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SHORT REPORT

Clinical spectrum and outcomes in families with coronal synostosis and *TCF12* mutations

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TCF12 **mutations have been reported very recently in coronal synostosis. We report several cases of familial coronal synostosis among four families harbouring novel *TCF12* mutations. We observed a broad interfamilial phenotypic spectrum with features overlapping with the Saethre–Chotzen syndrome. *TCF12* molecular testing should be considered in patients with unilateral- or bilateral-coronal synostosis associated or not with syndactyly, after having excluded mutations in the *TWIST1* gene and the p.Pro250Arg mutation in *FGFR3*.**
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INTRODUCTION

The aetiology of several cases of non-syndromic coronal craniosynostosis without abnormalities of the extremities remained genetically unexplained until fibroblast growth factor receptor 3 (*FGFR3*) and *TWIST1* mutations were characterised. The p.Pro250Arg (c.749C>G) mutation in *FGFR3,* which is associated with Muenke syndrome [MIM#602849],[1] is identified in about 70% of familial non-syndromic coronal synostosis.[2] This syndrome, in its complete phenotype, includes unilateral (plagiocephaly) or bilateral (brachycephaly) premature closure of the coronal sutures, hearing loss, broad toes, and carpal and tarsal fusions. The phenotype is however, variable, including non-penetrance, isolated craniosynostosis and features which overlap with other craniosynostosis syndromes (eg Crouzon syndrome, Saethre–Chotzen syndrome (SCS) and cloverleaf skull).[3,4] TWIST1 mutations, which are typically associated with SCS syndrome may also be identified in up to 46% cases of brachycephaly.[3] SCS typically presents with coronal synostosis, small ear with prominent crus, ptosis, syndactyly, and broad thumb and/or broad hallux. However, SCS also displays phenotypic variability depending on the type of mutation involved.[5]

The *TCF12* gene, which encodes a basic helix-loop-helix (bHLH) transcription factor, was recently identified by exome sequencing in cases displaying premature fusion of the coronal sutures.[6] Therefore, we tried to detect *TCF12* mutations in five families with coronal synostosis, after negative prior screening for *FGFR3* or *TWIST1* mutations, and found four positive families. We then reviewed the clinical manifestations and outcomes in these families, and further discussed their phenotype/genotype correlations.

MATERIALS AND METHODS

Blood samples were drawn for diagnostic purposes after obtaining informed consent and written permission to publish the clinical photographs. DNA was extracted using a QIAamp blood kit (Qiagen, Courtaboeuf, France) or phenol-chloroform extraction. *TCF12* (exons 7–21), *FGFR3* (exon 7), *TWIST 1* (Exon 1), *FGFR2* (Exons 4, 6, 8 (IIIa), 10 (IIIc), 11, 14, 15, 16, 17) were amplified and sequenced directly on both strands (Life technologies, Courtaboeuf, France) then analysed using Sescape (Life Technologies) and Alamut software (Interactive Biosoftware, Rouen, France). The NCBI reference sequences were as follows: *TCF12* (NM_207036.1), *FGFR3* (NM_000142.4), *TWIST1* (NM_000474.3), and *FGFR2* (NM_000141.4) for c.DNA, and for exon numbering *TCF12* (NM_207036.1), *FGFR3* (NG_012632.1), *TWIST1* (NG_008114.1), and *FGFR2* (NG_012449.1). The mutation nomenclature was based on HGVS nomenclature guidelines [http://www.hgvs.org/mutnomen]. The variants identified have been submitted to the ClinVar database [http://www.ncbi.nlm.nih.gov/clinvar].

CLINICAL REPORT

Family #1

The proband (II-5) was (Figure 1) a female born to unaffected parents (I-1 and I-2; father age 58) initially presenting with isolated left plagiocephaly (Figure 2a–c) defined by premature closure of one of the two coronal sutures. No ptosis, prominent ear crus, syndactyly, or single palmar crease was found. Weight, height and head circumference (HC) were 4200 g (97th percentile), 50 cm (50th percentile), and 35 cm (66th percentile) at birth and 5100 g (97th percentile), 56 cm (50th percentile), and 37 cm (25th percentile) at the time of diagnosis (2 months). She underwent surgery for plagiocephaly at 6 months of age. No additional signs of systemic disease were noticed during her infancy. Noticeably, there was no evidence of hearing loss on formal testing. A formal IQ assessment at the age of 4 years was within the normal range. No other surgery for craniosynostosis was required.

