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


Published in:
Journal of Medical Genetics

DOI:
10.1136/jmg.2010.087544

Link to publication

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)

Download date: 14 Apr 2019
ORIGINAL ARTICLE

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

Slimane Allali, Carine Le Goff, Isabelle Pressac—Diebold, Gwendoline Pfennig, Clémentine Mahaut, Nathalie Dagoneau, Yasemin Alanay, Angela F Brady, Yanick J Crow, Koen Devriendt, Valérie Drouin-Garraud, Elisabeth Flori, David Geneviève, Raoul C Hennekam, Jane Hurst, Deborah Krakow, Martine Le Merrer, Klaske D Lichtenbelt, Sally A Lynch, Stanislas Lyonnet, Kay MacDermot, Sahar Mansour, André Megarbane, Heloisa G Santos, Miranda Splitt, Andrea Superti-Furga, Sheila Unger, Denise Williams, Arnold Munnich, Valérie Cormier-Daire

ABSTRACT
Background Geleophysic dysplasia (GD, OMIM 231050) is an autosomal recessive disorder characterised 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 2 gene (ADAMTSL2).

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 (n=20, also including the 6 patients from our previous study), and group 2, patients without ADAMTSL2 mutations (n=19).

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 characterised by short stature, small hands and feet, stiff joints, thick skin, and pseudo-muscular hypertrophy. 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 brachymetacarpal/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. 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) which also includes acromicric dysplasia (AD) and Weill—Marchesani syndrome.

AD is distinct from GD by the absence of cardiac valvular disease, the presence of distinct x-ray 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.

Weill—Marchesani syndrome is characterised by ectopia lentis and microspherophakia and is either due to FBN1 mutations, responsible for the dominant form, or ADAMTS10 mutations, responsible for the autosomal recessive form.

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 TGFβ 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.

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.

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 centre 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, 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 Mann–Whitney test was used to compare means and a χ² 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).

Table 1 ADAMTSL2 mutations identified in our series

<table>
<thead>
<tr>
<th>Patients</th>
<th>Ethnic origin</th>
<th>Identified mutation</th>
<th>Position</th>
<th>ADAMTSL2 affected domain</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>France</td>
<td>c.[150G→T]+[1273C→T]</td>
<td>Ex 2/Ex 9</td>
<td>TSR 1/N glycan rich domain</td>
<td>p.[W50C]+[R425X]</td>
</tr>
<tr>
<td>4</td>
<td>France</td>
<td>c.[1148,1177del]</td>
<td>Ex 9?</td>
<td>N glycan rich domain</td>
<td>p.Asn383_Asp392del</td>
</tr>
<tr>
<td>5</td>
<td>Turkey</td>
<td>c.[493G→A]</td>
<td>Ex 5</td>
<td>CRD</td>
<td>p.[A165T]</td>
</tr>
<tr>
<td>7</td>
<td>France</td>
<td>c.[340G→A]</td>
<td>Ex 4?</td>
<td>CRD</td>
<td>p.[E114K]*</td>
</tr>
<tr>
<td>12</td>
<td>France</td>
<td>c.[475C→T]+[511T→C]</td>
<td>Ex 5</td>
<td>CRD</td>
<td>p.[R158W]+[C171R]</td>
</tr>
<tr>
<td>15</td>
<td>Japan</td>
<td>c.[2717C→T]</td>
<td>Ex 17?</td>
<td>PLAC</td>
<td>p.[P906L]</td>
</tr>
<tr>
<td>17</td>
<td>Pakistan</td>
<td>c.[661C→T]</td>
<td>Ex 6</td>
<td>Spacer</td>
<td>p.[R221C]</td>
</tr>
<tr>
<td>21</td>
<td>Pakistan</td>
<td>c.[661C→T]</td>
<td>Ex 6</td>
<td>Spacer</td>
<td>p.[R221C]</td>
</tr>
<tr>
<td>22</td>
<td>Italy</td>
<td>c.[715G→A]</td>
<td>Ex 7</td>
<td>Spacer</td>
<td>p.[A239T]</td>
</tr>
<tr>
<td>24</td>
<td>Pakistan</td>
<td>c.[661C→T]</td>
<td>Ex 6</td>
<td>Spacer</td>
<td>p.[R221C]</td>
</tr>
<tr>
<td>28</td>
<td>England</td>
<td>c.[215G→A]+[340G→A]</td>
<td>Ex 2/Ex 4</td>
<td>TSR1/CRD</td>
<td>p.[R720Q]+[E114K]</td>
</tr>
<tr>
<td>30</td>
<td>France</td>
<td>c.[1219C→T]+[1904C→T]</td>
<td>Ex 9/Ex 13</td>
<td>N glycan rich domain/TSR 3</td>
<td>p.[C407C]+[S635L]</td>
</tr>
<tr>
<td>32</td>
<td>England</td>
<td>c.[150G→T]+[1777C→T]</td>
<td>Ex 2/Ex 12</td>
<td>TSR 1/TSR 2</td>
<td>p.[W50C]+[R593C]</td>
</tr>
<tr>
<td>33</td>
<td>England</td>
<td>c.[150G→T]+[1777C→T]</td>
<td>Ex 2/Ex 12</td>
<td>TSR 1/TSR 2</td>
<td>p.[W50C]+[R593C]</td>
</tr>
</tbody>
</table>

