Homozygous Truncating Intragenic Duplication in *TUSC3* Responsible for Rare Autosomal Recessive Nonsyndromic Intellectual Disability with No Clinical or Biochemical Metabolic Markers

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Abstract Intellectual disability (ID), which affects around 2–3% of the general population, is classically divided into syndromic and nonsyndromic forms, with several modes of inheritance. Nonsyndromic autosomal recessive ID (NS-ARID) appears extremely heterogeneous with numerous genes identified to date, including inborn errors of

metabolism. The *TUSC3* gene encodes a subunit of the endoplasmic reticulum (ER)-bound oligosaccharyltransferase complex, which mediates a key step of N-glycosylation. To date, only five families with NS-ARID and *TUSC3* mutations or rearrangements have been reported in the literature. All patients had speech delay, moderate-to-severe ID, and moderate facial dysmorphism. Microcephaly was noted in one third of patients, as was short stature. No patients had congenital malformation except one patient with unilateral cryptorchidism. Glycosylation analyses of patients' fibroblasts showed normal N-glycan synthesis and transfer. We present a review of the 19 patients previously described in the literature and report on a sixth consanguineous family including two affected sibs, with intellectual disability, unspecific dysmorphic features, and no additional malformations identified by high-resolution array-CGH. A homozygous truncating intragenic duplication of the *TUSC3* gene leading to an aberrant transcript was detected in two siblings. This observation, which is the first reported case of *TUSC3* homozygous duplication, confirms the implication of *TUSC3* in NS-ARID and the power of the high-resolution array-CGH in identifying intragenic rearrangements of genes implicated in nonsyndromic ID and rare diseases.

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Introduction

Intellectual disability (ID), defined as an intelligence quotient below 70, is the most frequent handicap in children. ID affects 2–3% of the general population and is classically divided into syndromic (S-ID) and nonsyndromic (NS-ID) forms, with

several modes of inheritance (Ropers 2008). The vast majority of cases with moderate-to-severe ID results from chromosomal aberrations, rearrangements, submicroscopic deletions or duplications, and point mutations in numerous genes or candidate regions (Ropers 2008). Array-based comparative genomic hybridization (array-CGH) allows the detection of a de novo genomic abnormality in 15–20% of patients with major multiple malformations and intellectual disability (Miller et al. 2010; Jaillard et al. 2010; Cooper et al. 2011). Until 2011, a genetic cause was identified in only 10% of NS-ID and no more than 8 genes were implicated in non-syndromic autosomal recessive ID (NS-ARID) (Çalışkan et al. 2011; Musante and Ropers 2014). This number has increased significantly since the emergence of new-generation sequencing strategies (Rauch et al. 2012; Musante and Ropers 2014). These genes are involved in different pathways, thus showing the extreme functional diversity of pathological mechanisms involved in NS-ARID: synaptic proteolysis, regulation of mitochondrial energy metabolism, regulation of the I- κ B kinase/NF- κ B cascade, induction of long-term potentiation, GABAB receptor-mediated spikes and wave discharges in the thalamocortical pathway, regulation of polymerase II genes, mRNA metabolism, and N-glycosylation (Garshasbi et al. 2008, 2011; Musante and Ropers 2014). However, the genetic basis of ID is still unclear in a large number of affected patients, especially those with nonsyndromic intellectual disability, in whom there are no physical signs (Rauch et al. 2012).

Glycosylation is an important posttranslational modification in eukaryotic cells and has a significant impact on numerous biological processes. About half of the body's proteins contain carbohydrate chains essential for protein structure and function, and about 200–300 genes ($\approx 1\%$ of the human genome) have been found to be involved in glycosylation processes (Varki 1993; Morava et al. 2008). Many defects in asparagine-linked glycosylation (N-glycosylation) have been identified in congenital disorders of glycosylation (CDG) syndromes, often described as multi-systemic diseases. To date, more than 70 different genetic disorders of glycosylation have been described, mostly during the past 15 years, and this number is still growing (Freeze et al. 2012).