The proband's son (III-3) presented with brachycephaly at birth, with a birth weight of 3670 g (60th percentile), height 51.5 cm (70th percentile), and HC 33 cm (10th percentile). At 5 months of age, HC was 41.5 cm (25th percentile) (Figure 2d–g). Clinical examination showed that he had bicoronal synostosis. No extremity abnormalities were present. The child underwent surgical posterior skull vault decompression during his 5th month, and fronto-orbital advancement at 13 months.

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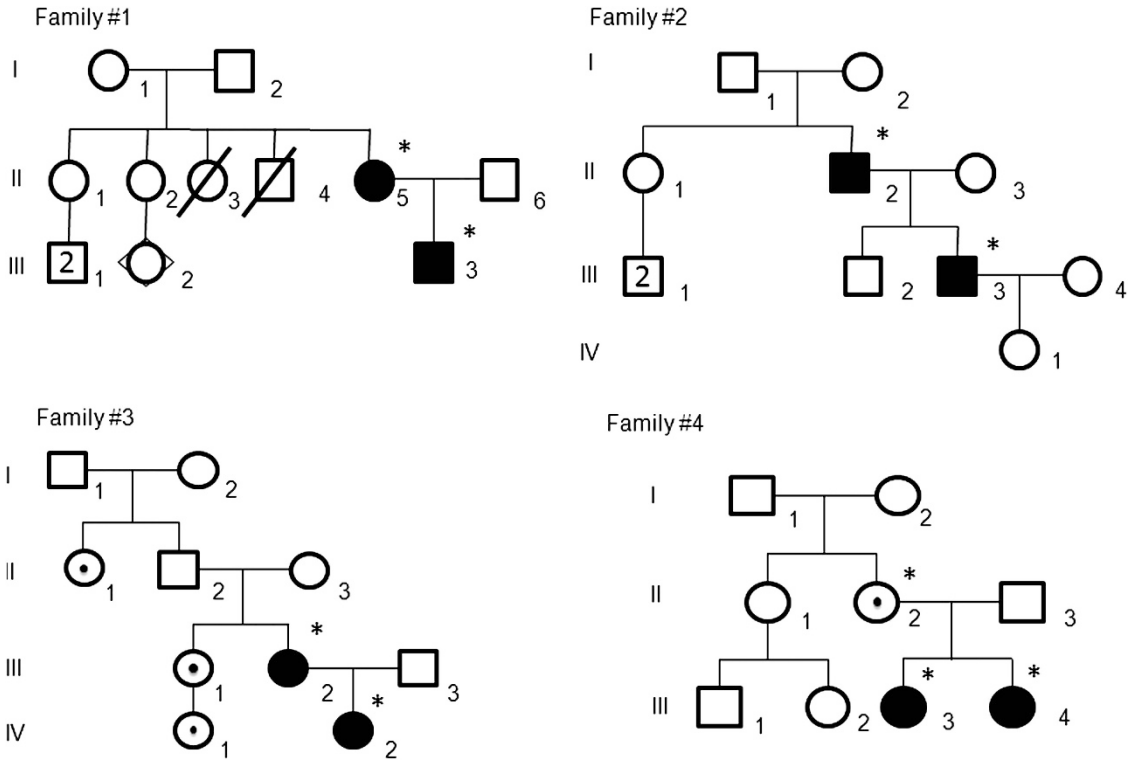

Figure 1 Pedigrees of the families with mutations in *TCF12*. Clear symbols denote unaffected individuals, and black symbols denote clinically-affected individuals either with or without craniosynostosis. In family #3, patients II-1, III-1, and IV-1 only showed syndactyly of the feet. *: available DNA. Dot: abnormal extremities without craniosynostosis.

