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Molecular screening of ADAMTSL2 gene in 33 patients reveals the genetic heterogeneity of geleophysic dysplasia

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Abstract

Background—Geleophysic dysplasia (GD, OMIM 231050) is an autosomal recessive disorder characterized by short stature, small hands and feet, stiff joints, and thick skin. Patients often present with a progressive cardiac valvular disease which can lead to an early death. In a previous study including six GD families, we have mapped the disease gene on chromosome 9q34.2 and identified mutations in the *A Disintegrin And Metalloproteinase with Thrombospondin repeats-like 2gene* (ADAMTSL2).

Competing interests None

Patient consent Obtained

Ethics approval This study was conducted with the approval of the Necker Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

Methods—Following this study, we have collected the samples of 30 additional GD families, including 33 patients and identified *ADAMTSL2* mutations in 14/33 patients, comprising 13 novel mutations. The absence of mutation in 19 patients prompted us to compare the two groups of GD patients, namely group 1, patients with *ADAMTSL2* mutations (n¼20, also including the 6 patients from our previous study), and group 2, patients without *ADAMTSL2* mutations (n¼19).

Results—The main discriminating features were facial dysmorphism and tip-toe walking, which were almost constantly observed in group 1. No differences were found concerning heart involvement, skin thickness, recurrent respiratory and ear infections, bronchopulmonary insufficiency, laryngo-tracheal stenosis, deafness, and radiographic features.

Conclusions—It is concluded that GD is a genetically heterogeneous condition. Ongoing studies will hopefully lead to the identification of another disease gene.

INTRODUCTION

Geleophysic dysplasia (GD, OMIM 231050) is a rare autosomal recessive disorder characterized by short stature, small hands and feet, stiff joints, thick skin, and pseudo-muscular hypertrophy.1 Facial features include round full 'happy' face (from the Greek geleos: '*happy*' and physis: '*nature*'), small nose with anteverted nostrils, long flat philtrum, long thin upper lip, broad nasal bridge, and narrow palpebral fissures. The radiological manifestations include brachymetacarpy/tarsy, delayed bone age, cone shaped epiphyses, shortened long tubular bones, and vertebral abnormalities (ovoid vertebral bodies, platyspondyly) (figure 1).

Patients often present with a progressive cardiac valvular disease, which may result in secondary hypertrophy and cardiac failure leading to death in the first years of life.[2] Progressive hepatomegaly, recurrent respiratory infections and tracheal stenosis leading to severe respiratory problems are also commonly observed.

GD belongs to the group of acromelic dysplasias (group 14 of the International Classification of Genetic Skeletal Disorders[3]) which also includes acromicric dysplasia (AD) and WeilleMarchesani syndrome.

AD[4] is distinct from GD by the absence of cardiac valvular disease, the presence of distinct xray abnormalities (internal notch of the femoral head, internal notch of the second metacarpal, and external notch of the fifth metacarpal) and autosomal dominant mode of inheritance. The molecular bases remain unknown.[5]

WeilleMarchesani syndrome is characterized by ectopia lentis and microspherophakia and is either due to *FBN1* mutations, responsible for the dominant form,[6] or *ADAMTS10* mutations, responsible for the autosomal recessive form.[7]

Studying a series of six GD families, we have mapped the disease gene on chromosome 9q34.2 and identified four distinct missense mutations and a nonsense mutation in the *A Disintegrin And Metalloproteinase with Thrombospondin repeats-like 2 gene* (ADAMTSL2). We also identified Latent TGFb Binding Protein 1 (LTBP1) as a partner of *ADAMTSL2* and found an enhanced transforming growth factor β (TGFβ) signalling in GD

fibroblasts, suggesting a role for ADAMTSL2 in the regulation of the bioavailability of TGFβ.[8]

We present here *ADAMTSL2* molecular screening in a series of 33 additional GD cases. The absence of mutation in a significant number of patients prompted us to compare the clinical and radiological features of mutated and non-mutated patients.

