ONCOMET
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Indian Referral Lab Detects 26% BRCA Mutation Incidence In A 77 Women Cohort

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BACKGROUND

- Hereditary cancer syndrome is a genetic disorder in which inherited genetic mutations in one or more genes predispose the affected individuals to the development of cancers and may also cause the early on set of these cancers.
- Cancer syndromes often show not only a high lifetime risk of developing cancer, but also the development of multiple independent primary tumors.
- Most of these syndromes are caused by mutations in tumors uppress or genes or DNA repair genes, oncogenes or genes involved in Angiogenesis.
- Breast cancer is one of the most common malignancies affecting women worldwide and is these cond most common malignant condition among women in India.
- Genetic predisposition for familial early onset of breast cancer accounts for approximately 5-10% of all breast cancers with mutations in BRCA1 and BRCA 2 being the major risk factors.
- The mutated BRCA1 / BRCA2 genes increase lifetime breast cancer risk to 85% and ovarian cancer risk to 60%, as compared to a 12% life time risk of breast cancer and 1% lifetime risk of ovarian cancer in women without mutations in BRCA1 and BRCA2

Who should undergo BRCA1/2 Mutation Testing

For all patients:
- Deleterious *BRCA1/2* gene mutation in a blood relative
- Personal history of ovarian, fallopian tube, and/or primary peritoneal cancer

For patients with breast cancer:
- One or more blood relatives diagnosed with breast cancer at ≤ 45 y
- Personal history of bilateral breast cancer at ≤ 50 y
- Personal history of triple-negative breast cancer at ≤ 60 y
- Personal history of male breast cancer

For patients without a personal history of breast or ovarian cancer:
- First- or second-degree blood relative with
  - Ovarian cancer
  - Breast cancer at ≤ 45 y
- ≥ 2 First-, second-, or third-degree blood relatives (in the same bloodline) with breast cancer, at least one of whom was diagnosed at ≤ 50 y

NCCN = National Comprehensive Cancer Network.
Data from Daly et al. J Natl Compr Canc Netw. 2016;[16]

AIMS OF THE STUDY

- The purpose of our study was to develop are liable assay for detection of germ line BRCA1/2 gene variation and to screen breast cancer patients and at risk individuals for the presence of gene variants.

PATIENTS AND METHODS

No. of Cases = 77 women (24-75 yr)
56 - hereditary breast/ovarian cancer patients
18 - sporadic breast cancer patients
3 - individuals with no personal or family history

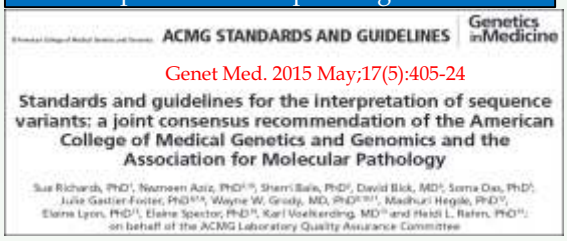
METHODS: PCR-SEQUENCING OF ALL THE EXONS OF BRCA1/ BRCA2

+ Patient Consent | PCR | 32 PCR – BRCA1, 48 PCR – BRCA2 | 80 Sequencing Reactions | Genetic Counseling

Pre-test & Post-test Counseling

RESULTS AND DISCUSSION

Interpretation of Sequencing Variants



ACMG STANDARDS AND GUIDELINES | Genetics in Medicine

Genet Med. 2015 May;17(5):405-24

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2-5], Sherri Bale, PhD[6], David Bick, MD[7], Soma Das, PhD[8], Julie Gastier-Foster, PhD[9,10], Wayne W. Grody, MD, PhD[5,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Terminology

Mendelian disease variant terminology
- Pathogenic
- Likely pathogenic ← (≥90% confidence)
- Uncertain significance (VUS)
- Likely benign
- Benign

Replace terms "mutation" and "polymorphism" with "variant"

