Determining the Clinical Significance of Silent *BRCA1* and *BRCA2* Sequencing Variants

Karla R. Bowles, Brian Morris, Dmitry Pruss, Jack Ji, Nanda Singh, Benjamin B. Roa, Richard Wenstrup

Myriad Genetic Laboratories, Inc., Salt Lake City, UT, USA

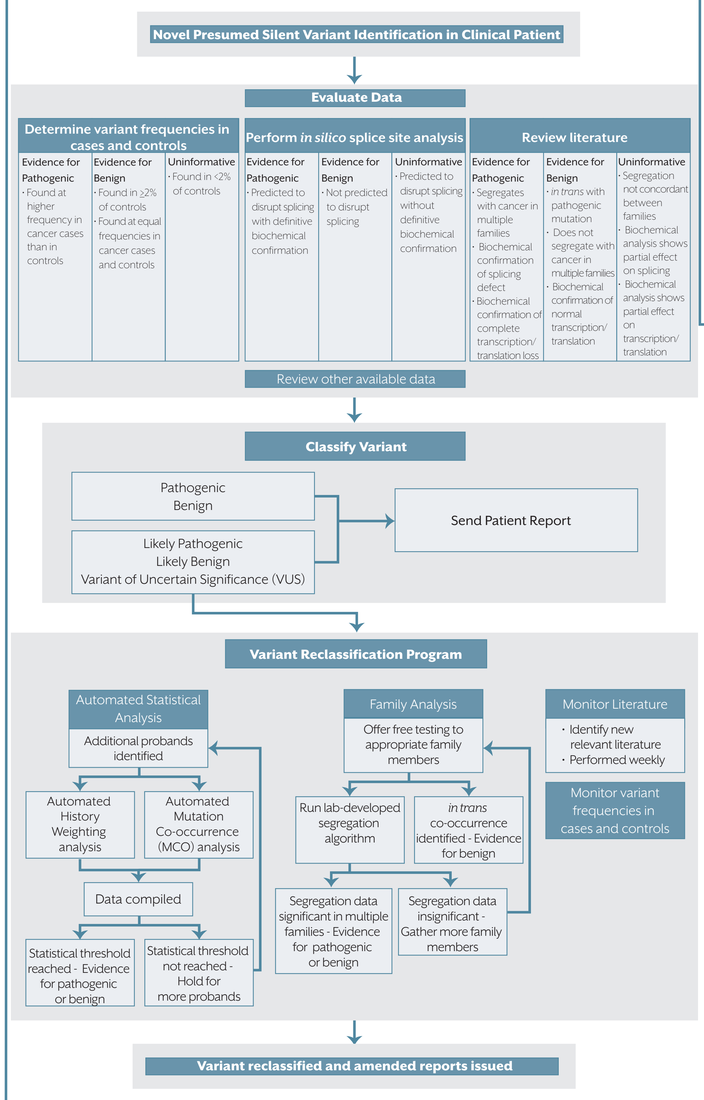
BACKGROUND

- Diagnostic sequencing analysis of the *BRCA1* and *BRCA2* genes may identify nucleotide changes that are predicted to be translationally silent.
- Analyses, such as the use of *in silico* mRNA splice site predictors and direct analysis of patient mRNA, may indicate that some variants cause abnormal mRNA splicing or production and potentially increased risk of breast and ovarian cancer.
- We describe the algorithms used by Myriad Genetic Laboratories to determine possible pathogenicity of presumed silent variants.

METHODS

- After informed consent, clinical diagnostic germline testing for *BRCA1* and *BRCA2* sequencing mutations was performed on extracted patient genomic DNA.
- Sanger sequencing analysis of *BRCA1* and *BRCA2* identified nucleotide changes predicted to result in translationally silent variants.
- *in silico* mRNA splice site analysis and scientific literature review identified variants which may result in abnormal mRNA splicing or production.
- Variant pathogenicity was further investigated through Myriad's variant classification and reclassification processes, which include multiple methods of variant evaluation (Figure 1, Table 1).

Figure 1. Basic algorithm used to classify presumed silent variants



RESULTS

- Sequencing analysis of >1 million patients identified >2000 unique presumed silent variants in *BRCA1* and *BRCA2*.
- *in silico* splice site analysis accurately identified some mutations lying at the last base of an exon, such as *BRCA1* c.4185G>A and *BRCA2* c.516G>A and c.9117G>A, as pathogenic splicing mutations (Tables 2-3, Figure 2).
- Splice site analysis identified *BRCA1* c.3699A>G (p.Lys1233Lys) and *BRCA2* c.9876G>A (p.Pro3292Pro) as potentially resulting in abnormal splicing but the history weighting algorithm strongly indicates these variants to be benign (Tables 2-3, Figure 2). Co-occurrences with pathogenic mutations have also been observed for both of these variants, providing additional support for their benign classifications.
- *BRCA1* c.75C>T (p.Pro25Pro) has been previously observed in a patient with decreased mRNA transcript levels and was postulated to be pathogenic.[4] However, in Myriad's patient population, this variant co-occurs *in trans* with known deleterious *BRCA1* mutations in five patients and has been found in the homozygous state in 14 patients, strongly indicating a benign classification. History weighting analysis confirms the benign nature of this variant.

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.

