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SHORT REPORT

A large-scale mutation search reveals genetic heterogeneity in 3M syndrome

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The 3M syndrome is a rare autosomal recessive disorder recently ascribed to mutations in the *CUL7* gene and characterized by severe pre- and postnatal growth retardation. Studying a series of 33 novel cases of 3M syndrome, we have identified deleterious *CUL7* mutations in 23/33 patients, including 19 novel mutations and one paternal isodisomy of chromosome 6 encompassing a *CUL7* mutation. Lack of

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mutations in 10/33 cases and exclusion of the *CUL7* locus on chromosome 6p21.1 in six consanguineous families strongly support the genetic heterogeneity of the 3M syndrome.

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The 3M syndrome (OMIM 273750) is an autosomal recessive condition characterized by pre- and postnatal growth retardation, facial dysmorphism, large head circumference, normal intelligence and skeletal changes including long slender tubular bones and tall vertebral bodies.[1–5] Studying a series of 29 families, we have previously mapped the disease locus to chromosome 6p21.1, and identified disease-causing mutations in the *CUL7* gene.[6]

Here, we report the molecular analysis of the *CUL7* gene in a series of 33 additional cases and the identification of mutations in 23/33 cases including one paternal isodisomy of chromosome 6. The absence of *CUL7* mutation in the other 10/33 cases and the exclusion of the 6p21.1 locus in consanguineous families support the genetic heterogeneity of the 3M syndrome.

Patients and methods

Patients

All patients included in this study fulfilled the diagnostic criteria for 3M syndrome, namely (1) severe pre- and postnatal growth retardation below −3 SD, (2) large head circumference and (3) facial dysmorphism (Figure 1a) that is, prominent forehead, anteverted nares and full lips. Thirty-three patients (16 boys and 17 girls) from 33 unrelated families were included, ranging in age from birth to 14 years. Among them, 19/33 patients were born to consanguineous parents.

Skeletal changes were not consistently present at birth but occurred in the course of the disease, namely delayed bone age, slender long bones and tall vertebral bodies (Figures 1b and 2). Two patients also presented with bilateral dislocation of hips (Figure 1) and two others had severe kyphoscoliosis.

This series also includes one pregnancy, terminated at 33 weeks of gestation. The foetus presented with severe growth retardation (weight <5th percentile, height <5th percentile), normal head circumference (>50th percentile), characteristic facial features, prominent heels and slender long bones, suggestive of the 3M syndrome (Figure 3a). Analysis of the femoral growth plate of this foetus showed an increased chondrocyte density and size in the resting and proliferative zones but no major abnormalities in the prehypertrophic and hypertrophic zones (Figure 3b). The placenta was reduced in size, with no major abnormality. Apart from reduced liver and lung sizes, no other anomaly was detected at autopsy.

Genomic sequencing

We obtained blood samples with written consent from the affected individuals and their unaffected relatives.