METHODS

Patients

Diagnosis of GD was assessed by a clinical geneticist. All patients included in the study fulfilled the diagnosis criteria for GD: (1) short stature (≤2 SD); (2) short hands and feet; (3) stiff joints; and (4) dysmorphic features. Cardiac valvular disease and thickened skin were not considered as mandatory criteria as these features were not present in 3/6 of our initial series.[8] Skeletal survey on all patients were requested to exclude features of acromicric dysplasia, specifically internal notch of the femoral head or of the second metacarpal and external notch of the fifth metacarpal.

Eleven patients were included in the study through the French reference center for constitutional bone disorders in Necker Hospital and 22 patients were diagnosed as GD by clinical geneticists from various countries (Belgium, UK, Germany, Netherland, Lebanon, Portugal, and Turkey). A total of 33 patients were included (21 males and 12 females), ranging in age from 2 months to 26 years and originating from Algeria, Canada, China, England, Italy, Japan, Lebanon, Morocco, Pakistan, Portugal, Russia, and Turkey. Five patients were offspring of consanguineous relationships (patient 5, 17, 21, 22, and 24); there was one sib pair (patient 32 and 33) and three cousins (patient 17, 21 and 24). Appropriate written informed consents regarding human study were obtained from all subjects.

Mutation analysis

ADAMTSL2 exon and flanking intron sequences were amplified from patient DNA by PCR using 21 couples of primers designed with the Primer 3 software. The amplicons were purified and sequenced using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Foster City, California, USA) on an automatic sequencer (ABI 3100).

Statistical analyses

A non-parametric ManneWhitney test was used to compare means and a χ^2 test was used to compare ratios in the two patients groups.

RESULTS

ADAMTSL2 sequence analysis performed in the 33 patients allowed us to identify 14 distinct mutations in 14 patients comprising 13 novel mutations (table 1). Mutations were present at the homozygous state in five cases while patients were compound heterozygous in six other cases. In three cases (4, 7, and 15) only a single heterozygous mutation, inherited from the mother, was detected.

The mutations were located throughout the gene (figure 2). Among them, one mutation was a nonsense mutation (p.[R425X]), one was a 30 bp deletion affecting the N glycanrich module (c.[1148_1177del]), and 11 were missense mutations (p.[W50C], p.[R72Q], p.[E114K], p.[R159W], p.[A165T], p.[C171R], p.[R221C], p.[A239T], p.[R593C], p.[S635L] and p.[P906L]). We also identified the p.[C407C] mutation which was predicted to alter splicing but mRNA was not available for this patient. Except for p.[E114K], none of these mutations had been previously described. All mutations cosegregated with the disease and were not identified in 200 control chromosomes. The missense mutations consistently involved residues conserved across species and across the ADAMTSL family members. The prediction program PolyPhen was queried for the reported missense mutations and all of them were predicted to have a damaging role.

The absence of mutation in 19/33 patients (58%) prompted us to compare the clinical and radiological features of the two groups of GD patients (table 2), namely group 1, patients with *ADAMTSL2* mutations (n¼20, also including the six patients from our initial study[8]), and group 2, patients without *ADAMTSL2* mutation (n¼19). Importantly, blinded comparison was made by clinical geneticists unaware of the molecular diagnosis.

