1709P - Cascade genetic testing utilized only in 31% of initial families with pathogenic variants in breast cancer genes

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

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

Genekor
Committed to Biotechnological Innovation

INTRODUCTION

Hereditary cancer predisposition syndromes are responsible for approximately 5-10% of all diagnosed cancer cases. Identification of genetic predisposition using germline testing, is usually followed by targeted variant testing in family members, ensuring a cost-effective approach for identifying high-risk individuals. Consequently, this has significant implications for treatment decisions, risk-reducing interventions, and cancer screening and prevention.

OBJECTIVES

The **aim** of this study is to examine the clinical use and implementation of **cascade family testing (CFT)** in the families of breast cancer patients with pathogenic/likely pathogenic variants (PVs/LPVs).

MATERIALS AND METHODS

We performed a retrospective analysis of 1785 individuals referred to our laboratory for genetic testing using a multigene panel. A capture-based method **NGS** technology was used for the analysis of 43 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. Cascade testing in 117 individuals was performed with **Sanger sequencing** or **MLPA**. The schematic representation of the workflow can be seen below:

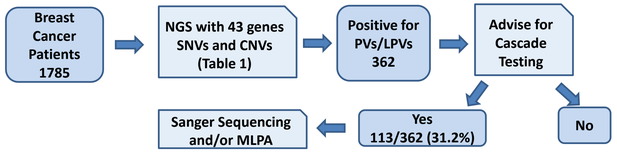

Figure 1. Schematic representation of the workflow used in this study.

Table 1. Genes analyzed.

Gene	Reference sequence	Gene	Reference sequence
APC	NM_000038	*MSH6**2	NM_000179
ATM *1	NM_000051	*MUTYH**2	NM_001128425
AXIN2	NM_004655	*NBN* *1	NM_002485
BARD1 *1	NM_000465	*NF1*	NM_000267
BMPR1A	NM_004329	*NTHL1*	NM_002528
*BRCA1**1,2	NM_007294	*PALB2**1,2	NM_024675
*BRCA2**1,2	NM_000059	*PMS2*	NM_000535
BRIP1 *1	NM_032043	*POLD1* (Exons 8-13)	NM_001256849
CDH1	NM_004360	*POLE* (Exons 1-14)	NM_006231
CDK4	NM_000075	*PPP2R2A**1	NM_002717
CDKN2A (p14ARF, p16INK4a)	NM_000077, NM_058195	*PTEN*	NM_000314
*CHEK2**1,2	NM_007194	*RAD50**1,2	NM_005732
*EPCAM**2	NM_002354	*RAD51B**1	NM_133509
FANCA *1	NM_000135	*RAD51C**1,2	NM_058216
*FANCL**1	NM_001114636	*RAD51D**1,2	NM_002878
FANCM *1	NM_020937	*RET*	NM_020975
HOXB13:c.251G>A p.(G84E)	NM_006361	*SMAD4*	NM_005359
MEN1	NM_000244	*SMARCA4*	NM_001128849
*MLH1**2	NM_000249	*STK11*	NM_000455
MRE11 *1	NM_005591	*TP53**2	NM_000546
*MSH2**2	NM_000251	*VHL*	NM_000551
MSH3	NM_002439		

*1 Genes of the homologous recombination (HR) complex

*2 Unless otherwise noted analysis of large rearrangement was performed on the following genes:
BRCA1, BRCA2, CHEK2, EPCAM (Exons 8, 9), MLH1, MSH2, MSH6, MUTYH, PALB2, RAD50 (Exons 1, 2, 4, 10, 14, 21, 23 and 25), RAD51C, RAD51D, and TP53.

RESULTS

In a cohort of 1785 patients with breast cancer, PVs/LPVs were found in 362 patients (20.3%). In specific, 52.2%, 25.1%, and 22.7% of positive patients had findings in high-, moderate- and low- penetrance cancer susceptibility genes, respectively. Although, CFT was advised in all families, 117 individuals from 113 families (**31.2%**) continued with genetic testing. The mean ages of probands and first-degree relatives tested were 46 and 40 years (p<0.0001), respectively. Of the first-degree relatives who underwent CFT, 70% were female and the 105/117 (**89.7%**) were asymptomatic (**Table 2**). The median time to cascade testing was 9 months.

Among the 117 tested individuals, 42.7%, 36.7% and 20.6% were offspring, siblings, and parents of probands, respectively **(Fig.2)**. In total, the familial PV/LPV was detected in 53.0% (62/117) of first-degree relatives tested. Our data suggest that CFT was mostly undertaken (104/113, 91.4%) in families with positive findings in high-risk genes, although these represent only **55.0%** (104/189) of initial families with PV/LPV in high-risk genes (**Fig 3**).

Table 2. Information of individuals who performed CFT.

