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ARTICLE

An unusual clinical severity of 16p11.2 deletion syndrome caused by unmasked recessive mutation of *CLN3*

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With the introduction of array comparative genomic hybridization (aCGH) techniques in the diagnostic setting of patients with developmental delay and congenital malformations, many new microdeletion syndromes have been recognized. One of these recently recognized microdeletion syndromes is the 16p11.2 deletion syndrome, associated with variable clinical outcomes including developmental delay, autism spectrum disorder, epilepsy, and obesity, but also apparently normal phenotype.
We report on a 16-year-old patient with developmental delay, exhibiting retinis pigmentosa with progressive visual failure from the age of 9 years, ataxia, and peripheral neuropathy. Chromosomal microarray analysis identified a 1.7-Mb 16p11.2 deletion encompassing the 593-kb common deletion (~29.5 to ~30.1 Mb; Hg18) and the 220-kb distal deletion (~28.74 to ~28.95 Mb; Hg18) that partially included the *CLN3* gene. As the patient's clinical findings were different from usual 16p11.2 microdeletion phenotypes and showed some features reminiscent of juvenile neuronal ceroid-lipofuscinosis (JNCL, Batten disease, OMIM 204200), we suspected and confirmed a mutation of the remaining *CLN3* allele. This case further illustrates that unmasking of hemizygous recessive mutations by chromosomal deletion represents one explanation for the phenotypic variability observed in chromosomal deletion disorders.
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INTRODUCTION

Common 16p11.2 microdeletion is defined as the presence of a 593-kb deletion at map position ~29.5 to ~30.1 Mb (Hg18), between two segmental duplications, suggesting NAHR as the causative mechanism.[1] Adjacent and more distal to this 16p11.2 deletion, there is a less frequently reported 220-kb 16p11.2 deletion (~28.74 to ~28.95 Mb;Hg18) that was referred to as the 'atypical 16p11.2 deletion'[2] or 'distal 16p11.2 deletion'.[3] Larger 16p11.2 deletions were also reported, including the distal deletion but not the common 16p11.2 deletion.[4–6] Finally, few cases encompassing both the distal and the common deletions were described in the literature.[4,7–9]

Recurrent 16p11.2 deletions are associated with variable clinical features, including delayed language development, learning difficulties/intellectual disability, social impairments with or without autism spectrum disorder and minor dysmorphic facial features without a consistent pattern. They can also be associated with a normal phenotype.

Here, we present a patient who harbors an uncommon 16p11.2 microdeletion with some features reminiscent of juvenile neuronal ceroid-lipofuscinosis (JNCL) or Batten disease (OMIM 204200), a progressive neurodegenerative disorder, caused by recessive mutations in the *CLN3* gene located on chromosome 16p11.2 and characterized by progressive visual failure from the early school years and deterioration in the early teenage years together with the onset of seizures, behavioral difficulties, until dementia. To the best of our knowledge, this is the first 16p11.2-deletion patient in whom unmasking of autosomal recessive gene mutation by a hemizygous deletion is demonstrated.

MATERIALS AND METHODS

Case history

The patient was the second child of non-consanguineous and healthy parents, with no relevant family history. The pregnancy and delivery were uneventful. His birthweight was 2830 g (−2 SD), length was 49 cm (−0.5 SD) and occipital-frontal circumference was 34 cm (−0.5 SD). He was hypotonic, was able to hold his head at 4 months, sat alone at 13 months, and was able to walk after 2 years. Speech development was also delayed. At the age of 9 years, acute visual impairment appeared with retinis pigmentosa and progressive visual loss. He presented recent ataxia and peripheral neuropathy.

On examination at the age of 16 years, his weight was 64.4 kg (+1 SD), his size was 1.68 m (median), and his head circumference was 55 cm (median). The patient had gynecoid obesity with purple stretch marks on the pelvic bones. No dysmorphic features was described. He reported sleeping troubles with very early wake-up. There was a bulimic symptomatology and he had

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Table 1 Clinical features of our patient compared with previously reported cases of 16p11.2 deletions and patients with classic JNCL