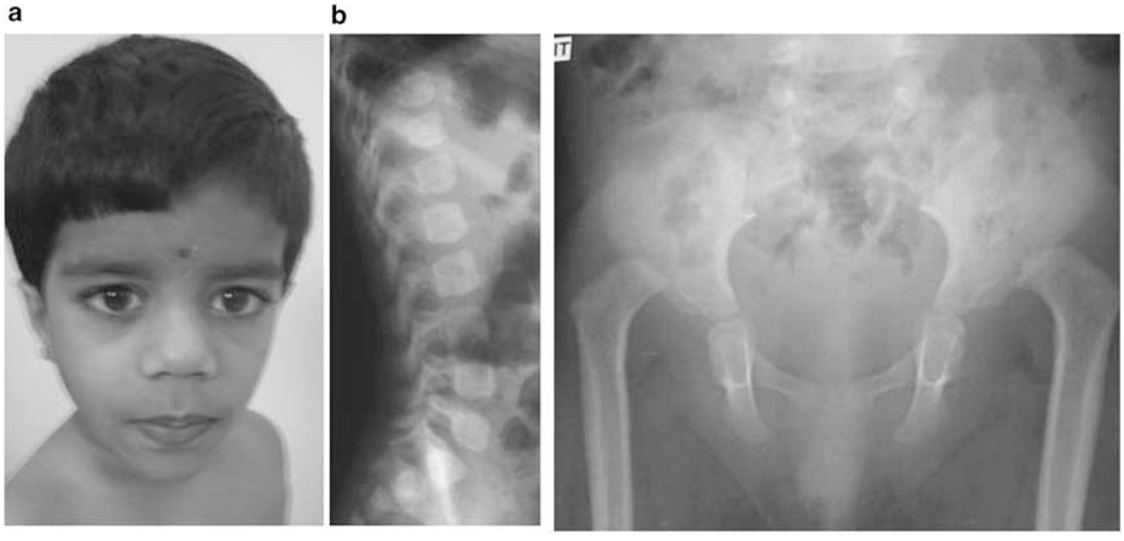

Figure 1 Facial and skeletal features of patient no. 14 at 3 years of age. (**a**) Note the round face, frontal bossing, short nose and full lips. (**b**) Note tall vertebral bodies and the hip dislocation.

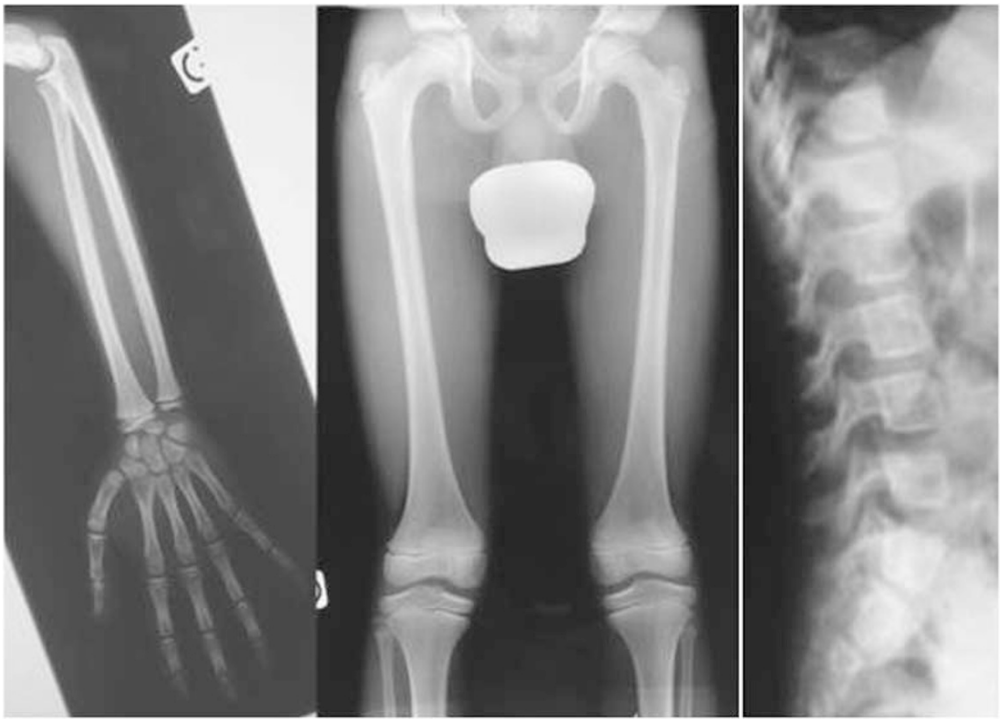

Figure 2 Skeletal features of a child at 7 years of age with 3M syndrome and without a CUL7 mutation. Note the long slender tubular bones and tall vertebral bodies.