	N=117
Female, n (%)	82 (70%)
Male, n (%)	35 (30%)
Mean Age, years	40
Asymptomatic, n (%)	105 (89.7%)
Symptomatic, n (%)	12 (10.3%)

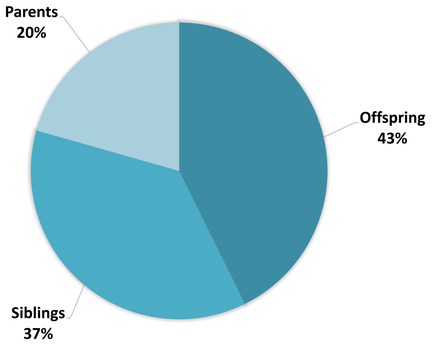

Figure 2. Percentage of first-degree relative categories undergoing cascade testing.

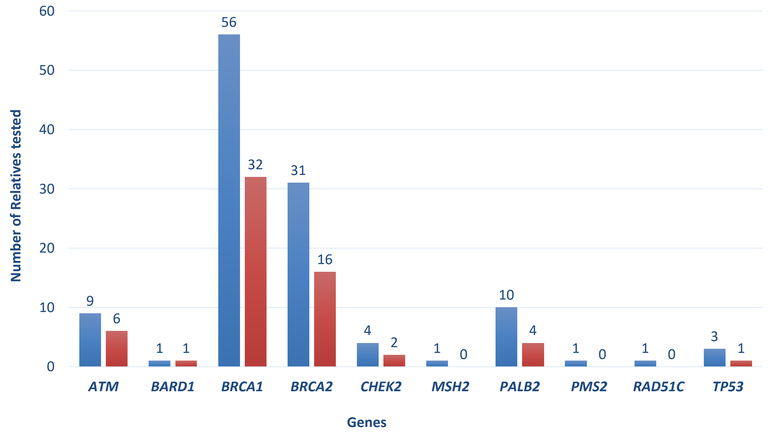

Figure 3. Number of relatives who pursued cascade testing with a negative (blue color) or positive (red color) results.

DISCUSSION

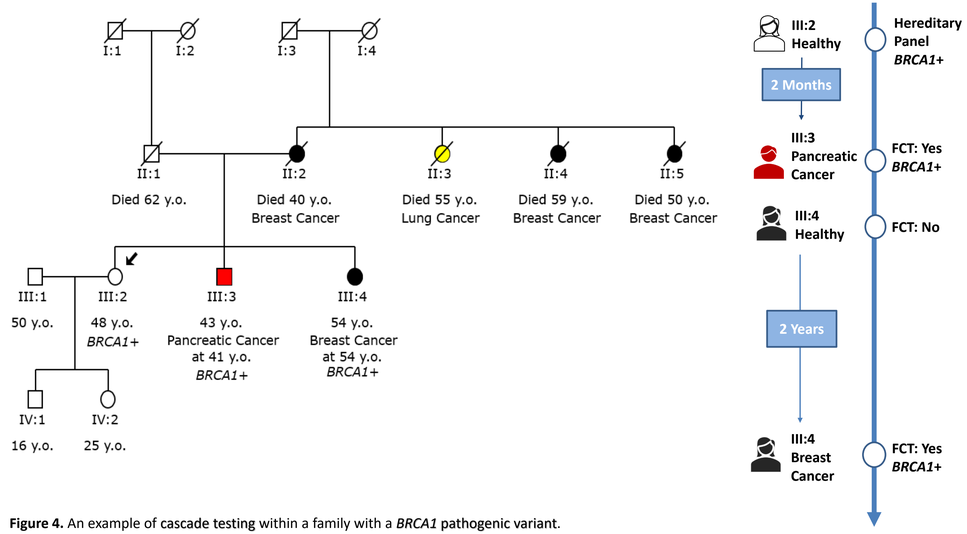

Figure 4. An example of **cascade testing** within a family with a *BRCA1* **pathogenic variant**.
In this family the initial proband (III:2) was tested positive for a pathogenic variant in the *BRCA1* gene. The reason of referral was the strong family history of breast cancer. After the announcement of the results the brother of the proband (III:3) who developed pancreatic cancer was tested positive for the specific variant in the *BRCA1* gene. The **sister of the proband (III:4)** who was healthy at that time did not consent to cascade family testing. **Two years later**, she (III:4) was **diagnosed with breast cancer** and she decided to be tested for the specific variant in the *BRCA1* gene. The analysis revealed that she carried the pathogenic variant in the *BRCA1* gene.

CONCLUSIONS

- Our results underline the fact that **CFT is underutilized, even** in families with PVs/LPVs **in high-risk genes**.
- Cascade testing can be **a powerful tool** for primary cancer prevention towards the identification of at-risk family members.
- Additional efforts should be targeted to the **implementation of genetic counseling approaches** leading to better family **education** and communication of genetic testing results.

REFERENCES

1. Frey MK, *et al*: Cascade Testing for Hereditary Cancer Syndromes: Should We Move Toward Direct Relative Contact? A Systematic Review and Meta-Analysis. *J Clin Oncol*, 2022
2. Schmidlen TJ, *et al:* The Impact of Proband Indication for Genetic Testing on the Uptake of Cascade Testing Among Relatives. *Front Genet*, 2022
3. Whitaker KD, *et al:* Cascade Genetic Testing for Hereditary Cancer Risk: An Underutilized Tool for Cancer Prevention. *JCO Precis Oncol*, 2021

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

Corresponding author: ziogasdc@gmail.com