Copy Number Variations (CNVs) and Hereditary Breast Cancer

Konstantinos Agiannitopoulos[1], Georgia Pepe[1], Eirini Papadopoulou[1], Georgios N Tsaousis[1], Despina Apostolopoulou[1], Nikolaos Tsoulos[1], Varvara Potska[1], Vassileios Venizelos[2], Christos Markopoulos[3], Rodoniki Iosifidou[4], Maria Vasilaki-Antonatou[5], Christos Christodoulou[2], Ioannis Natsiopoulos[6], Konstantinos Papazisis[7], Eleftherios Kabletsas[8], Amanta Psyrri[9], Sofia Karageorgopoulou[10], Stylianos Giassas[10], Dimitrios Ziogas[11], Efthalia Lalla[4], Anna Koumarianou[9], Christos Papadimitriou[12], Dimitrios Trafalis [13], Eleni Timotheadou[14] and George Nasioulas[1]

[1]Genekor Medical S.A, Athens, Greece, [2]Metropolitan Hospital, Athens, Greece, [3]Athens Medical Center, Athens, Greece, [4]Theagenio Anticancer Hospital, Thessaloniki, Greece, [5]Metropolitan General Hospital, Athens, Greece, [6]Interbalkan Medical Center of Thessaloniki, Thessaloniki, Greece, [7]Euromedica General Clinic, Thessaloniki, Greece, [8]University Hospital of Ioannina, Ioannina, Greece, [9]Attikon University Hospital, Athens, Greece, [10]IASO, General Maternity and Gynecology Clinic, Athens, Greece, [11]General Hospital of Athens "LAIKO", Athens, Greece, [12]General Hospital of Athens "ALEXANDRA", Athens, Greece, [13]Mediterraneo, Athens, Greece, [13]General Hospital of Thessaloniki "PAPAGEORGIOU", Thessaloniki, Greece

Genekor

Committed to Biotechnological Innovation

INTRODUCTION

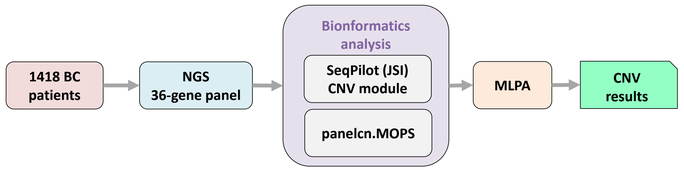
Breast cancer is the most frequently diagnosed cancer in women and about 10% of breast cancer cases are hereditary. *BRCA1* and *BRCA2* are the genes most frequently associated with Hereditary Breast Cancer, although there are numerous other genes, such as *PALB2*, *CHEK2* and *ATM*, that require to be considered as well. Germline Copy Number Variation (CNV) is a mutation type that is an important contributor to hereditary breast cancer. Nowadays, next-generation sequencing (NGS) technology has contributed to multi-gene panel analysis used in clinical practice.

OBJECTIVES

We investigate the performance of the CNV module of the commercial software suite SeqPilot (JSI Medical Systems) and the non-commercial tool panelcn.MOPS using NGS data of breast cancer samples.

MATERIALS AND METHODS

We performed a retrospective analysis of 1418 individuals referred to our laboratory for genetic testing using a multigene panel. A capture-based method NGS technology was used for the analysis of 36 genes involved in hereditary cancer predisposition. Sequencing was carried out using the Illumina NGS technology. Reads were aligned to the reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. The capture-based approach allowed for computational analysis of CNVs from NGS data. The schematic representation of the workflow can be seen below:



