

Tentative Clinical Diagnosis of Lujan-Fryns Syndrome—A Conglomeration of Different Genetic Entities?

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The clinical diagnosis of Lujan-Fryns syndrome (LFS) comprises X-linked intellectual disability (XLID) with marfanoid habitus, distinct combination of minor facial anomalies and nasal speech. However the definition of syndrome was significantly broadened since the original report and implies ID with marfanoid habitus. Mutations of three genes (*MED12*, *UPF3B*, and *ZDHHC9*) have been reported in “broadly defined” LFS. We examined these genes in 28 individuals with a tentative clinical diagnosis of LFS but we did not identify any causative mutation. By molecular karyotyping we detected other disorders, i.e., Phelan-McDermid syndrome and 16p11.2 microduplication, each in one patient. One affected individual was carrier of a different recurrent duplication on 16p11.2 that has been reported several times to the DECIPHER and ISCA databases in individuals with autism, intellectual disability (ID), and developmental delay. It may represent a new duplication syndrome. We also identified previously unreported de novo duplication on chromosome 12p13.31 which we considered to be disease-causing. X-exome sequencing of four individuals revealed private or non-recurrent mutations in *NKAP* and *LASIL* in one patient each. While LFS is defined as a form of XLID, there seem to be various conditions that have rather similar phenotypes. Therefore, the combination of ID and marfanoid habitus in a male patient is not sufficient for the diagnosis of LFS. We suggest that the diagnosis of LFS in patients with ID and marfanoid habitus should be made only in presence of specific facial features, nasal speech and obvious X-linked

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segregation of the disorder or an unambiguously pathogenic mutation in the *MED12*. © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

The combination of intellectual disability (ID) with marfanoid habitus and male gender was first described by Lujan et al. [1984] who reported on three unaffected sisters who had four affected sons. An X-linked genetic trait was assumed. Later, a pathogenic mutation in *MED12* was found in this family [Schwartz et al., 2007]. The patients presented with the specific set of minor facial anomalies (long narrow face, prominent tall forehead, prominent nasal bridge, highly arched palate, and small mandible), and nasal speech. Fryns and Buttiens described two pairs of two brothers who presented with similar clinical features [Fryns and Buttiens, 1987]. The authors suspected an X-linked intellectual disability (XLID). Later a distinct behavior profile associated with extreme was added to the typical feature of LFS [Van and Fryns, 2006]. Hence, the term Lujan-Fryns-syndrome (LFS) was coined referring to XLID in combination with marfanoid habitus. The OMIM database indicates *MED12* as the gene whose mutations are responsible for LFS (OMIM #309520) [Schwartz et al., 2007]. Tarpey et al. [2007] identified mutations in *UPF3B* in three families clinically diagnosed with LFS and in one family with FG syndrome. Raymond et al. [2007] identified mutations in *ZDHHC9* in three families with XLID and marfanoid habitus.

We collected DNA samples from 28 unrelated males with a tentative clinical diagnosis of LFS. In order to identify the underlying genetic aberrations we applied Sanger sequencing of *UPF3B*, *MED12*, and *ZDHHC9*, molecular karyotyping, and next generation sequencing of 7,591 coding exons of the X-chromosome. Not all patients were tested to the same extent.

MATERIALS AND METHODS

See Supplement 2.

RESULTS

Clinical Data

Inclusion criteria for this study were intellectual disability with a marfanoid habitus in male patients but absence of the diagnostic criteria of Marfan syndrome [Loeys et al., 2010] and low systemic score. All the patients were referred with a tentative clinical diagnosis of LFS. Table I summarizes the clinical features of 18 individuals of our cohort as well as of the carriers of pathogenic mutations in *MED12*, *UPF3B*, and *ZDHHC9* from previous publications. All 18 patients with the available clinical data had metabolic screening including serum and urine amino acids with normal results. 10 remaining patients presumably had amino acid screening as well (since this is a standard test during the diagnostic workup for ID patients that usually precedes genetic testing).

