Comparative Analysis of Variant of Uncertain Significance in *BRCA1* and *BRCA2* Amongst Four Institutions

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BACKGROUND: The interpretation of sequence variants identified through genetic testing in *BRCA1/2* genes is a necessary step in the reporting and counseling process. The likely pathogenic, uncertain, and likely benign categories include some level of uncertainty as to whether the variant is related to disease. This limits the clinical utility of the genetic testing. Much of the data regarding variants is proprietary or unpublished, presenting a barrier to reclassifying inconclusive results.

HYPOTHESIS: The compilation and characterization of variant of uncertain significant (VUS) in *BRCA1*/2 genes in patients with breast cancer who underwent genetic testing in 4 Sister Institutions: UT MD Anderson Cancer Center, AC Camargo Cancer Center, Barretos Cancer Hospital and Albert Einstein Hospital, Brazil will allow the classification of VUS by co-segregation analysis of probands and their family members.

METHODS: Each institution database was queried for patients who have a *BRCA1/2* variants. All data were merged and the variants considered as VUS were those classified as Class 3 by the IARC-LOVD database as well as whose clinical significance was “Uncertain” by CLINVAR. In regards to data from UTMDACC, variants were classified on the reports by the testing company (Myriad) based on available public databases and their own variant database.

RESULTS: By selecting the VUS of each institution, whose nucleotide variants leaded to amino acid changes, we were able to list 73 and 197 distinct VUS in *BRCA1* and *BRCA2* genes, respectively. Regarding the *BRCA1* gene, the majority of the VUS was found in exon 11 (66%). In exons 15 and 16 were found 5%. Regarding the *BRCA2* gene, 46% were detected in exon 11 and in exons 10 and 14 were detected 8 and 5%, respectively. The remaining VUS in both genes were detected throughout the other exons in lower frequency. In *BRCA1*, the most frequently changed amino acid was Arginine with 10%, followed by Asparagine with 8%. In *BRCA2*, Threonine with 12% followed by Serine with 10% were the most frequently changed amino acids. Proline, one of the most important amino acid that when altered can greatly impact

the protein structure was change in 4% and 7% in *BRCA1* and *BRCA2*, respectively. Interestingly, three VUSs in *BRCA1* and 7 in *BRCA2* were found in more than one Institution. One of the variants in BRCA2 (p.Asp1699Gly - c.5096A>G), reported in dbSNP with low frequency (0.02%), were found in three institutions. *In silico* and segregation analysis will be carried out to predict the pathogenicity of all VUS. In addition, for those variants classified as VUS after the *in silico* and segregation analysis, functional assays will be performed. The rate of common variants in these 4 institutions were low. Clinical and pathological characteristics of these patients will be presented at the meeting. The definition of pathogenicity will be important in regards to their personalized risk management options. This is one of the global approaches to identify women at increased breast and ovarian cancer risk via BRCA mutation classification.