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The Importance of the Detection of Large Genomic Rearrangements Predisposing Hereditary Cancer Syndromes: A Systematic Review

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Abstract

The precise identification of pathogenic germline variants in cancer predisposing genes is crucial for genetic screening and management of hereditary cancer syndromes. Next Generation Sequencing is now the gold standard test, currently in practice globally to identify these germline variants. However, large genomic rearrangements in cancer predisposing genes are usually missed by Next Generation Sequencing; hence they do not get reported in some patients. This may lead to underestimation of the frequency of the variants and lead to false-negative information, misleading the genetic diagnosis and early interventions in high risk individuals. These large genomic rearrangements have been characterized in several populations. The identification of variants in cancer predisposing genes for specific types of cancers provides the necessary information for the complete characterization of inherited cancer syndromes. The inclusion of tests for detection of large structural variants to diagnostic panels should be encouraged.

Keywords: Cancer predisposing genes; Proband; Hereditary cancer; Next-Generation Sequencing; Large structural variants; Single nucleotide variants

Introduction

Hereditary cancer syndromes

Cancer is one of the leading causes of morbidity and mortality worldwide. It is a malignant growth or tumor resulting from uncontrolled division of cells. Taken in the context of the family of the person affected with cancer, cancer falls into three groups- sporadic, familial, and hereditary [1]. It has been estimated that 5% to 10% of all cancers are inherited [2]. Inherited cancers have a definite pattern of transmission over several generations in a family. They are mostly caused by high-penetrant autosomal dominant Cancer Predisposing Genes (CPG s) such as *BRCA1* and *BRCA2* in hereditary breast and ovarian cancer and *APC* in Adenomatous Polyposis Coli. Similarly, each type of hereditary cancer syndrome has a pattern of clinical characteristics which is useful in the diagnosis.

In order to determine whether or not the cancers are likely to be hereditary, pedigrees must be reviewed. Genetic predisposition to cancer can be suspected as per the criteria made by National Comprehensive Cancer Network (NCCN) [https://www.nccn.org]. Criteria such as positive family history of cancer with multiple affected generations (1st, 2nd or 3rd degree relatives); early age of onset (below 50 years of age); multiple primary cancers in an individual belonging to a known hereditary cancer syndrome (e.g. breast and ovarian cancer, colorectal and endometrial cancer); clustering of rare cancers; bilateral involvement in paired organs (e.g. bilateral breast cancer) and unusual or atypical presentation of cancer (e.g. male breast cancer) can be found by constructing a pedigree (Figure 1). Individuals who meet one of the above criteria should be referred to analyze their genetic profiles.

An important benefit of testing CPG s is that it enables us to predict information related to the predisposing genes which allow accurate risk assessment since it confirms the diagnosis in the proband. Thus, enabling the first-degree relatives to be counseled about the risks and enables genetic screening of family members. If a cancer is detected early treatment and survival of the affected persons can be improved. Prevention usually involves surgical removal of the at-risk tissue such as in prophylactic mastectomy in breast cancer and is necessary to monitor or remove the respective organs in pre-symptomatic individuals at very high risk, such as the breast in *BRCA1*

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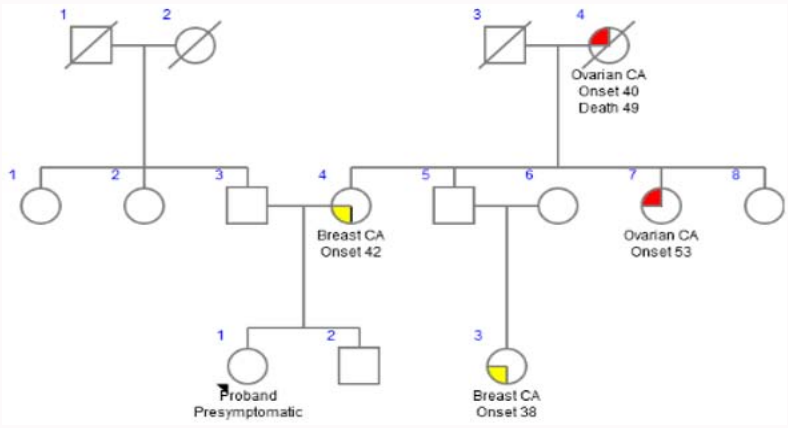

Figure 1: A classic pedigree of Hereditary Breast and Ovarian Cancer in a family.

mutation carriers; the kidney in FLCN mutation carriers; thyroid in RET mutation carriers; and the colon in *APC* mutation carriers, before they develop the cancer. Chemoprevention is also an attractive strategy to reduce the cancer risk or delay the development of cancer.