TUSC3, the *Ost3* *Saccharomyces cerevisiae* human ortholog, was initially identified as a 34 kD subunit in the yeast oligosaccharyltransferase complex (Kelleher and Gilmore 2006). The *TUSC3* gene, composed of 11 exons spanning ~ 224 Kbp of the genomic DNA on chromosome 8p22, encodes a predicted 348-amino-acid protein with five potential transmembrane domains. *TUSC3* is thought to encode a subunit of the ER-bound oligosaccharyltransferase (OTase) complex that mediates the central step in the N-linked protein glycosylation pathway (Garshasbi et al. 2008). Indeed, OTase is an oligomeric membrane-protein

complex that mediates the transfer of a preassembled high-mannose oligosaccharide onto asparagine residues of nascent polypeptides entering the lumen of the endoplasmic reticulum. Two mammalian OTase complexes composed of seven proteins differ by the presence of *TUSC3* or IAP proteins (Kelleher and Gilmore 2006). *TUSC3* appears ubiquitously expressed with greater expression in the fetal brain (Molinari et al. 2008) and interacts with the alpha isoform of the protein phosphatase 1 (PPPC1A; OMIM176875) catalytic subunit, which is involved in the modulation of synaptic plasticity, and in memory and learning processes in mice (Garshasbi et al. 2008). *TUSC3* has also been implicated in the Mg^{2+} membrane transport system with a possible role in learning capacities, working memory, and short- and long-term memory. Indeed, it has been shown that *TUSC3* and/or IAP's KO leads to a marked decrease in total and free magnesium concentration in human cells and that morpholino knockdown of IAP and *TUSC3* protein expression in zebra fish embryos results in early developmental arrest (Zhou and Clapham 2009). Interestingly, it has recently been proven that an increased concentration of magnesium in the brain leads to enhanced learning capacity, working memory, and short-term and long-term memories in rats (Slutsky et al. 2010). It had been suggested that disturbed Mg^{2+} levels caused by *TUSC3* impairment are in part responsible for the ID phenotype observed in patients carrying homozygous mutations in *TUSC3* (Garshasbi et al. 2011).

Reported patients with *TUSC3* mutations have normal results of glycosylation analyses, which could be explained by the compensation, at least partially in a tissue-specific manner, of *TUSC3* by *IAP*, the *TUSC3* paralog, coding for an OTase subunit and known to be involved in the N-glycosylation process (Kelleher et al. 2003; Garshasbi et al. 2008; Molinari et al. 2008). Another hypothesis would be that *TUSC3* and IAP protein function is essential for the glycosylation of proteins belonging to cellular subgroups in a specific tissue, mainly the central nervous system, which cannot be explored by usual serum glycosylation screening techniques. Normal glycosylation profiles in patients carrying a homozygous *TUSC3* deletion could also signify that *TUSC3* has no major role in the extracerebral N-glycosylation process (Garshasbi et al. 2008).

TUSC3 mutations or rearrangements have previously been reported in only five families, which were all but one consanguineous. The mutations lead not to aberrant DNA but to a reduced amount of transcript (Molinari et al. 2008; Garshasbi et al. 2008, 2011; Khan et al. 2011; Loddo et al. 2013).

We report a new family with NS-ARID secondary to a *TUSC3* rearrangement leading to an aberrant transcript and discuss the power of high-resolution array-CGH in

identifying intragenic rearrangements in genes implicated in nonsyndromic ID and rare diseases.

Clinical Report

Two siblings were referred to our unit for genetic investigations of ID. They were born to healthy consanguineous parents (Fig. 1a). The family history was unremarkable. In both cases, pregnancy and delivery were uneventful, and no postnatal infection, toxic exposure, or significant head trauma was noted.

Patient 1

Patient 1 was born at term with normal anthropometry. He presented significant developmental disability with independent sitting at 15 months, independent walking after 2 years, and disabled speech with very poor language. He was oriented towards a school for special needs. At 36 years of age, the physical examination was unremarkable with normal height, weight, and head circumference, except for facial dysmorphism, which included a long face with small deep set eyes (Fig. 1b). ID was estimated as severe with insufficient speech and behavioral disturbances but appeared to be nonprogressive. Neurological examinations were normal. Cardiac, abdominal, and renal ultrasound scans, skeletal X-rays, and brain MRI were normal as were funduscopy and electroencephalography.

