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ARTICLE

Expanding the clinical spectrum of *B4GALT7* deficiency: homozygous p.R270C mutation with founder effect causes Larsen of Reunion Island syndrome

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First described as a variant of Larsen syndrome in Reunion Island (LRS) in the southern Indian Ocean, 'Larsen of Reunion Island syndrome' is characterized by dwarfism, hyperlaxity, multiple dislocations and distinctive facial features. It overlaps with Desbuquois dysplasia, Larsen syndrome and spondyloepiphyseal dysplasia with dislocations ascribed to *CANT1, FLNB* and *CHST3* mutations, respectively. We collected the samples of 22 LRS cases. After exclusion of *CANT1, FLNB* and *CHST3* genes, an exome sequencing was performed in two affected second cousins and one unaffected sister. We identified a homozygous missense mutation in *B4GALT7*, NM_007255.2: c.808C>T p.(Arg270Cys) named p.R270C, in the two affected cases, not present in the unaffected sister. The same homozygous mutation was subsequently identified in the remaining 20 LRS cases. Our findings demonstrate that *B4GALT7* is the causative gene for LRS. The identification of a unique homozygous mutation argues in favor of a founder effect. *B4GALT7* encodes a galactosyltransferase, required for the initiation of glycoaminoglycan side chain synthesis of proteoglycans. This study expands the phenotypic spectrum of *B4GALT7* mutations, initially described as responsible for the progeroid variant of Ehlers–Danlos syndrome. It further supports a common physiopathological basis involving proteoglycan synthesis in skeletal disorders with dislocations.
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INTRODUCTION

Larsen of Reunion Island syndrome (LRS) [MIM 245600] has been described as a specific entity from Reunion Island in the south of the Indian Ocean in 1975.[1] The clinical manifestations of LRS include dislocations of large joints with ligamentous hyperlaxity, short stature and characteristic facial features, namely, round flat face, prominent forehead, prominent bulging eyes, under-eye shadows and microstomia. Radiological features include dislocations of knees, hips, elbows and fingers, advanced carpal ossification, widened metaphyses, particularly at the knees, and often aradioulnarsynostosis.[1,2]

LRS shares many clinical features with other conditions from the multiple dislocation group,[3] namely, Larsen syndrome (MIM 150250, 245600, LS),[4–6] spondyloepiphyseal dysplasia with dislocations (MIM 608637) and Desbuquois dysplasia (MIM 251450), ascribed to filamin B (*FLNB*),[7] carbohydrate sulfotransferase 3 (*CHST3*)[8] and calcium-activated nucleotidase 1 (*CANT1*) mutations,[9] respectively.

In 1992, Bonaventure *et al*[10] studied seven LRS children from three families from the Reunion Island, and excluded by linkage analysis four collagen candidate genes, namely, *COL1A1, COL1A2, COL3A1* and *COL5A2*.

The aim of our study was to identify LRS gene. Among the 53 LRS patients followed in our department in the last 20 years, only 22, originating from 20 distinct families, had detailed clinical data and were therefore included in the study. We first excluded *FLNB, CANT1* and *CHST3* by direct sequencing (data not shown). Considering the lack of multiplex families and the absence of consanguinity in our families, an exome sequencing strategy was undertaken, leading to the identification of *B4GALT7* as the causative gene of LRS.

SUBJECTS AND METHODS

Clinical and radiological features

The 22 cases fulfilled the diagnostic criteria for LRS, namely, (i) characteristic facial features, (ii) multiple joint dislocations (knees, elbows, hips) with hyperlaxity and (iii) severe short stature. The series included 11 males and 11 females, ranging in age from 4 to 46 years (Figure 1a–d). The clinical and radiological features of all patients are presented in Tables 1 and 2.

Molecular analysis

We selected two second cousins (one male and one female) and the unaffected sister of the affected male to perform exome sequencing (Figure 1e). For each subject, DNA samples from index cases and their parents and unaffected sibs (if any) were obtained following written informed consents. Genomic DNA was extracted from peripheral blood using standard procedure.