Genomic DNA was extracted from peripheral blood by standard procedures. For mutation detection, we used 23 primers to amplify the 25 coding exons of *CUL7* (primers available on request). We purified the PCR product with exonuclease I (ExoSAPIT; Amersham Bioscience) according to the manufacturer's instructions. Sequencing reactions were run on an ABI 3130 sequencer using a dye terminator cycle sequencing kit (Applied Biosystems) and analysed by sequencing analysis (Applied Biosystems).

Microsatellite analyses

Genotyping was performed using seven repeat-containing microsatellite markers from the *CUL7* region on chromosome 6p21.1 (markers available on request). Microsatellite analysis was performed in consanguineous families, in the affected foetus and his parents using markers of the ABI PRISM linkage mapping set (Applied Biosystems). HEX or FAM fluorescently labelled PCR products were run on an ABI 3130 sequencer and analysed using GeneMapper (Applied Biosystems).

FISH analyses

Lung cells from the affected foetus were submitted to FISH analyses. Bacterial artificial chromosome (BAC) clones were selected from the UCSC genome browser (www.genome.ucsc.edu) database and obtained from the Sanger Institute (www.sanger.ac.uk). FISH experiments were performed on interphase nuclei preparations using a bac RP11-653G5 clone corresponding to the *CUL7* gene on 6p21.1 and a control BAC RP11-39C2 clone on 6p12.3. BAC clone DNA was amplified by rolling circle amplification using TempliPhiTM Large Construct kit (GE Healthcare) following the manufacturer's instructions. BAC probes, RP11-653G5 and RP11-39C2, were labelled in red (tetramethyl rhodamine) and green (fluorescein isothiocyanate), respectively, by nick translation.

Results

Direct sequencing of *CUL7* identified deleterious mutations in 23/33 patients. Twelve out of 33 patients were homozygotes and 11/33 patients were compound heterozygotes. The mutations were located throughout the gene and included 8 missense, 19 nonsense and 6 splice-site mutations (Table 1, families 30–52). Among them, 19 were novel mutations (Table 1).

Only one mutation inherited from the father was detected in the affected foetus (I19: c.3645 + 1G > A). These findings were suggestive of either a deletion or a uniparental disomy. Microsatellite analyses of the foetus at the 3M locus was consistent with either homozygosity or hemizygosity, but FISH analysis using the probe RP11-653G5 (6p21.1) excluded a large-scale deletion (data not shown). Extensive microsatellite analysis of