Patient 2

Patient 2 was born at term with length 48 cm, weight 3,390 g, and occipitofrontal circumference (OFC) 33 cm. She presented developmental disability with sitting from 10 months and speech delay. She was oriented towards a school for special needs. At 29 years of age, physical examination was unremarkable with normal height, weight, and head circumference, except for facial dysmorphism, which included a long face, wide mouth, deep set eyes, and bulbous nose (Fig. 1c). Her gait was normal without ataxia. ID was estimated as moderate with insufficient speech; she was more autonomous than her brother and was able to hold a limited conversation, but never learned to read or write. Neurological examinations were normal. Cardiac, abdominal, and pelvic ultrasound scans, skeletal X-rays, brain MRI, and funduscopy were normal. She presented an epileptic stroke with an epileptic electroencephalogram. Ophthalmologic examinations were normal.

Standard metabolic screening and chromosomal analyses were normal. Molecular testing ruled out fragile X syndrome.

Methods

Genomic DNA of the affected siblings, their unaffected sister, and both parents was extracted from blood samples by conventional methods after informed consent had been provided.

Array-CGH

Array-CGH experiments were performed using the Agilent Human Genome CGH 105A oligonucleotide arrays (Agilent Technologies, Santa Clara, CA, USA; www.agilent.com), following the protocols provided by Agilent. The 105 K array has a genome-wide resolution of one probe each 21.7 kb on average. The array was analyzed with the Agilent scanner and the Feature Extraction software (v.9.5.1.3). A graphical overview was obtained using the DNA Analytics software (v4.0), using the statistical algorithms ADM-2 according to a sensitivity threshold at 6.0 and a moving average window of 5 pt. Mapping data were analyzed on the human genome sequence using ensemble (www.ensembl.org).

Molecular Investigations

Genomic Real-Time Quantitative PCR

Quantitative PCR (qPCR) was performed on genomic DNA, using an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). We designed 5 primer sets within *TUSC3*. All primer sequences used in this study are shown in Table 1. qPCR was carried out in a total volume of 20 µl containing 10 µl of SYBR Green Master Mix (Applied Biosystems), 0.4 mM of each primer, and 10 ng of genomic DNA. Thermal cycling conditions were 95°C for 20 s, followed by 40 cycles with 95°C for 3 s and 60°C for 30 s. The *RPPH1* gene was selected as the control amplicon. Validation experiments demonstrated that amplification efficiencies of the control and all target amplicons were approximately equal. All analyses were done in triplicate. The dosage of each amplicon relative to *RPPH1* and normalized to control male DNA was determined using the $2^{-\Delta\Delta C_t}$ method.

RNA Isolation and RT-qPCR

Total RNAs were isolated from PAXgene blood RNA tubes for patient 1 and a male control using RNeasy[®] mini kit (Qiagen). RNA was reverse transcribed through the use of random primers (Superscript, Invitrogen). Reverse transcriptase quantitative real-time PCR (RT-qPCR) was performed on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems). We designed three primer

Fig. 1 Consanguineous pedigree (a). Pictures of patient 1 (b) and patient 2 (c) showing similar facial dysmorphism

sets within *TUSC3* (Table 1). RT-qPCR was carried out in a total volume of 20 μ l containing 10 μ l of SYBR Green Master Mix (Applied Biosystems), 0.4 mM of each primer, and 5 μ l of cDNA. The thermal cycling conditions were 95°C for 20 s, followed by 40 cycles with 95°C for 3 s and 60°C for 30 s. The *ESD* and *ABL* genes were selected as control amplicons. Validation experiments demonstrated that amplification efficiencies of control and all target amplicons were approximately equal. All analyses were

done in triplicate. The dosage of each amplicon relative to *ESD* and *ABL* and normalized to control male cDNA was determined using the $2^{-\Delta\Delta C_t}$ method.

Reverse transcription PCR (RT-PCR) was performed using a forward primer in exon 7 of *TUSC3* and a reverse primer in exon 2 of *TUSC3*. RT-PCR was carried out in a total volume of 25 μ l containing 0.4 mM of each primer and 5 μ l of cDNA. RT-PCR products were electrophoresed on agarose gels, purified with NucleoSpin® Extract II kit

