Clinical Report

A Microduplication of *CBP* in a Patient With Mental Retardation and a Congenital Heart Defect

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Received 6 February 2007; Accepted 6 May 2007

Rubinstein–Taybi syndrome is a well-characterized genetic syndrome caused by haploinsufficiency of *CBP* in a majority of individuals. In 10% of cases a microdeletion in 16p13.3 affecting *CBP* is detected. We report on a patient with a de novo 345–480 kb micro-duplication the region, encompassing only *CBP* and *TRAP1*. This boy presented with various minor physical anomalies, moderate mental retardation, and an atrial septal defect, but none of the other typical

characteristics of the Rubinstein–Taybi syndrome, such as the broad thumbs and first toes or facial characteristics. This finding implicates *CBP* as one of the causative genes for the trisomy 16p13 syndrome, and indicates this is a contiguous gene syndrome. © 2007 Wiley-Liss, Inc.

Key words: Rubinstein–Taybi; *CBP*; duplication

How to cite this article: Thienpont B, Breckpot J, Holvoet M, Vermeesch JR, Devriendt K. 2007. A microduplication of *CBP* in a patient with mental retardation and a congenital heart defect. *Am J Med Genet Part A* 143A:2160–2164.

INTRODUCTION

Submicroscopic chromosomal imbalances are an important cause of congenital disorders. The recurrence of some of these chromosomal imbalances can be explained by non-allelic homologous recombination mediated by low copy repeats (LCRs). The identification of duplications as well as deletions of the same region in 17p11.2 suggested the existence of reciprocal imbalances to known recurrent microdeletions or microduplications [Lupski et al., 1991; Chance et al., 1993]. The lack of recognizable phenotypic selection criteria makes them difficult to identify. The identification of duplications reciprocal to the deletion of 22q11.2 (DiGeorge) [Ensenauer et al., 2003], 7q11.23 (Williams–Beuren) [Somerville et al., 2005], and 17p11.2 (Smith–Magenis) [Potocki et al., 2007] preceded the identification of the corresponding phenotype caused by these duplications, and some reciprocal duplications have been found to be benign variants [Baris et al., 2006].

To facilitate the detection of chromosomal imbalances causing recognizable syndromes as well as the discovery of their reciprocal imbalance, we enriched our whole genome micro-array with clones covering regions known to be dosage-sensitive upon haploinsufficiency or duplication (regardless of whether they were flanked or not by LCRs). Previous reports describe targeted micro-arrays dedicated to detect

copy number changes in regions flanked by LCRs. Although they have proven successful [Sharp et al., 2006], the present report highlights the importance of also looking for imbalances in other regions of the human genome, and primarily in regions that are known to be dosage sensitive.

CLINICAL REPORT

The patient is the first-born male child of healthy, nonconsanguineous Caucasian parents. Birth weight and length at term were 2,600 g (~3rd centile) and 44 cm (below 3rd centile). He was diagnosed with a type 2 atrial septal defect with displacement of the interatrial septum (partially overriding the inferior caval vein). His development was moderately delayed: there was little social contact at the age of 4 months and he walked independently at age 24 months. Now at age 15 years, he participates in special schooling for persons with a mild to moderate mental handicap. At examination, his weight was 62 kg, length 173 cm, and head circumference

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DOI 10.1002/ajmg.a.31893

FIG. 1. Patients' phenotype. Note the protruding ears, broad nose, synophrys, low frontal hair line, and camptodactyly of fingers 2–4.

55.4 cm (all near the 75th centile). Clinical evaluation showed minor anomalies including mildly protruding ears, a broad nose, hirsutism with synophrys and a low frontal hair line, mild cutaneous syndactyly of the fingers, camptodactyly of fingers 2–4, and incomplete extension of the elbows (Fig. 1). The family history is negative for similarly affected individuals.

MATERIALS AND METHODS

Array-CGH was performed as described [Menten et al., 2006], using a micro-array containing 3,625 BAC or PAC clones chosen genome-wide at 1 Mb intervals (provided by the Wellcome Trust Sanger Institute, Hinxton, UK), plus a set of 58 clones chosen in clinically important dosage-sensitive regions.