No consanguinity was found in group 2 whereas half of the families of group 1 were consanguineous. Antenatal history showed polyhydramnios and prenatal growth retardation in 91% and 76% of group 1 patients, versus 18% and 31% of group 2 patients, respectively. Birth term was 2 weeks earlier in group 1 (because of a higher proportion of induced deliveries due to prenatal growth retardation) and birth head circumference was normal for the term in both groups. The mean height was lower than _4 SD in both groups, but short stature was more severe in group 2 (_5.8 vs _4.1 SD). Tip-toe walking (restriction of dorsiflexion of the feet as a consequence of the extreme joint limitations and contractures) was almost constantly observed in group 1 whereas it was rarely reported in group 2 (88% vs 18%). Concerning facial dysmorphism, thin upper lip, long flat philtrum, and narrow palpebral fissures were much more frequent in group 1 than in group 2 (figure 3). No significant difference was found concerning heart involvement and skin thickness (which were observed in approximately 70% of both groups), recurrent respiratory and ear infections, bronchopulmonary insufficiency, laryngotracheal stenosis, high pitched voice, hepatomegaly, ophthalmologic symptoms, deafness, and radiographic features (delayed bone age, cone shaped epiphyses, shortened long tubular bones, abnormal femoral heads, platyspondyly, and ovoid vertebral bodies). One patient from group 1 had a severe systemic hypertension. However, the long term follow-up of patients from both groups did not reveal any difference in the course of the disease. Two mutated patients and five nonmutated patients died of cardiorespiratory failure (mean age 3.6 years).

DISCUSSION

We report here the identification of 14 *ADAMTSL2* mutations in 14/33 GD patients (42%), comprising 13 novel mutations located throughout the gene, with a majority of missense mutations involving highly conserved residues.

The absence of identified mutations in 58% of GD patients may have different explanations. First, only direct sequencing of *ADAMTSL2* was performed. One cannot exclude partial intragenic deletions or mutations in the introns or promoter region. This is probably the case for at least three patients (4, 7, and 15) where only a single heterozygous mutation was detected. However, the limit of our screening probably does not account for such a high proportion (58%) of non-mutated patients.

The absence of an identified mutation could be also due to overlapping diagnosis. Indeed GD is closely related to AD, which is the main differential diagnosis, and the distinction can be difficult especially in the absence of cardiac valvular disease.9 Importantly, recurrent sibs or consanguineous parents were never observed in the non-mutated patients group. However, all patients fulfilled the diagnosis criteria for GD and at least nine non-mutated patients presented with characteristic valvular cardiac disease.

Finally, we did not find any significant difference in the main clinical and radiological features characteristic of GD, namely cardiorespiratory involvement, skin thickness, laryngeal stenosis, hepatomegaly, natural history of the disorder, and severe outcome. By contrast, we found minor discriminating features including facial dysmorphism (thin upper lip, long flat philtrum and narrow palpebral fissures) and tip-toe walking, only consistently observed in the *ADAMTSL2* mutated group.

Our study supports the proposal that GD is a genetically heterogeneous condition, with *ADAMTSL2* mutations being identified in 42% of GD patients. Ongoing studies will hopefully lead to the identification of another GD gene presumably also involved in TGFβ bioavailability.

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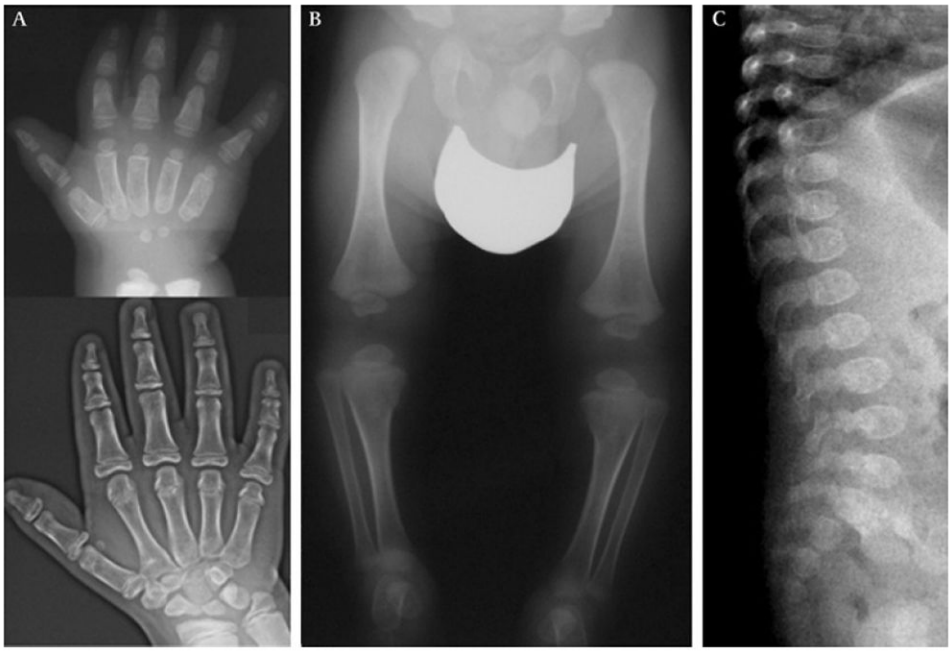