Table 1 Primer sequences

Primer set	Forward primer	Reverse primer	PCR product genomic localization (hg18)	PCR product size (bp)	Gene
<i>qPCR primers</i>					
1	TCTGTGCTAGGCCCCAGGTA	CCATCCTGCTACTGAACCCAAT	chr8:15441901+15441962	62	<i>TUSC3</i> (5'UTR)
2	GCAGCAGTAAGAACAGCTATGCA	AAATGCCGGGAAGTCTTATCTC	chr8:15463964+15464032	69	<i>TUSC3</i> (intron 1)
3	ACTCCTGGCGCTATTCACTG	CCCCTCATCATAGTCCACCATAC	chr8:15552609+15552676	68	<i>TUSC3</i> (exon 3)
4	GGAGCTGCTAGCCCAACCAT	TATTATAGGAAGAGAGAAACT GAGCCTTT	chr8:15624330+15624395	66	<i>TUSC3</i> (intron 6)
5	GTTTGTGGCAGAAATCACACATTATT	CCCTTTTCAATTTCGTGGTATC	chr8:15632575+15632643	69	<i>TUSC3</i> (exon 7)
<i>RT-qPCR primers</i>					
6	TCAGCGGCAGTGTCTGTGT	AGATGAATAGCGCCAGGAGTTC		80	<i>TUSC3</i> (exons 2–3)
7	AGATGAATAGCGCCAGGAGTTC	CAGGCCAAAGCAATGGTA		81	<i>TUSC3</i> (exons 4–5)
8	AGACTGGTTGGGCCATGGT	CACGGATATGTTCCACATCTG		79	<i>TUSC3</i> (exons 5–6)
Primer name	Forward primer	Gene	Primer name	Reverse primer	Gene
<i>RT-PCR primer</i>					
TUSC3-7F	GGCTCAGTTTGTGGCAGAAT	<i>TUSC3</i> (exon 7)	TUSC3-2R	GTTTCGAGGT GGTGCCTTTA	<i>TUSC3</i> (exon 2)

Fig. 2 (a) Sequencing results of the RT-PCR product showing an aberrant *N33/TUSC3* transcript. **(b)** Structure of the aberrant *N33/*

TUSC3 transcript—underlined, primer sequences; in bold, exon 7 and exon 2 sequences; third line, amino-acid translation; in red, amino-acid

(Macherey Nagel, SARL), and sequenced using the BigDye Terminator kit (Applied Biosystems).

Cell Culture

Primary fibroblasts from both patients, their parents, and controls were cultured at 37°C under 5% CO₂ in RPMI 1640/Glutamax (Invitrogen) supplemented with 10% of fetal calf serum (Invitrogen).

Biochemical Studies of the N-glycosylation Pathway

Transferrin Glycoform Study

Western blot analyses of serum transferrin, orosomucoid, alpha-antitrypsin, and haptoglobin were used to identify the loss of glycan chains leading to a decrease in the molecular weight. Polyacrylamide gel electrophoresis (SDS-PAGE) was done and followed by transfer onto nitrocellulose and revealed by a rabbit polyclonal anti-transferrin antibody as described previously (Seta et al. 1996).

Results

Array-CGH

An interstitial homozygous 178 kb amplification in the chromosome 8p22 locus was identified in both patients (Chr8:15403439–15581139, Hg19). The minimal region included exons 2 to 6 of the *TUSC3* gene. The mean log2ratio at +0.86 was compatible with homozygous duplication or triplication.

Two other amplifications located at 4q31.3 (Chr4: 151898224–152284954) and 9p24.1 (Chr9:7598025–8106493), with a minimal size of 386 and 508 kb, respectively, were detected in patient 1. The 4q31.3 and 9p24.1 duplications were inherited from the father and were absent in patient 2. Therefore, the patient's phenotype did not appear to be explained by these two genomic imbalances. Except for polymorphic regions, no other copy-number alterations were observed (data not shown).

Genomic Quantitative Real-Time PCR

By quantitative PCR, the homozygous 8p22 duplication was confirmed in both patients, and the parents turned out to be heterozygous. We performed qPCR on genomic DNA

in order to determine more precisely the extent of the duplication within the *TUSC3* gene. It appeared to start before the 5'UTR region and to end after exon 7.

Functional Analysis of the N33 Intragenic Duplication

RT-qPCR revealed a very low expression of *TUSC3* cDNA in peripheral blood. The method was therefore not sensitive enough to determine a difference of expression level between heterozygous, homozygous and normal individuals.

