

De novo mutations in the X-linked *TFE3* gene cause intellectual disability with pigmentary mosaicism and storage disorder-like features

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ABSTRACT

Introduction Pigmentary mosaicism (PM) manifests by pigmentation anomalies along Blaschko's lines and represents a clue toward the molecular diagnosis of syndromic intellectual disability (ID). Together with new insights on the role for lysosomal signalling in embryonic stem cell differentiation, mutations in the X-linked transcription factor 3 (*TFE3*) have recently been reported in five patients. Functional analysis suggested these mutations to result in ectopic nuclear gain of functions.

Materials and methods Subsequent data sharing allowed the clustering of *de novo* *TFE3* variants identified by exome sequencing on DNA extracted from leucocytes in patients referred for syndromic ID with or without PM.

Results We describe the detailed clinical and molecular data of 17 individuals harbouring a *de novo* *TFE3* variant, including the patients that initially allowed reporting *TFE3* as a new disease-causing gene. The 12 females and 5 males presented with pigmentation anomalies on Blaschko's lines, severe ID, epilepsy, storage disorder-like features, growth retardation and recognisable facial dysmorphism. The variant was at a mosaic state in at least two male patients. All variants were missense except one splice variant. Eleven of the 13 variants were localised in exon 4, 2 in exon 3, and 3 were recurrent variants.

Conclusion This series further delineates the specific storage disorder-like phenotype with PM ascribed to *de novo* *TFE3* mutation in exons 3 and 4. It confirms the identification of a novel X-linked human condition associated with mosaicism and dysregulation within the mechanistic target of rapamycin (mTOR) pathway, as

well as a link between lysosomal signalling and human development.

INTRODUCTION

Intellectual disability (ID) affects about 3% of individuals worldwide and raises significant issues in terms of diagnostic, management and genetic counselling. The presence of pigmentation anomalies in a patient with ID represents helpful clinical clues in order to narrow the range of aetiological hypothesis. Hypomelanosis of Ito (HMI, MIM #300337) is an unspecific term encompassing a heterogeneous group of disorders characterised by cutaneous hypopigmented whorls and streaks along Blaschko's lines and variable extracutaneous features affecting the musculoskeletal and nervous systems.¹ The cutaneous pattern therefore represents a non-specific hallmark of mosaicism in these neurocutaneous conditions. Genetic mosaicism is due to postzygotic mutations, either chromosomal rearrangements or point mutations, whereas random X inactivation in females leads to functional mosaicism.² Unravelling the molecular basis of pigmentary mosaicism (PM) is still a challenge due to clinical and genetic heterogeneity, technical difficulties in detecting mosaic mutations by classical sequencing approaches and the complexities of obtaining affected tissue. As part of a collaborative group, we recently reported *de novo* mutations in exons 3 and 4 of transcription factor E3 (*TFE3*) as the cause for HMI in four

unrelated individuals, including one male, as well as syndromic ID without pigmentary disorders in a female.³

TFE3 belongs to the MITF family of mammalian basic helix–loop–helix zipper transcription factors, together with *TFEB* and *TFEC*; all four can form homodimers or heterodimers with each other.⁴ Embryonic expression of *TFE3* orthologues *Tfe3a* and *Tfe3b* was demonstrated in the zebrafish in a wide range of tissues.⁵ *TFE3* subcellular localisation plays a crucial role in the regulation of cellular homeostasis and embryonic stem cell (ESC) differentiation. The phosphorylated *TFE3* is retained in the cytoplasm, whereas dephosphorylated protein translocates to the nucleus to promote the transcription of target genes involved in lysosomal biogenesis and autophagy.⁶ *TFE3* relocalisation to the nucleus is driven on various stressors, such as starvation,⁷ DNA damage,⁸ mitochondrial damage,¹⁰ Golgi stress¹¹ and pathogens¹² in an mTORC1-dependent manner, and oxidative stress¹³ or cadmium exposition¹⁴ in an mTORC1-independent manner. Moreover, lysosomal signalling-induced nucleocytoplasmic redistribution of *TFE3* is essential to the regulation of ESC renewal.³ By restricting nuclear localisation and activity of *Tfe3*, lysosome activity, the tumour suppressor protein folliculin and the Ragulator protein complex enable the exit from pluripotency and therefore drive differentiation. Conversely, enforced nuclear *Tfe3* enables ESCs to withstand differentiation.¹⁵ In humans, *TFE3* mutations have long been known in cancer. Gene fusions by translocations or other chromosomal rearrangements involving *TFE3* and five partner genes have indeed been reported to occur in a subset of renal cell carcinomas (RCCs), referred to as ‘*TFE*-fusion RCC’, and, more rarely, to lung sarcoma and perivascular epithelioid cell tumours.¹⁶ Beyond these data on *TFE3* function, by the report of a series of 17 individuals harbouring *de novo* mutations in exons 3 and 4 of *TFE3*, we emphasise their phenotype and bring additional clinical insight toward the recognition of this novel developmental disorder.

