Different genomic rearrangements account for 17% of *BRCA1/2* mutations in Greece

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Background: Most cases of breast cancer are sporadic. However, it is more common in some families due to their genetic background. Approximately 5-10% of breast cancer cases are hereditary.

According to recent studies, hereditary (germline) mutations in the *BRCA1* and *BRCA2* genes are responsible for 80% of hereditary breast cancer cases. Carriers of such mutations are usually members of families with at least 1-2 cases of breast cancer diagnosed before the age of 40 years.

Large genomic rearrangements account for approximately 5-30% of the mutations identified in the *BRCA1* gene and 10% of those identified in the *BRCA2* gene.

The scope to further delineate the extent and nature of mutations in the *BRCA1* and *BRCA2* genes, responsible for hereditary breast and ovarian cancer in Greek families.

Methods

Genomic DNA was isolated from whole peripheral blood of patients referred to our center for mutation analysis of the *BRCA1* and *BRCA2* genes.

Patients were included on the basis of affected family members, types of cancer present in the family and the age at diagnosis of breast cancer in the proband. The families were subdivided into high, medium and low risk depending on the number of affected family members, types of cancer diagnosed in the family and age at diagnosis of affected family members.

In total, 881 families have been analyzed by our group in the past 6 years. Mutation analysis in all cases included sequencing of the coding region and the splice sites of the two genes.

In addition, in 790 of the patients who were negative for *BRCA1/2* point mutations analysis for the presence of large genomic rearrangements was carried out by the use of Multiplex Ligation-dependent Probe Amplification (MLPA, MRC Holland)

Results

In total, a pathogenic mutation has been identified in 12% of the 881 patients analyzed. Of the 104 mutations identified in total, 17 (16.3%) were large genomic rearrangements, These were deletions of exons 8, 20, 21-23, 23, 23-24, 24 and the entire *BRCA1* gene, in addition to a duplication of exons 3-8 of the *BRCA1* gene. As far as the *BRCA2* gene is concerned deletions of exons 3, 4-18, 15 and the entire *BRCA2* gene were detected. All deletions were confirmed by use of other MLPA probe sets and relative quantitation by Real Time PCR. Three of the rearrangements identified, namely deletions of exon 20 and exons 23-24 of the *BRCA1* gene and deletion of exon 3 of the *BRCA2* gene, were identified in more than one unrelated families. In addition, the recurrent mutations 5382insC and G1738R, which have been previously identified as founder mutations in the Greek population, were identified in multiple unrelated families analyzed.

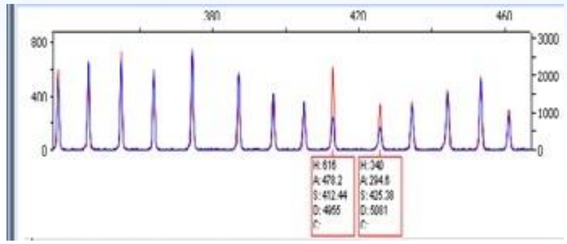

Figure 1a. Example of a chromatogram of a sample with deletion of exons 23-24 of the *BRCA1* gene.

Gene	Exon	Patient	Control
BRCA1	19	1,05	1,03
	23	0,52	1,02
	24	0,49	1,00
BRCA2	26	1,06	1,01

Table 1. Relative quantification of the sample under analysis compared to a normal control using Real-Time PCR

Gene	Chr pos	Length (nt)	MV36	Ratio	Ratio
BRCA1 probe 0763-L0268	17q21	148	17-038.5 BRCA1 exon 01A	1.19	0.98
BRCA1 probe 0764-L0269	17q21	157	17-038.5 BRCA1 exon 01B	1.08	1.07
BRCA1 probe 0765-L0270	17q21	166	17-038.5 BRCA1 exon 02	1.02	0.99
BRCA1 probe 0826-L0341	17q21	175	17-038.5 BRCA1 exon 03	0.99	1
BRCA1 probe 0767-L0272	17q21	184	17-038.5 BRCA1 exon 05	1.03	1.06
BRCA1 probe 0827-L0342	17q21	208	17-038.5 BRCA1 exon 06	1.12	1.11
BRCA1 probe 0769-L0274	17q21	217	17-038.5 BRCA1 exon 07	0.91	1.02
BRCA1 probe 1004-L0569	17q21	226	17-038.5 BRCA1 exon 08	0.99	1.11
BRCA1 probe 1005-L0581	17q21	235	17-038.5 BRCA1 exon 09	1.04	0.99
BRCA1 probe 0772-L0277	17q21	244	17-038.5 BRCA1 exon 10	1.05	1.09
BRCA1 probe 0830-L0345	17q21	268	17-038.5 BRCA1 exon 11A	0.92	0.99
BRCA1 probe 0774-L0279	17q21	277	17-038.5 BRCA1 exon 11B	1.01	1.03
BRCA1 probe 0775-L0280	17q21	286	17-038.5 BRCA1 exon 12	1.19	1.14
BRCA1 probe 2603-L2074	17q21	295	17-038.5 BRCA1 exon 13A	1.22	1.12
BRCA1 probe 11283-L12001	17q21	463	17-038.5 BRCA1 exon 13B	1	1.03
BRCA1 probe 0833-L0349	17q21	304	17-038.5 BRCA1 exon 14	1.03	0.94
BRCA1 probe 0778-L0347	17q21	328	17-038.5 BRCA1 exon 15	1.01	0.96
BRCA1 probe 0779-L0003	17q21	337	17-038.5 BRCA1 exon 16	0.93	1.02
BRCA1 probe 0780-L0283	17q21	346	17-038.5 BRCA1 exon 17	1.06	0.9
BRCA1 probe 0781-L0284	17q21	355	17-038.5 BRCA1 exon 18	1.09	1.12
BRCA1 probe 0782-L0285	17q21	364	17-038.5 BRCA1 exon 19	1.08	1.08
BRCA1 probe 0783-L0356	17q21	388	17-038.5 BRCA1 exon 20	1.05	1.06
BRCA1 probe 0784-L12004	17q21	398	17-038.5 BRCA1 exon 21	1.08	1.01
BRCA1 probe 0785-L0288	17q21	406	17-038.5 BRCA1 exon 22	1.22	1.04
BRCA1 probe 0786-L0289	17q21	415	17-038.5 BRCA1 exon 23	0.45	1
BRCA1 probe 2831-L13862	17q21	427	17-038.5 BRCA1 exon 24	0.52	0.94
Reference probe 0518-L0098	02q14	256	c	1	1.07
Reference probe 0673-L0117	03p21	454	c	0.97	0.96
Reference probe 0655-L0304	04q26	376	c	1.06	1
Reference probe 0797-L0093	05q31	127	c	0.95	0.94
Reference probe 6452-L05978	06p22	136	c	1.07	0.99
Reference probe 2946-L3265	07q31.2	198	c	0.98	1.04
Reference probe 0596-L0083	11p13	436	c	0.98	1.01
Reference probe 0495-L0303	12p12	316	c	1.14	0.9
Reference probe 4074-L03710	17q11.2	445	c	1.03	1

Figure 1b. MLPA analysis of the sample.

Conclusions:

Our results indicate that different large genomic rearrangements account for an important proportion (16.3%) of the mutations in the *BRCA1* and *BRCA2* genes, in Greek families at risk of carrying a germline mutation as judged by family / personal history.

The use of the available technologies for the identification of such mutational events is therefore necessary when carrying out complete analysis of the genes in high risk families of Greek background.

References:

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