Cancer predisposing genes involved in hereditary cancer syndromes

The discovery of genes predisposing hereditary cancer has been accompanied by technological advances in the characterization of the genetic mutations that predispose individuals to increase risk of cancer as well as by advances in therapeutic interventions and screening strategies that effectively address hereditary cancer risk. There is no definitive definition for CPGs [3]. Generally, they are genes that harbor genetic variants that predispose the variant carrier to the development of cancer. CPGs are known to have a broad range of functions. In 1997, Kinzler and Vogelstein [4] introduced two terms to distinguish CPGs, “gatekeeper genes” for genes that directly involve controlling the cellular proliferation, and "caretaker genes” for those that help to maintain the integrity of the genome [4]. Gatekeeper genes may further be divided into oncogenes (proto-oncogene) that positively effect on the growth and proliferation and tumor suppressor genes (anti-oncogenes) that maintain negative effect on tumor formation. Genetic predisposition to cancer has been found predominantly in tumor suppressor genes and less frequently in proto-oncogenes [1]. Variants in tumor suppressor genes is a common cause of autosomal dominantly inherited susceptibility to specific cancers-inherited retinoblastoma [5]; Familial Adenomatous Polyposis (FAP); Gorlin and Cowden syndromes; neurofibromatosis types 1 and 2. However, mutations in caretaker genes such as the DNA mismatch repair genes also have a contribution in inherited cancer susceptibility [6]. More than 100 such CPGs have been identified [3]. Some of the well-known CPGs include *BRCA1* and *BRCA2* in Hereditary Breast and Ovarian Cancer (HBOC); *APC* in Familial Adenomatous Polyposis (FAP); *MLH1*, *PMS2*, *MSH2*, *MSH6*, and *EPCAM* in Hereditary Non-Polyposis Colorectal Cancer (HNPCC); *BRCA1* and *TP53* in Li-Fraumeni Syndrome (LFS); *PTEN* in Cowden Syndrome (CS); *CDH1* in Hereditary Diffuse Gastric Cancer (HDGC); ATM in Ataxia Telangiectasia (AT) [7] Table 1 illustrates a few CPGs. They harbor different types of variants such as Single Nucleotide Variants (SNVs), small insertions/deletions (INDELS), and structural variants (large deletions and duplications) that can be employed as biomarkers for the diagnosis of hereditary cancers [8].

Approximately 7% of breast cancers and 10% of ovarian cancers are known to arise from inherited mutations in specific tumor suppressor genes *BRCA1* and *BRCA2* [9]. Women who carry mutations in these genes are estimated to have a 60% to 80% life time risk for breast cancer [10]. Personal and/or family histories of cancer combined with phenotypic clues have been used to guide the selection of genes that are most likely to have an underlying mutation [11].

Structural variants that predispose to hereditary cancer syndromes

Structural Variants (SV) refers to genomic alterations containing base pairs that differ between individuals and that are not Single Nucleotide Variants (SNPs). They are segments of DNA that are larger than 1 kb [12]. A better understanding of these mutational mechanisms used in the formation of structural variants is vital for improving the methods of mutation detection strategies use in the early detection of inheritance of cancer susceptibility. Various categories of mutational mechanism are known to give rise to genomic rearrangements: Errors in recombination, as in Homologous Recombination including, Non- Allelic Homologous Recombination (NAHR); Single Strand Annealing and Break Induced Replication; errors generated in DNA base repair as in Non-Homologous End Joining (NHEJ); microhomology-mediated end joining; or errors in replication, such as fork stalling and template switching or micro-homology mediated break-induced replication and serial replication slippage [13]. Out of these mechanisms’ homologous recombination, non-replicative non-homologous repair, and replication-based mechanism are the three main types of mechanisms known to cause SVs [13,14].

