DETERMINING THE CLINICAL SIGNIFICANCE OF *BRCA1* AND *BRCA2* INTRONIC AND EXONIC SPLICING VARIANTS

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BACKGROUND

- Nucleotide changes which are predicted by *in silico* splicing analysis to affect mRNA splicing are sometimes identified during diagnostic sequencing analysis of the *BRCA1* and *BRCA2* genes.
- *In silico* splicing analysis tools should be used with caution as their use can result in incorrect variant interpretation, potentially leading to inappropriate medical management decisions.
- We describe the algorithms used by our laboratory to determine possible pathogenicity of intronic and exonic variants predicted by *in silico* analysis to result in abnormal mRNA splicing.

METHODS

- Clinical germline testing for *BRCA1* and *BRCA2* sequencing mutations was performed on extracted patient genomic DNA, after informed consent was obtained.
- Sanger or next generation sequencing analyses of *BRCA1* and *BRCA2* identified nucleotide changes predicted by *in silico* splicing analysis to result in abnormal mRNA splicing.
- Using our variant classification and reclassification processes, which include multiple methodologies for variant evaluation and interpretation (Figure 1, Table 1), we further investigated the pathogenicity of these putative splicing variants.

Figure 1. Basic algorithm used to classify potential mRNA splicing variants

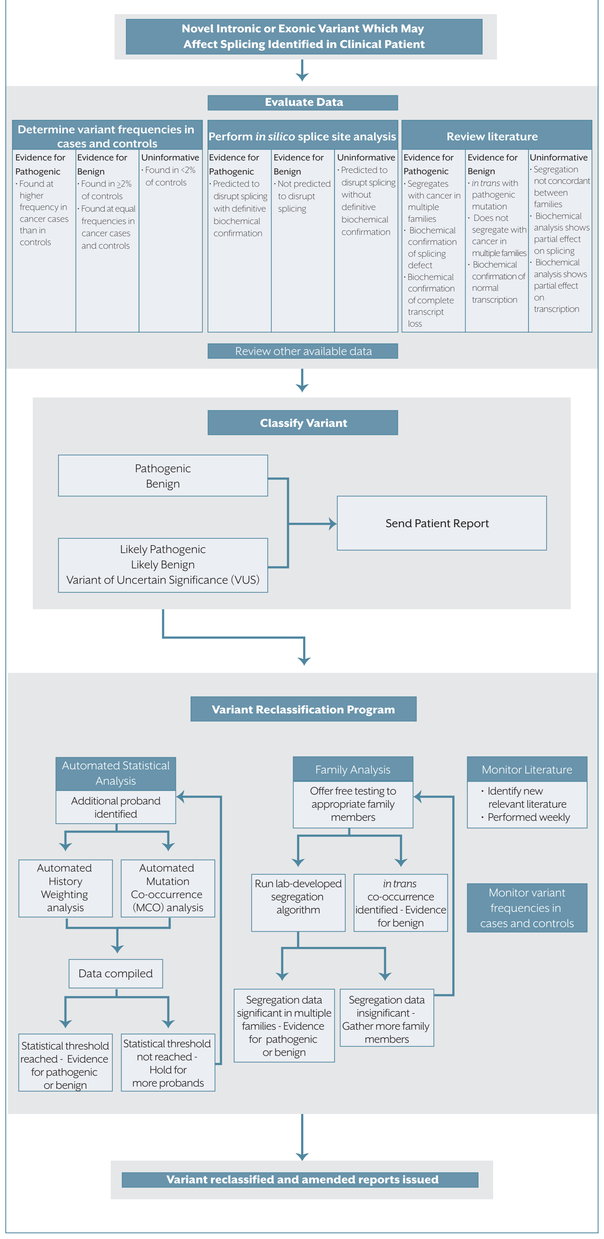


Table 1. Additional descriptions of select variant analysis methods

Method	Description/ Rationale for Use
In silico splice site prediction	Multiple splice site analysis programs, which estimate the impact of a particular variant on mRNA splicing, are available for public use. Myriad uses both publicly available and internally developed programs. BDGP results are provided for the variants discussed.[1]
History Weighting analysis	This statistical technique, developed and validated by Myriad, is based upon the premise that disease associated mutations will be observed more often in individuals at high risk for carrying a mutation, as determined by the severity of personal and family history, but the observation of benign variants should be independent of personal and family history.[2]
Mutation co-occurrence analysis	This statistical technique, developed and validated by Myriad is based on the observation that the primary genetic cause of disease in a family is usually attributable to a single pathogenic mutation. Therefore, variants found to co-occur with a pathogenic mutation in the same individual are less likely to be pathogenic themselves.[3]
