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LETTER TO THE EDITOR

Small patella syndrome: New clinical and molecular insights into a consistent phenotype

To the Editor:

Small patella syndrome (SPS, OMIM#147891) is a rare autosomal dominant disorder characterized by patellar agenesis or hypoplasia, abnormal ossification of the ischio-pubic junction, and feet abnormalities. This condition is caused by haploinsufficiency of *TBX4*,[1] encoding a T-box transcription factor involved in lower limb development and airway branching.[2,3] Ten different *TBX4* mutations have been reported so far in SPS patients.[1,4] In addition, 6 mutations were associated with childhood-onset pulmonary arterial hypertension (PAH), in patients who also had SPS features after clinical reassessment.[4] Loss of *Tbx4* at early stages of limb development in mice (E.9.5-10.5) results in hypoplastic fibula, femur and pelvis and thin anterior hindlimb digits.[2] Two hindlimb-specific *Tbx4* enhancers (HLEA and HLEB) have been described in mice.[5] Pitx1, directly involved in the hindlimb-type morphology, is required to maintain normal levels of *Tbx4* expression through these regulatory elements.[5]

Patients from 10 unrelated families with features suggestive of SPS were recruited from several clinical genetics departments (Table 1). They were referred mostly for recurrent knee subluxations and presented with characteristic skeletal features. The patellar defects were bilateral, the left side being slightly more affected. Diagnosis of SPS has been established *a posteriori* in 9 affected relatives among 6 families, raising the number of patients to 19.

Our study was performed using the Declaration of Helsinki protocol. Written informed consents were obtained from the patients. We performed Sanger sequencing of all *TBX4* coding exons and their flanking intronic regions (NM_018488.2, Hg19), and rearrangement screening by multiplex ligation-dependent probe amplification (P390-A1, MRC-Holland). In seven families we found *TBX4* anomalies predicted to cause loss-of-function or haploinsufficiency, confirming the clinical diagnosis of SPS. Of those, four were newly described (Table 1) and reported into LOVD Database. In three mutation-negative families, we performed Sanger sequencing of ortholog HLEA (chr17:59,521,403-59,521,530[Hg19]) and HLEB (chr17:59,611,720-59,612,278[Hg19]) regulatory regions identified in mouse, 180K array-CGH (comparative genomic hybridization) and the high-throughput targeted sequencing of 124 genes involved in limb development. Those additional analyses failed to identify any pathogenic variations.

SPS shows a consistent skeletal phenotype with constant patella and pelvic defects. Feet abnormalities are very frequent but may be mild and therefore overlooked. Our study raises some new features with incomplete penetrance: developmental dysplasia of the hip (DDH), spine deformities and dental issues.

DDH, observed in 4 probands, has not been described so far. Two patients needed surgical repair after orthopaedic treatment failure. We assume that pelvic ossification abnormalities could affect the morphology of the acetabular region and predispose to DDH.

Spine deformities were major findings in 2 probands (severe scoliosis in family 3 and kyphosis due to fused 8th-9th thoracic vertebrae and lombar-like morphology of S1 in family 8) and therefore definitely a condition to survey in SPS. Paediatric follow-up did not reveal any DDH or spinal deformities in the other probands.

Finally, dental examinations revealed *numerous caries of primary and permanent dentition and/or permanent teeth eruption delay* in four patients from two different families whereas none of their non-mutated relatives were affected. It is still unclear if this may be related or not to SPS.

Besides, only one proband suffers from *idiopathic PAH*, confirming its incomplete penetrance in SPS. She presented with exercise intolerance with cyanosis and syncope due to pulmonary hypertensive crises at 4 year old. Interestingly, the 3 individuals from family 3 are not affected with PAH while they share the same mutation with a previously reported SPS patient with PAH (patient 5 in Ref. 4). None of the other affected patients had clinical symptoms of childhood-onset PAH. Studies on larger cohorts are required to understand the full range of phenotypes and their penetrance, resulting from *TBX4* mutations.

The phenotypes observed in the mutation-negative patients appeared similar to those of the mutated patients. The SPS clinical picture appears consistent and easily recognizable among differential diagnoses. However, we sequenced a panel of 124 genes involved in limb development, revealing no pathogenic variation. Disruption of cis-regulatory modules appears to be a growing cause of limb malformations. We did not find any pathogenic sequence variation in the two *TBX4* enhancers. However, previous data in mice showed additional Pitx1 binding sites at the *Tbx4* locus suggesting that additional regulatory elements remain to be identified.[5]

Further explorations by high-throughput sequencing are required in the molecular delineation of SPS to look for *TBX4* deep intronic and regulatory regions, complex rearrangements or variants in other candidate genes.