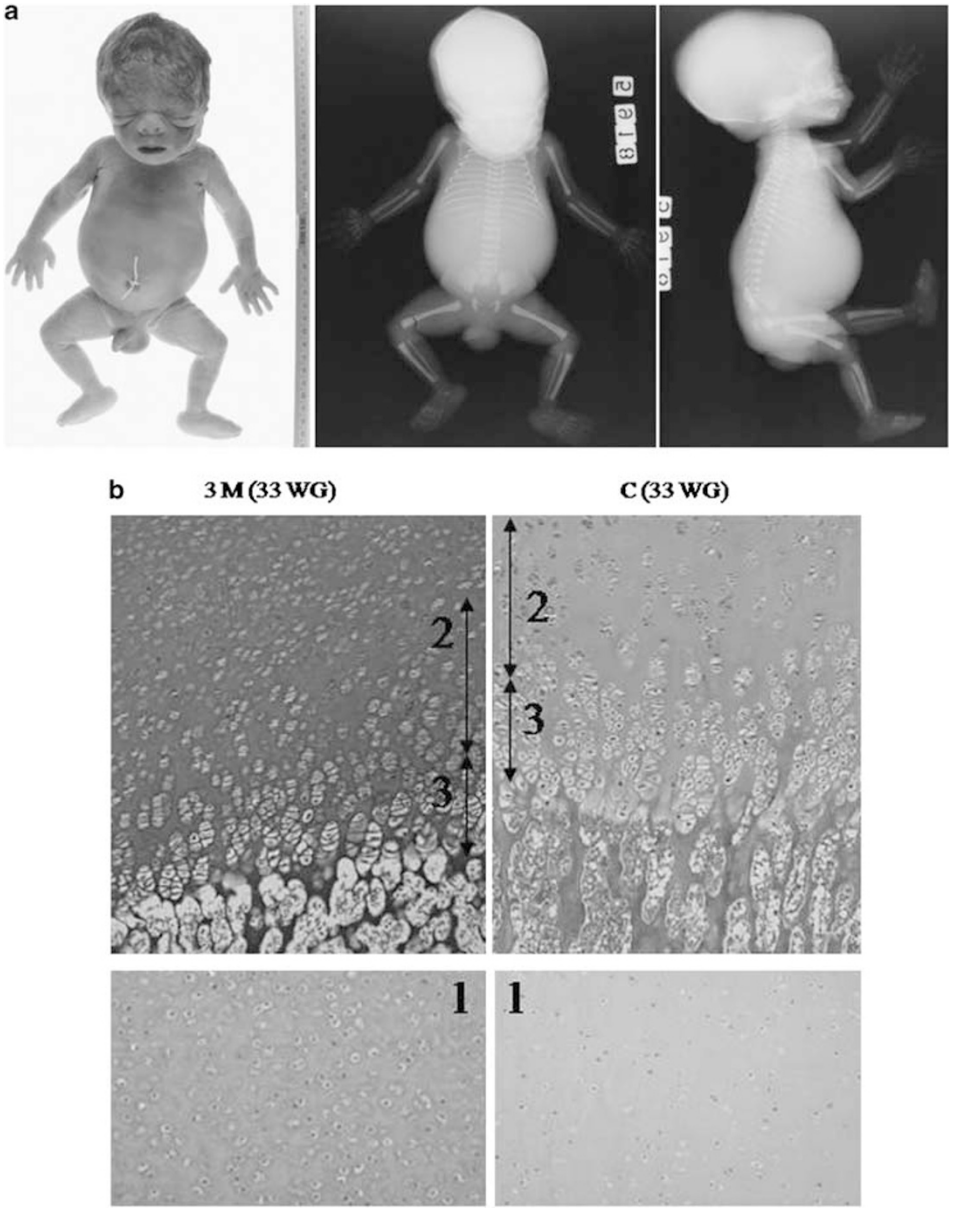

Figure 3 (**a**) Foetal case of 3M syndrome. The foetus presented with severe growth retardation, normal head circumference, characteristic facial features, prominent heels and long slender tubular bones suggestive of 3M syndrome. (**b**) Histological study of the femoral growth plate. Note the increased density and size of chondrocytes in the resting and proliferative zones and the defect in matrix production with no major abnormalities in the prehypertrophic or hypertrophic zones (1: resting zone; 2: proliferative zone; 3: hypertrophic zone).

chromosome 6 revealed that the foetus had complete isodisomy of chromosome 6, of paternal origin (data not shown). Finally, among the 10 cases without any *CUL7* mutations, 7/10 patients were born to related parents. Microsatellite analysis at the 3M locus revealed that 6/7 inbred children were heterozygotes at the 6p21.1 region, ruling out *CUL7* as the disease gene in these families (data not shown). No clinical or radiological differences were observed between patients with and without CUL7 mutation (Figures 1 and 2, respectively).

Discussion

Studying a large series of 33 additional cases of 3M syndrome, we identified *CUL7* mutations in 23/33 families. The combination of these data with our previous results supports the view that *CUL7* is the major disease gene in the 3M syndrome, accounting for 84% of our cases (52/62).