Exome sequencing

Exome sequencing was performed by IntegraGen (Evry, France). Enrichment was performed using 3 mg of genomic DNA and an Agilent SureSelect Human

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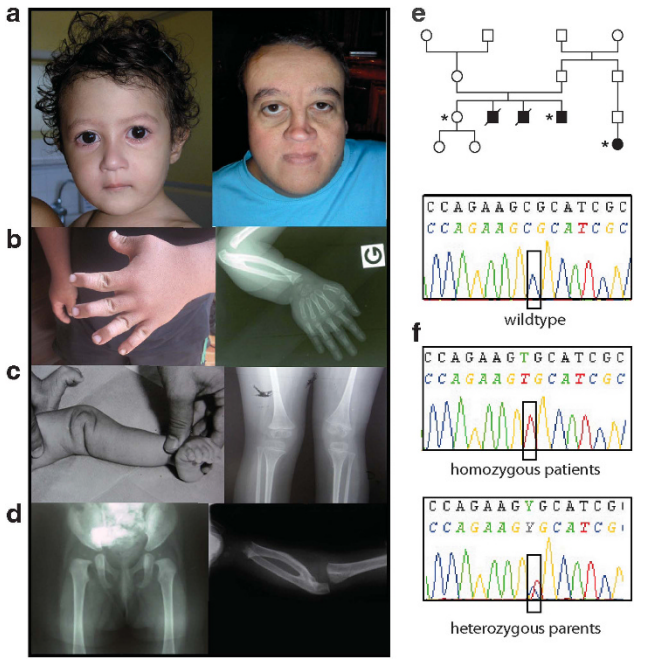

Figure 1 (**a**) Left: the female proband at 2 years of age, with round, flattened midface, prominent forehead, circles under eyes and microstomia, right: paternal uncle at 29 years of age with very small stature (122 cm) and obesity (BMI: 45.9). (**b**) Left: hand at 28 months of age with short fingers, right: X-Rays showing advanced carpal ossification (3½ years contrasting with the chronological age of 28 months), delta phalanx and proximal radioulnar synostosis. (**c**) Clinically unstable knee joint (left) confirmed by the femorotibial misalignment (right). (**d**) X-rays showing Swedish key appearance of the proximal femur (left) and radioulnar synostosis (right). (**e**) Pedigree of the family studied by exome sequencing. (**f**) Chromatograms showing the mutation in *B4GALT7* gene in control (wild type), affected (homozygous patients) and carrier (heterozygous parents) cases.

All Exon Kit V2, 46 Mb (Agilent Technologies, Santa Clara, CA, USA) in accordance with the manufacturer's protocols.[11] After sonication, DNA was fragmented and purified to yield fragments of mean size 150–200 bp. Each elute-enriched DNA sample was then sequenced on an IlluminaHiSEQ 2000 (Illumina Inc, San Diego, CA, USA) as paired-end 75b reads. Image analysis and base calling were performed using Illumina Real Time Analysis Pipeline version 1.9 with default parameters. On average, 7.4 Gb of sequence was generated for each subject to achieve 74 × and 90% (X10X) coverage. The bioinfomatics analysis of sequencing data was based on the Illumina pipeline (CASAVA 1.7). The alignment algorithm used was ELANDv2 (Illumina Inc) (performs multiseed and gapped alignments). Genetic variation annotation was performed using ANNOVAR software[12] including annotations (RefSeq hg19).

Sanger sequencing

A 666-bp fragment harboring the candidate mutation on chr5:g.177035995(hg Built 37.6) was amplified using primer couples with melting temperature of 58 °C (primers on request). Sequencing was performed using ABI PRISM 3130 BigDye terminator chemistry according to the manufacturer's instruction (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed using SeqScape v2.7 (Applied Biosystems).

Bioinformatics analysis

We predicted the effects of amino-acid substitution on protein stability and function on the basis of the following methods including in Annovar: SIFT, PolyPhen, PhyloP, LRT, MutationTaster, GERP + +.

