

Unusual Association of Two Germline Rearrangements in *CDC73/HRPT2* Gene: Complexity of Clinical Counseling in the Age of Genomic Medicine

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Case Report

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Abstract

Background: Primary hyperparathyroidism is a phenotype shared by several hereditary endocrine and non-endocrine cancer predisposition syndromes caused by heterozygous germline mutations deactivating tumor suppressor genes including *CDC73* (=HRPT2).

Objectives and Results: We describe the genetic investigation of a family in which the proband was a 16- year-old male patient suffering from primary hyperparathyroidism related to a unique parathyroid adenoma. His DNA analyses revealed a *CDC73* germline heterozygous deletion of exons 7 to 13 in the 1q31.2 locus of the chromosome 1. Genetic

investigations in his parents who were both asymptomatic showed that the father was not carrying the deletion. Two chromosomal rearrangements were carried by the mother, both affecting *CDC73*: the deletion previously identified in her son and a 626 kb duplication encompassing 6 contiguous genes: *UCLH5*, *TROVE2*, *GLRX2*, *CDC73*, *MIR1278* and *B3GALT2*. This duplicated segment was inserted into chromosome 20. Lack of symptoms in the proband's mother suggests that the *CDC73* extra copy is able to balance the loss of function caused by the deleted allele by allowing a normal level of parafibromin expression.

Conclusion: For the first time, we describe the association of two large and complex genetic events in *CDC73* gene complicating genetic counseling. Literature doesn't provide much information about potential consequences of these genes deletion or duplication, thus highlighting the importance of multidisciplinary in the practice of precision and genomic medicine. Moreover, this study demonstrates that copy number variations should be carefully investigated in routine diagnosis in order to deliver an accurate genetic counseling to those families.

Keywords: *CDC73*; *HRPT2*; Copy number variation (CNV); Primary hyperparathyroidism; Precision medicine

Introduction

Hyperparathyroidism-jaw tumor syndrome (HPT-JT), familial isolated hyperparathyroidism (FIHP) and parathyroid carcinoma are three phenotypes belonging to the spectrum of *CDC73*-related disorders. They all arise from structural variations that deactivate the tumor suppressor gene *CDC73* (also known as *HRPT2*, NM_024529.4). Diagnosis is based on the biochemical findings of primary hyperparathyroidism (PHPT), imaging studies, family history and detection of a heterozygous germline *CDC73* pathogenic variant. *CDC73*-related disorders display autosomal dominant inheritance with variable penetrance, the proportion of cases caused by *de novo* pathogenic variant being still unknown [1,2]. The *CDC73* gene, located to the 1q31.2 locus, comprises 17 exons, and encodes parafibromin, a component of the RNA polymerase II and PAF1 complex involved in transcriptional regulation and histone modifications [3]. *CDC73* pathogenic variations are mostly frame-shift or nonsense mutations leading to the occurrence of a truncated parafibromin [4]. In the absence of point mutations, *CDC73* intragenic deletions have to be searched in cases of high risk patients [5]. Here, we describe in a family two chromosomal rearrangements both affecting *CDC73*: a 7 exons deletion and a duplication of 6 genes including *CDC73*.

Case Report and Results

A 16 years old boy was initially referred for kyphosis investigations. Radiological screening incidentally

detected osteolytic lesions of 35 mm in left femur and of 20 mm in left greater trochanter. Biological tests (Figure 1A) revealed primary hyperparathyroidism. Echography detected a hypervascular left parathyroid nodule (15x15x20 mm) and technetium bone scan an osteolytic area in the left trochanter corresponding to a brown tumor (Figure 1B). Anatomopathological analysis after parathyroid surgery (February 2010) confirmed the presence of a unique parathyroid adenoma with oncocyctic cells. There was no familial history of endocrinopathy and his parents and sister were normocalcemic. In front of the early-onset of this PHPT, molecular genetic studies were performed after collection of informed consent. *CDC73* exonic regions and their flanking junctions were fully sequenced by Sanger sequencing [5] but no mutation was detected. Further analyses by qPCR (Taqman) and MLPA (P466-A1 kit, MRC Holland) (Figure 2A) left revealed a heterozygous deletion of exons 7 to 13. Moreover, according to the Knudson model, a second allele inactivation in the tumor was characterized (exon 2: c.142G>T, p.(Glu48*)). Thus, complete loss of function of *CDC73* was ascertained. Genetic investigations were performed in the kindred to discriminate *de novo* from familial mutation and to provide genetic counseling. DNAs from proband's father, mother and sister were analyzed by MLPA and qPCR. No deletion was found in father and sister. In proband's mother, 3 copies for exons 1 to 6 and 14 to 17 of *CDC73* gene were detected (Figure 2A) right suggesting an additional event. Comparative Genomic Hybridization (array-CGH) analysis (60K oligo probes (Agilent)) was performed in the mother and found a *CDC73* heterozygous deletion of exons 7 to 13 (80 kb;