In the majority of cases, germline variants in CPGs are single nucleotide variants [15]. But large structural variants are having also been recognized as having a significant impact on inter- individual genetic variation [16]. In the last decade, a number of studies have demonstrated that gene deletions/duplications are detectable in some cases which turn out to be negative for single nucleotide variants in CPGs. They have recently been identified and they account for a small but still significant proportion of affected cases and individuals who are at risk of hereditary cancers in several populations throughout the world. These variants are usually pathogenic because deletions or insertions of large genomic sequences within a coding region result in out-of-frame translation and usually lead to a mutant peptide of abnormal structure and/or function.

Problem associated with detection of structural variants

Genetic testing of a living relative who has had cancer must

Table 1: List of common cancer predisposing genes causing Hereditary cancers.

Chromosome region	Gene	Hereditary Cancer syndrome	Main cancer	Gene function	Reference
17q21	*BRCA1*	Hereditary Breast and Ovarian	Breast, ovarian	Tumor suppressor	[39]
13q12.3	*BRCA2*	Cancer Syndrome	Breast, ovarian, pancreatic, leukemia		
2p22-p21	*MSH 2*	Hereditary Non- polyposis	Colorectal, endometrial and ovarian	Mismatch repair	[40]
3p21.3	*MLH1*	colorectal cancer syndrome			
5q21-q22	*APC*	Familial polyposis coli	Colorectal, pancreatic hepatoblastoma	Tumor suppressor	[41]
22q12.1	*CHEK2*	Familial breast cancer	Breast, prostate	Tumor suppressor	[42]
17p13.1	*TP53*	Li-Fraumeni syndrome	Breast, sarcoma, adrenocortical carcinoma	Tumor suppressor	[43]
13q14.1-q 14.2	*RB1*	Hereditary retinoblastoma	Retinoblastoma Osteosarcoma	Tumor suppressor	[44]
11q13	*MEN1*	Multiple endocrine neoplasia type 1	Parathyroid adenoma, pituitary adenoma	Tumor suppressor	[45]
17q11.2	*NF1*	Neurofibromatosis type 1	Neurofibroma	Tumor suppressor	[46]
22q12	*NF2*	Neurofibromatosis type 2	Meningioma	Tumor suppressor	[47]

Table 2: Commonly use methods for the detection of large structural variants.

Method	Rearrangements that can be identified	Advantages	Drawbacks	Reference
Long Range PCR	deletions insertions duplications	Can detect recurrent variants Require only small volume of DNA	Low throughput unable to provide a genome-wide view of rearrangements.	[48,49]
Southern blotting	CNV	Detects small structural variants	Laborious, time-consuming requires large amounts DNA.	[50, 51]
aCGH	CNV	efficient method for the detection of structural variants	Expensive. Cannot identify Aberrations- balanced reciprocal translocations or inversions.	[52]
Quantitative multiplex PCR of short fluorescent fragments (QMPSF)	genomic deletions or duplications based on the simultaneous amplification of short genomic fragments	Rapid and sensitive	Requires proper training and prior experience	[53-55]
Real-time polymerase chain reaction (qPCR)	Deletions, duplications	Rapid	Not suitable for the detection of translocations or inversions or for genome-wide screening of rearrangements	[49,56]
MLPA	Can identify the precise location of the deletion or duplications	inexpensive, sensitive, relatively simple, and high-throughput method	Occurrence of a point mutation can give false positive results Falls positive results can occur when probes are designed outside the region of interest	[2,23,25,57]

be done first to identify the specific variant in a family. Once such a variant is identified testing of additional family members to determine whether they are at increased risk of developing cancer can be conducted.