To find the intragenic duplication at the RNA level, RT-PCR using a forward primer in exon 7 and a reverse primer in exon 2 of *TUSC3* was performed and amplified one fragment of approximately 250 bp in patient 1 and no specific fragment in a male control. This fragment was sequenced. It corresponded to exon 7, an inclusion of 95 bases 9,414 bp on the 3' side of *TUSC3* and exon 2 (Fig. 2a, b). Input of the chr8:15432761–15423779 region in the Human Splicing Finder (<http://www.umd.be/HSF/>) revealed a cryptic splicing site, a branch site, a CT-rich region, and an Exon Splicing Enhancer (ESE) as shown in Fig. 2c. Inclusion of this intergenic region leads to an aberrant transcript carrying the intragenic exon 2–7 duplication. Translation of this aberrant transcript leads to a premature termination codon (TGA) at position 289 as shown in Fig. 2b. Amino acids 277 to 297 are predicted to code for a transmembrane domain.

In summary, this intragenic duplication in *TUSC3* led to a premature termination codon and truncated protein with interruption in a transmembrane domain.

Glycosylation Analysis

Analysis of transferrin glycoforms by Western blot did not show any abnormal profiles in patient 1 and patient 2 compared with controls. The N-glycan structure of other serum glycoproteins was analyzed, but did not reveal any significant difference between patients and controls.

Discussion

We describe here a cohort of 21 patients harboring a homozygous *TUSC3* mutation, 19 affected members from 5 families previously described, and 2 new cases in the family we report here. All but one family were consanguineous. All of the reported 21 patients with homozygous *TUSC3* mutations/rearrangements, including our two patients, had speech delay, moderate-to-severe ID, and no

Fig. 2 (continued) translation of the intergenic included region leading to a premature stop codon; 4th line, amino-acid position. (c) Nucleotide sequence of the chr8:15432761–15423779 region analyzed with Human Splicing Finder—*underlined*, branch site, splice acceptor

site, and splice donor site consensus sequences; in *italics*, CT-rich region; in *bold*, predicted ESE; in *gray*, 95 bp intergenic included region

Table 2 Reported patients and methods of detection

	Molinari et al. (2008)	Garshasbi et al. (2008)	Garshasbi et al. (2011)	Khan et al. (2011)	Loddo et al. (2013)	Present study
Number of patients	2	7	3	6	1	2
Consanguinity	Yes	Yes	Yes	Yes	No	Yes
Main clinical features	ID (severe) Speech delay	Moderate facial dysmorphism ID (moderate) Speech delay Short stature (4/6) Microcephaly (1/6)	Moderate facial dysmorphism ID (severe) Speech delay Ankyloglossia Short stature (1/3) Microcephaly (2/3) NA	Moderate facial dysmorphism ID (severe) Speech delay (1/6) Short stature (3/6) Microcephaly (3/6) Normal (2/2) (CT scan) Homozygous deletion (170.673 kb)	Moderate facial dysmorphism ID (moderate) Speech delay Motor instability, anxiety, oppositional-defiant disorder Hypermetropia Left cryptorchidism Hypertensive hydrocephalus (MRI) Homozygous deletion (203 kb)	Moderate facial dysmorphism ID (severe) Speech delay
Brain imaging data	Normal (2/2) (MRI)	Normal (2/2) (MRI)	NA	Normal (2/2) (CT scan)	Left cryptorchidism Hypertensive hydrocephalus (MRI)	Normal (2/2) (MRI)
Molecular defect	Mutation c.787_788insC (exon 6)	Homozygous deletion (120–150 kb)	Mutation c.163C>T (exon 2)	Homozygous deletion (170.673 kb)	Homozygous deletion (203 kb)	Homozygous duplication (238 kb)
Array	SNP array Affymetrix GeneChip Human Mapping 10 K	SNP array Affymetrix Human Mapping 250 K (Nsp)	SNP array Illumina Linkage Panel IV (6 K)	SNP array Affymetrix 500 K NspI	SNP array Affymetrix GeneChip 6.0	Array-CGH Agilent Human Genome 105A 105 K
Strategy	Autozygosity mapping and sequencing analysis	Autozygosity mapping and CNV analysis	Autozygosity mapping and sequencing analysis	Autozygosity mapping and CNV analysis	Autozygosity mapping and CNV analysis	CNV analysis

ID intellectual disability, CT computed tomography, MRI magnetic resonance imaging, NA not available