	Large 16p11.2 deletion						*Common proximal 16p11.2 deletion*		*Atypical 16p11.2 deletion*	*Classic JNCL (1.02 kb deletion on CLN3 gene)*	
Clinical spectrum	*Ballif* et al., *2007*[4]	*Bochukova* et al., *2010*[7]	*Bochukova* et al., *2010*[7]	*Hanson* et al., *2010*[8]	*Sampson* et al., *2010*[9]	*Sampson* et al., *2010*[9]	*Ghebranious* et al., *2007*[1] *Bijlsma* et al. *2009*[2]	*Bachmann-Gagescu* et al., *2011* *Schaapveld* et al., *2011*	*Baarge-Schaapveld* et al., *2010*[6]	*Haltia, 2003*[15]	*Our patient*
Progression of vision loss until blindness	na	na	na	Patient too young to evaluate manifestations reliably	na	na	–	–		+ (Onset ~2–5 years)	+ (Onset 9 years)
Deterioration in cognitive skills, speech and mobility	na	na	na		na	na	–	–		+	+
Seizures	na	na	na		+	na	+ (Onset 1 year)	+		+ (Onset 9–18 years)	–
Premature death	na	na	na		na	na	–	–		+	na
Behavioral problems, extrapyramidal signs, and sleep disturbance	na	na	na		+	+	+	+		+ (Onset >10 years)	+
Attention-deficit disorder	na	na	na		+	na	+	+			+
Autism spectrum disorder	na	na	na		na	na	+	+		–	–
Developmental delay (learning difficulties/ intellectual disability, delayed language development)	+	+	+		+	na	+	+		–	+
Unusual facial morphology	na	na	na		+	na	–	+		–	–
Obesity	+	+	+		–	–	+	+		–	+
Neonatal hypotonia	na	na	na		na	na	–	+		–	+
Congenital anomalies of the kidney and urinary tract	na	na	na		+	na	–	+		–	–
Other major finding	na	na	na		+	+	–	–		–	–

Abbreviation: na, data not available.
[a]Severe kyphosis.
[b]Hirschprung disease and congenital joint contractures.

behavioral difficulties with aggressive crisis in case of frustration. He suffered from severe fatigability and chronic constipation.

Audiometric examination, metabolic exploration, and brain MRI did not reveal any anomaly. Retinal examination showed a pale optic disc with a narrowing of the vessels and bone spicular pigmentations in the peripheral retina. Vacuolated leukocytes were discovered on blood cell count without other hematological abnormalities (Table 1).

Classical and molecular cytogenetics

Chromosome analysis was performed on GTG-banded and RHG-banded metaphases prepared from cultured peripheral blood according to the standard protocols. Fluorescence *in situ* hybridization (FISH) was performed to confirm the genomic-copy-number aberration detected by array comparative genomic hybridization (aCGH). A commercially available probe, RP11-301D18 (29 776 143–29 961 746 – Hg18), located at 16p11.2, was obtained from BlueFISH, Amplitech (Compiegne, France) and was hybridized according to instructions provided by the manufacturer. At least 15 metaphase spreads and 100 interphase nuclei were analyzed under a fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

Microarray-based comparative genomic hybridization (aCGH) was performed on genomic DNA isolated from peripheral blood lymphocytes (Kit Nucleospin Blood L, Macherey-Nagel, Eurl, Hoerdt, France) using an Agilent 180K oligonucleotide microarray system, according to the manufacturer's protocol (Agilent 180 K Whole Human genome Oligo Microarray kit, Agilent Technologies, Santa Clara, CA, USA). The array was scanned and analyzed with Feature Extraction 10.5 software (Agilent Technologies). Control DNA consisted of a sex-matched pool of genomic DNA (Promega, Madison, WI, USA). Genomic-copy-number aberrations were identified using the ADM-II algorithm of DNA analytics 4.0.76 (Agilent Technologies).

Molecular genetics

Allele-specific PCR for detection of the major 1.02-kb deletion in the *CLN3* gene was performed according to Taschner *et al.*[10] by using a primer spanning the deletion (2.3INTF7) in combination with one primer within the deletion (2.3LR3) and another primer spanning the deletion junction (CLN3mut756R) (Figure 2a). Briefly, 50 ng of genomic DNA was amplified in a total volume of 25 μl at a final concentration of 50 mmol/l KC1, 1.5 mmol/l $MgCl_2$, 200 pmol/l dNTP, 0.004 U per 41 of SuperTaq (HT Biotechnology Ltd, Cambridge, UK) in the presence of 5 pmol of primers 2.3LR3 (5′-GGGGGAGGACAAGCACTG-3′) and 2.3INTF7 (5′-CATTCTGTCACCCTTAGAAGCC-3′), and 4 pmol of primer CLN3mut756R (5′-GGACTTGAAGGACGGAGTCT-3′). Primers were designed using the genomic *CLN3* sequence (Genbank Accession no. X99832).[11] Denaturation was for 3 min at 94 °C, followed by 35 cycles of amplification with denaturation for 1 min at 94 °C, annealing for 2 min at 56 °C, and extension for 1 min at 72 °C, with a final extension for 10 min. Twenty microliter of PCR samples were analyzed on a 2% agarose gel.

