**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.
- Variant pathogenicity was further investigated through Myriad’s variant classification and reclassification processes, which include multiple methods of variant evaluation (Figure 1, Table 1).

**RESULTS**

- Sequencing analysis of >1 million patients identified >2000 unique presumed silent variants in BRCA1 and BRCA2.
- A specific motif analysis identified some mutations lying at the last base of an exon, such as BRCA1 c.4185G>A and BRCA2 c.516G>A without evidence of splicing (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.755C>T (p.Pro25Pro) has been previously observed in a patient with decreased mRNA transcript levels and was postulated to be pathogenic. 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.

**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.

**REFERENCES**