Family #2

The first affected (Figure 1) member (II-2) was a male born to unaffected parents (I-1 and I-2; father's age: 34). He presented with brachycephaly, bilateral prominent ear crus, and short stature (161 cm). His first son (III-3) also displayed brachycephaly with bilateral prominent ear crus (Figure 2h). His birth HC was 33 cm (5th percentile). He underwent surgery for the brachycephaly when he was 6 months old. No further treatment was required.

Patient III-3 was re-assessed at age 31 and on clinical examination no abnormalities other than short stature (162 cm) and bilateral prominent ear crus were noticed. His brother (III-2), who was 6 years younger than III-3, also had isolated short stature (165 cm) without brachycephaly or prominent ear crus; their mother was 158 cm in height. Ptosis, syndactyly, and prominent ear crus were absent in this family. The palmar creases were normal.

Family #3

The female (Figure 1) proband (III-2) presented with brachydactyly at birth and was born to unaffected parents (II-2 and II-3; father's age: 31). She underwent surgery when she was aged 18 months. Her daughter (IV-2) also presented with brachycephaly after being born prematurely at 35 weeks of gestation. Birth weight and HC were 1550 g (0.15th percentile) and 32 cm (35th percentile), respectively. Her daughter underwent surgery during her 7th month.

Both the affected mother and her daughter displayed prominent ear crus. The mother also had dental malocclusion, with lateral deviation of the mandible. Both patients displayed brachydactyly with clinodactyly, membranous syndactyly of the second and third toes on both feet with a deviated great toe. Foot syndactyly was also present in a paternal aunt (II-1) as well as in the proband's sister (III-1) and her daughter (IV-1). These latter did not have craniosynostosis (Figure 1). The palmar creases were normal.

Family #4

The proband (III-3) was (Figure 1) a female affected with left plagiocephaly. Birth weight, height, and HC were 3185 g (40th percentile), 49 cm (10th percentile), and 32 cm (8th percentile), respectively. She underwent surgery by fronto-orbital advancement when she was 8 months old. The follow-up period was uneventful apart from a surgical opening of the naso-lachrymal ducts when she was 1 year old.

Her sister (III-4), who was born 4 years later, presented with bicoronal synostosis and mild hypertelorism. Birth weight, height, and HC were 3480 g (50th percentile), 50 cm (50th percentile), and 33.5 cm (25th percentile), respectively. As her sister, she underwent corrective surgery by a fronto-orbital advancement when she was 7 months old. At that stage, the intercanthal distance was 31 mm (97th percentile). The medical follow-up examination 18 months after surgery showed no recurrence of craniosynostosis or any other complications. No ptosis, syndactyly, or single palmar crease sign were observed in this family. The father showed no abnormalities. Both the girls and their mother (II-2) presented with brachydactyly (Figure 2i). The mother also underwent surgery for naso-lachrymal duct stenosis in her infancy, but had no craniosynostosis. Her birth weight was 3760 g (75th percentile) for 51 cm length (60th percentile) and her head circumference was 37 cm, 8 days after birth. Her father was 25 years old at her birth.

Families 1, 2, and 4 were of French ancestry while family 3 was of Portuguese ancestry.

