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ARTICLE

Large deletions encompassing the *TCOF1* and *CAMK2A* genes are responsible for Treacher Collins syndrome with intellectual disability

Marie Vincent[1,7], Corinne Collet[2,7], Alain Verloes[3], Laetitia Lambert[4], Christian Herlin[5], Catherine Blanchet[6], Elodie Sanchez[1], Séverine Drunat[3], Jacqueline Vigneron[4], Jean-Louis Laplanche[2], Jacques Puechberty[1], Pierre Sarda[1] and David Geneviève*,[1]

Mandibulofacial dysostosis is part of a clinically and genetically heterogeneous group of disorders of craniofacial development, which lead to malar and mandibular hypoplasia. Treacher Collins syndrome is the major cause of mandibulofacial dysostosis and is due to mutations in the *TCOF1* gene. Usually patients with Treacher Collins syndrome do not present with intellectual disability. Recently, the *EFTUD2* gene was identified in patients with mandibulofacial dysostosis associated with microcephaly, intellectual disability and esophageal atresia. We report on two patients presenting with mandibulofacial dysostosis characteristic of Treacher Collins syndrome, but associated with unexpected intellectual disability, due to a large deletion encompassing several genes including the *TCOF1* gene. We discuss the involvement of the other deleted genes such as *CAMK2A* or *SLC6A7* in the cognitive development delay of the patients reported, and we propose the systematic investigation for 5q32 deletion when intellectual disability is associated with Treacher Collins syndrome.
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INTRODUCTION

Mandibulofacial dysostosis (MFD) is defined by abnormal craniofacial development, particularly of the first and second branchial arches. MFD is characterized by malar and mandibular hypoplasia, often associated with cleft palate, conductive hearing loss, and, less frequently, choanal atresia, visceral or skeletal malformations as well as intellectual disability (ID). MFD is not a distinct and specific genetic disease, but is in fact composed of a group of clinically and genetically heterogeneous disorders. Among the several types of MFD, Treacher Collins syndrome (TCS, OMIM 154500) is the most frequent. TCS is also genetically heterogeneous and to date three genes have been implicated in explaining ~80% of the genetic anomalies in TCS patients (namely *TCOF1*,[1] *POLR1D*[2] and *POLR1C*[2]). The remaining genes are still unknown. Heterozygous intragenic deletions/duplications of *TCOF1* have been recently described and are a rare cause of TCS.[3–4] Mutations in *EFTUD2* have been identified in patients with MFD and microcephaly[5] (MFDM, OMIM 610536), characterized by progressive and severe microcephaly, ID and additional malformations, such as choanal and aural atresia, cleft palate, congenital heart defect and esophageal atresia.[6] However, Gordon *et al*[6] and Luquetti *et al*[7] reported *EFTUD2* mutations in patients without microcephaly, suggesting that *EFTUD2* could be responsible for MFDM as well as other forms of MFD.[6]

We report for the first time two patients with MFD and ID due to the deletion of several genes including *TCOF1* and *CAMK2A*, and discuss the involvement of these genes in the phenotype of the patients.

MATERIALS AND METHODS

Informed consent was obtained from the parents of the patients for molecular genetic analysis, and publication of the clinical data and pictures.

DNA was extracted from the patient's whole blood sample using the QIAamp DNA Blood Midi Kit (Qiagen, Courtaboeuf, France) according to the supplier's protocol.

The 27 coding exons of *TCOF1* and at least 60 bp of intronic sequence flanking the exons were amplified and sequenced with Life Technologies reagents and equipment, see Supplementary data for details.

MLPA analysis was performed using the Salsa MLPA Kit P310 TCOF1 (MRC-Holland, Amsterdam, the Netherlands) and carried out as described by the manufacturer with capillary electrophoresis on an ABI 3130 DNA Analyzer (Life Technologies, Courtaboeuf, France), GeneMapper software version 4.0 (Life Technologies) and MRC Coffalyzer MLPA-Dat Software (MRC-Holland). Additional information about commercially available probe sets is available at http://www.mrc-holland.com.