In trans co-occurrence and homozygosity analyses	Homozygous or compound heterozygous *BRCA1* and *BRCA2* pathogenic mutations are generally presumed to be embryonically lethal (*BRCA1/2*) or to result in severe phenotypes such as Fanconi anemia (*BRCA2*), although some exceptions have been identified. Therefore, a homozygous observation of a variant or, an *in trans* co-occurrence of a particular variant with a pathogenic mutation, provides evidence that a variant may be benign.[3]

1. Berkeley Drosophila Genome Project (BDGP). www.fruitfly.org/seq_tools/splice.html
2. Pruss D et al. Breast Cancer Res Treat, Epub ahead of print, 2014.
3. Eggington JM, et al. Clin Genet. 2014 Sept;86(3):229-37. PubMed PMID: 24304220.

Table 2. BDGP Splice Site Analysis Results

Gene	Variant	Wild Type Score	Variant Score
BRCA1	c.135-1G>T	0.97 (Acceptor)	<0.10 (Acceptor)
BRCA1	c.81-13C>A	0.52 (Acceptor)	0.25 (Acceptor)
BRCA1	c.3699A>G (p.Lys1233Lys)	<0.10 (Alternate Donor)	0.98 (Alternate Donor)
BRCA1	c.4185G>A	0.95 (Donor)	0.38 (Donor)
BRCA2	c.7977-1G>C	0.98 (Acceptor)	<0.10 (Acceptor)
BRCA2	c.9501+3A>T	0.99 (Donor)	0.41 (Donor)
BRCA2	c.9117G>A	0.57 (Donor)	<0.10 (Donor)
BRCA2	c.9876G>A (p.Pro3292Pro)	0.10 (Alternate Acceptor)	0.85 (Alternate Acceptor)

RESULTS

- INTRONIC VARIANTS: Sequencing analysis of >1 million patients identified ~1500 unique intronic variants in *BRCA1* and *BRCA2*.
  - *In silico* splice site analysis accurately identified pathogenic mutations lying within the consensus splice junctions at the +1/+2 or -1/-2 intronic positions as pathogenic splicing mutations (Tables 2-3, Figure 2).
  - *In silico* splice site analysis of 456 benign intronic variants indicated that ~4.6% of these variants may negatively affect normal mRNA splicing, resulting in a 25%-75% estimated decrease in wild type donor or acceptor strength (Figure 3). However, analysis of some of these variants, such as *BRCA1* c.81-13C>A and *BRCA2* c.9501+3A>T, using other classification methodologies confirms their benign classifications.
- EXONIC VARIANTS:
  - *In silico* splice site analysis accurately identified pathogenic mutations lying at the consensus last nucleotide of certain exons, such as *BRCA1* c.4185G>A and *BRCA2* c.9117G>A, as pathogenic splicing mutations.
  - *In silico* splice site analysis of 3380 benign exonic variants indicated that ~4% of these variants have the potential to result in the creation of or significant strengthening of (> 0.10 absolute score increase with a final score >0.50) an alternative donor or alternative acceptor. However, variants such as *BRCA1* c.3699A>G (p.Lys1233Lys) and *BRCA2* c.9876G>A (p.Pro3292Pro) have proven to be benign using other classification evidence.