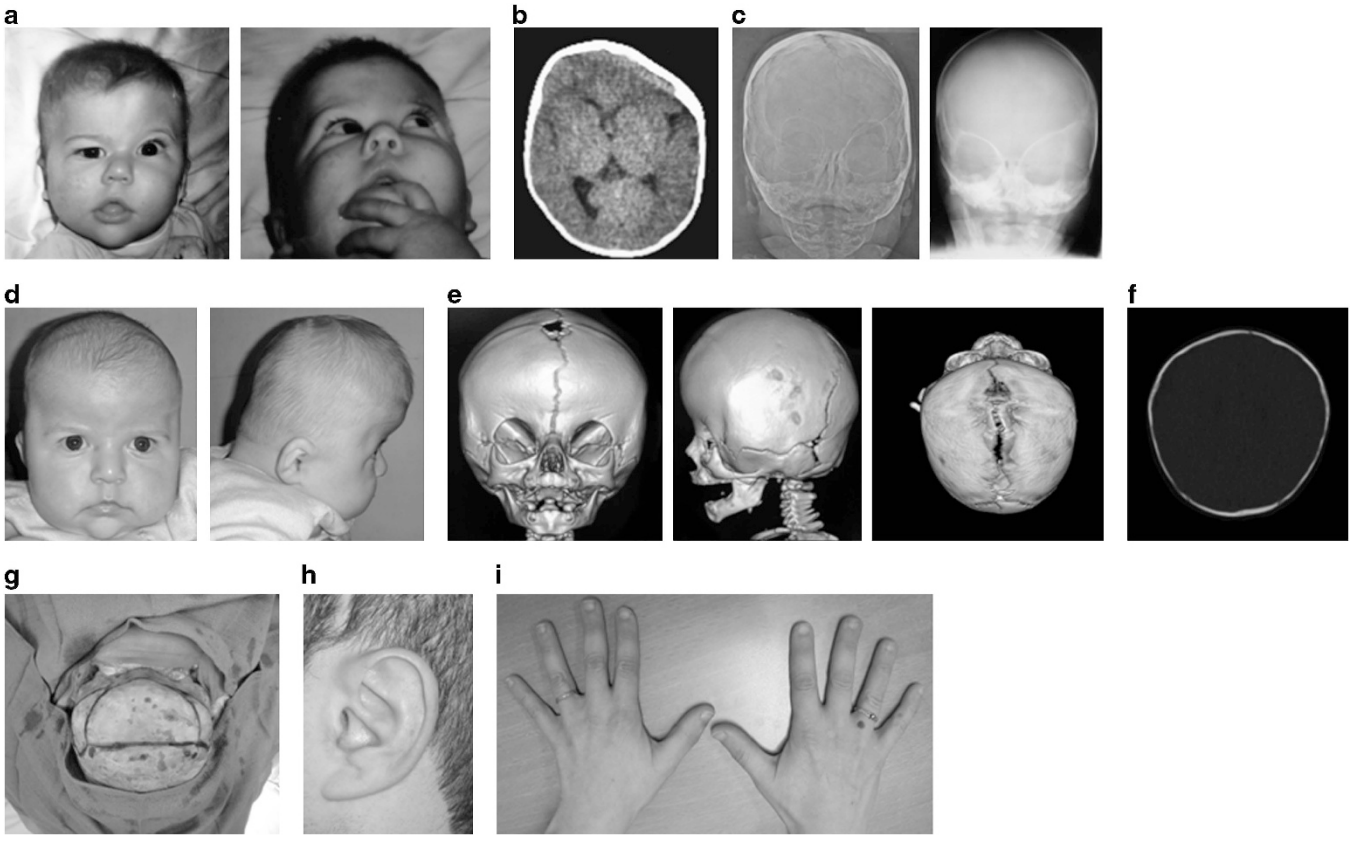

Figure 2 Clinical phenotypes. Family #1. Patient II-5 (**a**–**c**) at age 6 months. She presented with left plagiocephaly secondary to the early fusion of the left coronal suture: the two frontal photographs show unilateral coronal synostosis (**a**); computed tomography (CT) showing the left frontal deformation (**b**); posterior and anterior facial X-ray (**c**) with the characteristic harlequin's eye at the time of diagnosis (**c**-left) and at the time of surgery (**c**-right); note also the deviation of the sagittal suture. Family #1. Patient III-3 (**d**–**g**), son of II-5, at age 5 months. Frontal and lateral pictures showing bicoronal synostosis (**d**); 3D-CT showing the bilateral fusion of the coronal sutures (**e**); 2D CT image showing the fusion of the coronal sutures (**f**), intra-operative picture showing the deformation at surgery and the planned osteotomy lines in blue (**g**). Family #2, patient II-2. Small-sized ear with prominent crus (**h**). Family #4, patient II-2. brachydactyly (**i**). A full colour version of this figure is available at *The European Journal of Human Genetics* online.