RESULTS

Both algorithms are specifically developed for CNV analysis of sequencing data reporting 99–100% sensitivity and up to 100% specificity for the prediction of CNVs up to the level of a single gene exon. All CNVs detected with these algorithms were then verified experimentally using the MLPA technique as an orthogonal assay. At least one pathogenic/likely pathogenic variant was identified in 289 samples (20.4%). CNVs accounted for 10.4% (30/289), referring to the deletion of one or more exons of a gene. Interestingly, 50% of deletions were single exon and approximately 36% of CNVs were detected in genes other than *BRCA1/2*. In specific, of the 30 CNVs detected, 60% occurred in *BRCA1*, 3.3% in *BRCA2*, 20% in *CHEK2*, 6.7% in *FANCA*, 6.7% in *PMS2*, and 3.3% in *ATM*. The majority of CNVs in *BRCA1* were deletions of exons 19, 22, and 22-23 whereas deletions of exons 9-10 were the most common deletions in *CHEK2*. Detailed information of all CNVs detected is provided in **Table 1**.

Table 1. Pathogenic Copy Number Variations (CNVs) identified in this study

Gene	HGVS nomenclature	Other nomenclature	# detected
ATM	NM_000051:c.(-30+1_-29-1)_(331+1_332-1)del	deletion of exons 2-4	1
BRCA1	NM_007294:c.(5467+1_5468-1)-(*1_?)del	deletion of exon 23	7
BRCA1	NM_007294:c.(5406+1_5407-1)_(*1_?)del	deletion of exons 23-24	5
BRCA1	NM_007294:c.(5193+1_5194-1)-(5277+1_5278-1)del	deletion of exon 19	6
BRCA2	NM_000059:c.(6841+1_6842-1)_(7007+1_7008-1)del	deletion of exons 12-13	1
CHEK2	NM_007194:c.(908+1_909-1)_(1095+1_1096-1)del	deletion of exons 9-10	4
CHEK2	NM_007194:c.(792+1_793-1)_(846+1_847-1)	deletion of exon 7	2
FANCA	NM_000135:c.(1626+1_1627-1)_ (2852+1_2853-1)del	deletion of exons 18-29	1
FANCA	NM_000135:c.(893+1_894-1)_(1359+1_1360-1)del	deletion of exons 11-14	1
PMS2	NM_000535: c.(903+1_904-1)_(988+1_989-1)del	deletion of exon 9	1
PMS2	NM_000535:c.(705+1_706-1)_(2006+1_2007-1)del	deletion of exons 7-11	1

RESULTS

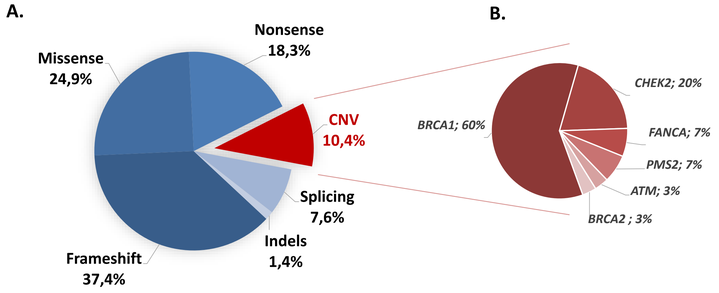


Fig.1. A. Distribution of mutation types for the pathogenic/likely pathogenic variants identified in 289 individuals with positive findings. **B.** Distribution of genes with CNVs.