Table 3. Variants determined by *in silico* splicing analysis (BDGP) to possibly result in abnormal splicing

Gene Variant	Transcript Analysis	# Myriad Probands	# Probands with a Pathogenic Mutation *(in trans)*	History Weighting Algorithm Call	Mutation Co-occurrence Algorithm Call	Final Interpretation
BRCA1 c.135-1G>T	Abnormal splicing[1]	164	0 (0)	Pathogenic	No Call	Pathogenic
BRCA1 c.81-13C>A	No Data	168	11 (1)	Benign	Benign	Benign
BRCA1 c.3699A>G (p.Lys1233Lys)	No Data	47	3 (1)	Benign	No Call	Benign
BRCA1 c.4185G>A	Abnormal Splicing[2]	11	0 (0)	No Call	No Call	Pathogenic
BRCA2 c.7977-1G>C	Abnormal Splicing[3]	118	0 (0)	Pathogenic	No Call	Pathogenic
BRCA2 c.9501+3A>T	No Data	316	18 (5)	Benign	Benign	Benign
BRCA2 c.9117G>A	Abnormal Splicing[4]	150	0 (0)	Pathogenic	No Call	Pathogenic
BRCA2 c.9876G>A (p.Pro3292Pro)	No Data	136	11 (1)	Benign	Benign	Benign

1. Tesoriero AA, et al. Hum Mutat 26:495, 2005. 2. Claes K, et al. Genes, Chromosomes & Cancer 37:314-320, 2003. 3. Myriad Genetic Laboratories, Inc. Internal data. 4. Peelen T et al. British J Cancer 82:151-6, 2000; Bonatti F et al. Cancer Genet Cytogenet 170:93-101, 2006.

Figure 2:

Raw history weighting algorithm graphs illustrating classification calls for select BRCA1 and BRCA2 variants. Deleterious (red) and benign (green) control distributions with corresponding variant-specific history weighting scores (blue) are indicated for each variant. The Log History Weighting Score is plotted on the x-axis and the Number of Control Variants is plotted on the y-axis.

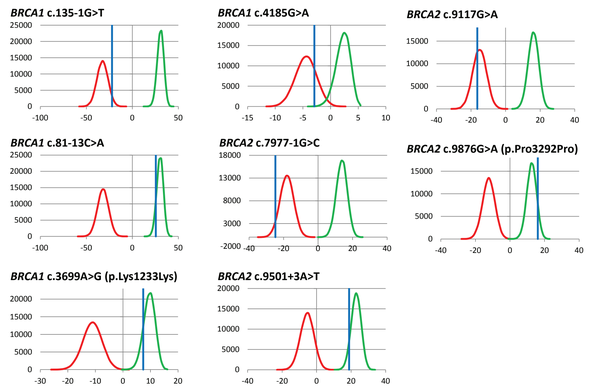
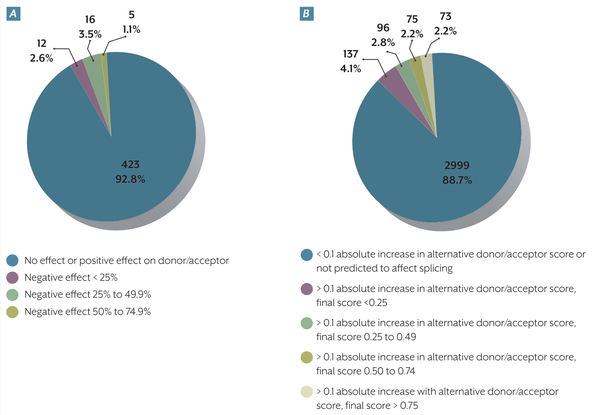


Figure 3:

A) In silico splice site analysis of 456 benign intronic variants indicated that ~4.6% of these variants may negatively affect normal mRNA splicing, resulting in a 25%-75% estimated decrease in wild type donor or acceptor strength.

B) In silico splice site analysis of 3380 benign exonic variants indicated that ~4% of these variants have the potential to result in the creation of or significant strengthening of (> 0.10 absolute score increase with a final score >0.50) an alternative donor or alternative acceptor.



CONCLUSIONS

- We have developed and implemented a robust classification and reclassification program for variants predicted by *in silico* analysis to result in abnormal mRNA splicing.
- *In silico* mRNA splicing analysis may indicate that particular variants negatively affect mRNA splicing. However, many of these predictions are either inaccurate or splicing effects are small and do not result in significant increases in cancer risk.
- *In silico* predictors should be used with caution and results rigorously verified by other independent methods before being used in the clinical setting in order to ensure correct test result interpretation and appropriate clinical management.

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