Sequencing Results

In 28 individuals with a clinical diagnosis of LFS we screened for mutations in *MED12*, *UPF3B*, and *ZDHHC9* by conventional Sanger sequencing. In seven cases we analyzed every single exon of all three genes. In 21 individuals *ZDHHC9* and *UPF3B* were sequenced completely while for *MED12* sequence analysis was restricted to exons 21 and 22 as these two exons comprised the sites of all previously reported mutations [Schwartz et al., 2007; Graham et al., 2008] (Table II). As a result of this effort we found two intronic variants in *UPF3B* (NM_080632.2:c.470-54_470-52delTCT) and *ZDHHC9* (NM_001008222: c.167-46_167-39delATATAGG), each in one individual only. Being intronic and sufficiently far away from splicing sites, we did not rank them as disease-causing mutations. In summary, we did not find any mutation in these three genes that we would consider pathogenic.

Twenty-seven individuals showed a marfanoid habitus but did not meet the minimum Ghent score for the diagnosis of Marfan syndrome. Accordingly, no molecular diagnostic for this trait was applied. For one individual mutation in *FBN1*, *TGFBR1*, and *TGFBR2* were excluded.

Molecular Karyotyping Results

Array CGH was applied in 11 patients and 1 patient was analyzed in an external laboratory. DNA qualities of the remaining 16 samples were insufficient for array CGH. In one individual, only frequent variants (benign polymorphisms) were found. All others had copy number variants (CNVs) that were either unknown, suspected to be pathogenic, or certainly pathogenic (Table III). These classifications were made after comparing our data set with those available in different databases such as The Database of Genomic Variants (mainly benign variants), ISCA (all types of CNVs with a rating from the uploader), DECIPHER (mainly unknown and pathogenic CNVs), and ECARUCA (mainly microscopically visible aberrations) [Feenstra et al., 2006; Firth et al., 2009; Macdonald et al., 2014]. Additionally, we compared the set of CNVs found in the patients to our in-house database which contains entries from 930 individuals.

EVALUATION OF ARRAY CGH RESULTS

Benign CNVs/ CNVs of Unknown Pathogenicity

One patient showed only known polymorphisms or benign variants. Five patients carried CNVs of unknown pathogenicity (Table III; detailed information in supplementary note).

Pathogenic CNVs

Patient 5436 carried a heterozygous deletion on 22q13. This aberration affected *SHANK3* and neighboring genes. Haploinsufficiency of *SHANK3* is a known cause of Phelan-McDermid syndrome (OMIM #606232 [Durand et al., 2007; Gauthier et al., 2010; Phelan and McDermid, 2012]).

Two duplications were found in the genome of propositus 15975. One of them was located on chromosome12 comprising

TABLE I. Clinical Features of 18 Individuals in Comparison With the Carriers of Pathogenic Mutations in MED12, UBF3B, and ZDHHC9 (The Remaining 10 Individuals From our Cohort Were Referred for Genetic Testing With a Tentative Diagnosis of LFS but Without Detailed Clinical Information)

Clinical features	Present cohort (carrier/information available)	MED12 mutation, LFS cohort (Schwartz et al., 2007), (Callier et al., 2013)	UPF3B – LFS cohort (Laumonier et al., 2010; Tarpey et al., 2007) – S98	ZDHHC9 (Masarel-Paulet et al., 2014; Raymond et al., 2007)
ID	18/18	8/8	12/12	10/10
Marfanoid habitus ^a	18/18	6/8	8/11 ^c	6/10
Macrocephaly/large head (>75th centile)	8/18 ^b	4/7 ^b	5/11	2/10
Behavioral disorders	10/18	7/8	4/12	2/10
Tall stature (>97thcentile/ +>2SD)	9/18	4/7	2/12	3/10
Nasal speech	5/14	3/8	4/12	0/10
Long, narrow face	14/18	7/8	6/12	3/10
Prominent nasal bridge	12/18	7/8	6/12	2/10
High and narrow palate	9/18	5/6	6/12	0/10
Short philtrum	7/18	7/8	0/12	0/10
Micrognathia/ retrognathia	6/18	5/8	0/12 ^d	0/10
Chest deformity	5/18	2/8	5/12	3/10
Agenesis of corpus callosum	1/9	3/5	2/5	2/2
Cardiac defect	6/18	0/8	1/12	0/10
Seizures	4/15	2/8	0/12	1/10
Hypotonia	5/18	2/8	3/12	3/10

