Hereditary Breast/Ovarian Cancer: A systematic screening of DNA repair genes in 300 consecutive patients

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INTRODUCTION

In about 25% of cases, hereditary breast and ovarian cancer (HBOC) is caused by mutations in the BRCA1 or BRCA2, both components of DNA repair pathways. In recent years, additional genes of the DNA repair system such as *CHEK2, ATM, BRIP1, PALB2, RAD51C* and others have been implicated in HBOC. Interestingly, many of these genes had been identified first (with biallelic mutations) in Fanconi Anemia patients. We set out to study the contribution to HBOC of 30 DNA repair genes contained within the TruSight Cancer panel (Illumina™).

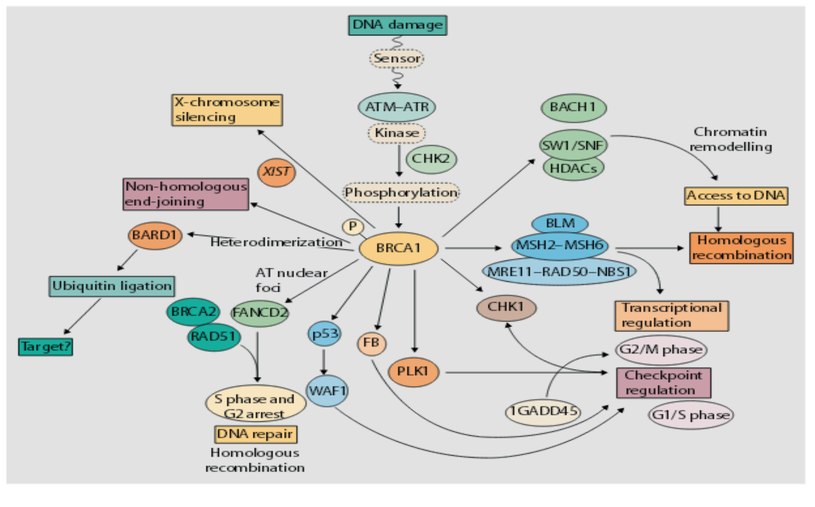

Figure 1: Schematic representation of the BRCA1-/BRCA2-signal pathway.

(Gadzicki et al. Medgen 2007, Bd.19: 202-209).

METHODS

All 300 patients fulfilled the inclusion criteria defined by the German Consortium for Breast and Ovarian cancer. Target enrichment was performed with the Illumina TruSight cancer panel which includes 94 genes associated with a predisposition towards cancer.

Next generation sequencing data were generated on a MiSeq (Illumina). Variants were identified and analysed by GensearchNGS software (PhenoSystems). Copy number variation analysis was carried out with the NextGENe CNV detection tool (Softgenetics).

RESULTS

In all samples, data analysis showed an even coverage of at least 50-fold across 98,5% of all coding regions investigated. We focused on protein truncating mutations since functional data on other variants in these genes are scarce. 45 patients (15%) carried a mutation in *BRCA1* or *BRCA2*. In addition to *BRCA1* and *BRCA2*, we detected truncating mutations in the following genes: *ATM, CHEK2, PALB2, NBN, BRIP1, PTEN, CDH1, FANCA, FANCI, FANCM, ERCC2, XPC, RECQL4* and *MSH6*. Altogether we found 9,6% deleterious mutations in genes other than *BRCA1* and *BRCA2*.

Table 1: Protein truncating mutations found in DNA repair genes by NGS in 300 patients

Gene	Frequency	HGVS nomenclature	Consequence	Mutation type
BRCA1	25x			
BRCA2	20x			
CHEK2	4x	c.1100del	p.Thr367Metfs*15	fs
		c.1297C>T	p.Gln433*	nonsense
		c.444+1G>A	p.?	splice
ATM		c.7308-2A>C	p.?	splice
		c.1718del	p.Lys573Argfs*4	fs
		c.790del	p.Tyr264Ilefs*12	fs
		c.8291dup	p.Ser2764Argfs*5	fs
		c.7327C>T	p.Arg2443*	nonsense
		c.5319+2T>C	p.?	splice
NBN	2x	c.657_661del	p.Lys219Asnfs*16	fs
PALB2		c.18G>T,	p.?	splice
		c.509_510del	p.Arg170Ilefs*14	fs
BRIP1		c.507G>A	p.?	splice?
PTEN		c.170dup,	p.Leu57Phefs*6	fs
MSH6		c.3991C>T,	p.Arg1331*	nonsense
FANCA		c.1716-2A>C	p.?	splice
FANCI		c.3853C>T	p.Arg1285*	nonsense
FANCM	2x	c.5101C>T	p.Gln1701*	nonsense
	2x	c.5791C>T,	p.Arg1931*	nonsense
XPC		c.537-1G>C	p.?	splice
ERCC2		c.2009dup	p.Lys671Glnfs*103	fs
RECQL4		c.419del	p.Pro140Glnfs*40	fs
CDH1		c.1679C>G	p.?	splice