METHODS

Patients

We clustered 17 patients with *de novo* *TFE3* variants from 13 cohorts using the GeneMatcher tool.¹⁷ Patients 1, 2 and 7 were from a research cohort (Mosaïc Undiagnosed Skin Traits And Related Disorders, M.U.S.T.A.R.D.) compiled in the genetics department of Dijon Hospital to identify molecular bases of PM that included 26 individuals. Twelve patients were investigated for diagnostic purposes because of ID (patients 3–5, 8 and 10–17). Patients 6 and 9 had a clinical diagnosis following the identification of the gene; patient 9 previously had whole-exome sequencing (WES) done, which had been considered as negative and was interpreted again to focus on *TFE3*. We obtained written informed consent for diagnostic or research purposes testing from all subjects or their legal representatives. We extracted genomic DNA from fresh skin, cultured skin fibroblasts and blood samples using the Gentra Puregene Blood and Tissue Extraction Kit (Qiagen). We assessed genomic DNA integrity and quantity by agarose gel electrophoresis, NanoDrop spectrophotometry and Qubit fluorometry (Thermo Fisher).

Whole-exome sequencing

Routine trio WES was applied by all participants according to their own diagnostic protocols and platforms.¹⁸ Candidate *de novo* mutational events were identified by focusing on protein-altering and splice-site changes (1) supported by at least three reads and 10% of total reads in the proband; (2) absent in both

parents, as defined by variant reads representing less than 5% of total reads; (3) at base-pair positions covered by at least four reads in the entire trio; and (4) present at a frequency less than 1% in the Single Nucleotide Polymorphism database dbSNP (build 147) and 0.1% in the Exome Aggregation Consortium (<http://exac.broadinstitute.org/>). For patient 6, the general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (<http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/>).

TFE3 sequencing

For Patient 6, we amplified regions of interest using custom intronic primers and long-range polymerase chain reactions with the PrimeSTAR GXL DNA Polymerase (Takara Bio, Saint-Germain-en-Laye, France). We pooled, purified, and quantified PCR amplicons from each affected individual. We prepared libraries using the Nextera XT DNA Sample Preparation kit (Illumina, Paris, France), which involves simultaneous fragmentation and tagging of DNA fragments by *in vitro* transposition. We performed paired-end sequencing reactions of 150 bp reads on a MiSeq platform using 300-cycle reagent kits (v2; Illumina, Paris, France).

Determination of the X-chromosome inactivation (XCI) pattern

XCI studies were performed in four of the seven females, in blood and fibroblasts for patients 1 and 3, in fibroblasts only for patient 2 and in blood only for patient 7.

XCI pattern was estimated at the *HUMARA* locus as described previously²⁰ on DNA extracted from uncultured fibroblasts. Fluorescent PCR products were analysed by capillary electrophoresis on an ABI3130XL genetic analyser (Applied Biosystems), and peak areas were generated with the GeneMapper software (Applied Biosystems). Skewing was defined as greater than 85% of one X allele active.

RESULTS

We describe a series of 17 patients carrying a *de novo* mutation in *TFE3*, 5 of them being previously published with limited clinical information.³ Twelve were females and five were males; their age ranged from 12 months to 22 years. Five were referred for HMI, five for syndromic ID and five for suspicion of storage disorder.

Clinical data

The clinical features are summarised in table 1. Additional information can be found in online supplementary data 1.

Neonatal course was remarkable for nine patients: history of jaundice, hepatomegaly or feeding difficulties was reported for three patients each, hypoglycaemia for two and cholestasis for one. All these features were transient.

Developmental delay, usually severe and noticeable from the first months of life, was a constant feature in all the individuals. Only 6 patients were able to walk at the time of the study, whereas 11 were still unable to walk. All patients were non-verbal, except for two older patients who could speak a few words. Neurological examination was abnormal in 12 individuals and consisted in truncal hypotonia, associated with lower limb spasticity (6 individuals) or ataxia (2 adults). Behavioural issues such as autistic features and sleeping disturbance were noted for 11 patients. Eleven patients developed epilepsy, onset in the first decade and characterised as intractable in three of them. Brain MRI was normal in 10 individuals and abnormal

Table 1 Clinical and molecular features of the 17 patients with an *TFE3* mutation