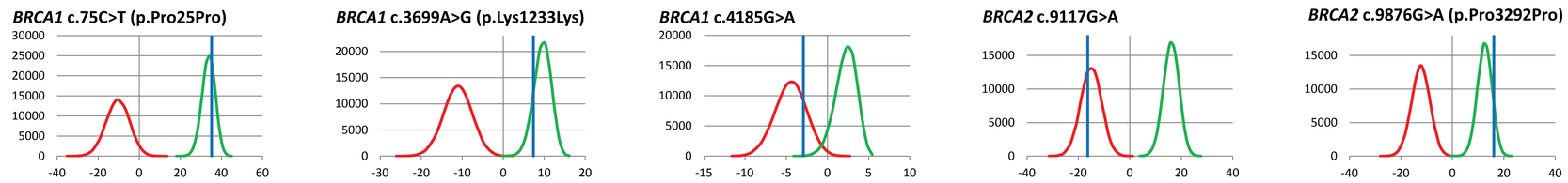


Table 2. BDGP Splice Site Analysis Results

Gene	Variant	Wild Type Score	Variant Score	Interpretation
BRCA1	c.75C>T (p.Pro25Pro)	1.00 (Donor)	1.00 (Donor)	No change
BRCA1	c.3699A>G (p.Lys1233Lys)	<0.10 (Alternate donor)	0.98 (Alternate donor)	Variant may result in strong alternative splice donor
BRCA1	c.4185G>A	0.95 (Donor)	0.38 (Donor)	Variant disrupts splice donor
BRCA2	c.516G>A	0.98 (Donor)	0.42 (Donor)	Variant disrupts splice donor
BRCA2	c.9117G>A	0.57 (Donor)	<0.10 (Donor)	Variant disrupts splice donor
BRCA2	c.9876G>A (p.Pro3292Pro)	<0.10 (Alternate acceptor)	0.85 (Alternate acceptor)	Variant may result in strong alternative splice acceptor

Table 3. Summary of Variant Data

Gene	Variant	BDGP Splice Interpretation	Transcript Analysis	# Myriad Probands	# Probands with a Pathogenic Mutation (*in trans*)	# Homozygous Observations	History Weighting Algorithm Call	Mutation Co-occurrence Algorithm Call	Final Interpretation
BRCA1	c.75C>T (p.Pro25Pro)	Splicing not affected	Decreased transcript levels[4]	692	48 (5)	14	Benign	No Call	Benign
BRCA1	c.3699A>G (p.Lys1233Lys)	Possible abnormal splicing	No Data	47	3 (1)	0	Benign	No Call	Benign
BRCA1	c.4185G>A	Possible abnormal splicing	Abnormal splicing[5]	11	0 (0)	0	Pathogenic	No Call	Pathogenic
BRCA2	c.516G>A	Possible abnormal splicing	Abnormal splicing[6]	6	0 (0)	0	No Call	No Call	Pathogenic
BRCA2	c.9117G>A	Possible abnormal splicing	Abnormal splicing[7,8]	139	0 (0)	0	Pathogenic	No Call	Pathogenic
BRCA2	c.9876G>A (p.Pro3292Pro)	Possible abnormal splicing	No Data	113	11 (1)	0	Benign	Benign	Benign

Table 1. Additional descriptions of select variant analysis methods

Method	Description/Rational 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. Results from the Berkeley Drosophila Genome Project are provided for the variants discussed.[1]
mRNA transcript level analysis	Multiple methodologies are available to measure a specific variant's effect on mRNA transcription. Care must be taken when interpreting this data as the effect of partial transcript reduction on cancer risk is not known, and attributing a transcription level defect to a specific variant (rather than a nearby undetected variant) is complex. Myriad does not typically consider this type of data
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 analysis	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, homozygous observations of a variant or *in trans* co-occurrences of a particular variant with a pathogenic mutation provide evidence that a variant may be benign.[3]

CONCLUSIONS

- The majority of predicted silent variants identified during DNA sequencing represent benign variants, but some variants may result in abnormal mRNA transcription or splicing and increased cancer risk.
- We have developed a rigorous algorithm that can be used to clinically classify sequence variants, including variants initially presumed to be silent.
- While the use of *in silico* splice site analyses may predict some presumed silent variants to result in abnormal splicing, these tools should be used cautiously and predictions rigorously verified by other methods.
- Analysis of mRNA transcription levels to determine pathogenicity should be used with extreme care as transcript levels may not correlate directly with variant pathogenicity and clinical outcome. It may also be difficult to determine whether transcription level effects are due to the variant in question or a nearby unidentified variant.

REFERENCES

1. Berkeley Drosophila Genome Project (BDGP). www.fruitfly.org/seq_tools/splice.html.
2. Pruss D, et al. Submitted, 2014.
3. Eggington JM, et al. Clin Genet, Epub ahead of print, 2014.
4. Walsh T, et al. Platform presentation. ASHG Annual Meeting, 2013.
5. Claes K, et al. Genes, Chr, & Ca, 37:314-320, 2003.
6. Hansen TVO, et al. Breast Cancer Res Treat, 119:547-550, 2010.
7. Peelen T, et al. British J Cancer, 82:151-6, 2000.
8. Bonatti F et al. Cancer Genet Cytogenet, 170:93-101, 2006.

Presented at ACMG - March 28, 2014