ESHG

BRIEF COMMUNICATION

Two unrelated cases with biallelic *CHEK2* variants:a novel condition with constitutional chromosomal instability?

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Constitutional heterozygous mutations in *CHEK2* gene have been associated with hereditary cancer risk. To date, only a few homozygous *CHEK2* mutations have been reported in families with cancer susceptibility. Here, we report two unrelated individuals with a personal and familial cancer history in whom biallelic *CHEK2* alterations were identified. The first case resulted homozygous for the *CHEK2* c.793-1 G > A (p.Asp265Thrfs*10) variant, and the second one was found to be compound heterozygous for the c.1100delC (p.Thr367Metfs*15) and the c.1312 G > T (p.Asp438Tyr) variants. Multiple cytogenetic anomalies were demonstrated on peripheral lymphocytes of both patients. A literature revision showed that a single other *CHEK2* homozygous variant was previously associated to a constitutional randomly occurring multi-translocation karyotype from peripheral blood in humans. We hypothesize that, at least some biallelic *CHEK2* mutations might be associated with a novel disorder, further expanding the group of chromosome instability syndromes. Additional studies on larger cohorts are needed to confirm if chromosomal instability could represent a marker for *CHEK2* constitutionally mutated recessive genotypes, and to investigate the cancer risk and the occurrence of other anomalies typically observed in chromosome instability syndromes.

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INTRODUCTION

A hereditary cancer syndrome is present when an individual has an increased cancer risk due to an inherited genetic variant. Among the known monogenic cancer risk factors, dominant mutations in *CHEK2* gene, which is involved in the preservation of genomic integrity, have been associated to breast, prostate, colorectal, thyroid, gastric and kidney cancers [1]. *CHEK2* codes for the checkpoint kinase 2 (CHK2) protein, that is involved in cell cycle regulation through the ATM-CHK2-p53 pathway [1]. In response to DNA damage (Double Strand Breaks, DSBs), CHK2 prevents the entry into mitosis, leading to cell cycle arrest. In addition, CHK2 phosphorylates BRCA1 and allows this protein to intervene in the Homologous Recombination (HR) repair of DSBs to restore cell survival after DNA damage [2]. *CHEK2* alterations are most frequently found in heterozygosity [1], but few cases of homozygous *CHEK2* mutations have been described in patients with hereditary cancer syndromes [3–7].

Here we present two unrelated individuals with personal and familial history of different neoplasms, carrying biallelic *CHEK2* alterations: one homozygous case for the *CHEK2* NM_007194.3:c.793-1 G>A (p.Asp265Thrfs*10), and one compound heterozygous case for the c.1100delC (p.Thr367Metfs*15) and the c.1312 G>T (p.Asp438Tyr). Both cases presented with chromosomal instability in peripheral lymphocytes.

MATERIALS AND METHODS

The probands (Fig. 1: Family A case III:2; Family B case III:4) were selected from those patients attending an outpatient service at San Camillo-Forlanini hospital (Rome, Italy). They gave informed consent for the genetic analyses, approved by local ethic committees in accordance with the principles of the Declaration of Helsinki. Genomic DNA and RNA from peripheral blood were extracted by standard methods. The probands were tested for 113 genes related to cancer susceptibility by the TruSight Hereditary Cancer Panel on NextSeq550Dx sequencer (Illumina, San Diego, USA). Sequencing reads were aligned to the human reference genome (UCSC hg19) by BWA (v0.7.7-isis-1.0.2). Variant calling was performed by GATK Variant Caller (v1.6-23-gf0210b3). DNA changes were filtered by MAF < 0.05 (GnomAD v2.1) and classified according to ACMG-AMP criteria [8]. Filtered variants were validated and tested in other family members by Sanger sequencing. The RNA from case III:2 (Family A) was retro-transcribed and then sequenced by Sanger technique.

Cytogenetic analysis was performed on heparinised peripheral blood sample. Two cell cultures were set up for each patient. In the first one, lymphocyte analysis was performed by standard methods: whole blood was incubated for 72 h in a culture medium containing phytohemagglutinin (PHA). The second cell culture was performed by a synchronization of the cell cycle: whole blood was incubated for 48 h in a culture medium. Then, a methotrexate solution (Amethopterin, 10-5 M, Top Syncro) was added. Thymidine was supplemented after 17 h. Finally, colcemid solution was added, and cells were resuspended in hypotonic solution, fixed and G-stained. For each cell culture, 100 metaphases were studied with Nikon microscope and Genikon software.