Sanger DNA sequencing has been the standard method for detecting variants in clinical practice for years. Yet the utilization of Sanger sequencing is very limited when analyzing multiple genes from several patients simultaneously, because sequencing has to be conducted serially in one gene at a time. This is costly and time consuming. Furthermore, Sanger sequencing is protracted, laborious and also has a comparatively low throughput to the newer parallel sequencing approaches. Next Generation Sequencing (NGS) technology is the most recent evolution in genomic technology; a high-throughput process of DNA sequencing by providing a base-by-base view of the genome, NGS can identify SNVs and INDELS [17]. NGS panel testing for inherited cancer susceptibility has gained wide acceptance as a useful diagnostic tool in routine clinical practice as it enables simultaneous testing of all the CPGs in a cost effective and timely manner [18]. Several studies which assessed the frequency of variants among patients referred for inherited cancer risk assessment using NGS panels have recently been published [19-21]. Certain structural variants that can evade both read-depth analyses (by having a breakpoint within a small assay target) and INDEL detection (by being too large or by having a breakpoint outside of the targeted regions) will be missed by NGS [22]. Taking HBOC as an example, and focusing on structural variants, many large structural variants have been found in *BRCA1* and *BRCA2* genes in patients with HBOC. In some countries, large structural variants are found in a high proportion of patients affected with HBOC. In Northern Italy, large deletions account for approximately one-third of the pathogenic *BRCA1* variants [23]. Similarly, in the Netherlands, large deletions account for 30% of all deleterious variants in *BRCA1* [24,25]. Unfortunately, there is no single technique that can identify all mutations in the CPGs that could predispose to hereditary cancer syndromes. This raise the question that some families, for whom variant testing has so far yielded a negative result by NGS, may harbor large deletions and rearrangements that provides a promising outlook for those engaged in clinical practice. There may be several reasons for negative results in an affected proband whose family has been identified as having certain type of hereditary cancer. Affected relative in the family could be a sporadic case who does not carry the affected CPG that circulate within the family. Secondly, technical limitations also may responsible for false-negative results. Methods employed for mutation screening usually focus the detection of sequence alterations, such as point mutations, small deletions, and insertions. Hence the loss of partial or entire exons will not be detected by these methods. If, a strategy is added to detect large structural variants that may have been missed by NGS, can increase the detection rate of variants in hereditary cancer families.

Table 3: Structural variants identified in western countries.

Country	CPG	SV Identified	Prevalence	Reference
Australia	*BRCA1*	Del.ex.3, ex.5, ex. 21-23	2%	[2]
	BRCA2	Del.ex 1-2, ex. 14-16		
Mexico	*BRCA1*	Del.ex. 9-12 , ex.18-19, Dup.ex.8-10	28%	[58]
Czech Republic	*BRCA1*	Del.ex.1A/1B-2,ex.5-14,ex.11-12,ex.18-19, ex.20,ex. 21-22	6%	[48,59]
		Del.ex 1-17, ex.5-10,ex.13-19,ex.18-22, ex.21-24		
Netherlands	*BRCA 1*	Del. ex 8, ex 13, ex 20-22, ex 22; Dupl. ex 13, ex 21-23	7% to 9%	[24,25]
Portugal	*BRCA1*	Del.ex.1-22, ex.8-13, ex.15-16, ex.11-15	9.60%	[60,61]
		Dup.ex.3-8, ex.18-20, ex. 3		
Netherlands	*APC*	Del.ex. 1-15,1-5, 4-5, 6-15, 7-13, 9-15	8%	[62]
Spain	*BRCA2*	Del. ex 2, ex 10-12, ex 15-16;	1.50%	[63,64]
		Dupl. ex 20		
Germany	*BRCA1*	BRCA1: Del. ex 1A/1B-2, ex 5, ex 5-7, ex 17; Dupl. ex 13.	1.8% to 5.7%	[65,66]
USA	*BRCA1*	Del. ex 14-20, ex 22, ex 13; Dupl. ex 13, Del. ex 9-12	12.70%	[2,55]

Table 4: Structural variants identified in the populations in Asian countries in the world.