RESULTS

We focused our analyses on non-synonymous variants located in coding sequence by assuming that synonymous variants were less likely to be pathogenic. DNA variants were filtered on the basis of the presence of variants in the same gene in the two affected individuals and on their absence in the unaffected sister. First, a total of 117 310 variants (single-nucleotide polymorphisms and small insertions/deletions) was identified; after using various computational filtering criteria including the hypothesis of a recessive inheritance of rare mutations, 4146 homozygous variants present in the two patients and not in the healthy sister were found. After filtering with Exome Sequencing Project database, only seven synonymous variants were selected and dbSNP 137 NonFlagged allowed to retain an unique variant in *B4GALT7* gene: it was predicted to have a functional effect by the five functional prediction algorithms in Annovar. This mutation was confirmed by direct sequencing in the two affected individuals, although their parents were heterozygous (Figure 1f). The *B4GALT7* mutation was submitted to the corresponding LOVD database at http://databases.lovd.nl/shared/variants/0000031236. We sequenced *B4GALT7* gene in the other 20 patients: all were homozygous for this mutation.

A total of 500 control cases (chosen among the same ethnic group 'white creoles' than that of our patients) was studied. No control case was homozygous for the mutation. In this ethnic group, the allelic frequency was 2%, corresponding to a prevalence of 1/2500 births.

DISCUSSION

We report here the identification of a homozygous *B4GALT7* mutation c.808C>T p.(Arg270Cys) in 22/22 LRS and confirm that LRS is a distinct condition from previously reported skeletal disorders with multiple dislocations. The *B4GALT7* mutation was submitted to the corresponding LOVD database at http://databases.lovd.nl/shared/variants/0000031236

The identification of a unique homozygous *B4GALT7* mutation c.808C>T p.(Arg270Cys) in our cohort supports a founder effect. Our patients belong to the same ethnic group named 'white creoles', representing about 15.5% of the population from Reunion Island.

With a total of 22 patients ranging from 4 to 46 years, our cohort of patients with Larsen of Reunion Island constitutes the largest one ever reported in the literature, leading to define key features for clinical diagnosis (Tables 1 and 2). Indeed, LRS must be considered in the presence of multiple dislocations (21/21, 100%), severe pre- and postnatal dwarfism (19/19, 100%) with height often inferior to −6 SD (growth hormone therapy was administered in none of the cases), advanced carpal ossification (11/15, 73%) and facial features. All the patients presented with high forehead, hypertelorism, protruding eyes with under-eye shadows, hypoplastic midface and microstomia with protruding lips; a thin prominent nasal bridge tented to appear with age. Progeroid aspect was absent in all cases. Learning difficulties were reported in more than a half of the patients; however, repeated surgeries were also leading to significant difficulties in attending school daily. Other frequently observed features were a Swedish key appearance of the proximal femora (9/19, 47%) and radioulnar synostosis (10/21, 48%) without progressive severe arthritic changes. By contrast, progressive scoliosis, accessory ossification center at the base of the proximal phalange of the second digit and/or a delta phalanx of the thumb, which are hallmarks of Desbuquois dysplasia, were not observed in LRS. In addition, platyspondyly and intervertebral space narrowing, which are characteristic of spondyloepiphyseal dysplasia with dislocations, were never observed in LRS.

Table 1 Clinical features of the 22 LRS patients

Patients	Age	Gender	Birth length (cm)	Adult height (cm)	BMI	Facial dysmorphism 22/22	Cutaneous hyperextensibility 21/22	Learning difficulties 12/22	Glaucoma 5/21	Pectus carinatum 5/22	Bifid thumb 2/22	Cleft palate 1/22	Scoliosis, kyphosis 6/22
1	32	M	43	113	38	+	+	−	−	−	−	−	−
2	22	M	42	130	32.5	+	+	+	−	−	−	−	−
3	15	M	46	132 at 14 years	25.2	+	+	−	−	−	−	−	−
4	29	F	42.5	122	45.9	+	+	+	−	−	−	−	−
5	13	M	43	127	16.7	+	+	+	−	+	−	−	−
6	36	F	39	127	24.8	+	+	−	−	−	−	−	+
7	11	M	41	111 at 10 years	14.6	+	+	+	+	+	−	−	−
8	46	F	?	112	32.8	+	+	+	−	−	−	+	+
9	15	M	39	138	39.3	+	+	+	−	−	+	−	−
10	21	M	38	121	17	+	+	+	+	−	−	−	−
11	19	F	41	131	17.1	+	+	−	+	−	−	−	−
12	35	F	42	?	17.5	+	+	+	−	+	−	−	−
13	25	M	40	128	20	+	+	−	Megalocornea	−	−	−	−
14	Deceased	M	43	132	20.8	+	+/−	+	−	−	−	−	−
15	24	F	39	117	41.1	+	+	+	+	−	−	−	+
16	34	F	38.5	120	?	+	+	−	−	−	+	−	+
17	22	F	41	127	?	+	−	−	−	−	−	−	+
18	29	F	45	120	19.8	+	+	−	−	−	+	−	+
19	25	F	?	?	31.25	+	+	−	+	−	−	−	−
20	29	M	42	133	?	+	+	+	−	+	−	−	−
21	21	M	44	131	26.3	+	+	+	−	−	−	−	−
22	4	F	38	−	?	+	+	−	?	+	−	−	−