^aDefined as asthenic build with slender long extremities (excess of span over height, although span/height ratio not obligatory >1.05), but with negative wrist and/or thumb sign, and low systemic score for Marfan syndrome.

^bOne patient was reported as microcephalic (OFC < 3rd centile).

^cDefined as slender build in Tarpey et al. span/height ration reported in four patient and was normal.

^dFour patients reported with prominent jaw.

ALG10B and *CPNE8*. The impact of this variant is unknown. The other duplication detected on chromosome 16 is likely to be the cause of ID in this individual. It is known as the cause of 16p11.2 duplication syndrome (OMIM #614671) initially discovered in individuals with autism. Later on, this variant was also found in ID [Fernandez et al., 2010; Shinawi et al., 2010]. The habitus may also

be influenced by this variant with duplication carriers on average being thinner than the general population [Jacquemont et al., 2011]. Parental DNA for segregation analyses was not available in patient 15975.

Likely Pathogenic CNV

We identified two variants that appeared to be likely pathogenic. Patient 14952 carried a duplication on chromosome 16p11.2 which was different from the one found in propositus 15975. The region is flanked by segmental duplications and recurrent deletions have been reported to be associated with developmental delay and obesity [Bachmann-Gagescu et al., 2010]. The DECIPHER and ISCA databases confirm the expectation that the duplication of the region itself is also a recurrent event (Suppl. Fig. S2). The reported phenotypic appearance of duplication carriers varies from unaffected states (in parents) to syndromic states with autism, developmental delay, ID, facial and skeletal abnormalities, hearing impairment, microcephaly, and seizures. Similar to other microduplications, duplications at 16p11.2

TABLE II. Overview of Sanger Sequencing, x, Analysis was Carried out

Number of individuals	ZDHHC9 {9 exons}	UPF3B {11 exons}	MED12 {45 exons}	MED12 {exons 21 and 22}
7 ^a	x	x	x	
21	x	x		x

No Disease Causing Mutation was Detected.

^aThe entire X-exome was additionally analyzed in four of these individuals.

TABLE III. Molecular Karyotypes [hg19] of 12 Individuals Diagnosed With LFS

Individual	Platform*	Array CGH result [hg19] according to ISCN* nomenclature	Pathogenic CNVs	NGS
3938	1	arr 3q22.3[138,213,075–138,825,057]x3pat	Unknown	Yes
5436	2	arr 22q13.31q13.33[44,574,802–51,224,402]x1dn	Yes, OMIM #606232	No
6942	2	arr 2q31.2[178,549,945–178,577,359]x3pat, Xq21.2[85,801,865–85,834,994] x2mat	Unknown	Yes
13847	2	arr 11q13.1[63,998,912–64,035,535]x1pat, 22q12.3[33,025,202–33,159,102] x1pat	Unknown	Yes
14952	1	arr 16p11.2[28,824,594–29,044,917]x3	Likely	No
14960	2	arr 2q37.1[233,013,128–233,036,991]x3mat	Unlikely	No
15961	1	arr [1-22] x2, (XY)x1	No	Yes
15970	1	arr 18p11.32[85,432–861,334] x3, 18p11.32[2,830,218–2,908,341]x1	Unknown	No
15972	1	arr 4q13.3[75,357,826–75,534,551] x1, 6p25.1p24.3[7,019,716–7,282,298] x3, 6p22.1[27,100,342–27,107,586] x3, 16p13.3[841,466–882,321]x3	Unknown	No
15975	1	arr 12q11q12[37,983,386–39,555,002] x3, 16p11.2[29,307,833–30,332,722]x3	Yes OMIM #614671	No
20594 ¹	3	arr 12p13.31[6,736,798–7,241,598]x3dn	Likely	No