MOLECULAR ANALYSIS

Molecular screening of *FGFR2*, *FGFR3*, and *TWIST1* did not detect any mutation in the exons or splice-site junctions of the genes but different heterozygous mutations were identified in *TCF12* (Figure 3). The ClinVar Accession numbers of the variants are SCV000115325 to SCV000115328.

In family #1, sequencing of *TCF12* in patients II-5 and III-3 revealed a heterozygous duplication in exon 16, c.1366dupA predicting p.(Ile456Asnfs*3) in the activation domain 2 of the protein. In family #2 (patients II-2, III-3), a heterozygous deletion, c.1071delG, which predicts p.(Ser358Glnfs*39), was also located in the sequence encoding the activation domain 2. In family #3, a heterozygous mutation, c.1838G>A, which predicts p.(Arg613His), was found in patients III-2 and IV-2, within the b-HLH-encoding domain. The missense mutation p.(Arg613His) was classified as probably damaging by the Polyphen software, with a score of 0.999 (maximum 1), and as deleterious by the Mutation Taster, SHIFT and Align GVGD software. This missense mutation was not found in 1000 Genomes (http://www.1000genomes.org/) and in the Exome Variant Server (http://evs.gs.washington.edu/EVS/). In family #4, one c.1000_1001delCA heterozygous deletion, which predicts p.(Gln334Aspfs*3) was detected within the activation domain 2 in patients II-2, III-3, and III-4.

In families #1 (II-5 and III-3), #2 (II-2, III-3), and #3 (III-2, IV-2), the mutation segregated with craniosynostosis. However, in family #4, the mother (II-2) of the two affected daughters (III-3, III-4) harboured an identical *TCF12* deletion but did not present any synostosis (Figure 2).

DISCUSSION

We report here several familial cases of coronal synostosis associated with mutations in *TCF12* in four out of five families analysed. The implication of *TCF12* in coronal synostosis has been recently discovered using an elegant-sequencing exome approach conducted in a large cohort of patients.[6] All but one of the affected members of the four families displayed isolated coronal synostosis at birth, either brachycephaly or plagiocephaly. In families #2, #3, and #4, the major extracranial features of SCS were also present, such as prominent ear crus (51–91%), ptosis (45–100%), and brachydactyly (45–79%),[7] while these features were absent in family 1.

Thus, it appears that *TCF12* mutation-associated syndrome overlaps with SCS and displays phenotypic variability. Indeed, the mother of the two affected sisters from family #4 did not display coronal synostosis while she carried the same *TCF12* deletion as her daughters. Such intra and interfamilial variabilities are also apparent in SCS patients. The absence of clinical signs in the first generation families (DNAs unavailable for mutation screening) suggested incomplete penetrance of the disease, as already reported by Sharma *et al*,[6] or the occurrence of *TCF12 de novo* mutations. The fifth family examined, whose members displayed isolated brachycephaly, was negative for *TCF12* mutations (as well as for *TWIST1* and