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	F	F	F	F	F	F
Age	20 years	20 years	3 years and 2 months	18 months	10 years	29 months
Pregnancy	High serum markers, normal foetal karyotype	Uncomplicated	Oligohydramnios, third trimester IUGR	Uncomplicated	Uncomplicated	Uncomplicated
Term (WG), birth weight/length/OFC (SD)	36, -1/+0.5/NA	-1	41, -2/NA/NA	41, +2/>+3/+2	38, +1/-1/-1	Term, 0/+0.5/+0.5
Neonatal features	-	-	Hypoglycaemia	Plagiocephaly	Jaundice, transient neonatal hepatomegaly	Feeding difficulties
DD/ID	Severe	Severe	Severe	Severe (no interaction, low head control)	Severe	Severe (head control)
Age of walking	Not acquired	40 months	Not acquired	Not acquired	Not acquired	Not acquired
Speech	No speech	Few words	No speech	No speech	No speech	No speech
Epilepsy (age of onset)	+7 months	+10 years	+17 months	-	+6 years	-
Developmental regression (age)	PM regression (7 m.)	PM regression (9 m.)	Regression (infancy)	-	-	-
Neurological phenotype	Hypotonia	Hypotonia	-	Severe truncal muscular hypotonia, distal hypertonia	Hypotonia, lower limb spasticity	-
Behavioural issues	-	-	-	Autistic behaviour (stereotypic movements)	NA	Sleep disturbance
Brain MRI	Normal	Normal	Normal	Normal	Short corpus callosum	Hydrocephaly (acqueductal stenosis)
Facial phenotype				High forehead		
Widely spaced eyes	+	+	+	+	+	-
Anteverted nares/short nose	Bifid nose	+/+	+/+	+/NA	+/+	+/+
Flat nasal bridge	+	+	+	+	+	+
Coarseness	+	+ protruding tongue	+	+	+	+
Hypertrichosis	+	+	+	-	+	+
Thick lips	+	+	+	-	+	-
Pink and full cheeks	+	+	+	+	+	+
Almond-shaped eyes	+	+	+	+	+	+
Thick earlobes	+	+	+	NA	+	+
Length (SD)	-4.5	-4	-1.4	+1.4	-4	-1
BMI (centile)	25 (80th)	30 (92th)	17.5 (90th)	19.9 (95th)	19	18 (90th)
OFC (SD)	NA	0	-1	+0.7	0	0
Orthopaedic anomalies						
Flat feet	+	+	-	-	-	-
Hyperlordosis	+	+	-	-	-	-
Hip dislocation	+	-	Unilateral dysplasia	-	-	-
Genu valgum	-	+	-	-	-	-
Club feet	-	+	-	-	-	-
Skeletal X-rays	ND	Normal	ND	ND	Metaphyseal enlargement	Short metacarpals and long bones
Abnormality of skin pigmentation	Blaschko's lines	-	Blaschko's lines	-	Blaschko's lines	Blaschko's lines
Umbilical hernia	-	-	+	-	-	-
Eye findings	-	Strabismus	Strabismus, impaired vision	Hyperopia	Abnormality of the VEP	Strabismus, depigmented iridian macule
Hearing loss	-	-	-	Unconclusive hearing test	+	+
Other	Aortic insufficiency, body asymmetry	-	-	-	Clubbing of fingers	Lateral semicircular canal dysplasia, laryngomalacia
Recurrent infections	-	-	Chronic upper airways infections	-	NA	+
Metabolic workup	ND	Normal	Normal alpha-L-iduronidase activity	Alanine-lysine ratio in plasma<3, elevated lactate in blood	Muscle biopsy: fat accumulation, irregular fibre size	Normal
Chromosome X inactivation	Blood: skewed (89%) Fibroblasts: random	Fibroblasts: skewed	Blood: skewed (>97%) Fibroblasts: random	ND	ND	ND

Continued

Table 1 Continued

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
<i>TFE3</i> variant	c.356A>Cp.Gln119Pro	c.557C>Tp.Pro186Leu	c.560C>Tp.Thr187Met	c.559A>Cp.Thr187Ala	c.572T>Cp.Leu191Pro	c.572T>Cp.Leu191Pro
	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Sex	F	F	F	F	F	F
Age	4 years and 3 months	14 years	10 years	22 years	9 years	12 months
Pregnancy	Ureteral dilatation, gestational diabetes	Uncomplicated	Uncomplicated	Tegretol	Uncomplicated	NA
Term (WG), birth weight/length/OFC (SD)	38, +2/0/0	39, 0/0/NA	39, +1/NA/NA	36+6, 0/NA/NA	36+6, -0.5/-1/-1.8	39, -1/-1/-1
Neonatal features	Neonatal hypotonia, feeding difficulties	-	Neonatal jaundice	Feeding difficulties	-	-
DD/ID	Severe (sat at 12 months)	Severe	Severe	Severe	Severe	Significant DD
Age of walking	Not acquired	6 years	30 months	7 years (walks with assistance)	Not acquired	Not acquired
Speech	No speech	No speech	3 years (single words)	No speech	No speech	No speech
Epilepsy (age of onset)	-	+4 years	+10 years	+10 years	One seizure (5 years)	-
Developmental regression (age)	-	Loss of words (infancy)	-	Loss of babbling (9 m.)	-	-
Neurological phenotype	-	Hypotonia	-	Increased reflexes, tremor of the upper extremities, wide-based gait	Hypotonia, mild spasticity (left side)	Hypotonia
Behavioural issues	Hand stereotypies, sleep disturbance, autism	Happy behaviour, sleep disturbance	Autistic behaviour (stereotypies)	Autistic behaviour (stereotypies)	-	-
Brain MRI	Normal	Normal	Normal	Abnormal myelination	Normal	ND
Facial phenotype				Broad forehead		
Widely spaced eyes	-	+	+	-	-	+
Flat nasal bridge	+	+	+	+	+	NA
Anteverted nares/short nose	+/+	+/+	+/NA	+/+	+	+
Coarseness	-	+	+	+, protruding tongue	+	-
Hypertrichosis	-	+	-	-	+	-
Thick lips	-	+	+	+	+	+
Pink and full cheeks	+	+	+	+	-	-
Almond-shaped eyes	+	+	-	+	-	+
Thick earlobes	+	+	+	+	+	+
Length (SD)	+2.5	-3	-2.8	-3.5	-2.5	+1
BMI	15.4 (50th)	22.4 (85th)	25 (90th)	21	17.5	95th
OFC (SD)	+1	-0.5	+0.5	NA	NA	+0.5
Orthopaedic anomalies						
Flat feet	+	+	-	+	-	-
Hyperlordosis/scoliosis	-	+	-	-/+	-	-
Hip dislocation	-	-	-	-	-	-
Genu valgum	-	-	-	+	-	-
Club feet	-	-	-	+	+Left	-
Skeletal X-rays	Normal	Normal	ND	ND	ND	ND
Abnormality of skin pigmentation	Blaschko's lines	Blaschko's lines	Blaschko's lines	Large hyperpigmented region on the abdomen	Blaschko's lines	+
Umbilical hernia	-	-	-	-	-	-
Eye findings	-	Hyperopia	-	-	-	-
Hearing loss	-	-	-	-	-	+Conductive
Recurrent infections	-	-	-	-	-	-
Other	Histiocytofibroma (4 years)	Patent ductus arteriosus, gastro-oesophageal reflux	-	Chronic constipation	Body asymmetry	-
Metabolic workup	ND	Hypercholesterolaemia. Normal lysosomal activities in leucocytes	MPS, OGS, UAA, OA screening: normal	Normal, skin biopsy negative for GM1, Niemann-Pick, and Gaucher diseases	ND	ND
X inactivation	Blood : skewed (91%)	ND	ND	ND	ND	
<i>TFE3</i> variant (NM_006521.4)	c.602A>Cp.Gln201Pro	c.560C>A; p.Thr187Lys	c.780+1G>A	c.551A>Gp.Glu184Gly	c.560C>Tp.Thr187Met	c.570C>Gp.His190Gln
	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	