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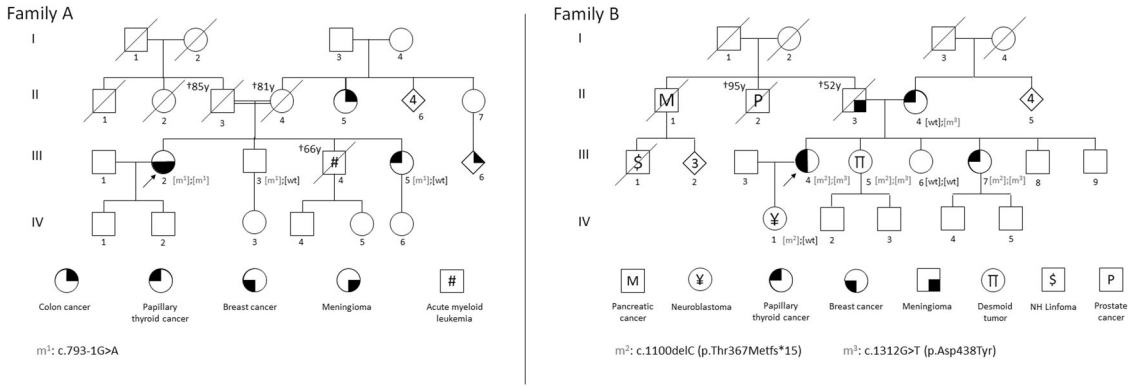

Fig. 1 Pedigree of family A and family B. The clinical legend is given under the pedigree. m: mutation †: age at death; y: years. The arrows indicate the probands. (FAMILY A). The proband had a 65-year-old healthy brother (III:3), a 69-year-old sister (III:5) who was treated for a papillary thyroid carcinoma at age 46, and a brother (III:4) affected by acute myeloid leukemia who deceased at age 66. At 49 years of age, the proband was diagnosed with hormone receptor positive, HER2 negative right breast cancer with axillary lymph nodes metastases. Five years later, an ipsilateral malignant breast lesion of 6 mm was identified. Unfortunately, seven years later from the second tumor event, an inoperable locoregional lymph nodes recurrence was detected by global FDG-PET-TC. Furthermore, a meningioma was surgically removed in the woman when she was 50 years old. The tumor recurred within a few years together with the appearance of a second meningeal lesion. The patient also suffered from multinodular goitre in presence of normal thyroid function test results. (FAMILY B). The proband's family history included: a papillary thyroid carcinoma in the mother and in one sister, detected at the age of around 70 and 47 years respectively; a meningioma in the father removed before the age of 50 years; a thoracic desmoid tumor in another sister diagnosed at age 43; and a neuroblastoma in the only daughter which manifested at the age of 18 months.

Table 1. Results of the karyotype analyses.

Family	Pt.	CHEK2 alteration		Cell colture condition (methotrexate addition)	Metaphases with chromosomal alterations (%)					
		Variant	Zygosity		Total rearrangements	translocation	inversion	deletion	2 rearrangements	≥ 3 rearrangements
A	III:2	c.793-1 G > A	Hmz	y	25[¥]	4	-	-	2*	19
				n	23	6	-	1	3[†]	13
	III:5	c.793-1 G > A	Htz	y	0	-	-	-	-	-
				n	0	-	-	-	-	-
B	III:4	c.1100delC	cmp Htz	y	10[¥]	5	2	-	-	3
		c.1312 G > T		n	5	-	-	1	-	4
	III:5	c.1100delC	cmp Htz	y	3[¥]	-	1	1	-	1
		c.1312 G > T		n	2	1	-	1	-	-
	III:7	c.1100delC	cmp Htz	y	3[¥]	-	-	2	1	-
		c.1312 G > T		n	2	1	1	-	-	-
	IV:1	c.1100delC	Htz	y	3[¥]	-	1	-	1	1
				n	2	-	1	1	-	-

Pt. patient, *Hmz* homozygous, *cmp* compound, *Htz* heterozygous, *y* yes, *n* not
*Both showing one translocation and one deletion
[†]Two/3 metaphases showed one translocation and one deletion; one/3 metaphases showed 2 translocations
[¥]Metaphases with the chromosomal alterations are showed in Supplementary Fig. 2

RESULTS

Family A

The proband was an Italian 63-year-old woman. She was born to a consanguineous mating. Both her mother and father survived old age (81 and 85-years respectively), without any cancer. Her personal and familial history of neoplasms is described in Fig. 1. The proband was found to carry the *CHEK2* c.793-1 G>A variant in homozygosity, while one of her brother and her sister were found to be heterozygous carriers. cDNA sequencing of the proband revealed, as previously demonstrated by Agiannitopoulos and colleagues [9], that the presence of the c.793-1 G>A allele led to the loss of the first nucleotide of exon 7, determining the formation of a premature termination codon ten residues downstream (p.Asp265Thrfs*10) (Supplementary Fig. 1A, B). Karyotype analysis on 100 metaphases of the proband revealed different chromosomal alterations (Table 1 and Supplementary Fig. 2). Karyotype analysis on 100 metaphases of case III:5 did not show any chromosomal alteration.