Figure 1.

Skeletal manifestations of geleophysic dysplasia. (A) Hand x-rays of patient 3 at age 3 years (top) and age 10 years (bottom). Note the very small hand with short and plump tubular bones and cone shaped epiphyses. Note also the carpal ossification delay. (B) Hip and lower limbs x-ray of patient 4 at age 8 months. Note the small capital femoral epiphyses and the shortened long tubular bones. (C) Anteroposterior view of the spine of patient 12 at age 1 year. Note the ovoid vertebral bodies.

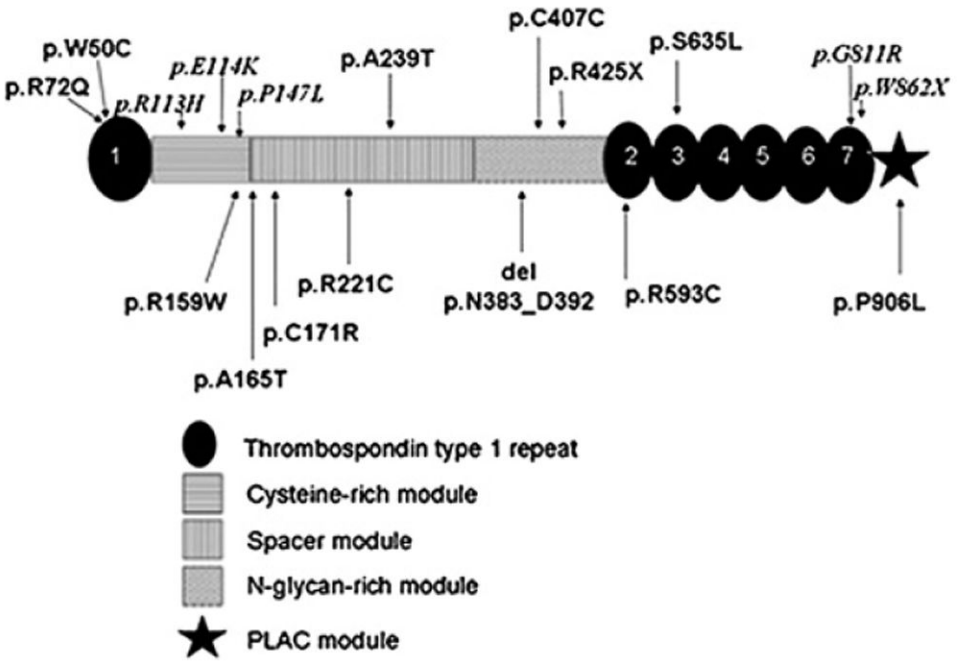

Figure 2.

ADAMTSL2 mutations. In italics: *ADAMTSL2* mutations previously identified (Le Goff *et al*[8]). In non-italics: novel mutations.