Continued

Table 1 Continued

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	M	M	M	M	M	
Age	22 years	4 years 5 months	6 years 6 months	7 years	5 years	
Pregnancy	Gestational diabetes	Uncomplicated	NA	NA	Uncomplicated	
Term (WG), birth weight/length/OFC (SD)	41, -0.5/-1/-1	NA, -1/NA/NA	NA	33, NA/NA/NA	Term, -1/NA/+1	
Neonatal features	Hepatomegaly (4 months)	-	-	Jaundice	Cholestasis, hepatomegaly, hypoglycaemia	
DD/ID	Severe	Severe	Severe	Severe (sat 4 years)	Severe	
Age of walking	Not acquired	30 m	3y	Not acquired	Not acquired	
Speech	No speech	No speech	NA	No speech	No speech	
Epilepsy (age of onset)	+ (<1 year)	-	-	+ (NA)	+ (day 1)	
Developmental regression (age)	-	-	-	-	-	
Neurological phenotype	Spastic tetraplegia, ataxic gait	Hypotonia	-	Hypotonia, spasticity	Hypotonia	
Behavioural issues	Autistic behaviour (stereotypies, self-injuries) Sleep disturbance	Autistic behaviour	Autism	Autistic behaviour, sleep disturbance	Sleep disturbance	
Brain MRI	Unspecific changes in paraventricular myelination, generalised cerebral atrophy, retrocerebellar arachnoid cyst	Normal	Normal	Arachnoid cyst, Dandy-Walker malformation	Hydrocephaly, periventricular white matter lesions	
Facial phenotype	Flat face				Prominent forehead	
Widely spaced eyes	+	+	-	+	+	
Anteverted nares/short nose	+	+/NA	-/NA	+/+	+/+	
Flat nasal bridge	+	+	+	+	+	
Coarseness	+	+	+	+	+	
Hypertrichosis	+	+	-	+	-	
Thick lips	+	-	+	+	-	
Pink and full cheeks	+ (full cheeks)	+	+	+	+	
Almond-shaped eyes	+	+	-	+	+	
Thick earlobes	+	+	-	+	+	
Length (SD)	-4	+2	-1	-2.63	-4.5	
BMI (centile)	23	16	21.3 (>95th)	20 (>95th)	16.6 (80th)	
OFC (SD)	-1	0	+1	+1.9	-0.4	
Orthopaedic anomalies						
Flat feet	+	-	-	-	+	
Hyperlordosis	+	+	-	-	NA	
Hip dislocation	Hip dysplasia	-	-	-	+	
Genu valgum	+	-	-	-	NA	
Club feet	-	-	-	+	-	
Skeletal X-rays	Advanced bone age	ND	ND	ND	Normal (thorax)	
Abnormality of skin pigmentation	-	-	-	Blaschko's lines	Blaschko's lines	
Umbilical hernia	+	-	-	NA	+	
Eye findings	Strabismus, severe myopia, retinal degeneration	Oculomotor apraxia	Strabismus	Cortical visual impairment, strabismus, amblyopia	-	
Hearing loss	-	-	-	+	+	
Other	Clubbing of fingers, splenomegaly, anteriorly displaced anus, kidney asymmetry	Interstitial lung disease, chronic diarrhoea	Posterior plagiocephaly	Chronic lung disease (4 m.), sleep apnoea syndrome	Bronchomalacia, cardiac left ventricle dilatation, hypospadias, clubbing of fingers	
Recurrent infections	Recurrent lung infections	-	-	+ (neutropenia)	+	

Continued

Table 1 Continued

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Metabolic workup	ND	Normal	ND	ND	Normal metabolic screening, fat and glycogen accumulation, decreased muscular ATP production and substrate oxidation	
<i>TFE3</i> variant	c.566A>G, p.(Tyr189Cys)	c.350G>A p.Arg117Gln	c.350G>A: p.Arg117Gln Mosaic (88%)	c.560C>T p.Thr187Met	c.560C>G p.Thr187Arg Mosaic (65%)	

BMI, body mass index; DD, developmental delay; ID, intellectual disability; IUGR, intrauterine growth retardation; m., months; NA, not applicable; ND, not done; OFC, occipitofrontal circumference; PM, Psychomotor; *TFE3*, transcription factor 3; VEP, visual evoked potential; WG, weeks of gestation.

in 6 patients (hydrocephaly, short corpus callosum, Dandy-Walker malformation, arachnoid cyst, periventricular white matter lesions, delayed myelination and cerebral atrophy). The sensory anomaly was congenital hearing loss (5 patients), and ophthalmological anomalies (10 patients) consisted of strabismus, hyperopia, retinal degeneration, depigmented macule on the iris, oculomotor apraxia or impaired vision of cortical origin.