- BRCA 1/2 mutation screening as say was successfully developed using gold standard Sanger sequencing methodology and has passed CAP proficiency assessment.
- Seventy four out of 77 cases either had HBOC history or were sporadic breast / ovarian cancer cases and fulfilled NCCN criteria for screening of BRCA mutations. Three women with no personal or family history of cancer opted to undergo BRCA testing.
- Out of 77 cases analyzed pathogenic mutations were detected in 20 cases (26%) with 13 cases (16.9%) showing BRCA2 mutation and 7 cases (9.1%) with BRCA1 mutation. Amongst the 56 HBOC cases 16 (28.6%) had mutation and 4/18 (22.2%) sporadic breast cancer cases showed presence o fmutation.
- Most of the pathogenic mutations (19/20) detected in BRCA1/2 were insertion, deletions or substitution mutations which lead to stop codon and premature chain termination. One case showed presence of in tronic mutation leading to splicing defect. Three novel mutations (classified as' Likely Pathogenic') were identified in the study.
- Other Indian studies have reported mutation frequency of 13% to 50% with higher prevalence in HBOC category. However, the global data indicate lower frequency of 7.5% to 25% in different populations.
- We have observed VUS frequency of7 .8% (6/77) with 7.14% in HBOC cases. The VUS frequency was found to be 7%-15% oreven higher in other countries.
- All the negative cases should be further tested for large deletions and duplications by Multiplex Ligation Probe Assay (MLPA) technology to have more comprehensive BRCA1/2 analysis.
- Targeted (single) mutation testing rather than comprehensive BRCA testing should be offered to the at risk family members when BRCA1/2 mutation is already identified in the index case in the family.
- BRCA1/2 testing should be shifted on Next Generation Sequencer (NGS) to make the assay costeffective and affordable for the patients.

Table 1: List of Mutations Identified In BRCA1/2 Genes

Sr. No.	Age (yr)	Gene	Category	Mutation Identified	Effect
1	21	BRCA1	HBOC	c.68_69delAG, p.Glu23Valf-	Pathogenic
2	26	BRCA1	HBOC	c.1504_1508delTTAAA	Pathogenic
3	29	BRCA2	HBOC	c.5292 G>T, E1725X	Pathogenic
4	30	BRCA1	HBOC	c.4412delG, p.Gly1471Alafs*1504	Pathogenic
5	30	BRCA2	HBOC	c.5410_5411delGT, p.Val1804Lysfs*2	Pathogenic
6	34	BRCA1	HBOC	c.5292 G>T, E1725X	Pathogenic
7	34	BRCA2	HBOC	c.5410_5411delGT, p.Val1804Lysfs*2	Pathogenic
8	36	BRCA2	HBOC	c.68_69delAG, p.Glu23Valfs*17	Pathogenic
9	40	BRCA1	Sporadic	c.2766delA, p.Thr922fs	Likely Pathogenic
10	40	BRCA2	HBOC	c.2397_2398delA, p.Gly800Valfs*10	Pathogenic
11	41	BRCA2	HBOC	c.6952C>T, p.Arg2318*	Pathogenic
12	45	BRCA1	HBOC	c.5251 C>T, p.Arg1751X	Pathogenic
13	46	BRCA2	HBOC	c.5164_5165delAG, p.Ser1722Tyr fs	Pathogenic
14	50	BRCA2	Sporadic	c.925delT	Pathogenic
15	51	BRCA2	HBOC	c.9257-3_9258delTAGGA	Pathogenic
16	51	BRCA2	HBOC	c.9257-2A>A/T (Intronic)	Pathogenic
17	60	BRCA2	HBOC	c.5682 C>G, Y1894X	Pathogenic
18	63	BRCA2	Sporadic	c.3865_3868delAAAT, p.Lys1289Alafs	Likely Pathogenic
19	69	BRCA1	HBOC	c.516delA, p.Gln172His fs*233	Pathogenic
20	75	BRCA2	Sporadic	c.7505 G>A, R2502H	Likely Pathogenic

Table 2: List of Variants of Unknown Significance (VUS) Identified In BRCA1/2 Genes

Sr. No.	Age (yr)	Gene	Category	VUS
1	25	BRCA2	HBOC	c.5986 G>A, p.Ala1996Thr
2	40	BRCA1	HBOC	c.2706 A>C
3	50	BRCA2	Sporadic	c.6935 A>T
4	65	BRCA2	Sporadic	c.6935 A>T,p.Asp2312Val
5	66	BRCA1	HBOC	c.3776 A>C, N1259T
6	67	BRCA2	HBOC	c.7884 A>G, p.I2628M c.9409 A>T p.T3137S

CONCLUSIONS

- Our study results indicate that BRCA1/2 mutation has a significant role in breast cancer despite the fact that a considerable proportion of the HBOC may be due to genes ot her than BRCA1/2 mutations. Comprehensive analysis comprising of BRCA1/2 gene sequencing and large deletion / duplication should offered to the patients.
- The study should be extended to alarger cohort of patients to understand the frequency of BRCA1/2 mutations in Indian patients.
- Further, Indian specific BRCA gene data base should be created comprising of all the identified SNPs, pathogenic variants andV US.
- Pre-and Post-test Genetic Counseling forms an integral part of BRCA mutation screening.
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REFERENCES:

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- ° Hirotsuet al., 2015, Mol Genet Genomic Med.; 3(2): 121–129°Claeset al., 2004, Br J Cancer;90(6):1244-51