Failure to identify *CUL7* mutations in 10 patients and exclusion of the 6p21.1 region in six consanguineous families argue in favour of genetic heterogeneity. This observation is in contrast with clinical homogeneity of the

Table 1 CUL7 mutations identified in families with 3M syndrome

Family	*Ethnic origin*	*Consanguineous parents*	*No. of affected children*	*Nucleotide change*	*Amino-acid change*	*Mutated exon (s) intron*
1	Tunisia	Yes	1	c.4450_4451delTG	V1484GfsX68	Ex24
2	Tunisia	Yes	2	c.4450_4451delTG	V1484GfsX68	Ex24
3	Tunisia	Yes	1	c.4450_4451delTG	V1484GfsX68	Ex24
4	Tunisia	Yes	1	c.4450_4451delTG	V1484GfsX68	Ex24
5	Morocco	Yes	1	c.3136delC	L1046WfsX94	Ex16
6	Morocco	Yes	3	c.1234_1235del-4del	—	I4 and Ex5
7	France	Yes	3	c.3970C>T	Q1324X	Ex21
8	France	No	1	c.3167_3168CC>GA	S1056X	Ex16
				c.4342T>C	W1448R	Ex23
9	France	No	2	c.2725G>A	G909R	Ex13
				c.4318C>T	R1440X	Ex23
10	Algeria	Yes	2	c.4450_4451delTG	V1484GfsX68	Ex24
11	Syria	Yes	2	c.2213_2235del	V738AfsX34	Ex10
12	Tunisia	No	1	c.923T>G	V308G	Ex4
				c.3624_3639 del	F1210TfsX1	Ex19
13	Madeira Island	Yes	2	c.4780_4781insG	E1594GfsX18	Ex26
14	Sri Lanka	Yes	1	c.4333C>T	R1445X	Ex23
15	Sri Lanka	Yes	1	c.4333C>T	R1445X	Ex23
16	Sri Lanka	Yes	1	c.4333C>T	R1445X	Ex23
17	Turkey	Yes	1	c.2706_2707dupGG	A903Gfs21	Ex13
18	Arab	Yes	1	c.3733_3828 del	—	Ex20, intron20, and Ex21
19	Turkey	Yes	2	c.1938dupG	T647DfsX32	Ex8
20	Germany	Yes	1	c.3041T>G	L1014R	Ex16
21	Germany	No	1	c.3041T>G	L1014R	Ex16
22	Austria	No	2	c.920_929del	L307PfsX28	Ex4
				c.3044T>G	L1014R	Ex16
23	Austria	No	1	c.2111G>A	W704X	Ex9
24	Italy	Yes	5	c.4391A>C	H1464P	Ex23
25	Italy	Yes[a]	1	c.4391A>C	H1464P	Ex23
26	Italy	Yes	1	c.462delT	G155Efs15	Ex2
27	Suriman	No	1	c.3608dupG	A1204SfsX14	Ex19
				c.3907C>T	Q1303X	Ex21
28	Bengali	Yes	1	c.2710C>T	R904X	Ex13
29	Brazil	Yes	5	c.4717C>T	R1573X	Ex25
30	Algeria	No	1	**c.3645+1G>A** and complete isodisomy of chromosome 6	—	**In19**
31	Algeria	Yes	1	c.4450_4451delTG	V1484GfsX68	Ex24
32	Algeria	Yes	1	c.4449_4450delGT	V1484GfsX68	Ex24
33	Morocco	Yes	1	c.4449_4450delGT	V1484GfsX68	Ex24
34	Morocco	Yes	1	**c.3646-18G>A**	—	**In20**
35	North Africa	Yes	1	**c.2660+1G>T**	—	**In12**
36	North Africa	Yes	1	**C.2581C>T**	**P861S**	**Ex12**
37	Turkey	Yes	1	**c.1938dupG**	**T647DfsX32**	**Ex8**
38	France	No	1	**c.3750del+3753_3762del**	**A1251LfsX2**	**Ex20**
					V844WfsX12	**Ex12**
				c.2530delG		
39	Italy	No	1	**c.4186C>T**	**R1396X**	**Ex22**
				c.2700delC	**Y900X**	**Ex13**
40	Italy	No	1	**c.3538C>T**	**Q1180X**	**Ex19**
				c.3750delA+3753_3762del	**A1251LfsX2**	**Ex20**
41	Italy	No	1	c.4449_4450delGT	V1484GfsX68	Ex24
				c.1570-3C>A	—	**In6**
42	Italy	Yes	1	c.4391A>C	H1464P	Ex23
43	Turkey	No	1	**c.4607C>T**	**S1536L**	**Ex25**
				c.3645+29G>A	—	**In20**
44	Germany	No	1	**c.2242C>T**	**Q748X**	**Ex10**
				c.3044T>G	L1014R	Ex16
45	Germany	Yes	1	c.4717C>T	R1573X	Ex25
46	Oman	Yes	1	**c.4406A>G**	**Q1469R**	**Ex23**
47	USA	No	1	**c.1338C>G**	**Y446X**	**Ex5**
				c.2112G>A	W704X	Ex9
48	Austria	No	1	c.923T>G	V308G	Ex4
				c.3722_3749dup	**V1252fsX22**	**Ex20**
49	India	Yes	1	**c.3379_3380delTG**	**W1127EfsX38**	**Ex18**
50	Pakistan	Yes	1	**c.3379_3380delTG**	**W1127EfsX38**	**Ex18**
51	Argentina	No	1	**c.1676dupT**	**I560DfsX11**	**Ex7**
				c.3646-18G>A	—	**In20**
52	Brazil	No	1	c.3136delC	L1046fsX94	Ex16
				c.279G>T	**Q93H**	**Ex2**