Facial dysmorphism shared among the patients consisted in coarseness, flat nasal bridge, short nose with anteverted nares, widely spaced eyes, almond-shaped eyes, thick lips, facial hypertrichosis, fleshy earlobe, and full and pink cheeks (figure 1). Twelve patients had pigmentation anomalies, located on Blaschko's lines for 10 of them (figure 2). One was diagnosed at 4 years old with histiocytofibroma. Moderate to severe postnatal growth retardation affected 10 patients, who had a length between -2.0 and -4.5 SD. Obesity affected 13 individuals. Skeletal anomalies were frequent (11 individuals) and consisted of flat or clubfeet, hyperlordosis, scoliosis, hip dislocation, limitation of elbow extension and genu valgum. Recurrent infections of the upper airways were noted in five patients; one had a documented neutropenia. Early-onset chronic interstitial lung disease was reported in two patients; nail clubbing was noted

in two individuals. Visceral malformations consisted of congenital heart defect (left ventricle dilatation, aortic insufficiency and patent ductus arteriosus) in three patients, umbilical hernia in three individuals, lateral semicircular canal dysplasia, posterior plagiocephaly, sleep apnoea syndrome, anteriorly displaced anus and hypospadias in one individual each.

Molecular results

The characteristics of the 13 different *de novo* *TFE3* variants identified in the 17 unrelated individuals are summarised in table 2. All but one were missense variants, affecting nine different aminoacids. One was a splice donor mutation. This mutation was reported a few weeks ago in a patient with a similar phenotype.²¹ Two variants were localised in exon 3 and 11 in exon 4 (figure 3). All were absent from public databases and were predicted to be pathogenic by prediction softwares.

The putative mosaicism was assessed through X inactivation studies in females and analysis of the exome sequencing data in males, by checking the total number of reads covering the variant, as well as the number of reads supporting the presence of the variant (table 1). Allele frequencies in females were always



Figure 1 Facial phenotypes of seven patients. (A–C) Patient 5, aged 6 months (A,B) and 3 years (C). (D) Patient 8, aged 5 years. (E,F) Patient 2, aged 5 and 20 years. (G–I) Patient 3, aged 1 year (G) and 3 years (H,I). (J,K) Patient 13, aged 22 years. (L–O) Patient 6, aged 8 months (L), 20 months (M) and 3 years (N,O). (P,Q) Patient 10 aged 22 years.



Figure 2 Cutaneous features. (A) Patient 3: umbilical hernia, widely spaced nipples and hypopigmentation on the left side of the abdomen. (B) Patient 17: hypotonia, umbilical hernia and widely spaced nipples. (C) Patient 8: Blaschko's lines on the back. (D) Patient 6: hypopigmentation on the left side of the abdomen. (E) Patient 7: Blaschko's lines on the abdomen and right side of the trunk. (F) Patient 13: hand; note clubbing of thumbnail and loose skin. (G) Patient 17: Blaschko's lines on the left side of the abdomen. (H) Patient 17: hand; note tapering fingers and wrinkled skin. (I) Patient 1: linear hypopigmentation on the back. (J) Patient 6: Blaschko's lines on the back. (K) Patient 3: Blaschko's lines on the right lower limb. (L) Patient 6: Blaschko's lines on the right lower limb. (M,N) Patient 7: linear hyperpigmentation on the lower limbs. (O) Patient 11: Blaschko's lines on the back.

consistent with a constitutional heterozygous mutation. X inactivation was skewed in blood of the female patients 1, 3 and 7 and in fibroblasts of Patient 2. X inactivation was random in fibroblasts of Patient 1 and 3. Regarding the male patients, the mutation was identified in 65% of the reads for Patient 17 and 88% of the reads for Patient 15 (106/120). No mosaicism was detected in the blood of Patient 13, 14 and 16 despite the presence of pigmentary manifestations in Patient 16.

DISCUSSION

TFE3 functions in signalling of the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1). The PIK3-AKT-mTOR pathway plays a role in the regulation of cellular growth,

proliferation, survival and metabolism. Overactivation of the mTOR signalling is responsible for neurocutaneous disorders and cancers.²² Somatic mutations in *TSC1*, *TSC2*, *AKT3*, *PIK3CA* and *MTOR* are responsible for focal cortical dysplasia type II (MIM607341),^{23–25} hemimegalencephaly²⁶ and megalencephaly.²⁷ The phenotype ascribed to germline *TSC1/TSC2*, *PTEN*, *MTOR* and *PK3R2/AKT3/CCND2* mutations – respectively in tuberous sclerosis (TS, MIM 191100), Cowden syndrome (CS, MIM 158350), Smith-Kingsmore syndrome (MIM 616638) and Megalencephaly, Polymicrogyria, Polydactyly and Hydrocephalus syndrome 1, 2 and 3 (MPPH1/2/3, MIM 603387/615939/615938) – is characterised by the association of ID, epilepsy, brain malformations and skin tumours. Similarly, all

Table 2 Molecular data of the 13 *de novo* *TFE3* mutations: characteristics, inheritance, frequency in the public database GnomAD, prediction scores regarding pathogenicity (Polyphen, Grantham and CADD (Combined Annotation Dependant Deletion) scores); the transcript is NM_006521.5