Molecular karyotyping was carried out on Agilent CGH arrays: ¹design 14963 [244 k], ²design 21850 [2 × 400 k], ³design 14968 [2 × 105 k]. Only unknown, rare, or pathogenic copy number variants are listed. If parental information was available, the karyotype was amended by “mat”=maternal, “pat”=paternal, or “dn”= de novo (Please refer to Supplement 1 including affected genes and available detailed clinical information).

*International Standing Committee on Human Cytogenetic Nomenclature. et al., 2013.

appear to be quite variable in their penetrance and expression [Firth, 1993; Brunetti-Pierri et al., 2008].

A heterozygous de novo duplication on chromosome 12 was detected in patient 20594. Although this variant is rather small (~500 kb) it comprises 35 genes and thus may be involved in the pathogenesis of the patient’s features. The databases do not include any comparable duplication. The smallest overlapping duplication in DECIPHER (249483) is about 6 times as large and contains 99 genes and the smallest overlap in ISCA is about ten times as large.

NEXT GENERATION SEQUENCING (NGS) RESULTS

In four patients with unremarkable or familial copy number variants we sequenced all coding regions on the X chromosome (Table IV). In total, we found five single nucleotide variants with unknown pathogenicity. None of them have been described before. All of them were inherited from the mother as confirmed by Sanger sequencing.

Evaluation of Single Nucleotide Variants From NGS Analyses

In individuals 13847 and 3938 we found only previously reported polymorphisms.

In the X-exome of individual 6942 we detected three variants that were previously not reported as polymorphisms. Two of them were located within *LANCL3* and *SLC16A2* that are known to relate to XLID [Visser et al., 2013]. However, both variants were also detected in an unaffected uncle and thus were unlikely to be pathogenic (Fig. 1). The third variant, NM_024528.3:c.998G>A, p.Arg333Gln. (NG_021260.1:g.18682G>A), was found in *NKAP* and had a CADD PHRED like score of 26.4 (recommended cut off for potentially pathogenic variants is >15; [Kircher et al., 2014].

NKAP seems to be involved in T cell development [Pajeroski et al., 2009]. Immunodeficiency was not observed in the carrier of this variant.

Two unknown variants were detected in individual 15961, NM_012084.3:c.1576A>G (NG_016456.1:g.6653A>G) in *GLUD2* which was also detected in the unaffected brother, thus not being likely to cause ID (Fig. 1) and NM_001170649.1:c.646A>T (NG_016369.1:g.10060A>T) in *LASIL* which had a very low CADD PHRED like score of 0.17 predicting a “benign” effect. Although this variant is not listed in Exome Aggregation Consortium (ExAC: Cambridge, MA (URL: <http://exac.broadinstitute.org>); accessed April 2015), a different nucleotide change at the same position NM_001170649.1:c.646A>G resulting in the same amino acid change (p. Asn216Asp) has been reported in ExAC with the frequency of 0.00002279 in two individuals. However, one of the carriers is male. Since several of the projects contributing to ExAC data were studying patients with autism, schizophrenia and bipolar disorders we cannot assume by default that the carrier of *LASIL:c.646A>G* variant is a healthy man. GeneMANIA (<http://www.genemania.org>) reports a physical interaction between the gene products of *LASIL* and *PNKP*. Mutations in the latter have been associated with microcephaly, seizures, and developmental delay [Reynolds et al., 2012]. Although this variant was predicted to be “benign” we cannot exclude that the *LASIL* variant in individual 15961 has an impact on that interaction. However, it appears unlikely that this variant alone is causative for the phenotype.