chr1:193,110,996-193,190,959; GRCh37/hg19) associated with a heterozygous duplication encompassing *CDC73* and 5 contiguous genes (*UCHL5*, *TROVE2*, *GLRX2*,

MIR1278, *B3GALT2* ; 626 kb: chr1:192,805,274-193,431,181; GRCh37/hg19) (Figure 2B). FISH analysis located this extra-copy inserted into chromosome 20.

Date	Calcemia mmol/L	PTH pg/ml	25OH Vitamin D ng/ml	Phosphatemia mmol/L	Calciuria mmol/24h
Normal ranges	2,18-2,58	11-54	26-65	0,8-1,5	2, 5-6,2
December-09	4,2	870		0,58	12
February 2010	2,12	8			
July 10	2,37	22	17		
September 10	2,48		44		
January 12	2,41	19,5	22,4		

Figure 1: Primary hyperparathyroidism investigations in the proband.

1A: Phosphocalcic metabolism investigations before and after surgery (February 2010).



1B: Technetium bone scan (January 2010) detected the presence of an osteolytic area in the left femur (white arrow).

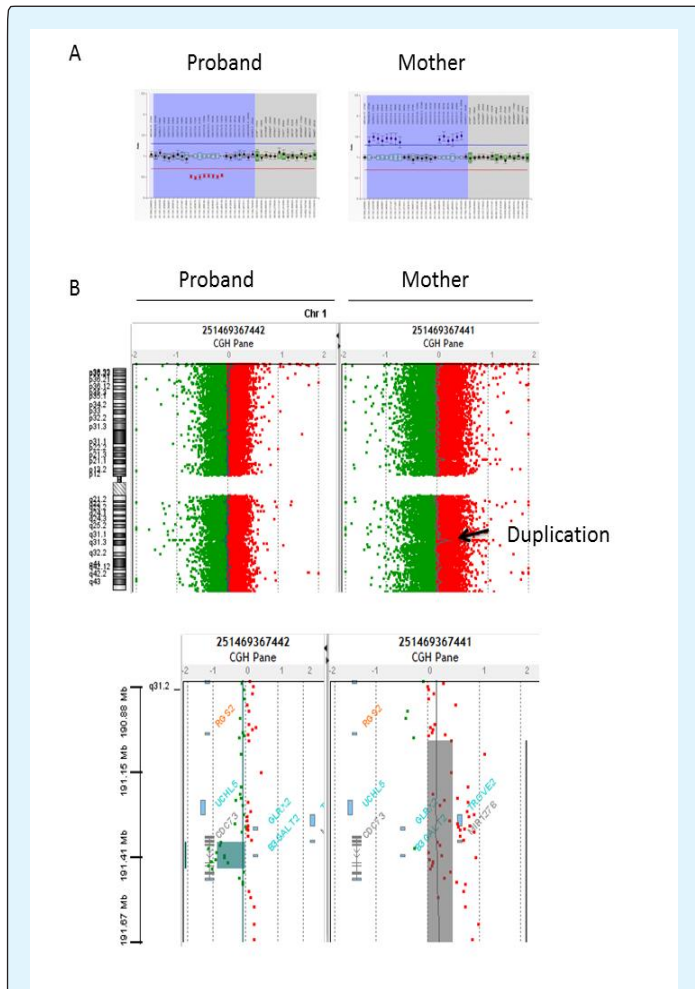


Figure 2: Rearrangement studies. MLPA profiles of the proband and his mother.