Patient	Genomic position	cDNA	Protein	Exon	Function	Status	GnomAD	Polyphen	Grantham score	CADD score
1	g.48896810T>G	c.356A>C	p.Gln119Pro	3	Missense	Germline	0	0.94	76	16.33
13	g.48896816C>T	c.350G>A	p.Arg117Gln	3	Missense	Germline	0	0.991	43	25.5
14	g.48896816C>T	c.350G>A	p.Arg117Gln	3	Missense	Mosaic	0	0.991	43	25.5
2	g.48895945G>A	c.557C>T	p.Pro186Leu	4	Missense	Germline	0	1	98	25.8
3, 11, 15	g.48895942G>A	c.560C>T	p.Thr187Met	4	Missense	Germline	0	1	81	21.6
16	g.48895942G>C	c.560C>G	p.Thr187Arg	4	Missense	Mosaic	0	1	71	20.7
4	g.48895943T>G	c.559A>C	p.Thr187Pro	4	Missense	Germline	0	1	58	16.5
5, 6	g.48895930A>G	c.572T>C	p.Leu191Pro	4	Missense	Germline	0	0.994	98	24.1
7	g.48598900G>T	c.602A>C	p.Gln201Pro	4	Missense	Germline	0	0.995	76	15.13
8	g.48895942G>T	c.560C>A	p.Thr187Lys	4	Missense	Germline	0	1	78	21.6
9	g.48895721C>T	c.780+1G>A	–	Intron 4	Splice site	Germline	0	–	–	–
10	g.48895951T>C	c.551A>G	p.Glu184Gly	4	Missense	Germline	0	1	98	17.81
12	g.48895936T>C	c.566A>G	p.Tyr189Cys	4	Missense	Germline	0	0.995	194	19.800
17	g.48895932G>C	c.570C>G	p.His190Gln	4	Missense	Germline	0	0.998	24	15.81

TFE3, transcription factor 3.

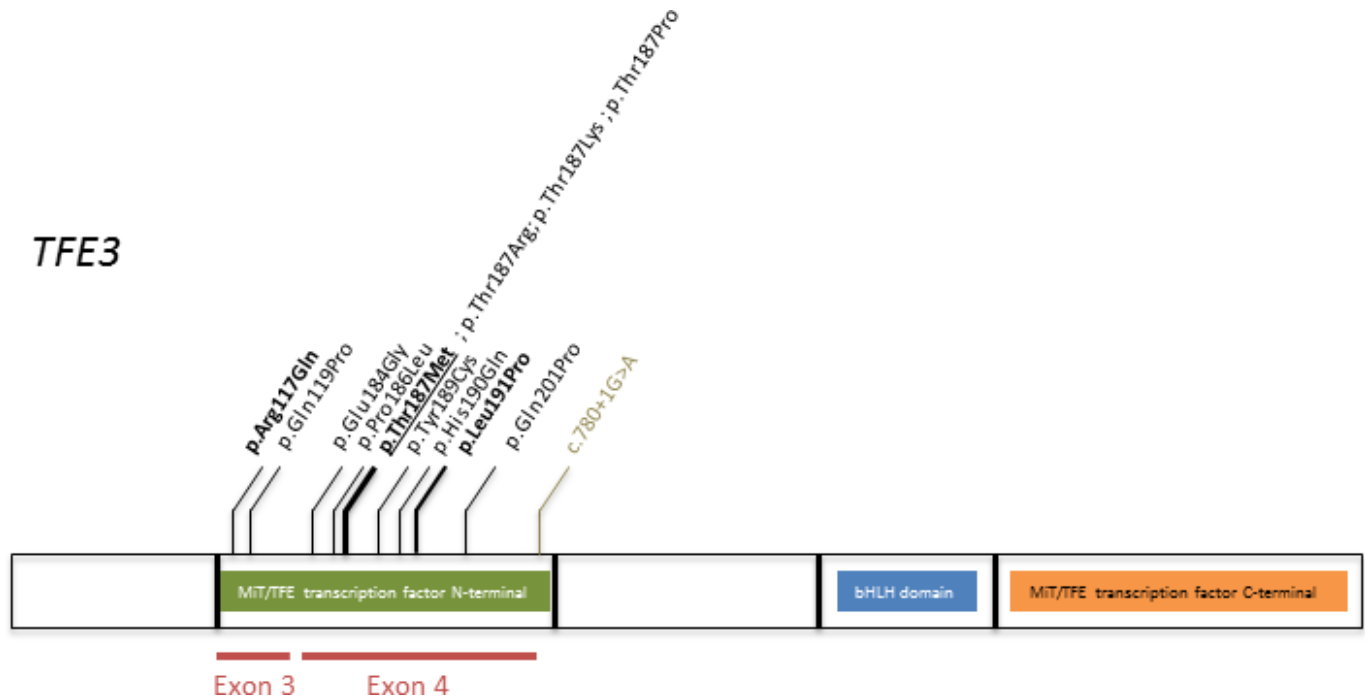


Figure 3 *TFE3* protein and localisation of the missense variants identified. In bold are variants identified in two patients. In bold and underlined is the variant identified in three patients. In green is the intronic variant.

the individuals harbouring a *de novo* *TFE3* mutation reported in the series presented with a severe neurodevelopmental disorder. Delayed psychomotor development was constant; the youngest patient to acquire independent walking was 30 months old, and more than half of the patients aged over 18 months (57%), did not acquire walk at the last examination. Conversely to patients with *MTOR*, *AKT3* or *PTEN* mutation, none of the patients described in this series had macrocephaly. Brain imaging was abnormal in 35% of the patients. Hydrocephaly and corpus callosum dysgenesis, identified in respectively three and one individual, were previously reported in patients with mosaic gain of function *MTOR* mutations.^{28 29} One patient had surgery to remove an early-onset histiocytofibroma. However, no other skin tumour was reported, either in this patient or in any other from the series.