TABLE 1 Phenotypic and genotypic data of our patients series

	Family 1				Family 2	Family 3			Family 4		Family 5		Family 6	Family 7	Family 8		Family 9	Family 10		
	III3	II1	III2	III5	II2	II1	II2	I1	II2	I2	III1	II2	III1	III1	III2	III1	I1	II1	I1	Total
Sex (F/M)	F	F	F	F	F	F	F	F	F	M	M	M	F	F	M	F	F	F	M	—
Origin	France				UK	France			France		France		France	Switzerland	France		UK	Belgium		—
Age at diagnosis (years)	15	40	17	7	13	20	16	48	11	38	8	40	11	26	15	19	42	5	34	—
Familial (f)/Sporadic (s)	f	f	f	f	s	f	f	f	f	f	f	f	f	f	f	f	f	f	f	—
Patella abnormalities	+	+	+	+	+	+	+	NA	+	+	+	+	+	+	+	+	+	+	+	18/18
Absent	+	−	−	−	−	−	−	NA	+	−	+	−	−	−	−	+	−	−	−	4/18
Small or bipartite	−	+	+	+	+	+	+	NA	+	+	−	+	+	+	+	−	+	+	+	15/18
Laterally placed /dislocated	−	−	−	−	+	−	−	NA	+	−	−	−	+	+	−	−	+	−	−	5/18
Pelvic abnormalities	+	+	NA	+	+	+	+	+	+	+	+	NA	+	+	+	+	+	NA	NA	15/15
Absence or abnormal ossification of the ischio-pubic junction	+	+	NA	+	+	+	+	+	+	+	+	NA	+	+	+	+	+	NA	NA	15/15
Infra acetabular axe cut notches	+	−	NA	−	−	+	+	−	−	−	+	NA	−	−	−	−	−	NA	NA	4/15
Elongated femoral neck	+	−	NA	+	−	−	+	−	+	−	+	NA	+	+	−	−	−	NA	NA	7/15
Developmental hip dysplasia	+	−	NA	−	+	−	−	−	+	−	−	NA	−	−	−	−	+	NA	NA	4/15
Feet abnormalities	+	+	+	+	+	+	+	+	+	+	+	+	NA	+	+	+	+	NA	NA	16/16
Wide space between I and II	+	+	−	−	NA	+	+	+	−	+	+	+	NA	−	+	+	+	NA	NA	11/15
Short IV and V rays	+	+	+	+	NA	−	+	+	+	−	+	−	NA	+	+	+	+	NA	NA	12/15
Pes planus	−	−	−	−	+	+	+	−	−	−	+	+	NA	−	−	−	NA	NA	NA	5/15
Syndactyly of toes	−	+	+	−	NA	−	−	+	−	−	−	−	NA	−	−	−	NA	NA	NA	3/14
Ball-and-socket ankle joint	+	−	NA	NA	NA	−	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1/3
Other characteristics	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−	NA	NA	NA	8/16
Poor dental health	+	+	−	+	NA	−	−	NA	+	+	−	−	−	−	−	−	NA	NA	NA	5/14
Short stature	+	+	+	+	+	−	−	NA	−	−	−	−	−	−	−	−	NA	NA	NA	5/15
Spine deformities	−	−	−	−	NA	+	+	NA	−	−	−	−	−	−	+	−	NA	NA	NA	3/14
Pulmonary hypertension	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	1/19
TBX4 mutation	c.1112dupC				c.748C>T	c.1164dupC			c.1062T>G		c.901C>T		c.932C>A	c.(?_-45)_(401 +1_402-1?)del	—		—	—		—
Peptide	p.(Pro372Serfs*14)				p.(Arg250Trp)	p.(Arg389Glnfs*30)			p.(Tyr354*)		p.(Gln301*)		p.(Ser311*)	p.0?	—		—	—		—
Mutation type	Frameshift				Missense	Frameshift			Nonsense		Nonsense		Nonsense	Deletion	—		—	—		—
Location	Exon 8 (putative transactivation domain)				Exon 6 (DNA-binding domain)	Exon 8 (putative transactivation domain)			Exon 8 (putative transactivation domain)		Exon 7		Exon 7	Exon 1 to 3 (DNA-binding domain)	—		—	—		—
Predicted effect	?				LOF	?			?		HI		HI	HI	—		—	—		—
Reported previously/novel	Reported previously				Reported previously	Reported previously			Novel		Novel		Novel	Novel	—		—	—		—

F, female; f, familial; HI, haploinsufficiency; LOF, loss-of-function; M, male; NA, not available; s, sporadic.

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