIP1	c.890A>G
45-49	M	14	45669125	45669127	GAG	-	FANCM	c.6061_6063del
		6	35424011	35424013	GGA	-	FANCE	c.736_738del
45-49	F	13	32953550	32953550	G	A	BRCA2	c.8851G>A
		16	23637715	23637715	G	A	PALB2	c.2590C>T
45-49	F	16	89877377	89877377	G	A	FANCA	c.386C>T
		16	14041570	14041570	T	C	ERCC4	c.2117T>C
40-44	M	16	89871709	89871709	C	T	FANCA	c.688G>A
		3	10085255	10085255	-	TGGA	FANCD2	c.1077_1078insTGGA
		16	3640461	3640461	G	A	SLX4	c.3178C>T
45-49	F	13	32907407	32907407	A	G	BRCA2	c.1792A>G
		17	59761303	59761303	C	T	BRIP1	c.3104G>A
40less	M	13	32907407	32907407	A	G	BRCA2	c.1792A>G
		17	56774172	56774172	G	A	RAD51C	c.523G>A
40-44	M	17	59924505	59924505	A	G	BRIP1	c.584T>C
		14	45605397	45605397	G	A	FANCM	c.163G>A
		16	3656645	3656645	A	G	SLX4	c.590T>C

ant details

protein	predictionConsensus	databasePresence
p.E769Q	Benign	unreported rare variants
p.N106S	Damaging	unreported rare variants
p.M1458I	Benign	novel
p.C625S	Damaging	unreported rare variants
p.I2490T	Benign	present in FAmutDB/ClinVar/BIC
p.R173C	Damaging	present in FAmutDB/ClinVar/BIC
p.Q1701X	Damaging	unreported rare variants
p.R1053C	Benign	unreported rare variants
p.Q355fs	Damaging	novel
p.G2353R	Damaging	present in FAmutDB/ClinVar/BIC
p.M525V	Damaging	unreported rare variants
p.L57F	Benign	unreported rare variants
p.M160I	Benign	present in FAmutDB/ClinVar/BIC
p.F257C	Benign	present in FAmutDB/ClinVar/BIC
p.Q26R	Indeterminate	present in FAmutDB/ClinVar/BIC
p.I208V	Indeterminate	unreported rare variants
p.G700E	Damaging	unreported rare variants
p.Q1216H	Benign	unreported rare variants
p.A895T	Benign	present in FAmutDB/ClinVar/BIC
p.Y42C	Benign	present in FAmutDB/ClinVar/BIC
p.Q690R	Benign	novel
p.R525C	Benign	unreported rare variants
p.R173C	Damaging	present in FAmutDB/ClinVar/BIC
p.C758S	Damaging	unreported rare variants
p.P370fs	Damaging	novel
p.D219G	Benign	present in FAmutDB/ClinVar/BIC
p.I275T	Damaging	unreported rare variants
p.E787K	Indeterminate	present in FAmutDB/ClinVar/BIC
p.M1I	Damaging	novel
p.A280V	Damaging	unreported rare variants
p.S10P	Indeterminate	unreported rare variants
p.I1421F	Indeterminate	present in FAmutDB/ClinVar/BIC
p.P370fs	Damaging	novel
p.I275T	Damaging	unreported rare variants
p.R162Q	Damaging	present in FAmutDB/ClinVar/BIC
p.G340R	Indeterminate	present in FAmutDB/ClinVar/BIC
p.K458E	Damaging	present in FAmutDB/ClinVar/BIC
p.Q686K	Benign	present in FAmutDB/ClinVar/BIC
p.L554P	Damaging	present in FAmutDB/ClinVar/BIC
p.A2951T	Damaging	present in FAmutDB/ClinVar/BIC
p.Q740H	Damaging	present in FAmutDB/ClinVar/BIC
p.K770R	Benign	unreported rare variants
p.T1143I	Damaging	unreported rare variants

p.V709M	Damaging	unreported rare variants
p.M525V	Damaging	unreported rare variants
p.I2490T	Benign	present in FAmutDB/ClinVar/BIC
p.Q65H	Damaging	present in FAmutDB/ClinVar/BIC
p.P386H	Benign	present in FAmutDB/ClinVar/BIC
p.L332H	Indeterminate	present in FAmutDB/ClinVar/BIC
p.E1593D	Benign	present in FAmutDB/ClinVar/BIC
p.L146V	Damaging	unreported rare variants
p.L57F	Benign	unreported rare variants
p.R576T	Damaging	present in FAmutDB/ClinVar/BIC
p.F612L	Benign	novel
p.H478R	Indeterminate	present in FAmutDB/ClinVar/BIC
p.A3029T	Damaging	present in FAmutDB/ClinVar/BIC
p.R1060W	Indeterminate	present in FAmutDB/ClinVar/BIC
p.T126R	Benign	unreported rare variants
p.E2856A	Damaging	present in FAmutDB/ClinVar/BIC
p.A166T	Damaging	unreported rare variants
p.I1421F	Indeterminate	present in FAmutDB/ClinVar/BIC
p.Q247K	Damaging	unreported rare variants
p.L526V	Indeterminate	unreported rare variants
p.L57F	Benign	unreported rare variants
p.M525V	Damaging	unreported rare variants
p.Q147R	Benign	present in FAmutDB/ClinVar/BIC
p.L763F	Indeterminate	present in FAmutDB/ClinVar/BIC
p.P370fs	Damaging	novel
p.R381S	Indeterminate	novel
p.G1235V	Damaging	unreported rare variants
p.K297R	Indeterminate	present in FAmutDB/ClinVar/BIC
p.2021_2021del	Damaging	novel
p.246_246del	Damaging	novel
p.A2951T	Damaging	present in FAmutDB/ClinVar/BIC
p.P864S	Indeterminate	present in FAmutDB/ClinVar/BIC
p.A129V	Benign	unreported rare variants
p.I706T	Damaging	present in FAmutDB/ClinVar/BIC
p.V230I	Benign	unreported rare variants
p.T359fs	Damaging	novel
p.R1060W	Indeterminate	present in FAmutDB/ClinVar/BIC
p.T598A	Benign	present in FAmutDB/ClinVar/BIC
p.R1035H	Indeterminate	present in FAmutDB/ClinVar/BIC
p.T598A	Benign	present in FAmutDB/ClinVar/BIC
p.A175T	Damaging	present in FAmutDB/ClinVar/BIC
p.L195P	Benign	present in FAmutDB/ClinVar/BIC
p.D55N	Damaging	unreported rare variants
p.V197A	Benign	present in FAmutDB/ClinVar/BIC

Supplemental Figure 1. Enrolled patients under 50 years of age with incident HNSCC and reason for exclusion from sequencing analysis.



*Includes 5 patients with upper neck lymph node metastasis with unidentified primary site.
**Includes 3 patients with hypopharyngeal cancer.