Pigmentation anomalies, along Blaschko's lines or, for one patient, as a large hyperpigmented area, were present in a majority of the individuals (71%) in the series, including 40% of the males and 83% of the females. PM along Blaschko's lines is highly suggestive of genetic mosaicism.³⁰ Genomic mosaicism is defined by the presence of at least two cell populations with different genotypes in an individual originating from one zygote and mainly occurs through post-zygotic event, whereas females can present with functional (epigenetics) mosaicism due to X inactivation.² PM is a classical feature of X-linked male-lethal genodermatosis, such as incontinentia pigmenti (IP, MIM #308300), focal dermal hypoplasia (FDH, MIM #305600), chondrodysplasia punctuata type 2 (Conradi-Hunermann-Happle syndrome, CDPX2, MIM #302960) and linear skin defects with multiple congenital anomalies (LSDMCA1, MIM #309801). In these conditions, the overwhelming predominance of affected females is a consequence of the male lethality, and the PM a manifestation of the functional mosaicism occurring in females. Similarly, the majority of individuals with *de novo* *TFE3* variants in our cohort were females (sex ratio female:male was 12:5 (2.4)). The study of X-inactivation on

non-cultured fibroblasts was consistent with functional mosaicism in two affected females with PM who harboured random X-inactivation, whereas a third female without PM had skewed X-inactivation. In IP, FDH and CDPX2, most hemizygous males die in utero; however, there have been reports of surviving males^{31–33} with an estimated prevalence around 10% in FDH and IP.^{32 34} The majority of them are explained by post-zygotic mutations or chromosomal anomalies (Klinefelter syndrome). Non-mosaic males have also been reported in FDH and IP – respectively about 17% and 45% of the affected males harbour a non-mosaic variant.^{32 35} In our series, males represented 29% of the patients with a *de novo* *TFE3* variant. A mosaic variant was identified in blood for half of them; none had Klinefelter syndrome. Interestingly, mosaicism was detected in only one out of the two males with PM, and one male with a mosaic variant had no pigmentation anomalies noted on examination. It is still possible that subtle pigmentation anomalies were missed on examination. Moreover, somatic mosaicism can be difficult to detect; recent studies have shown that a large proportion of *de novo* mutations presumed to be germline had in fact occurred as post-zygotic event.³⁶ In the males of this cohort, WES was performed on leucocytes-derived DNA and no other tissue was studied; therefore, it is possible that a low mosaicism was not detected. Finally, it is probable than *TFE3* mutations account for a significant proportion of patients with HMI. Indeed, in this population, the high frequency of ID, epilepsy, coarse facial features has long been emphasised in the literature.³⁷

By its ability to bind the coordinated lysosomal enhancement and regulation (CLEAR) sites in the promoter region of target genes, *TFE3* is involved in the control lysosomal biogenesis, autophagy and endocytosis.⁸ Several patients of the series indeed had clinical and biochemical features that pointed toward an inborn error of metabolism. Lysosomal storage disorder was suspected due to the variable association of coarse facial features (88%), skeletal anomalies (65%) – flat or clubfeet, hyperlordosis, hip dislocation, limitation of

elbow extension, genu valgum, scoliosis-, postnatal growth retardation (59%), history of speech or developmental regression (29%) congenital hearing loss (29%), recurrent upper airways infections (29%) neonatal liver anomalies such as hepatomegaly and cholestasis (18%), upper airways infections (24%), umbilical hernia (18%), sleep apnoea syndrome (6%) and aortic insufficiency (6%). Other metabolic anomalies observed in the series were obesity, defined in children by body mass index (BMI) ($\text{weight}/\text{height}^2$) above the WHO curve, present in the oldest patients (76%), neonatal transient hypoglycaemia (12%), and hyperlactataemia (6%). Dysregulation of lipid metabolism, via suppression of thermogenesis and decreased lipolysis, thus leading to increased adipose tissue, was previously observed in adipose-specific TFE3 transgenic mice.³⁸ Similarly to lysosomes, mitochondrias have a key role in cellular metabolism, including autophagy; recent data demonstrate that mitochondrial and lysosomal metabolisms are interrelated.³⁹ Muscle biopsy, performed in two individuals from this cohort, showed fat and glycogen accumulation, muscular fibre size irregularity, without evidence of mitochondrial dysfunction. Interestingly in the more recent data, evidences showing that *TFE3* plays a role in the regulation of the circadian oscillations of the expression of genes involved in autophagy and lipid metabolism, and that *Tfe3* knock-out mice had abnormal circadian behaviour.⁴⁰ Indeed, in our series, five patients (29%) were noted to have sleep disturbance: this could be due to circadian rhythms alteration. Finally, *TFE3* has been shown to be involved in the regulation of innate immune response in macrophages via the FLCN-AMPK signalling axis,⁴¹ and of B-lymphocytes activation.⁴² Along these lines, four patients of the series (24%) had a history of recurrent infections, associated with documented neutropenia in one of them. As shown in table 3, a summary of the frequency of the features observed in the cohort, facial dysmorphism was constant and strikingly similar among the patients: more than two-third had anteverted nares, broad flat nasal bridge, almond-shaped and widely spaced eyes, puffy cheeks and coarse facial features (thick lips and fleshy earlobes); more than half had facial hypertrichosis. All individuals presented with at least four of the above features. One patient had an extreme facial phenotype of hypertelorism, bifid nose and bilateral cleft lip and palate. Whether these frontonasal dysplasia features may be associated with the *TFE3* mutation remains unclear; no other mutation in known genes was found in Patient 1's exome sequencing data.