Families 1–29 published in Huber *et al*[6]; families 30–52: presently reported series of 3M families; the novel mutations are in bold.
[a]This family comes from the same village as 24.

3M syndrome. Indeed, no distinctive feature was observed in patients, whether or not *CUL7* mutations were observed. These data suggest that a second disease-causing gene is closely related to the *CUL7* pathway.

CUL7 belongs to the cullin family and plays a scaffold role in the E3 ubiquitin ligase complex, in which CUL7 interacts with both a heterodimer (composed of Skp1 bound to a member of the F-box protein family named Fbx29) and the ROC1 RING-finger protein.[7–11] The exact function and specific substrates of CUL7 are unknown. At high expression levels, CUL7 may interfere with the function of other cullins by sequestering ROC1 in the cytoplasm.[12] On the other hand, in normal cells, the growth-promoting activity of CUL7 is attributed to its ability to bind p53,[12] and the increased p53 activity could partly explain the delayed growth of Cul7−/− mouse embryo fibroblasts.[17] Finally, in cells expressing T antigen, CUL7 acts as a tumour suppressor,[7,13] and T antigen may inhibit CUL7 activity by inhibiting the ubiquitination of a substrate or its binding to CUL7.[14]

We have shown earlier that nonsense and missense *CUL7* mutations (R1445X and H1464P) impaired the ability of CUL7 to recruit ROC1, suggesting that impaired ubiquitination may play a role in the pathogenesis of prenatal growth retardation in humans.[6] In addition, the association of pre- and postnatal growth retardation with skeletal changes in 3M syndrome suggests that CUL7 may play a specific role in the endochondral ossification process. In keeping with this, analysis of the femoral growth plate of the 3M foetus revealed an increased in chondrocyte density and a defect in matrix production in the resting and proliferative zones. These preliminary data suggest that CUL7 is involved in chondrocyte growth and proliferation. However, one cannot exclude the responsibility of the paternal isodisomy of chromosome 6 in the phenotype observed in the foetus. Apart from low birth weight, none of the other features described in the paternal isodisomy of chromosome 6 was present in the foetus (ie, macroglossia, heart defect and, obviously, neonatal diabetes).[15,16] The prenatal growth retardation and growth plate anomalies might be due to the combination of the paternal isodisomy of chromosome 6 and 3M syndrome.

Additional studies are ongoing and will hopefully lead to the understanding of CUL7 function as well as to the identification of its partners.

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