2A: "Salsa MLPA probemix P466-A1 *CDC73*" kit (MRC Holland). Each box plot corresponds to a probe hybridization on each *CDC73* exon (blue area) in addition to control probes on other chromosomes on the right (grey area). Normal hybridization ratio is comprised between the two horizontal bars (mean \pm 2sd, blue and red bars). A signal with a ratio around 0.5 suggests a heterozygous deletion and a ratio around 1.5 suggests a heterozygous duplication.

2B: Array CGH analysis of the proband and his mother's DNA (Agilent oligonucleotide array 60K; up: complete view of the chromosome, Bottom: zoom on the affected region). The right panel highlights the duplicated region in the mother DNA whereas the left zoom shows the deletion of exons 7 to 13 in the proband's DNA.

Discussion

Four other cases of partial or whole *CDC73* deletion have also been described in literature [5-10]. So *CDC73* gross deletions are not that uncommon, with an estimated frequency around 7% [5]. Interestingly, one study [10] described in 1q32 locus a large germline deletion affecting the 6 contiguous genes we found duplicated in the proband's mother DNA. Indeed, literature highlights the inherent potential of this region, corresponding to a conserved haplotype block structure across mammals [11], to be involved in breakage and recombination events. Large duplications mostly result in direct tandem or interstitial insertion mechanism [12]. In our study, insertion into chromosome 20 was quite unexpected but explains the familial segregation of the disease, especially the absence of *CDC73* mutated allele in sister's proband for chromosome 1 and 20. These results raised the issue of the consequence of the extra *CDC73* gene copy, the proband's mother being still asymptomatic at the age of 56. Haploinsufficiency resulting from the partially deleted allele might be properly balanced by the *CDC73* extra-copy. However, annual phosphocalcic metabolism monitoring and cervical echography every 2 to 3 years were recommended, the long term consequences of the association of such unusual events remaining unknown. Here, four modes of chromosomal segregation could be expected and have to be explained during genetic counseling, as well as their potential functional consequences:

- Apart from genotypes identified in the proband and his mother, we could observe a complete normal profile for the offspring or duplication of the 626 kb locus without the 80 kb segmental deletion.
- The pathogenicity of most duplications is not only explained by an extra-copy of one or several genes leading to triplosensitivity but by disruption or misregulation of genes that span duplication breakpoints.
- In addition to *CDC73*, 5 other genes are involved in the proband's mother rearrangement: *UCHL5*, *GLRX2*, *TROVE2*, *MIR1278* (3 copies) and *B3GALT2*, an overlapping *CDC73* gene in intron 10 (2 copies). Large CNV, larger than 400 or 500 kb, have been described in control populations [13,14], but both gene density and size are strongly anticorrelated with allele frequency.

Thus, size alone can't be used as a predictor of pathogenicity [13]. The 626 kb CNV identified in our study wasn't reported in literature thus leading us to search for diseases individually associated with these

genes. *UCHL5* encodes a deubiquitinating enzyme, *GLRX2* a glutathione-dependant oxydoreductase involved in oxydative stress response, *TROVE2* a RNA-chaperone involved in congenital heart block and Sjogren's syndrome, *MIR1278* encodes a microRNA expected to be implicated in expression regulation of various genes and *B3GALT2* a beta-1,3-galactosyltransferase involved in glycoprotein and glycolipid biosynthesis. No particular diseases are reported in databases as typically associated with these genes. Interestingly, *B3GALT2* overlaps *CDC73* gene in intron 10 and is included in the proband's deleted region. Cascon, et al. [10], who found all these genes deleted in one patient without troubles out of HPT, suggested that they are haplo sufficient. This hypothesis is in agreement with the absence of additional symptom for the proband and his mother. *CDC73* is considered as a medically actionable gene [15], involving that incidental events found in this gene during exome analyses have to be reported. Our work highlights the difficulties of precision medicine which will be amplified in the coming years by growing number of whole exome and genome sequencing studies. It thus raises the issue of genetic counseling complexity for these incidental events not yet reported in publications and emphasizes the importance of multidisciplinary support. Literature should collect such data about these complex genetic events to create a medical bank [16,17], as described by Aronson, et al. [18], to which refer to regarding the patient support, genetic counseling and medical monitoring of associated pathologies. In conclusion, our work describes for the first time the association of two genetic rearrangements affecting *CDC73* gene. This case report highlights the importance of cooperation between the patient, his family and the medical multidisciplinary team in the age of precision medicine.

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