Multigene panel testing results for hereditary breast cancer in 1325 individuals: implications for gene selection and considerations for guidelines

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Background

The application of the Next Generation Sequencing (NGS) technology has facilitated multigene panel testing for hereditary breast cancer (BC) in clinical practice. We performed a retrospective analysis of individuals referred for testing in our lab aiming to investigate the contribution of included genes and evaluate current genetic testing guidelines in BC.

Methods

In total, **1141 BC patients and 184 unaffected individuals** with family history (FH) of BC were referred from physicians for testing using a multigene panel. Genomic DNA was enriched for targeted regions of 33 genes panel (***APC, BMPR1A, BRCA1, BRCA2, CDH1, CDK4, CDKN2A, EPCAM, MEN1, MLH1, MSH2, MSH6, MUTYH, PALB2, PMS2, PTEN, RET, SMAD4, STK11, TP53, VHL, ATM, BRIP1, CHEK2, NBN, RAD51C, RAD51D, BARD1, CHEK1, MRE11 (MRE11A), NF1, RAD50, RAD51B***) and sequencing was carried out using the Illumina NGS technology. The presence of large genomic rearrangements (LGRs) was investigated computationally and by Multiplex Ligation-dependent Probe Amplification (**MLPA**). All clinically significant observations were confirmed by orthogonal technologies.

Results

Table 1. Demographic and clinical characteristics for all tested individuals.

Demographic	**No.**	**%**
Total individuals	1325	100
Female	1316	99.3
Male	9	0.7
Age at diagnosis (years)		
Mean ± SD	44.9 ± 11.1	
Median	43	
Range	22-86	
Age at testing (years)		
Mean ± SD	47.0 ± 11.2	
Median	45	
Range	23-88	
Ethnicity		
Greek	754	56.9
Romanian	384	29.0
Turkish	187	14.1
Clinical status		
Affected	1141	86.1
Unaffected	184	13.9

A pathogenic variant (PV) was identified in 22% (291/1325) of analyzed individuals and in specific in 23.2% of BC patients and 14.1% of unaffected individuals (*P = 0.006*). Among individuals with PVs, **49.1%** were located in the BRCA1/2 genes whereas **8.6%**, **22.7%** **and 19.6%** occurred in other **high**, **moderate and low risk** genes respectively. Notably, 21 of the 291 positive individuals (7.2%) carried clinically significant variants **in two different** genes and 6.5% had a large genomic rearrangement (**LGR**).

Results

Table 2. Frequency of Pathogenic, Likely Pathogenic and Variants of Uncertain Significance (VUS) among tested individuals.

Category	Individuals	% VUS rate	% Positive rate	%Positive rate in gene categories% (positive individuals/total individuals)			
				BRCA1* and *BRCA2	Other high-risk genes	Genes with moderate risk	Genes with low/unknown risk
Total individuals	1325	38.3%	22.0%	10.8%	1.9%	5.0%	4.3%
Greek	754	38.9%	20.6%	9.2%	1.6%	5.2%	4.6%
Romanian	384	33.6%	27.9%	14.6%	2.6%	6.3%	4.4%
Turkish	187	45.5%	15.0%	9.1%	1.6%	1.6%	2.7%
Affected individuals	1141	37.5%	23.2%	11.5%	2.1%	5.3%	4.3%
Unaffected individuals	184	43.5%	14.1%	6.0%	0.5%	3.3%	4.3%