DISCUSSION

LFS is defined as a syndromic XLID associated with marfanoid stature, distinct facial features (long narrow face, small mandible

TABLE IV. X-exome Sequencing Data From Four Individuals

No.	Gene	Variant of unknown significance	CADD score	Mat. inherit.
3938		<i>n/a</i>		
6942	<i>LANCL3</i>	NM_198511.2:c.504C>G NG_016368.1:g.5806C>G [p.Asp168Glu]	19.85 ^b	Yes
	<i>SLC16A2</i>	NM_006517.3:c.1711G>A NG_011641.1:g.115173G>A p.[Val571Ile]	23.1 ^b	Yes
	<i>NKAP</i>	NM_024528.3:c.998G>A NG_021260.1:g.18682G>A [p.Arg333Gln]	26.4 ^b	Yes
13847 ^c		<i>n/a</i>		
15961	<i>GLUD2</i>	NM_012084.3:c.1576A>G NG_016456.1:g.6653A>G [p.Met526Val]	0.002 ^a	Yes
	<i>LAS1L</i>	NM_001170649.1:c.646A>T NG_016369.1:g.10060A>T [p.Asn216Asp]	0.17 ^a	Yes

This table shows previously unreported variants and CADD PHRED like scores of each variant:

^aBenign [CADD PHRED like score <15]

^bDeleterious [CADD PHRED like score >15]. Dark grey background indicates variants that were not found in male relatives.

^cAccording to Kirchner et al., 20144, see previous page.

and prominent forehead), and behavior problems [Van and Fryns, 2006]. Notably, the diagnosis of LFS is often used as a definition of syndromic ID with marfanoid habitus not taking into account other specific features present in the original reports. In a cohort of 28 males clinically diagnosed with LFS we did not find any pathogenic mutation in the genes that have previously been reported with this syndrome.

Table I compares the clinical features reported in the patients included in the present study with the clinical presentation in patients from the family originally reported by Lujan et al. with proven mutations in *MED12* as well as patients with mutation in *UPF3B* and *ZDHHC9*. The two consistent features present in all groups are intellectual disability and marfanoid habitus. However, the definition of marfanoid habitus differs between the studies. Tarpey et al. used the descriptive term slender build. Tall stature was reported for 9 out of 18 the individuals of our cohort. This feature was reported for 4 out of 7 individuals with a mutation in *MED12* while it was rarer in *UPF3B* (2/12) and *ZDHHC9* (3/10) mutation carriers. Moreover, *ZDHHC9* mutation carriers show neither the distinct facial phenotype nor the behavior abnormalities that were reported to be common in LFS. LFS typical facial features were also not present in the majority of patients with *UPF3B* mutations. Our cohort shows considerable overlap with the *MED12* mutation carriers except for several specific minor anomalies such as short philtrum, high narrow palate and small mandible. The more careful dysmorphological assessment of the male ID patients presented with marfanoid habitus may potentially increase the diagnostic yield of *MED21* mutations. Lack of LFS specific features in patients with *UBF3B* and *ZDHHC9* mutations suggests that these are rather two separate forms of XLID than a part of LFS spectrum.

A similar diagnostic approach was chosen in another recent study [Callier et al., 2013]. In a group of 100 individuals with “marfanoid syndromes and intellectual disability” the same genes (*MED12*, *UPF3B*, and *ZDHHC9*) were analyzed [Callier et al., 2013]. However, the inclusion criteria differed from criteria used in the present study. Patients with specific cardiac and ophthalmological features of Marfan syndrome were included into the cohort. Consequently, the genes *TGFBR1*, *TGFBR2*, and *FBN1* were sequenced to search for variants that might be responsible for the marfanoid phenotype/ Marfan syndrome. Five patho-

genic mutations in the Marfan syndrome gene *FBN1* and only one amino acid change with unclear consequences in *MED12* were identified [Callier et al., 2013]. Interestingly, one of the patients with *FBN1* mutation also carried a de novo microdeletion 17q21.31 that was the major ID cause. We strongly suspect that the remaining *FBN1* positive patients should have additional unrevealed changes responsible for the ID component. Therefore, we would conclude that patients with marfanoid habitus but lacking major criteria for Marfan syndrome do not generally require nor *FBN1* neither *TGFBR1/TGFBR2* testing.

