Molecular detection of BRCA1 and BRCA 2 mutations in Iran

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Introduction

Germ line mutations in breast cancer susceptibility genes 1 and 2 (BRCA1 and BRCA2) are reported in breast cancer patients in a varying degree based on ethnicity and being familial versus sporadic. BRCA1 mutations are reported in up to 45% of familial and 2–30% of sporadic breast cancer patients. BRCA2 mutations are reported to account for a comparable proportion of the familial cases. Carrier individuals transmit the gene to 50% of their offspring's. Carriers have higher life long risk of developing breast cancer.

Material and Methods

Familial cases of breast cancer were recruited and entire coding region of BRCA1 and 2 genes were sequenced. Results were checked against Breast Cancer
Information Core database to find pathologic sequence variation.
(http://research.nhgri.nih.gov/projects/bic/Member/index.shtml).

Results

Sequencing analysis of BRCA1 and 2 genes four families revealed 48 variations within coding region and intron-exon boundaries, with 17 to 23 variations in each case (Table 1). Eleven of these variations have been reported to be non-pathogenic in the BIC database. Each case carried 2 to 8 variations of this kind. Thirteen unknown variation (4-9 per case) and 24 unreported variations were also found (3-10 per case).
These cases are under further bioinformatics studies to determine their possible deleterious effect.
Codon, (CD) 694AGC>AGT, CD1183AAA>AGA, CD1436TCT>TCC and IVS18+73 G>A were found in all cases in homo or heterozygous state. BRCA1 exons 11, 13 and 9 have been shown to 52.09% of variations. BRCA2 exons 10, 11 and 17 have been shown to have 21.88% of variations (Table 2-3).

Conclusion

Further molecular study and bioinformatics investigation is needed to elucidate the molecular picture of BRCA1 and BRCA2 genes in Iranian patients with familial breast cancer.
In cases where several missense mutations are seen it is needed to further analyze them to determine the disease causing mutation. Sometimes this can be found by the pattern of inheritance in the affected cases or bioinformatics analysis. In other cases a missense mutation can not be found as homozygote since the mutation should be seen as heterozygote. Other methods can also be used to determine the deleterious mutation among several missense mutation. One of the main method could be population study.

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Table 2: Most common variations and their frequencies in our patients

	Exon	Variation	Allele	%
1	BRCA1.Exon-13	CD1436 TCT>TCC Ser>Ser	7	7.291667
2	BRCA1.Exon-11	CD694 AGC>AGT Ser>Ser	6	6.25
3	BRCA1.Exon-18	IVS18+73	6	6.25
4	BRCA1.Exon-11	CD1183 AAA>AGA Lys>Arg	6	6.25
5	BRCA1.Exon-11	CD771 TTG>CTG Leu>Leu	5	5.208333
6	BRCA1.Exon-11	CD1038 GAA>GGA Glu>Gly	5	5.208333
7	BRCA2.Exon-10	CD599 TTT>TCT Phe>Ser	4	4.166667
8	BRCA1.Exon-2	IVS1-235 G>A	4	4.166667
9	BRCA1.Exon-16	CD1613 AGT>GGT Ser>Gly	4	4.166667
10	BRCA1.Exon-2	IVS1-134 T>C	4	4.166667
11	BRCA1.Exon-11	CD1140 GGT>AGT Gly>Ser	4	4.166667
12	BRCA2.Exon-17	IVS16-14 T>C	3	3.125

Table 3- Frequencies of variations seen in each exon in our patients

	Exon	Allele	%
1	BRCA1.Exon-11	30	31.25
2	BRCA1.Exon-13	11	11.46
3	BRCA2.Exon-10	9	9.38
4	BRCA1.Exon-2	9	9.38
5	BRCA2.Exon-11	8	8.33
6	BRCA1.Exon-18	8	8.33
7	BRCA2.Exon-17	4	4.17
8	BRCA1.Exon-16	4	4.17
9	BRCA2.Exon-12	3	3.13

Table 1: List of polymorphism and their appearance in each case in an affected individual in a family