CNV detection was performed using array-CGH Agilent (Agilent Technologies, Les Ulis, France) 180k for patient 1 and array-CGH Agilent 244k for patient 2. Experiments followed standard and manufacturer's recommendations. DNA sequence information refers to the public UCSC database NCBI37 (Hg19). Genome assemblies were NCBI136/hg18 UCSC for patient 1 and GRCh37/hg19 for patient 2.

[1]Département de Génétique Médicale, CHRU Montpellier, Faculté de Médecine de Montpellier-Mimes, Université Montpellier 1, Montpellier, France; [2]Service de Biologie Moléculaire, Hôpital Lariboisière, Paris, France; [3]Département de Génétique Médicale, Hôpital Robert Debré, Paris, France, Université Denis Diderot Sorbonne-Paris, INSERM U676, Département de Génétique, Sart Tilman University Hospital, Liège, Belgium; [4]Service de Génétique Médicale, CHRU de Nancy-Brabois, Nancy, France; [5]Service de Chirurgie Plastique Infantile, CHRU Montpellier, Montpellier, France; [6]Service d'Oto-rhino-laryngologie, CHRU Montpellier, Montpellier, France
[7]These authors contributed equally to this work.
*Correspondence: Professor D Geneviève, Departement de Génétique, CHU Montpellier, Hopital Arnaud de Villeneuve, 371, avenue du Doyen Gaston Giraud, 34295 Montpellier cedex 5, France. Tel: + 33 4 67336564; Fax: + 33 4 67336052; E-mail: d-genevieve@chu-montpellier.fr
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RESULTS

Clinical reports

Patient 1. She was born at term after a normal pregnancy, to unrelated and unaffected Belgian parents. Birth weight was normal (3100 g, −0.5 SD). Other parameters were unknown. Family history was uneventful. Diagnosis of MFD was made at birth based on malar hypoplasia, down-slanted palpebral fissures, microtia and micro-retrognathia. The baby had bilateral conductive hearing loss (−60 dB on the right side and −70 dB on the left side), which was treated with bone-anchored hearing aid implants. She walked at 18 months and spoke with a delay initially attributed to hearing loss. However, she had persistent learning difficulties despite the hearing aid. She was given special schooling and acquired the basics of reading and writing, but had attention deficit, major difficulties in logical-mathematical skills and persistent clumsiness. She also had difficulties in social interaction, but did not present objective autistic features. At the last evaluation at the age of 14 years, growth parameters and head circumference were normal (weight: 55.5 kg, mean (M); height: 160 cm, M; and OFC: 55.5 cm, M). Her facial features fit the diagnosis of MFD, namely down-slanted palpebral fissures with mild colobomatous cleft of the lower lid, small mandible with class III malocclusion and low-set and dysplastic ears (Figure 1a). The voice was high pitched and hypernasal.

Patient 2. He was born at term after a pregnancy marked by maternal smoking (10 cigarettes per day). BW was 2800 g (−2 SD), BL was 50 cm (M) and BOFC was 35 cm (M). The low weight was attributed to smoking during pregnancy and normalized quickly. Parents were unrelated and unaffected, of French origin with an uneventful family history. TCS was diagnosed at 6 days of life based on facial features. The neonatal period was marked by feeding difficulties due to craniofacial malformation and required nasogastric tube. Newborn neurological examination was normal. The baby had bilateral moderate conductive hearing loss, which was treated with auditory prostheses. He spoke no words at the age of 26 months, and only 3 words at 3 years. The speech delay was initially thought to be due to neglect to wear the hearing aids, but he thereafter developed additional learning difficulties. The patient had a neuro-psychological evaluation at 8.5 years. Performance IQ was at 71, working memory was at 58. He could neither read nor write. He presented behavioral disorders, violence and aggressiveness, and required treatment with risperidone. He attended special classes in an institute for patients with hearing impairment. At the last evaluation (Figure 1b) at age 9 years and 11 months, growth, weight and head circumference were in the normal range (135 cm, 30 kg and 54.5 cm). He presented with facial features of MFD, namely down-slanted palpebral fissures, malar hypoplasia, micrognathia and microtia, and mild ID.