Table 2: CNV analysis of NGS data from a patient with a deletion of exon 1-5 (2-7) in BRCA1

Chr	Gene	CDS	Start	End	Length	Log2Ratio	Original Cov(S;C)	Normalized Cov(S;C)
chr17	BRCA1; -	5	2440863175	2440863315	141	-1.063	591;1471	591;1235
chr17	BRCA1; -	4	2440863921	2440864010	90	-1.157	324;861	324;723
chr17	BRCA1; -	3	2440865509	2440865587	79	-1.047	260;640	260;537
chr17	BRCA1; -	2	2440874779	2440874833	55	-0.952	200;461	200;387
chr17	BRCA1; -	1	2440883070	2440883150	81	-1.101	218;557	218;467

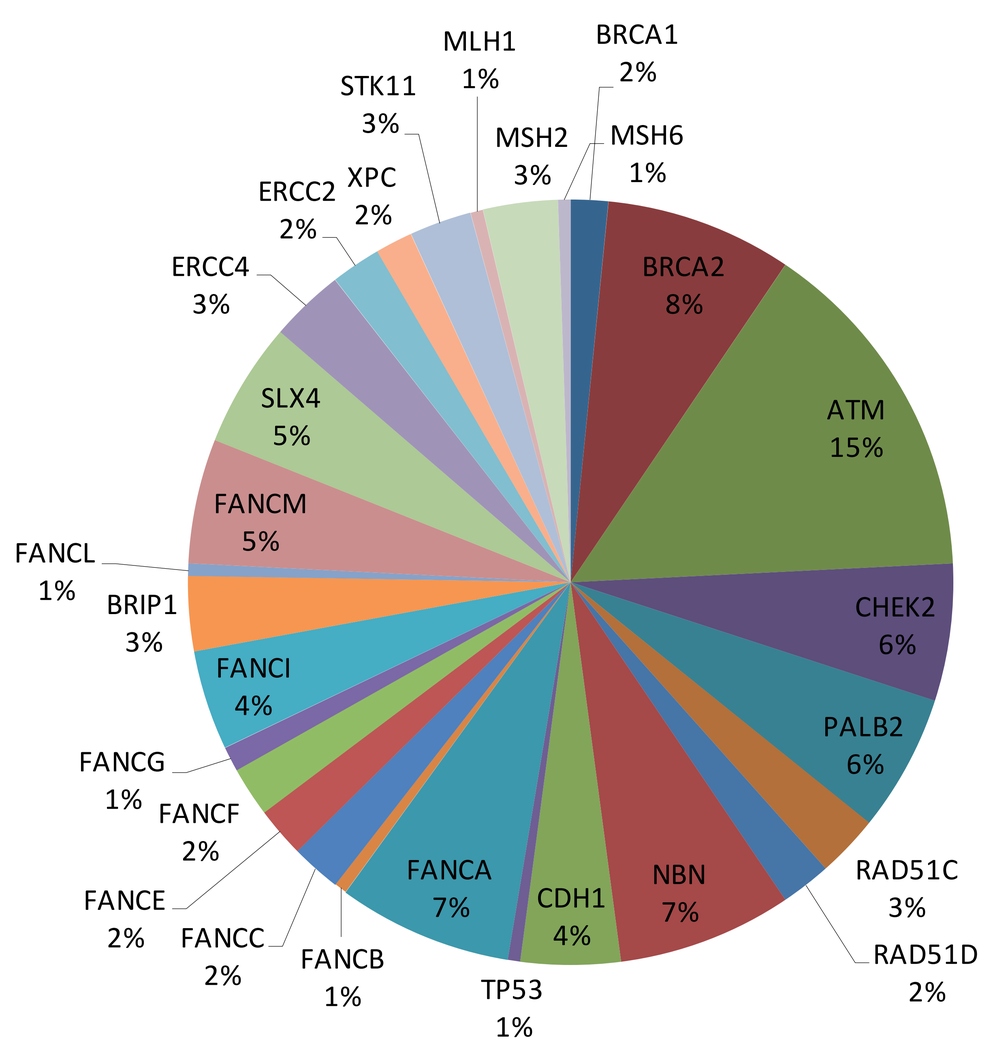

Figure 2: Relative distribution of VUS (IARC class 3) detected with NGS in 300 HBOC patients. No VUS was found in the *PTEN* gene.

A variant of uncertain significance (VUS) was identified in at least 1 of the 30 genes tested in 147 individuals (49%), for a total of 193 variants. CNV analysis revealed deletions or duplications only in *BRCA1* and not in any of the other genes studied.

CONCLUSION

Mutation screening by NGS was able to identify monoallelic, likely pathogenic mutations in DNA repair genes other than *BRCA1/2* in a significant number of HBOC cases. However, the causative association to HBOC and the prospective tumor risks for many of these mutations and genes have yet to be determined. Of note, extending the analysis to a larger number of genes proportionally increases the number of unclassified variants and the workload to survey and classify them.

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