RESULTS

Classical and molecular cytogenetics

By using GTG and RHG bandings, the patient was initially judged to have a normal 46,XY karyotype at 550 band level. aCGH analysis revealed a ~1.7-Mb interstitial deletion of chromosome 16p11.2 (28 399 426–30 098 210 – Hg18), stretching from oligonucleotide A_16_P40598212, the first-deleted oligonucleotide, to oligonucleotide A_16_P20443172, the last-deleted oligonucleotide (Figure 1a), and excluded the presence of any other genomic imbalance, except copy number variations (CNV) previously described in the normal population according to the Database of Genomic Variants (http://projects.tcag.ca/variation) (data not shown).

Metaphase FISH analysis using RP11-301D18 probe demonstrated a hemizygous deletion of chromosome 16p11.2, which is consistent with aCGH results. Parental karyotype and FISH using the same probe showed no 16p11.2 deletion or other rearrangement involving this locus, indicating a *de novo* occurrence (data not shown).

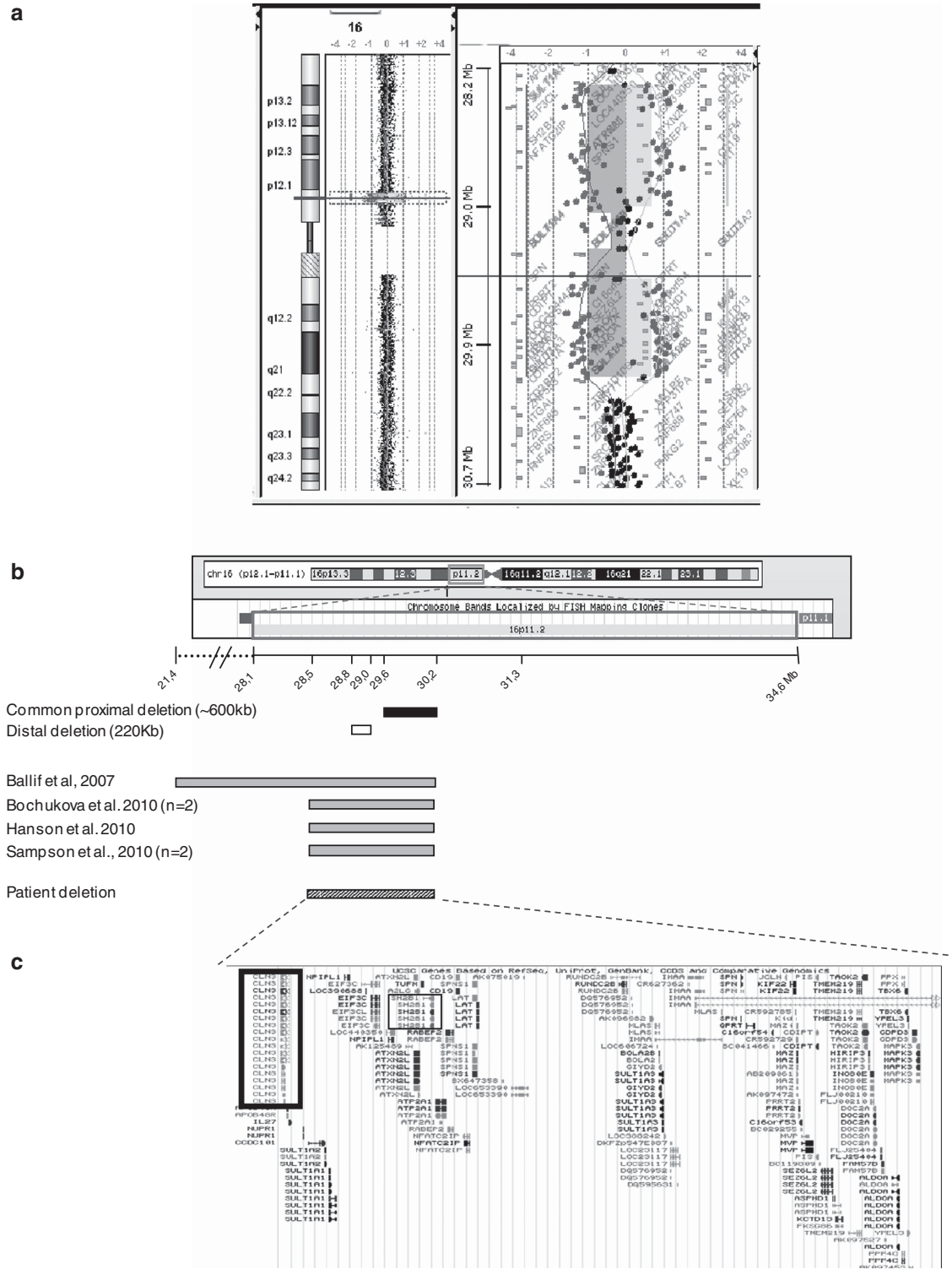

Figure 1 (**a**) Oligo array-CGH of whole chromosome 16 with ideogram illustrating deletion and enlargement of the ~1.7-Mb interstitial deletion of chromosome 16p11.2 (28, 491, 925–30, 190, 709), stretching from oligonucleotides A_16_P40598212 to A_16_P20443172 (hg18 – NCBI Build 36.1). (**b**) Schematic representation of the 16p11.2 chromosomal region. The figure shows the