TFE3 is a highly conserved protein, intolerant to loss of function as supported by data from the GnomAD browser⁴³ (probability of being loss-of-function intolerant (pLI) evaluated at 0.98, observed:expected ratio=0.06) and to missense variants ($Z=2.15$). Moreover, *TFE3* does not, or only in a few tissues, escape X inactivation, suggesting that *TFE3* gene dosage is crucial to cell function.^{44 45} In vitro, Villegas *et al* recently showed that the absence of either *TFE3* exon 3 or 4 resulted in a nuclear gain-of-function *Tfe3* allele in ESCs, indicating that both exons 3 and 4 are required for *Tfe3* inactivation.³ Nuclear localisation and resistance to differentiation were proved in *Tfe3* knock-out (K.O.) ESCs expressing murine *Tfe3* alleles (Gln118Pro and Pro185Leu, corresponding to mutations Gln119Pro and Pro186Leu identified in individuals referred to as patients 1 and 2 in this series). Based on the analysis of *TFE3* secondary structure,⁴⁶ indicating that residues 110–215 are predicted to form a domain of two stable alpha helices that might be disrupted by mutations in exons 3 and 4, and the observation of a similar phenotype in patients

Table 3 Frequency of the clinical features observed in the series

	Number of patients	Frequency (%)
Neurodevelopmental abnormality	17/17	100
Intellectual disability	17/17	100
Inability to walk	11/17	65
Inability to speak	14/17	82
Epilepsy	11/17	65
Muscular hypotonia	10/17	59
Autistic behaviour/autism	8/17	47
Spasticity	6/17	35
Sleep disturbance	6/17	35
Developmental regression	5/17	29
Ataxia	2/17	12
Brain imaging abnormality	6/16	38
Abnormal myelination	2/16	13
Hydrocephaly	2/16	13
Arachnoidal cyst	2/16	13
Hypoplasia of the corpus callosum	1/16	6
Cerebral atrophy	1/16	6
Facial dysmorphism	17/17	100
Anteverted nares	16/16	100
Broad flat nasal bridge	16/16	100
Almond-shaped palpebral fissures	16/17	94
Fleshy earlobes	15/16	94
Coarse facial features	15/17	88
Full cheeks	15/17	88
Widely spaced eyes	12/17	71
Thick lips	12/17	71
Facial hypertrichosis	10/17	59
Growth abnormality	13/17	76
Obesity	13/17	76
Postnatal growth retardation	10/17	59
Abnormality of skin pigmentation	12/17	71
Abnormality of the skeletal system	11/17	65
Flat feet	7/17	41
Hyperlordosis	5/6	33
Clubfeet	4/17	24
Genu valgum	3/16	20
Congenital hip dislocation	2/17	12
Hip dysplasia	2/17	12
Abnormality of the eye	10/17	59
Strabismus	6/17	35
Hyperopia	2/17	12
Abnormality of the visual evoked potentials	2/17	12
myopia		
Oculomotor apraxia	1/17	6
Retinal degeneration	1/17	6
Neonatal features	9/17	53
Hepatomegaly	3/17	18
Feeding difficulties	3/17	18
Prolonged neonatal jaundice	3/17	18
Hypoglycaemia	2/17	12
Recurrent infections	5/17	29
Abnormality of the respiratory system	4/17	24
Interstitial lung disease	1/17	6
Chronic lung disease	1/17	6
Bronchomalacia	1/17	6
Laryngomalacia	1/17	6

Continued

Table 3 Continued

	Number of patients	Frequency (%)
Cardiac anomaly	3/17	18
Aortic insufficiency	1/17	6
Patent ductus arteriosus	1/17	6
Abnormal left ventricle morphology	1/17	6
Other		
Hearing loss	5/17	29
Clubbing of fingers	3/17	18
Umbilical hernia	3/17	18
Sleep apnoea syndrome	1/17	6

harbouring mutations in exons 3 and 4, it was suggested that Tfe3 exons 3 and 4 form a Rag binding fold whose structural integrity is indispensable for lysosome-mediated cytoplasmic Tfe3 inactivation.³ In this series, the recurrent mutations Arg117Gln, Leu191Pro and Thr187Met were present in respectively two, two and three patients. The aminoacid in position 187 was mutated in five patients. In addition, 13 of the described mutations were localised between positions p.184 and p.201: this proximity could account for the absence of obvious genotype–phenotype correlation. The canonical splice site variant in intron 4 identified in patient 9 might lead to in-frame exon skipping of exon 4. The clinical picture of the patient with this splice site variant perfectly fits with the syndrome described here. As a consequence, we raise the hypothesis of a gain-of-function effect of this variant.

In conclusion, *de novo* mutations in exons 3 and 4 of the X-linked gene *TFE3* are responsible for a neurocutaneous disorder with specific and recognisable facial dysmorphism, lysosomal storage disorder-like features and PM. This series unravels *TFE3* as a major gene responsible for HMI and for a rare cause of syndromic ID. Furthermore, we provide clinical and molecular data on a previously unidentified lysosomal storage disorder, in which new insights, especially biochemical features, will probably be investigated further, together with the description of more patients. Further delineation of this phenotype will indeed allow a better understanding of the link between lysosomal signalling and development. Finally, the evidence for mosaicism in this recently described disorder highlights the importance of considering mosaic variants on next-generation sequencing reports in diagnostic, including for patients without suggestive phenotype.

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