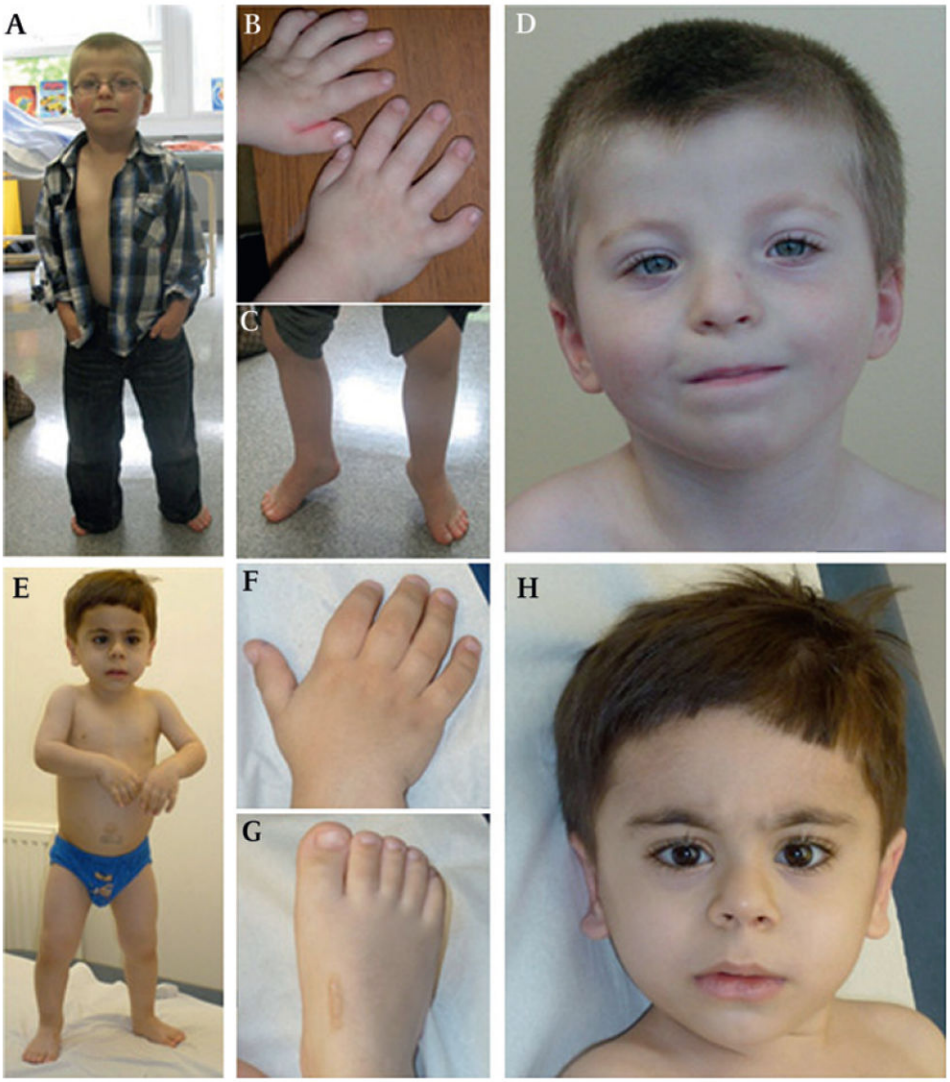

Figure 3.

Clinical manifestations of geleophysic dysplasia in patient 28 (mutated in *ADAMTSL2*) and patient 31 (not mutated in *ADAMTSL2*). (A, E) Note the short stature, small hands and feet, stiff joints and pseudomuscular hypertrophy (patient 28 at age 9 years and patient 31 at age 4 years). (B, F, G) Note the very small hands and feet. (C) Note the tip-toe walking. (D, H) Note the common facial features, including round full face, small nose with anteverted nostrils and long philtrum (patient 28 at age 6 years and patient 31 at age 4 years). Note also the thin upper lip, and narrow palpebral fissures present only in patient 28 (mutated in *ADAMTSL2*). Informed consent was obtained to publish the photographs in this figure.