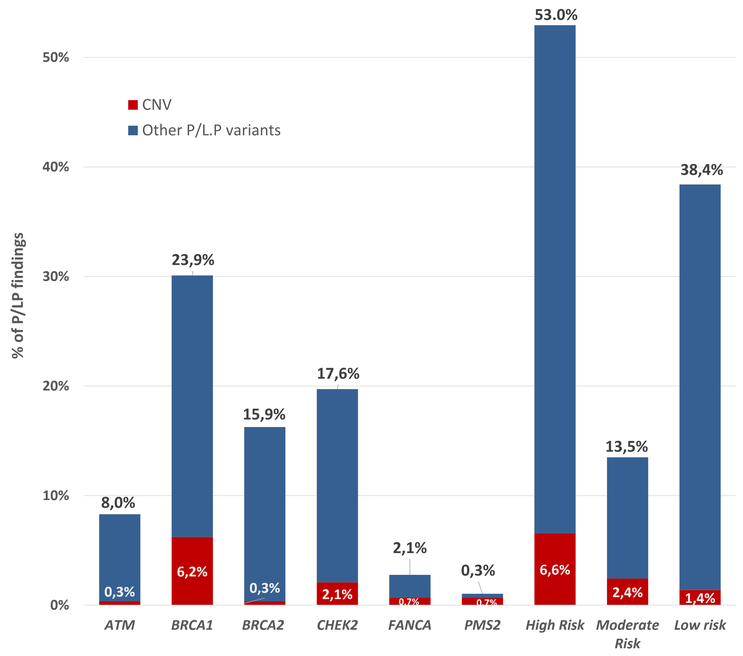


Fig 2. Percentages of observed CNVs in cancer predispositions genes. CNVs are shown in red, and other P/LP variants in blue.

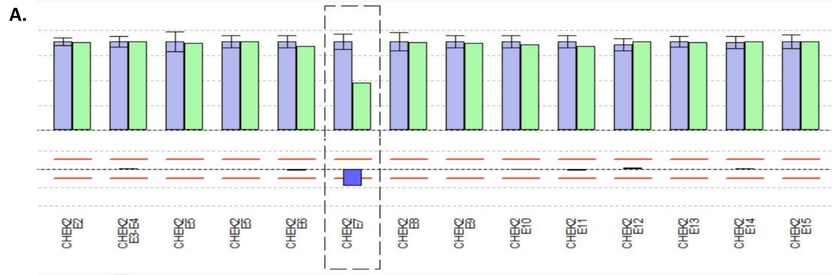


Fig 3. A. Copy Number Variations (CNVs) detection with NGS using SeqPilot. Visualization of *CHEK2* germline **exon 7 deletion** in JSI SeqPilot SeqNext software. The upper histogram shows the relative coverage of every target region of interest (ROI) of the patient sample in green and the average relative target coverage of control samples in blue. The lower histogram shows the ratio of the relative coverage of target ROIs calculated from patient versus controls. If the ratio exceeds the defined limits indicated by red lines, the bars change from light blue to dark blue, indicating a genomic change. A lower limit (lower red dotted line) of 75% (deletion) and an upper limit (upper red dotted line) of 135% (duplication) was used. Data are expressed as means ± SD of control samples.
B. Representative plots of the MLPA analysis by Coffalyser.Net showing the probe ratios with 95% confidence intervals as error bars for all exons of the *CHEK2* gene. MLPA analysis of the proband showing heterozygous **deletion of exon 7** in the *CHEK2* gene.

CONCLUSIONS

- **CNV analysis should not be restricted to *BRCA1/2.***
- **A significant proportion of CNVs (36%) occurs in additional breast cancer predisposition genes.**
- ***In silico* CNV detection tools provide a cost-effective and feasible methodology for the identification of CNVs from NGS experiments.**
- **Comprehensive genetic testing that includes full sequencing and CNV analysis in hereditary breast cancer patients facilitates personalized management decisions.**

REFERENCES

1. Lepkes L, *et al:* Performance of In Silico Prediction Tools for the Detection of Germline Copy Number Variations in Cancer Predisposition Genes in 4208 Female Index Patients with Familial Breast and Ovarian Cancer. *Cancers (Basel)* 2021.
2. Schmidt A, *et al:* Next-Generation Sequencing-Based Detection of Germline Copy Number Variations in BRCA1/BRCA2: Validation of a One-Step Diagnostic Workflow. *J Mol Diagn* 2017, 19(6):809-816.
3. Moreno-Cabrera JM, *et al:* Screening of CNVs using NGS data improves mutation detection yield and decreases costs in genetic testing for hereditary cancer. *J Med Genet,* 2020.
4. Tsaousis GN, *et al:* Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. *BMC Cancer, 2019, 19(1):535*