Fluorescent in situ hybridization (FISH) using the cosmid RT100 [Petrij et al., 2000] and real time quantitative PCR (qPCR) were performed as described [Menten et al., 2006]. Oligonucleotides used for qPCR are listed in Table I. Polymorphic repeat lengths were analyzed by PCR on 50 ng of genomic DNA using Expand Long Template (Roche, Mannheim, Germany) and buffer three according to the manufacturer's instructions. The following polymorphic repeats inside the duplicated region were analyzed: D16S2622, D16S3027, D16S3065, MS1, MS2, and MS4 [Coupry et al., 2002].

RESULTS

In the absence of an etiological diagnosis, DNA from the proband was subjected to array-CGH

TABLE I. Primer Pairs Used for qPCR Analysis

Name	Forward	Reverse
A	CCTTCATTGCCACTTGTGATACTG	TCAACGCTTTCCACTGACAAA
B	GGTCCAGGGCTCTTAGTTTAGTT	AGTAGGTTCCCTTCACACATG
C	GCCAAGCACTGCCAAGAAA	TGGCGGAGCTTGTGTTTGA
D	CGGCCGAGAGCATCCA	TTCCGATTCGAGGGAGATTCT
E	TCTCATCCATCAGCCCAACTG	GCAGCCAGCAAGGGAAGAC
F	CCAGATTCCACAGCACAGA	GATGGCCAAGTTGGATGCTT

analysis. This showed a duplication of two cosmid (RT100 and RT102) mapped to *CBP* (Fig. 2A–D) [Petrij et al., 2000]. qPCR analysis confirmed the presence of the duplication in the patient, and showed his mother does not carry the duplication

(Fig. 2C). Paternal DNA was not available. Sequential rounds of qPCR revealed the duplication is between 345 and 480 kb in size, spanning *TRAP1* and *CBP* in their entirety, and part of *ADCY9* and *DNASE1* (Fig. 2D). FISH on metaphase chromosome spreads

FIG. 2. **A:** Molecular karyotype. X-axis: clones ordered by chromosome (p → q-arm); Y-axis: log₂ (Cy5/Cy3); unfilled dots: known polymorphic clones; dotted line: 4 × standard deviation (SD) threshold; full line: log₂(1.5) – 2 × SD threshold [Vermeesch et al., 2005]. **B:** Partial molecular karyotype for chromosome 16. Duplication of clones RT100 and RT102. Plot legend as in (A), except X-axis: clone position [Mb]. **C:** qPCR analysis and data processing was performed as described [Menten et al., 2006]. Intensities (indicated at the base) were normalized by comparison to three normal controls. X-axis: qPCR primer pairs (sequences in Table I); Y-axis: relative copy number. **D:** Map of the *CBP* region. Physical location of polymorphic markers (underlined), qPCR primer pairs (italics), known genes (black arrow) and duplicated microarray clones (gray boxes). The shaded and the black bar below indicate, respectively the potentially duplicated and the duplicated area in the present patient. Results from polymorphic marker analysis for D16S2622 (**E**) and MS2 [Coupry et al., 2002] (**F**) showing that the duplicated allele (*) from the patient (upper box) is inherited from his mother (lower box).

from the proband showed a signal on both chromosomes 16, one weak and one stronger. The majority of interphase nuclei showed three signals (data not shown). To determine the parental origin of this duplication, marker analysis was performed. None of the six polymorphic markers inside the duplicated region showed more than two alleles, strongly suggesting the duplication arose intrachromosomally. Markers D16S2622 and MS2 showed that the duplicated allele corresponds to the maternal allele (Fig. 2E,F). Since qPCR analysis demonstrated that the mother does not carry the duplication (Fig. 2C), we conclude that it arose *de novo*.

DISCUSSION

We report on a patient with moderate mental retardation, a congenital heart defect, and various minor physical anomalies. Array-CGH analysis of DNA from this patient revealed a duplication of the region encoding the CREB-binding protein (*CBP*). qPCR and marker analyses showed this duplication was maternal and intrachromosomal in origin and occurred *de novo*.