Family B

The proband was a 65-year-old Italian woman who was diagnosed with papillary thyroid cancer at the age of 48. She had been previously diagnosed with unilateral metachronous breast carcinoma respectively at age 34 and 47. Both breast lesions resulted hormone receptor positive and HER2 negative. In addition, in the patient a hamartoma in the right middle lung lobe was incidentally found after the first diagnosis of breast cancer. The proband's family is described in Fig. 1. The proband as well as two out of her three sisters were found to carry the *CHEK2* c.1100delC (p.Thr367Metfs*15) and the c.1312 G>T (p.Asp438Tyr) variants in compound heterozygosity, while the daughter and mother were monoallelic carriers of respectively the c.1100delC and the c.1312 G>T. Karyotype analysis on 100 metaphases from the proband, her sisters and her daughter, revealed in each, different proportions of chromosomal alterations (Table 1 and Supplementary Fig. 2). At physical examination, both the probands did not show any remarkable phenotypic features.

Table 2. Genetic and clinical features of cases reported with homozygous CHEK2 variants.

Reference	Pt	Sex	Condition	Age at first cancer onset (y)	CHEK2 homozygous alteration			Founder effect	Genome instability by karyotype from peripheral blood
					Nucleotide level	Protein level	Protein domain		
Janiszewska et al., [6]		M	myelodysplastic syndrome	66	c.444 + 1 G >A	p.Glu149Lysfs*	FHA	Poland	n.e.
Kaczmarek-Rys et al., [7]	1	F	papillary thyroid carcinoma	33	c.470 T>C	p.Ile157Thr	FHA	Poland	n.e.
	2	F	papillary thyroid carcinoma	34	c.470 T>C	p.Ile157Thr	FHA		n.e.
	3	F	papillary thyroid carcinoma	59	c.470 T>C	p.Ile157Thr	FHA		n.e.
Paperna et al., [3]	1	M	intestinal polyps, thymoma, breast cancer, prostate cancer, left renal cell carcinoma, angiomyolipoma of the right kidney, sigmoid gastrointestinal stromal tumour	35	c.499 G>A	p.Gly167Arg	FHA	-	+
	2	F	acute myeloid leukemia, hypocellular bone marrow	21	c.499 G>A	p.Gly167Arg	FHA		+
Present report		**F**	**multiple breast cancers, multiple meningiomas**	**49**	**c.793-1 G> A**	**p.Asp265Thrfs*10**	**kinase**	**-**	+
Van Puijenbroek et al., [5]		M	sigmoid carcinoma	52	c.1100delC[§]	p.Thr367Metfs*15	kinase	northern Europe	+ (mouse) [21]
Paperna et al., [3]		F	serous ovarian carcinoma	72	c.1283 C>T	p.Ser428Phe	kinase	Ashkenazi Jewish	-
Kukita et al., [4]	1	M	multiple primary lung cancer, colon cancer, prostate cancer	59	c.1420 C>T	p.Arg474Cys	kinase	-	n.e.
	2	F	multiple primary lung cancer, uterine myoma, breast cancer	38	c.1420 C>T	p.Arg474Cys	kinase	-	n.e.

Pt patient, *M* male, *F* female, *y* years old, *FHA* forkhead-associated domain; +: presence; -: absence; *n.e.* not evaluated; the findings regarding the here described homozygous variant are indicated in bold
[§]homozygosity for the c.1100delC mutation was also reported in three case series [10, 22, 23]

DISCUSSION

In this study, we described two unrelated cases with biallelic pathogenic variants in *CHEK2*. The proband of family A was homozygous for the c.793-1 G>A and developed multiple breast cancer and multiple meningiomas. The proband of family B, as well as two of her sisters, were found compound heterozygotes for the c.1100delC (p.Thr367Metfs*15) and the c.1312 G>T (p.Asp438Tyr), and developed different neoplasms including, breast, thyroid and desmoid tumor. To date, six others different *CHEK2* variants have been reported in homozygosity in patients with multiorgan tumorigenesis (Table 2).