*This mutation was previously identified (Le Goff et al*).
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 glycan-rich module (c.[1148_1177del]), and 11 were missense mutations (p.[W50C], p.[R72Q], p.[E114K], p.[R159W], p.[A165T], p.[R159W], p.[A165T], p.[C171R], p.[R221C], p.[A239T], p.[R593C], p.[S635L] and p.[P563X]). 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, with a majority of missense mutations involving highly conserved residues. The prediction program PolyPhen was queried for the reported missense mutations and all of them were predicted to have a damaging role.

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 non-mutated patients died of cardiorespiratory failure (mean age 5.6 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. 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.

Author affiliations
1Department of Genetics, INSERM U781, Université Paris Descartes, Hôpital Necker, Paris, France
2Genetics Unit, Department of Pediatrics Hacettepe, University School of Medicine, Ankara, Turkey
3North West Thames Regional Genetics Service, Northwick Park Hospital, Harrow, UK
4Genetic Medicine, University of Manchester, Manchester Academic Health Science Centre, Central Manchester Foundation Trust University Hospitals, Manchester, UK
5Department of Medical Genetics, Leuven University Hospital, Leuven, Belgium
6Department of Medical Genetics, Hôpital Charles Nicolle, Rouen, France
7Department of genetics, Strasbourg hospital, Strasbourg, France
8Service de Génétique Médicale, Hôpital Arnaud de Villeneuve, Montpellier, France
9Department of Pediatrics, Academic Medical Center, University of Amsterdam, The Netherlands
10Department of Clinical Genetics, Oxford Radcliffe Hospitals, UK
11Cedars Sinai Medical Center, Los Angeles, California, USA
12Department of Medical Genetics, University Medical Center, Utrecht, The Netherlands
13National Center for Medical Genetics, Dublin, Ireland
14SW Thames Regional Genetics Service, St George’s University of London, London, UK
15Unité de génétique médicale, Université Saint Joseph, Beyrouth, Lebanon
16Department of Medical Genetics, Lisboa, Portugal
17Institute of Human Genetics, Newcastle, UK
Acknowledgements We thank all patients and their families for their contribution to this work. We thank all the clinical geneticists for their participation and availability.

Funding The Medical Research Foundation (FRM, to SA) and by French National Research Agency (ANR) award R09183KS (to VC-D). YJC acknowledges the Manchester NIHR Biomedical Research Centre.

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.

REFERENCES

Correction

Prof David St Clair and Dr Zosia Miedzybrodzka should have been co-corresponding authors on this paper. Their contact details are shown below:

Prof David St Clair, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK; d.stclair@abdn.ac.uk
Dr Zosia Miedzybrodzka, University of Aberdeen, Department of Genetics, Polwarth Building Foresterhill, Aberdeen, Scotland, UK; zosia@abdn.ac.uk

J Med Genet 2011;48:421. doi:10.1136/jmg.2009.066852corr1
Molecular screening of ADAMTSL2 gene in 33 patients reveals the genetic heterogeneity of geleophysic dysplasia

Slimane Allali, Carine Le Goff, Isabelle Pressac–Diebold, et al.

doi: 10.1136/jmg.2010.087544

Updated information and services can be found at:
http://jmg.bmj.com/content/48/6/417.full.html

These include:

References
This article cites 9 articles, 2 of which can be accessed free at:
http://jmg.bmj.com/content/48/6/417.full.html#related-urls

Article cited in:
http://jmg.bmj.com/content/48/6/417.full.html#ref-list-1

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Molecular genetics (1079 articles)
Epidemiology (534 articles)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/