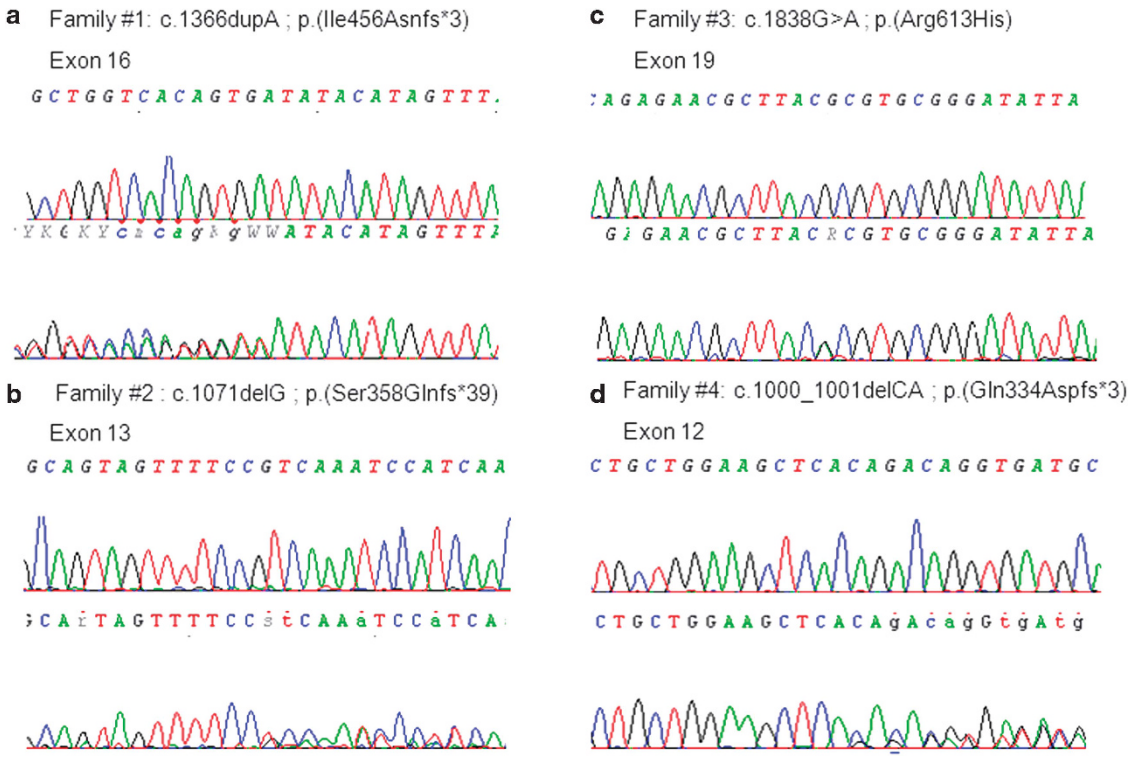

Figure 3 Mutations of the *TCF12 gene*. (**a**) Family #1: heterozygous duplication, c.1366dupA, (**b**) Family #2: heterozygous deletion, c.1071delG, (**c**) Family #3: heterozygous mutation, c.1838G>A, and (**d**) Family #4, heterozygous deletion, c.1000_1001delCA.

p.Pro250Arg in *FGFR3*) suggesting the implication of a new gene to be discovered.

The follow-up after surgery was uneventful in the four families. None of the patients displayed any intellectual disability. All the adult patients have a normal working and social life. On the basis of our experience, patients with *TCF12* mutations seem to have a good prognosis. Moreover, compared with those with SCS, they seem to present a lower risk of requiring reoperation,[5] but this small sample precludes any definitive conclusion. It can be added that none of the *TCF12*-mutated patients required new surgery for recurrence of raised-intracranial pressure (mean follow up time = 17.2 years (1.25–40)).

The missense mutations, deletions, and duplications of *TCF12* found in our families were all included within the sequences encoding either the AD2 or the bHLH domains, as previously reported.[6] However, these mutations have not been previously reported and no mutation hot spot is apparent after compiling this study and the previous publication.[6]

In family #3, the mutation targeted the bHLH domain p.(Arg613His). Interestingly, this latter family displayed further extra-cranial skeletal features of SCS, such as brachydactyly with clinodactyly, membranous syndactyly of the second and third toes on both feet as well as a deviated great toe and prominent ear crus. Our observation is consistent with the hypothesis according to which the mutant TCF12 protein can form heterodimers with bHLH E-proteins, such as TWIST1, whose functionality would be disturbed by TCF12 mutations.

In conclusion, most of the features related to *TCF12* mutations resemble those of SCS[8] with a large clinical spectrum. The close relationship between *TCF12* and *TWIST1* raises the question whether TCF12 mutations could be responsible for other types of abnormal sutures, as sometimes occurs in SCS. However, Sharma *et al*[6] did not find any mutation in *TCF12* in 93 cases of isolated metopic, sagittal, or lambdoid synostosis.

Finally, we confirm the interest of *TCF12* analysis in case of isolated plagiocephaly or brachycephaly, even in the absence of any other phenotypic abnormalities. *TCF12* should be added to the list of putative genes to screen in all coronal suture abnormalities, together with *FGFR3* and *TWIST1*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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