Our probands' history of neoplasms reflects what has been already reported in women with biallelic *CHEK2* mutations, in whom the risk for invasive breast cancer is higher compared to monoallelic *CHEK2* deleterious variant carriers [10, 11]. Nevertheless, even if a recent study reported that in a large cohort of cases carrying *CHEK2* alterations, the prevalence of breast and colorectal cancer was higher in the biallelic cohort compared to the heterozygous cohort, the difference was not statistically significant [12]. However, some evidence suggests possible genotype-phenotype correlations that might modulate the penetrance of *CHEK2* alterations [12, 13]. For instance, in family A, the *CHEK2* c.793-1 G > A variant, when present in heterozygosity, seems to display incomplete penetrance as highlighted by case III:3.

The present probands developed neoplasms other than breast cancer. While thyroid cancer has been already associated to *CHEK2* mutations [7], meningeal tumors have not been previously associated to these mutations. Nevertheless, 22q deletions, which is often observed in meningiomas as somatic mutations, frequently involve codeletion of *NF2* and *CHEK2*. When this happens, the meningioma tends to show chromosomal instability further probing a possible role of CHK2 in genomic instability [14]. Remarkably, in family B, the father of the proband developed a meningioma, but his genotype remains unknown.

Interestingly, lymphocytes of both probands exhibited multiple chromosomal structural rearrangements. The addition of the methotrexate to the cell culture seemed to increase the rate of chromosomal rearrangements (Table 1). This finding is in line with the known role of methotrexate in inducing DNA damage [15]. In the heterozygous carrier of the c.793-1 G>A, the karyotype analysis did not show any alteration, suggesting that the expression of the wild type allele might protect against genomic instability. The homozygosity for the c.793-1 G>A, compared to the c.1100delC/c.1312 G>T compound heterozygosity, seemed to associate to a higher chromosomal rearrangements rate. While the c.1100delC and the c.793-1 G > A are established pathogenic *CHEK2* variants, the functional role of the c.1312 G>T is still uncertain, with some studies reporting a residual CHK2 activity [16]. We assume that the c.1100delC variant in the context of CHK2 dimerization might hinder the residual kinase activity of the c.1312 G>T variant. We hypothesize that the c.1312 G>T is a low penetrance allele that, when acting together with a pathogenic *CHEK2* variant, modulates its function also according to inter-individual differences. Indeed, between the three compound heterozygous cases, only one had a significant rate of chromosomal imbalances. Moreover, as for case II:4 in family B, we cannot exclude that the c.1312 G>T might per se increase the cancer susceptibility.

Reciprocal translocations generally result from a Double Strand Break followed by the swapping of chromosomal arms between heterologous chromosomes and subsequent repair of the DSB. Two major pathways for DSBs repair have been described: HR and non-homologous end joining (NHEJ) [17]. Furthermore, single strand annealing (SSA) and alternative end joining (alt-EJ) have been reported as parallel and more error-prone repairing processes [17]. The exact contribution to genome rearrangements [18]. In the context of a biallelic *CHEK2* alteration, HR could be compromised [2] and then superseded by other repair processes [19].

We suggest that genome instability, revealed by multiple rearrangements in peripheral blood karyotype, could be a marker of *CHEK2* biallelic germline alterations, thus expanding the group of chromosomal instability syndromes. These are recessively inherited conditions associated with defects in DNA repair and increased cancer risk [20], including: Fanconi anemia, ataxia telangiectasia, Nijmegen syndrome and Bloom syndrome [20]. In patients with biallelic mutations, these syndromes are diagnosed by a personal/familial cancer history, as well as by small body size, congenital anomalies, and quite distinct chromosomal alterations. Notably, the heterozygous carriers may have an increased cancer risk, but do not show neither congenital anomalies nor karyotype alterations [20].

In conclusion, we propose to perform karyotype analysis in biallelic carriers of *CHEK2* constitutional changes. In addition, *CHEK2* should be sequenced in those individuals with multiple chromosomal rearrangements at karyotype analysis, as well as in all patients with oncological personal/family history and a clinical phenotype including the anomalies observed in chromosome instability syndromes.

DATA AVAILABILITY

The data generated and analysed during this study can be found within the published article.

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AUTHOR CONTRIBUTIONS

BI: conception of the study and drafting the manuscript; SE: acquisition of data and drafting the manuscript; MS and AF: clinical genetic evaluation and revising the manuscript; MC: acquisition of data; VM: clinical genetic evaluation and revising the manuscript; FA: genetic counselling; RV: clinical assessment; SF: clinical assessment; CMP: analysis and interpretation of data; BF: analysis and interpretation of data; GB: acquisition of data; DGG: acquisition of data; BS: analysis and interpretation of data; DD: analysis and interpretation of data; GP: revising the article critically for important intellectual content.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

Informed consent was obtained from all subjects, and study procedures were compliant with the revised Helsinki Declaration of 2000. The study, part of a research project about genetic disorders, was approved by the San Camillo Forlanini Hospital Ethics Board.

ADDITIONAL INFORMATION

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