Table 1

ADAMTSL2 mutations identified in our series

Patients	Ethnic origin	Identified mutation	Position	*ADAMTSL2* affected domain	Protein
2	France	c.[150G→T]+ [1273C→T]	Ex 2/Ex 9	TSR 1/N glycan rich domain	p.[W50C]+[R425X]
4	France	c.[1148_1177del]	Ex 9/?	N glycan rich domain	p.Asn383_Asp392del
5	Turkey	c.[493G→A]	Ex 5	CRD	p.[A165T]
7	France	c.[340G→A]	Ex 4/?	CRD	p.[E114K]*
12	France	c.[475C→T]+[511T→C]	Ex 5	CRD	p.[R159W]+[C171R]
15	Japan	c.[2717C→T]	Ex 17/?	PLAC	p.[P906L]
17	Pakistan	c.[661C→T]	Ex 6	Spacer	p.[R221C]
21	Pakistan	c.[661C→T]	Ex 6	Spacer	p.[R221C]
22	Italy	c.[715.G→A]	Ex 7	Spacer	p.[A239T]
24	Pakistan	c.[661C→T]	Ex 6	Spacer	p.[R221C]
28	England	c.[215G→A]+ [340G→A]	Ex 2/Ex 4	TSR1/CRD	p.[R72Q]+p.[E114K]
30	France	c.[1219C→T]+ [1904C→T]	Ex 9/Ex 13	N glycan rich domain/TSR 3	p.[C407C]+p.[S635L]
32	England	c.[150G→T]+ [1777C→T]	Ex 2/Ex 12	TSR 1/TSR 2	p.[W50C]+[R593C]
33	England	c.[150G→T]+ [1777C→T]	Ex 2/Ex 12	TSR 1/TSR 2	p.[W50C]+[R593C]

*This mutation was previously identified (Le Goff *et al*[8]).

Table 2

Comparison of geleophysic dysplasia (GD) patients with *ADAMTSL2* mutations (group 1) and without *ADAMTSL2* mutation (group 2)

	Group 1: mutated (n=20)	Group 2: non- mutated (n=19)	p Value
Consanguinity	10/20	0/16	0.003
Polyhydramnios	10/11	2/11	0.003
Prenatal growth retardation	13/17	4/13	0.03
Birth term (weeks of amenorrhoea)	37.5±2.1 (3 NA)	39.7±1.1 (5 NA)	0.004
Birth length (cm)	44.6±3.0 (5 NA)	48.9±1.6 (8 NA)	0.0004
Birth head circumference (cm)	33.5±1.7 (4 NA)	35.7±1.1 (11 NA)	0.002
Cardiac valvular anomaly	14/18	9/13	0.89
Pulmonary arterial hypertension	4/11	5/9	0.42
Cardiac surgery	3/14	4/9	0.47
Recurrent respiratory and ear infections	8/12	8/15	0.75
Bronchopulmonary insufficiency	8/16	7/15	0.62
Laryngotracheal stenosis	6/13	7/14	0.99
Deceased (of cardiorespiratory failure)	2/20	5/19	0.36
Deafness	8/13	6/13	0.69
Ophthalmologic symptoms	8/13	3/12	0.15
Mild mental retardation	6/15	5/17	0.80
Height (SD)	−4.1±1.0 (6 NA)	−5.8±2.4 (3 NA)	0.016
Head circumference (SD)	−0.9±1.7 (11 NA)	0.0±1.2 (5 NA)	0.15
Tip-toe walking	14/16	2/11	0.001
Thickened skin	10/15	9/13	0.99
Pseudomuscular hypertrophy	11/14	7/14	0.24
Hepatomegaly	5/17	9/16	0.22
High pitch voice	6/11	1/7	0.22
Round full face	14/15	9/16	0.05
Small nose	11/16	13/15	0.41
Broad nasal bridge	14/15	11/15	0.33
Long flat philtrum	18/19	10/17	0.027
Thin upper lip	18/18	6/15	0.005
Narrow palpebral fissures	13/15	7/17	0.03
Delayed bone age	12/13	12/13	0.99
Cone shaped epiphyses	5/8	8/12	0.99
Shortened long tubular bones	15/15	9/11	0.33
Small and/or irregular femoral epiphyses	8/10	6/12	0.31
Platyspondyly	5/8	2/9	0.22
Ovoid vertebral bodies	6/10	7/11	0.99

NA, not available.