chromosomal 16 ideogram and the distance in Mb from p-telomere with location of the patient deletion (hatched box) and deletions previously found on reported cases (grey boxes). The black box indicates the common proximal deletion (~ 600 kb). The white box indicates the distal deletion (~220 kb). (**c**) The region of interest showing the positions of implicated genes consistent with the UCSC Genome Browser. *CLN3* and *SH2B1* genes are highlighted in black. The full colour version of this figure is available at *European Journal of Human Genetics* online.

Molecular genetics

Mutation analysis of the *CLN3* gene in the patient revealed on the remaining allele a 1.02-kb deletion (c.461_677del, NM_001042432.1), previously reported as the major causative deletion in JNCL. The father of the patient was found to be a heterozygous carrier of this mutation. No mutation in the *CLN3* gene was detected in the mother

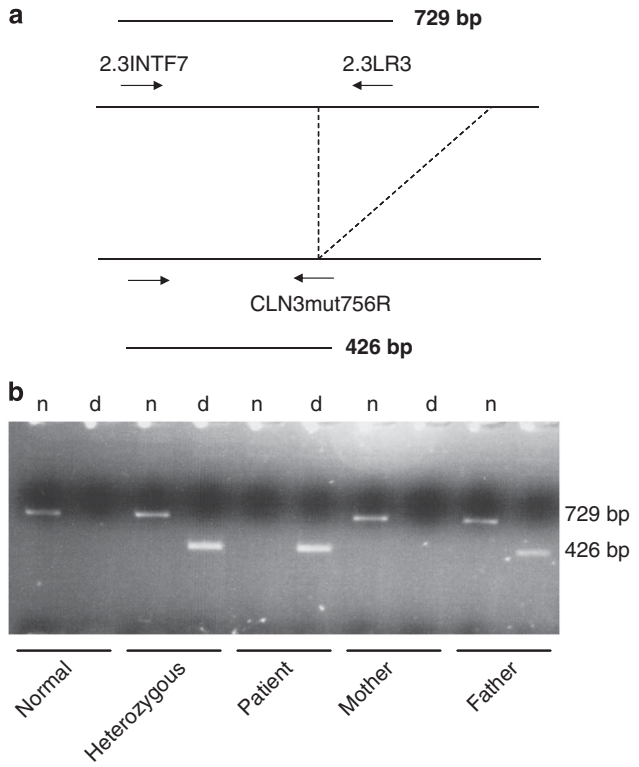

Figure 2 (**a**) Direct detection of the major deletion in the *CLN3* gene by allele-specific PCR. (**b**) The patient has the 426-bp major deletion fragment. The mother of the patient has the 726-bp wild-type fragment. The father of the patient has both. n: amplification of the wild-type allele. d: amplification of the deleted allele.

(Figure 2b), suggesting that submicroscopic deletion of 16p11.2 occurred *de novo* on the maternal allele.