Abbreviations: BMI, body mass index; F, female; M, male.
+, present; −, absent; ?, unknown.

Table 2 Radiological findings of the 22 LRS patients

Patients	Joint dislocations	Advanced bone age 11/15	Bifid thumbs 2/17	Brachy mesophalangy of II–III–IV fingers 9/17	Phalangeal dislocation 12/17	Swedish key 9/19	Radioulnar synostosis 10/21	Genu recurvatum 7/20
1	Bilateral hips and elbows, right shoulder, right wrist	±	−	+	+	−	+	?
2	Elbows, left shoulder	?	−	−	−	−	−	−
3	Elbows, right wrist, shoulders	+	−	+	+	+	−	Right
4	Elbows, left patella	+	−	+	+	+	+	−
5	Left shoulder	−	−	−	+	−	−	−
6	Right knee, shoulders, fingers	?	−	−	+	+	+	Bilateral
7	Right knee	?	−	−	+	+	−	−
8	Right hip, right knee	+	−	+	+	−	+	Bilateral
9	Bilateral elbows and knees	−	+	+	+	+	−	−
10	Elbows, patella, bilateral wrist, right shoulder	+	−	−	+	+	+	−
11	Elbows	+	−	−	+	+	−	Bilateral
12	Left hip, knees	−	−	?	?	+	−	−
13	Left knee, fingers	+	−	+	−	−	+	−
14	Shoulders, hips, knees	?	?	?	?	?	+	Bilateral
15	Elbows, right hip, patella, fingers	+	−	−	−	−	−	Bilateral
16	Knees, hips	?	+	?	?	−	−	Bilateral
17	Knees	−	−	+	−	−	+	−
18	Knees, elbows	+	+	+	+	−	+	−
19	Hips, elbows, knees	+	−	+	−	+	−	−
20	Left shoulder, knees, elbows	?	?	?	?	?	+	−
21	Knees, elbows, fingers	+	−	−	+	−	−	−
22	?	?	?	?	?	?	?	?

+, present; −, absent; ?, unknown.

Our series of mutations in *B4GALT7* constitutes the largest one ever recorded in the literature; it gives us the opportunity to also revise the clinical phenotype related to *B4GALT7* mutations. Only four patients have been reported[13–16] with *B4GALT7* mutations including either compound heterozygous mutations c.[557C>A];[617T>C] and c.[808C>T];[122T>C] or the homozygous c.[808C>T] also

identified in LRS patients. The clinical manifestations of these four patients have been recently reviewed[16] and included wrinkled loose facial skin, curly fine hair, scanty eyebrows and eyelashes, short stature and development anomalies of forearm and elbows with proximal radioulnar synostosis. Importantly, progeroid facial appearance was not consistently observed[15] questioning the name of 'progeroid type of Ehlers–Danlos syndrome'.[16] The detailed description of the 22 LRS patients confirms the absence of progeroid symptoms and highlights the frequency and the severity of large joint dislocations requiring repeated surgical procedures. The joint hypermobility is present in all patients reported with *B4GALT7* mutations and particularly severe in the young girl reported by Faiyaz-Ul-Haque *et al*.[15] She was firstly suspected of Larsen syndrome but joint dislocations have never been reported in the previous reports. These clinical differences might be explained by different functional consequences of the reported mutations with variable quantitative effect on glycosaminoglycan (GAG) biosynthesis. Whereas the c.557C>A p.(Arg186Asp) mutation does not affect GAG biosynthesis severely, the c.617T>C p.(Leu206Pro) mutation leads to a complete inhibition and the c.808C>T p.(Arg270Cys) mutation to a significant reduction of GAG biosynthesis.[17] Nevertheless, the phenotypic differences between the patients reported by Faiyaz-Ul-Haque *et al*[15] and LRS patients who are sharing the same mutation may have other explanations. Given the insular peopling pattern and the high level of homozygosity in the white creoles population, the interaction with other variants in close linkage disequilibrium to *B4GALT7* could be hypothesized as well as the involvement of modifier genes.