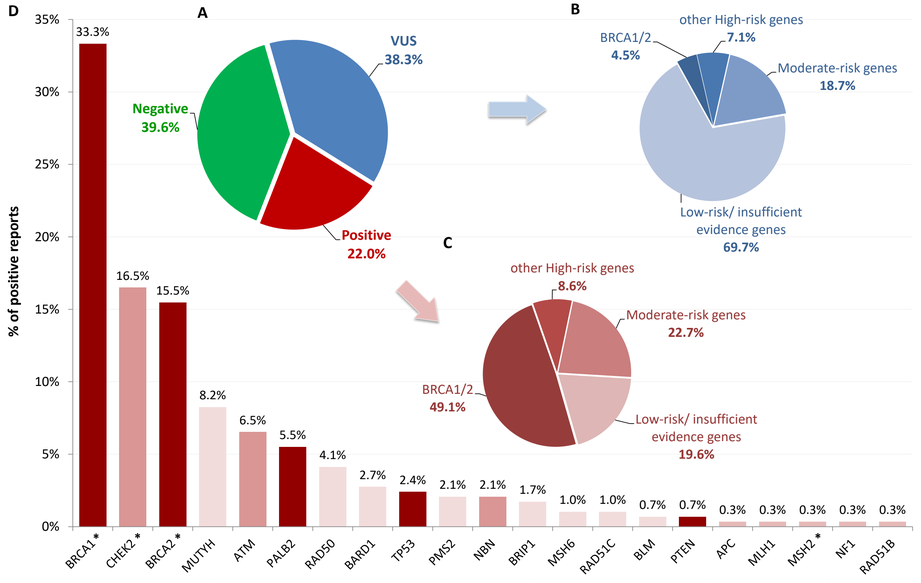

Figure 1. A. Panel testing outcomes for **1325** individuals with personal and/or family history of Breast cancer, **B.** Percentage of VUS identified stratified by gene risk category, **C.** Percentages of gene categories in individuals with positive findings, **D.** Percentages of genes in individuals with positive findings.,* genes with LGRs.

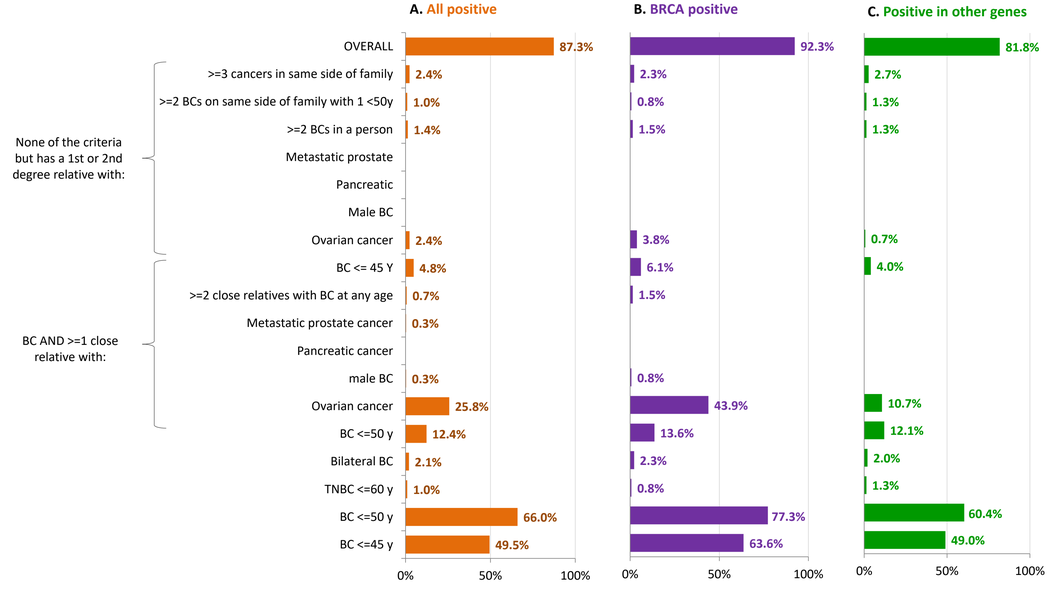

Figure 2. Breakdown of criteria for further genetic risk evaluation in NCCN guidelines for Genetic/Familiar High-Risk Assessment: Breast and Ovarian. The percentage of individuals fulfilling each criterion is compared in the subpopulations of all individuals with positive results (**A**) compared to BRCA-positive (**B**) and non-BRCA positive individuals (**C**). This retrospective analysis of positive individuals showed that 88.3% of BC patients met the NCCN criteria for further genetic risk evaluation compared to 80.8% of unaffected individuals with FH of BC (*P = 0.269*). In BRCA-positive cases (**B**), NCCN criteria were met in 92.3% of the referrals compared to 81.8% in individuals positive (**C**) for other genes (*P = 0.008*).

Conclusions

Extended multigene panel testing in hereditary BC facilitates towards the detection of nearly twice as many individuals that could benefit from personalized management. In our cohort, currently used selection criteria for HBOC failed to identify only **12.7%** of individuals positive for pathogenic variants, suggesting strong selection strategies from physicians. However, our results indicate that selection criteria perform better for the identification of BRCA-positive BC patients and should be revised to facilitate towards the inclusion of BC patients with PVs in other genes.

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Conflicts of interest: The authors declare that they have no competing interests

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