Molecular study. Direct sequencing of *TCOF1* was negative in both patients. MLPA of *TCOF1* showed a complete deletion of the gene in the two patients (Figures 2a and 2b). For patient 1, MLPA of both parents showed no deletion of *TCOF1*. For patient 2, only the DNA of the mother was available and MLPA of *TCOF1* was normal. CGH array in patient 1 showed a deletion of 1 Mb, encompassing *TCOF1*,

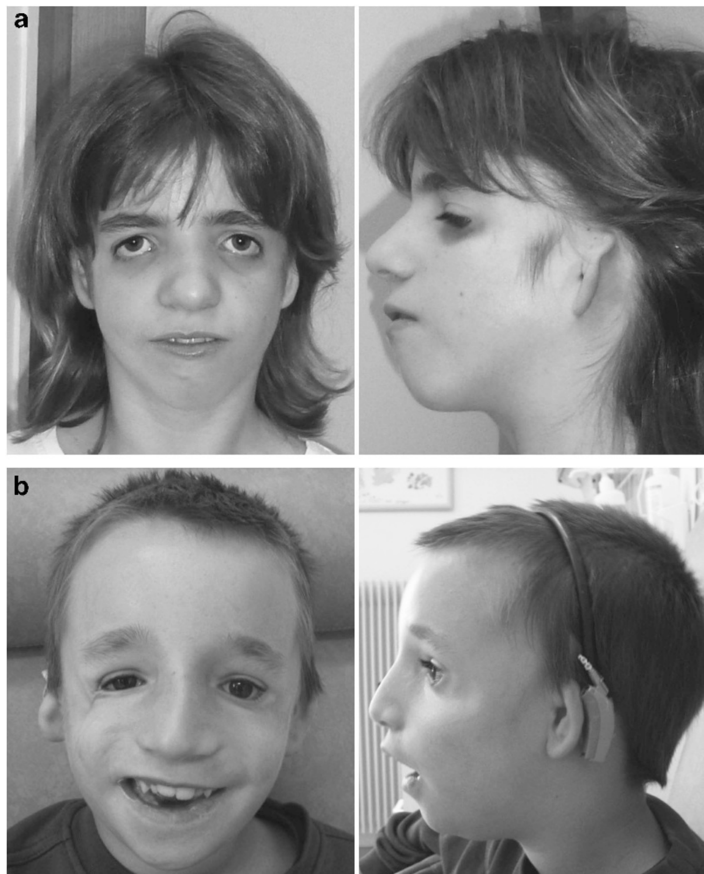

Figure 1 (**a**) Front and lateral view of patient 1, at the age of 14 years. Note down-slanted palpebral fissures, coloboma of the lower lid, malar hypoplasia and micrognathia. (**b**) Front and lateral view of patient 2, at the age of 9 years. Note down-slanted palpebral fissures, malar hypoplasia, micrognathia and microtia with audioprosthesis.