Mutations in *CBP* [Petrij et al., 1995] and in *EP300* [Roelfsema et al., 2005], and micro-deletions of the *CBP* region [Breuning et al., 1993] cause Rubinstein-Taybi syndrome (RSTS). This is a rare congenital disorder affecting about one in 125,000 newborns [Hennekam et al., 1990]. Its main clinical features are developmental delay, heart defects, a characteristic face, and broad great toes and thumbs [Rubinstein and Taybi, 1963; Hennekam, 2006]. A duplication of only exon 1 of *CBP* has been described in one patient with classical signs of RSTS, suggesting this to be an intragenic duplication causing haploinsufficiency [Roelfsema et al., 2005]. The duplication found in the present patient encompasses *CBP* and *TRAP1* entirely. The duplication of *TRAP1* most likely does not influence the patient's phenotype for two reasons; mutations in *TRAP1* are not yet described in association with human malformations, and because some RSTS patients carry a microdeletion affecting *CBP* as well as *TRAP1*, without overt phenotypic difference. These findings suggest *TRAP1* is not dosage-sensitive upon haploinsufficiency.

Duplication of the subtelomeric part of the short arm of chromosome 16 causes a recognizable phenotype characterized by psychomotor and growth retardation, microcephaly, seizures, cardiac and hand anomalies and a specific facial appearance [Kokalj-Vokac et al., 2000]. The most telomeric band (16p13.3) is about 6 Mb in size and contains about 260 genes, making it particularly gene-rich. Duplication of this region has been found to be one of the more frequent subtelomeric duplications, occurring in seven patients out of a group of almost 12,000

selected for a variety of indications [Ravnan et al., 2006]. In seven patients, submicroscopic duplications of this region have already been sized beyond banding resolution using array-CGH: five subtelomeric duplications were delineated [Martin et al., 2002; Wong et al., 2005], one interstitial duplication was reported [de Ravel et al., 2005] and one interstitial duplication was found in an array-CGH screening study [Friedman et al., 2006]. All duplications encompass *CBP*, except one unbalanced translocation between 16p and 17p. Comparison with the present patient indicates that duplication of *CBP* causes a subset of the clinical features associated with the 16p duplication phenotype, namely congenital heart defects and developmental delay. Other features commonly associated with subtelomeric 16p duplications (growth delay and microcephaly) were not found in the three patients carrying interstitial duplications. We conclude that one or more genes telomeric to *CBP* are also dosage-sensitive, and that their imbalance contributes to the more severe phenotype of other patients with a duplication of 16p13. Further support for this notion is provided by the absence of large deletions that span both *CBP* and *PKD1*, a gene located about 1.6 Mb more telomeric on 16p. This was suggested to indicate the presence of one or more hemizygous-lethal genes in the intervening gene-rich interval [Hennekam et al., 1993]. Array-CGH analysis of other cases will prove essential for further dissection of this and other contiguous gene syndromes into their different causative facets.

Not surprisingly, the duplication of *CBP* in this patient affects similar parts of the body as haploinsufficiency in RSTS (brain, heart). In general, duplications cause a less severe phenotype than deletions [Devriendt and Vermeesch, 2004], as is observed here. We would not suggest a remarkable "reciprocal phenotype," as has been suggested e.g. for Williams-Beuren syndrome and its reciprocal 7q11.23 duplication [Somerville et al., 2005].

CBP encodes a protein functioning in chromatin remodeling [Ogryzko et al., 1996]. Another gene involved in chromatin remodeling, *MECP2*, is known to cause Rett syndrome upon deletion or mutation [Amir et al., 1999]. Recently duplications of this gene were shown in patients with severe to profound MR and progressive spasticity [Van Esch et al., 2005]. As for *MECP2*, maintaining *CBP* levels within narrow limits seems essential for its proper functioning. This shows again that chromatin remodeling is exquisitely sensitive to dosage perturbations.

ACKNOWLEDGMENTS

The authors thank the patient and his mother for their kind cooperation and Dr. Els Schollen for help with the marker analysis.

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