Gene	Exon	Mutation	Clinically Important (BIC)	Affected # 1	Affected # 2	Affected #3	Affected # 4
BRCA1	2	IVS1-115 T>C	Unknown	Hetero			
	2	IVS1-134 T>C	Not Reported		Hetero	Homo	Hetero
	2	IVS1-235 G>A	Not Reported			Homo	Homo
	3	IVS2-18 C>A	Not Reported		Hetero	Hetero	
	8	IVS7-34 C>T (rs799923)	No		Hetero		
	9	IVS8-58 Deletion T	No		Hetero		
	9	IVS8-64 Deletion T	Unknown	Homo			
	10	IVS10+21 G>A	Not Reported				Hetero
	11A	CD356 CAG>CGG Gln>Arg (rs1799950)	Unknown				Hetero
	11C	CD693 GAC>AAC Asp>Asn (rs4986850)	No	Hetero			
	11C	CD694 AGC>AGT Ser>Ser (rs1799949)	Unknown	Homo	Hetero	Homo	Hetero
	11C	CD771 TTG>CTG Leu>Leu (rs16940)	Unknown	Homo		Homo	Hetero
	11D	CD1038 GAA>GGA Glu>Gly (rs16941)	No	Homo		Homo	Hetero
	11D	CD871 CTG>CCG Leu>Pro (rs799917)	No		Hetero		Hetero
	11E	CD1140 GGT>AGT Ser>Gly (rs2227945)	Unknown	Homo		Homo	
	11E	CD1183 AAA>AGA Lys>Arg (rs16942)	No	Homo	Hetero	Homo	Hetero
	11F	IVS11+80 deletion TTAA	Not Reported				
	13	CD1436 TCT>TCC Ser>Ser (rs1060915)	Unknown	Homo	Hetero	Homo	Homo
	13	IVS12-22 G>A	Not Reported			Hetero	
	13	IVS13+60 T>A	Not Reported			Hetero	Hetero
	13	CD1403(nt 4327) AAC>GAC Asn>Asp	Not Reported			Hetero	
	14	CD2414 TCA>TCG Ser>Ser	Not Reported				
	14	IVS14+34 C>A	Not Reported				Hetero
	15	IVS14-63 G>C	Not Reported	Homo			
	16	CD1613 AGT>GGT Ser>Gly (rs1799966)	No	Homo	Hetero		Hetero
	16	CD1652 ATG>ATA Met>Ile (rs1799967)	Unknown				
	18	IVS18+73	Unknown	Homo	Hetero	Homo	Homo
	18	IVS18+51 G>A	Not Reported				Hetero
	19	IVS18-26 A>G (rs80358109)	Unknown				Hetero
	22	IVS22-4 T>A	Not Reported	Hetero			
	22	IVS20-102 C>A	Not Reported			Hetero	
	22,23	IVS21-66 T>C	Not Reported				
BRCA2	2	Nt 203 (5'UTR) G>A	No				
	3	IVS2-6 deletion A	Not Reported		Homo		
	4	IVS4+67 A>C	Unknown				
	8	IVS8+56 C>T (rs2126042)	Unknown				
	9	IVS9+65 del. T	Unknown				
	9	IVS8-58 del. T	Not Reported				Hetero
	10A	CD372 CAT>AAT His>Asn	No	Hetero	Hetero		
	10B	CD599 TTT>TCT Phe>Ser	Unknown	Homo	Homo		
	10B	IVS10+12 deletion T	Unknown	Hetero			Hetero
	10B	IVs10+48 A>T	Not Reported		Hetero		
	11A	IVS11-74 T>C	Not Reported				Hetero
	11C	CD1132 AAA>AAG Lys>Lys (rs1801406)	No		Hetero		Hetero
	11D	CD1366 CTT>CAT Leu>His	Not Reported		Hetero		
	11D	CD1367 AAA>AAG Lys>Lys	Not Reported			Hetero	
	11F	CD2171 GTG>GTC Val>Val	Unknown			Homo	
	11.G	CD1621 TGT>GGT Cyt>Gly	Not Reported				Hetero
	12	IVS12+112 C>G	Not Reported			Homo	
	12	IVS12+151 A>G	Not Reported				Hetero
	14	CD2414 TCA>TCG Ser>Ser (rs1799955)	No	Hetero	Hetero		
	17	IVS16-14 T>C (rs9534262)	No	Hetero			Homo
	17	CD2653 CTT>CCT Leu>Pro (rs80359022)	Unknown				Hetero
	19	IVS18-70 T>A	Not Reported	Hetero			
	19	IVS19-71 T>A	Not Reported		Hetero		