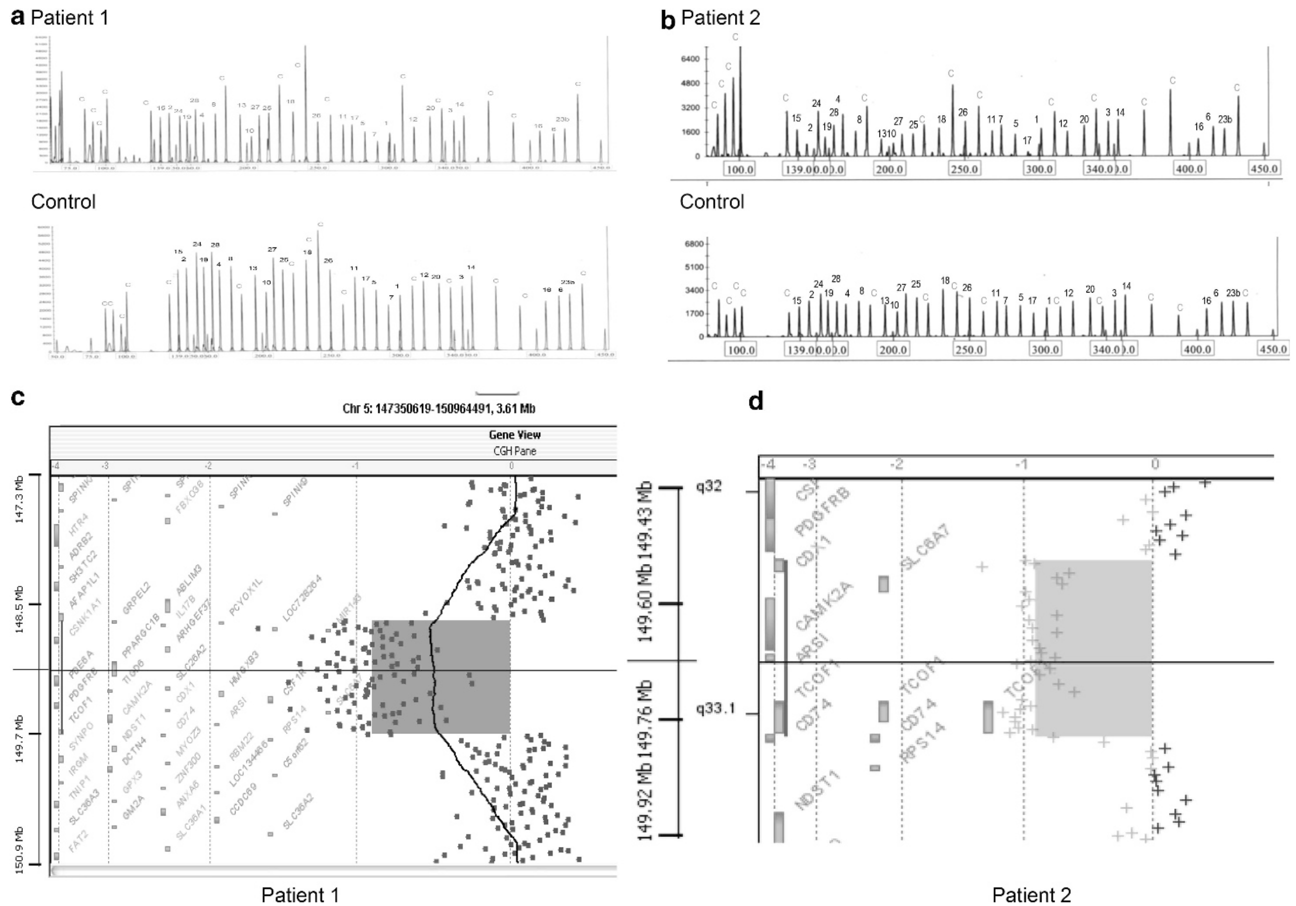

Figure 2 (**a**) MLPA analysis of patient 1, showing a half-dose for all exons of *TCOF1*, revealing a total deletion of the gene, c correspond to internal controls. (**b**) MLPA analysis of patient 2, showing a half-dose for all exons of *TCOF1*, revealing also a total deletion of the gene. (**c**) CGH array of patient 1, revealing a deletion of 1 Mb encompassing *TCOF1*, *GRPEL2*, *PCYOX1L*, *IL17B*, *CSNK1A1*, *FLJ41603*, *PPARGC1B*, *PDE6A*, *SLC26A2*, *TIGD6*, *CSF1R*, *PDGFRB*, *CDX1*, *SLC6A7*, *CAMK2A*, and *ARSI*. (**d**) CGH array of patient 2, revealing a deletion of 262 000 bp encompassing *TCOF1*, *CDX1*, *SLC6A7*, *CAMK2A*, *ARSI* and *CD74*.