B4GALT7 is directly involved in the biosynthesis of proteoglycans. These macromolecules consist in GAG polymer chains attached to core proteins. The synthesis of GAG chains is initiated by the formation of a tetrasaccharide linker region attached to a serine residue of the core protein. The synthesis of this linker region starts by the transfer of xylose onto a serine residue of the core protein catalyzed by xylosyltransferases and, subsequently, two galactose residues are added by galactosyltransferase I (B4GALT7, encoded by *B4GALT7* gene [MIM 604327])[18,19] and galactosyltransferase II (B3GALT6, encoded by *B3GALT6*)[20] followed by the transfer of glucuronic acid by glucuronosyltransferase I (GlcAT-I, encoded by *B3GAT3*) [MIM 606374].[21] After this tetrasaccharide linkage region synthesis, the GAG chain is modified by epimerization and sulfation.

Previous functional studies have shown that the c.808C>T p.(Arg270Cys) mutation identified in *B4GALT7* causes a reduction in galactosyl transferase activity, directly involved in the tetrasaccharide linker synthesis. A lack of decorin and biglycan synthesis and reduced epimerization of the chain of decorin glycosaminoglycans have been demonstrated in p.(Arg270Cys)-mutant fibroblasts from progeroid Ehlers–Danlos patient.[22] This deficiency results in a loss of cohesion of the tissue, which has been further demonstrated by the ultrastructural study showing abnormal connective tissue.[23]

In the last few years, the impairment of proteoglycan synthesis has been involved in a few human connective tissue disorders, namely, (1) SED with dislocations due to *CHST3* mutations responsible for a defect in carbohydrate chondroitin 6-sulfotransferase with defective sulfation of chondroitin proteoglycan,[8,24] (2) Desbuquois dysplasia due to *CANT1* mutations responsible for a defect in GAG synthesis,[9,25] (3) a chondrodysplasia with joint dislocations due to mutations in *IMPAD1* (inositol monophosphatase domain-containing protein 1) encoding the Golgi resident nucleotide phosphatase gPAPP, and responsible for a defect in sulfated substrates,[26] (4) 'a Larsen-like' phenotype with cardiac defects due to *B3GAT3* (galactosyltransferase) mutations[27] and affecting, through the linker synthesis defect, dermatan heparan and chondrotin sulfate proteoglycans and (5) very recently, a spondyloepimetaphyseal dysplasia with joint laxity type 1 and a spectrum of disorders affecting a broad range of skeletal and connective tissues characterized by lax skin, muscle hypotonia, joint dislocation and spinal deformity due to *B3GALT6* mutations[28] (Supplementary Table).

In conclusion, our study demonstrates that LRS shares a common physiopathological basis with the other multiple dislocation disorders involving proteoglycan synthesis from the linker synthesis (*B4GALT7, B3GAT3, B3GALT6*) to GAG chain addition and modification (*CANT1*, *CHST3*, *IMPAD1)*. It also highlights that LRS constitutes a specific entity, and expandes the clinical spectrum of *B4GALT7* mutations, supporting the screening of *B4GALT7* in the presence of severe pre- and postnatal growth retardation, joint dislocations, radioulnar synostosis and advanced carpal bone age, without progeroid appearance, especially in a patient from Reunion Island.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

FC, BD and VC-D wrote the paper; FC, M-LJ and HR provided the clinical data; PM, CP and JV performed PCR and Sanger sequencing; FC and PM performed the exome analysis; VC-D, AM and FC analyzed the data.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)