Country	CPG	Ethnicity	SV Identified	Prevalence	Cancer/s in the family	Reference
Singapore	*BRCA1*	Indian	Del. ex. 13-15	3%	Breast, Ovarian	[31]
		Chinese	Dup. ex. 13		Nil	
	BRCA2	Chinese	Dup. ex. 4-11		Nil	
Korea	*BRCA1*	Korean	Del. ex.13–15	0.80%	Breast	[32]
Malaysia	*BRCA1*	Indian	Del. ex. 13-15	8%	Nil	[33]
		Chinese	Del. ex. 1-14		Ovarian	
	BRCA2	Indian	Del. ex. 14-16	4%	Breast	
South China	*BRCA1*	Chinese	Del. ex. 1-12	6.90%	Bone, leukemia, liver, pancreas	[34]
			Del. ex. 17-20		Esophagus, stomach	
	BRCA2		Del. ex. 15-16	5.00%	Esophagus,	
			Del. ex. 21		Nil	
Pakistan	*BRCA1*	Punjabi	Del. ex.1-2	3.30%	Liver, abdomen, bone	[67]
			Del. ex. 20-21		Stomach, brain, uterus	
			Del. ex. 21-24		Lung, Leukemia	
Sri Lanka	**No studies have been done so far.**					

Methods to detect LGR missed by NGS

Several approaches have been used to detect large structural variants in CPG s that are missed by NGS. They include southern blot; long-range PCR; fluorescence in situ hybridization-based methods; real-time PCR; array Comparative Genomic Hybridization (aCGH) [26,27], and Multiplex Ligation Probe Amplification assay (MLPA) [28]. A summary of the most commonly used methods for the identification of large structural variants in patients affected with Hereditary Cancer Syndromes are stated in Table 2.

Structural variants identified in different populations

There is a difference in the degree of large genomic rearrangements found in different ethnic groups and populations (Table 3). There are some founder mutations that have been identified in certain populations predisposing to various hereditary cancer syndromes [29]. These mutations are located within a genomic region and segregate as a unit due to linkage disequilibrium. These mutations are inherited and often remain restricted to one or a few populations or specific geographic regions [30]. The founder effect has been used to explain the high frequencies of disease-associated mutations in specific populations. A 510 bp deletion of exon 22 (IVS21-36del510) and a 3835 bp deletion of exon 13 (IVS12-1643del3835), are founder mutations in Dutch breast cancer patients and represent 36% of all *BRCA1* mutations in this population [24]. Identification of founder mutations in the various ethnic groups is important for the improvement of genetic counseling as it makes possible to use a more specific approach to molecular testing that would also be cheaper and quicker. A less expensive mutation detection strategy might also allow to extend genetic counseling and testing to families with a low hereditary history.

In some countries, as shown in Table 3, large structural variants are found in a high proportion of patients with hereditary cancer syndromes. In Northern Italy large deletions account for approximately one third of the pathogenic *BRCA1* mutations [23]. Similarly, in Netherlands, large deletions account 30% of all deleterious mutations in *BRCA1* [24,25].

The prevalence of large genomic rearrangements in Asians is still

unknown since studies done in these populations are limited. Few large genomic rearrangements have been reported from Singapore [31]; Korea [32]; Malaysia [33]; South China [34] and they are mainly focused on *BRCA1* and *BRCA2* in Hereditary breast and ovarian cancer, as mentioned in Table 4 [33,35-38].

Conclusion

In conclusion, the identification of variants in CPGs for specific types of cancer provides the necessary information for the complete characterization of inherited cancer syndromes. The inclusion of tests for detection of large structural variants to diagnostic panels should be encouraged. The close observation of families at risk has significantly enriched our knowledge in distinct phonotypical features, age of onset and survival rates for each hereditary cancer syndrome and provided the opportunity to further understand the molecular basis of hereditary cancer. The deficiency in the knowledge and understanding of the molecular mechanism associated with inherited predisposition to cancer has resulted in sub optimal management, follow- up and surveillance of individuals in the Asian countries. Hence there is an urgent need for more research into large structural variants in CPG s in Asian countries.

Authors Contributions

PW conceived the review, acquired the data from literature and drafted the manuscript. Remaining authors, KW and VHWD read and approved the final version of the manuscript to be published.

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