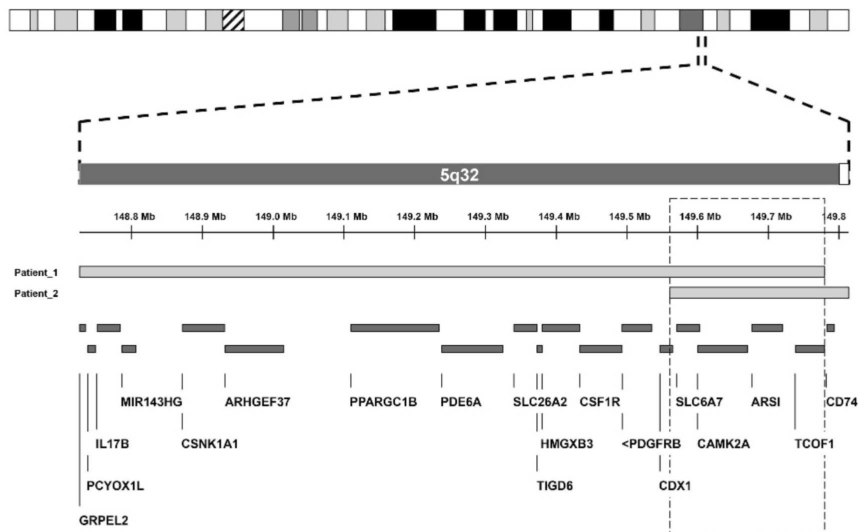

Figure 3 Schematic representation of the 5q32 deletions with their content of genes by the patients reported.

GRPEL2, PCYOX1L, IL17B, CSNK1A1, FLJ41603, PPARGC1B, PDE6A, SLC26A2, TIGD6, CSF1R, PDGFRB, CDX1, SLC6A7, CAMK2A, and *ARSI* (Figure 2c). CGH array in patient 2 showed a deletion of 262 kb, encompassing *TCOF1, CDX1, SLC6A7, CAMK2A, ARSI* and *CD74* (Figure 2d). The common deleted genes in the two patients are *TCOF1*, *CAMK2A*, *CDX1*, *ARSI* and *SLC6A7* (Figure 3).

No patient with similar deletion was reported in the Decipher Database.

DISCUSSION

MFD is a clinically and genetically heterogeneous group of disorders associated with craniofacial features, visceral and/or skeletal malformations and sometimes ID. The clinician's orientation towards the diagnosis of a specific kind of MFD is based on different combinations of these clinical features. Usually the observation of ID in a patient with MFD encourages the clinician to consider a diagnosis other than TCS. Among the etiologies of MFD with ID, the MFDM or MFD Guion-Almeida type was recently linked to mutations in *EFTUD2*.[5–7] Here, we report on two patients with MFD, mild ID and normal OFC, sharing a deletion of *TCOF1*, *CAMK2A*, *CDX1*, *ARSI* and *SLC6A7*. In an attempt to determine which genes (or combination of genes) are responsible for the phenotype observed in the two patients, we considered the known functions of the genes located in the minimal critical region.

TCOF1 encodes for a protein called treacle, which is part of a complex implicated in ribosomal RNA biogenesis and is involved in the proliferation and differentiation of neural crest cells in the first and second branchial arches during early embryogenesis.[8] To our knowledge, mutations in *TCOF1* are responsible for the facial features observed in TCS, but have never been reported to cause ID. However, it has been shown recently that *Tcof1*± mice exhibit reduced brain size as a consequence of defects in neural progenitor maintenance.[9] We believe that deletion of *TCOF1* is responsible for the facial features observed in the two patients we reported on. We also believe that the role of *TCOF1* in ID observed in the two reported patients is unlikely. Indeed, even if *Tcof1* is involved in the control of brain size in mice, patients with haploinsufficiency of *TCOF1* by point mutation or intragenic deletion/duplication usually do not present with ID or microcephaly. However, we cannot rule out a combinatorial effect of the deletion of *TCOF1* with the other deleted genes.