DISCUSSION

Here, we reported on a 16-year-old patient with developmental delay, behavioral difficulties, and obesity consistent with 16p11.2 deletion. He also exhibited neurodegenerative features from the age of 9 years. The interstitial deletion of chromosome 16p11.2 (28 491 925–30 190 709 – Hg18) revealed by aCGH encompassed the 220-kb deletion and extended through the common 593-kb 16p11.2-deleted region (Figure 1b). Five patients with this 1.7-Mb deletion have been described in the literature (Figure 1b).[7–9] All patients had developmental delay except for one patient who was too young to evaluate this reliably.[8] In addition to the developmental delay, one patient was hyperactive and two had behavioral problems (Table 1). The deleted region includes the *SH2B1* gene, known to be involved in leptin and insulin signaling, which is consistent with hyperphagia and gynecoid obesity of our patient. Indeed, several authors provided evidence that CNV of *SH2B1* may significantly contribute to human obesity, as deletion carriers exhibited hyperphagia and severe insulin resistance.[6,7] Of interest, besides the patient reported here, three out of the five patients mentioned in Figure 1b for whom we have information about the body mass index, had obesity in addition to their developmental delay. Neurodegenerative features have not been described in 16p11.2 patients with or without 16p11.2 common deletion. As aCGH excluded the presence of any other pathogenic genomic imbalance, we suspected that, in addition to causing clinical features of 16p11.2-deletion syndrome, the deletion identified on chromosome 16 unmasked a hemizygous gene mutation, leading to the complex phenotype of our patient.

The deletion contained 58 protein-coding genes, including the *CLN3* gene (Figure 1c). The *CLN3* gene encodes a 438-amino-acid glycosylated membrane protein almost certainly located in the lysosome and in additional locations in the neurons.[12] The function of CLN3 remains unknown but proposed functions include lysosomal acidification, sequestration of lysosomal enzymes, degradation of proteins and small molecule transport, organelle fusion, and apoptosis.[13,14] Mutations in *CLN3* cause JNCL, the most common of a group of inherited, autosomal recessive, progressive, and ultimately fatal neurodegenerative disorders that share the clinical features of progressive loss of vision, myoclonic seizures, loss of cognitive function, pyramidal and extrapyramidal motor dysfunctions, and the pathologic features of progressive neuronal loss, with an accumulation of lipofuscin-like autofluorescent storage material in the cytoplasm of neurons and other cells (Table 1).[15] The presence of fingerprint patterns in the lysosomes of different tissues and the finding of vacuolated lymphocytes are considered as pathognomonic for JNCL.

Vacuolated lymphocytes on peripheral blood film testing were present in our patient, which strongly strengthens our hypothesis that the distinct neurological features of the patient could be derived from a hemizygous *CLN3*-gene mutation associated with the submicroscopic 16p11.2 deletion, even if the patient did not present all JNCL features, especially myoclonic seizures (Table 1). Mutation analysis of the *CLN3* gene in the patient by allele-specific PCR revealed a 1.02-kb deletion on the remaining allele (NG_008654.1: g.10373_11338del). This common mutation is a c.461_677del (NM_001042432.1) and deletes 217 bp of coding sequence, including exons 7 and 8 (Figure 2b). Forty-one mutations and five polymorphisms have been described in *CLN3* (http://www.ucl.ac.uk/ncl/cln3.shtml – accessed on 19 November 2007). Approximately 85% of JNCL patients are homozygous for this 1.02-kb deletion, resulting in a frameshift and a premature stop. The remaining patients are mostly compound heterozygotes for the 1.02-kb deletion and for one of the many other mutations described so far.[16] In some patients, the second mutation has not yet been identified,[12] suggesting the interest of using aCGH to detect 16p11.2 microdeletion on the remaining allele.

This case provides further evidence that phenotypic variability among patients with similar deletion syndromes may, in part, be due to hemizygous expression of a recessive mutation on the non-deleted homolog. Indeed, a review article by Coman and Gardner[17] highlights that this mechanism has already been demonstrated by several authors.[18–23] However, to our knowledge, this is the first report of JNCL caused by an unmasked mutation of *CLN3* in a 16p11.2-deletion syndrome. This report illustrates the importance of searching for *CLN3* mutation when larger 16p11.2 deletion is associated with neurodegenerative features.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA ARCHIVING

The data were submitted to DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (http://decipher.sanger.ac.uk/).

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