Fig. 3 Schematic representation of the mutations in *TUSC3* reported in the literature and in our patients

or moderate facial dysmorphism. Among the 20 patients for whom the values were available, 6 (30%) had microcephaly, 6 (30%) had short stature, and none of them had congenital malformation except one patient who presented left cryptorchidism. Clinical features are summarized in Table 2. Among the patients who had brain imaging, only one had brain abnormalities (hypertensive hydrocephalus, surgically treated at age 3 months, and mild enlargement of the frontal subarachnoid spaces and ventricles on the follow-up brain MRI) and none of them had the cerebellar abnormalities classically described in CDG (Table 2) (Molinari et al. 2008; Garshasbi et al. 2008, 2011; Khan et al. 2011; Loddo et al. 2013). The majority of patients (16 patients, 4 families) were identified by array-CGH analysis (Table 2). Other families were identified by homozygosity mapping and direct sequencing of *TUSC3* (Molinari et al. 2008; Garshasbi et al. 2008, 2011; Khan et al. 2011; Loddo et al. 2013) (Fig. 3).

The family we describe here is the sixth with mutations identified in the *TUSC3* gene. Since more than two thirds of the genes responsible for NS-ARID were reported in only one family, *TUSC3* appears to be one of the genes most frequently implicated in NS-ARID, (Table 2) (Musante and Ropers 2014). Interestingly, the present *TUSC3* mutation was an intragenic duplication leading to genomic imbalance identified by high-resolution 105 K

array-CGH. It is the first reported case of *TUSC3* duplication, which was found in the homozygous state, due to parental consanguinity. Because of the absence of specific signs of inborn errors of metabolism and syndromic ID, next-generation technologies including array-CGH and exome sequencing will be the only way to detect such patients and expand the clinical and biological spectrum of such pathologies. Indeed, our patients, like the other patients previously described, had normal glycosylation analyses using Western blot and isoelectric focusing, which classically permit the detection of type Ia and II CDG syndromes (Molinari et al. 2008).

Since its development, high-resolution array-CGH has shown its ability to detect intragenic copy-number variations (CNVs) and has highlighted the implication of new genes in ID, in particular in autosomal dominant familial ID (Thevenon et al. 2012). Differentiating between benign and pathogenic CNVs could be a challenge, as it depends on the size, the genes involved, and the mode of inheritance. The constitution of databases will be of help (Cooper et al. 2011). Intragenic rearrangements have been shown to contribute enormously to allelic heterogeneity in certain diseases and have been, for example, implicated in one third of patients with Cohen syndrome in the *VPS13B* gene (Knijnenburg et al. 2009; Balikova et al. 2009; El Chehadah-Djebbar et al. 2011).

The identification of a sixth family with mutations in the *N33/TUSC3* gene not only supports the hypothesis that mutations leading to an abnormal OTase are responsible for NS-ARID but also adds to the list of N-glycosylation disorders with a normal transferrin isoelectric focusing (IEF) profile (GCS1-CDG (IIb), SLC35C1-CDG (IIc) et SLC35A1-CDG (IIf) (Lefeber et al. 2011; Freeze et al. 2012).

In conclusion, this report confirms that patients carrying *TUSC3* mutations/rearrangements cannot be diagnosed by classical CDG screening analyses and emphasizes the power of high-resolution array-CGH in identifying intragenic rearrangements in genes implicated in nonsyndromic ID, in particular when diagnosis using usual metabolic screening and gene-by-gene sequencing analysis methods is difficult.

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Conflict of Interest

S. El Chehadeh, C. Bonnet, P. Callier, M. Béri, T. Dupré, M. Payet, C. Ragon, A.L Mosca-Boidron, N. Marle, F. Mugneret, A. Masurel-Paulet, J. Thevenon, N. Seta, L. Duplomb, P. Jonveaux, L. Faivre, and C. Thauvin-Robinet declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki of 1975, as revised in 2000. Informed consent for inclusion in the study was obtained from all patients.

The study was performed according to ethical recommendations of the French National Ethics Committee.

Additional informed consent was obtained from all patients for whom identifying information is included in this article.

Contributions of Individual Authors

Conception and design, conduct, report and review, and clinical follow-up of patients: S. El Chehadeh, L. Faivre, C. Thauvin-Robinet.

Technical aspects (array-CGH, RT-PCR, functional analyses, metabolic analyses): C. Bonnet, M. Béri, M. Payet, C. Ragon, P. Jonveaux, L. Duplomb, P. Callier, A.L Mosca-Boidron, N. Marle, F. Mugneret, A. Masurel-Paulet, J. Thevenon, T. Dupré, N. Seta.

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