The *CAMK2A* gene (OMIM 114078) encodes the alpha subunit of calcium/calmodulin-dependent protein kinase II (CaM kinase II), which is present in abundance in the brain where it constitutes a major constituent of the postsynaptic density.[10] Autophosphorylation of *CAMK2A* after NMDA receptor activation seems to be essential for contextual long-term memory formation.[11] Different models have shown that *CAMK2A* is necessary for hippocampal place cell stability *ex vivo* and for spatial learning, long-term memory, decreased fear response and increase in defensive aggression *in vivo*.[12] *CAMK2A* may modulate the synaptic events required for the consolidation of memory traces in cortical networks and may have a critical role in plasticity and learning.[10] Mice heterozygous for a null mutation of *Camk2a* have profoundly dysregulated behaviors and impaired neuronal development in the dentate gyrus.[13] No heterozygous variant predicting to lead to haploinsufficiency in *CAMK2A* has been identified in Exome Variant Server (EVS; http://evs.gs.washington.edu). Integrating all these data, we suggest that *CAMK2A* could be responsible for the ID observed in the two reported patients.

The *SLC6A7* gene (OMIM 606205) encodes a neurotransmitter L-Proline transporter and is a member of the γ-aminobutyric acid (GABA) neurotransmitter gene family.[14] It is expressed mostly in the hypothalamus and the hippocampus.[15] To date, this gene has been only described as a susceptibility gene for asthma.[14] Based on the known function of this gene (gene belonging to the GABA neurotransmitter family and expressed in the brain), we can only speculate that this gene could participate to the ID observed in the two reported patients. However, two frameshift variants (with unknown modification of function using polyphen web resource) have been observed in EVS.

The caudal-type homeobox transcription factor 1 gene (*CDX1*, OMIM 600746) is known to be required for anterior–posterior regional identity in Drosophila. In humans, it is an essential transcription factor for intestinal differentiation, and may contribute to intestinal metaplasia in the stomach.[16] No digestive problems were observed in the patients and only one nonsense variant with unknown modification of function using polyphen web resource was observed in EVS. We suggest that this gene is not involved in the phenotype of the two reported patients.

The *ARSI* gene (OMIM 610009) encodes for arylsulfatase I, which catalyzes the hydrolysis of sulfate esters and is involved in hormone biosynthesis, modulation of cell signaling, and degrading of macromolecules.[17] The pattern of expression of the *ARSI* gene is mainly restricted to embryonic tissue and some cancer cell lines,[18] suggesting that *ARSI* is also not involved in the phenotype of the patients. In addition, no heterozygous variant predicting to lead to haploinsufficiency was observed in EVS for *ARSI*.

In conclusion, we describe for the first time, two patients with MFD and ID and for whom a deletion encompassing *TCOF1* and *CAMK2A* has been identified. Based on the knowledge of the deleted genes in the two patients, we hypothesize that *CAMK2A* is in part responsible for ID and *TCOF1* is responsible for the facial features. However, we cannot rule out a combinatorial effect with the other deleted genes. To our opinion, the genetic disorder observed in the two patients is to be considered as a contiguous gene deletion syndrome due to the combination of the effect of the loss of the *TCOF1* gene and other genes such as *CAMK2A*. We suggest investigating for 5q32 deletion covering *TCOF1* and *CAMK2A* if TCS is associated with ID without microcephaly, and to analyze *EFTUD2* if TCS is associated with ID and microcephaly.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)