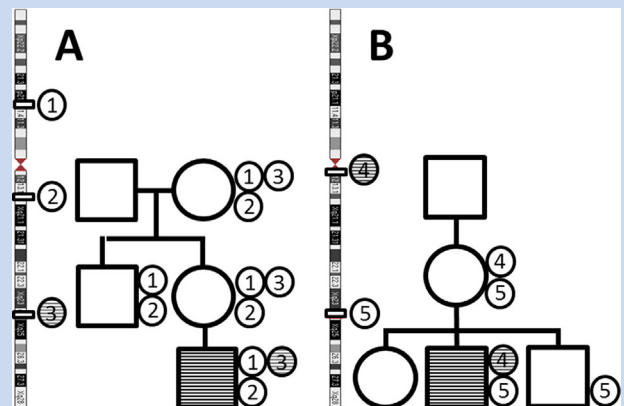


FIG. 1. Segregation analyses of variants detected by X-exome sequencing. All individuals in one pedigree were tested for the family-specific variants. Hatched square represents individuals affected with LFS; white circles depict mutations that are not disease causing because they appear in unaffected males; hatched circles indicate that pathogenicity in male carriers cannot be excluded. Numbers correspond to mutations. A: Individual 6942: [1] *LANCL3*, NM_198511.2:c.504C>G, p.[Asp168Glu]. [2] *SLC16A2*, NM_006517.3:c.1711G>A, p.[Val571Ile]. [3] *NKAP*, NM_024528.3:c.998G>A, p.[Arg333Gln]. B: Individual 15961: [4] *LAS1L*, NM_001170649.1:c.646A>T, p.Asn216Asp, [5] *GLUD2*, NM_012084.3:c.1576A>G, p.Met526Val. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>]

The limited clinical data presented for the patient with likely pathogenic *MED12* variant does not allow full comparison with the features of p.Asn1007Ser mutation carriers [Schwartz et al., 2007]. However reported long and thin habitus, pectus excavatum, joint laxity and hypernasal speech are features consistent with LFS.

Molecular karyotyping of Callier et al. group revealed 16 CNVs that were considered pathogenic. Twelve of those were previously known. 80 individuals had neither any pathogenic CNV nor a pathogenic mutation in any of the genes mentioned above. One of the CNVs was a de novo gain on chromosome 16p11.2 spanning both variants on 16p11.2 that we detected in patients 14952 and 15975. No other overlap exists with the CNVs reported in the present paper.

The 220 kb duplication on chromosome 16p11.2 that we detected in individual 14952 (arr[hg19] 16p11.2 (28,824,594–29,044,917)x3) may represent a novel microduplication syndrome. The ISCA and the DECIPHER databases host several similar entries at this region. Within the DECIPHER database these cases overlap with the 16p11.2p12.2 microduplication syndrome but they are rated “unknown” regarding their pathogenicity. It seems probable that the 220 kb duplication itself already represents a potentially pathogenic copy number variant. The phenotype of individuals carrying this aberration is highly variable ranging from unaffected parents across autism to developmental delay, ID, facial and skeletal abnormalities, hearing impairment, microcephaly, and seizures (according to ISCA and DECIPHER reports). A highly variable phenotype-genotype correlation has also been described for other microduplication syndromes [Wentzel et al., 2008]. The reciprocal event has previously been described and the deletion of this region has been associated with developmental delay and obesity [Bachmann-Gagescu et al., 2010]. In keeping with the genotypic reciprocity, obesity is reciprocal to the slender build of individuals with marfanoid habitus. Notably, a correlation of microdeletion and microduplication with opposing body mass indices has been described before at the adjacent 16p11.2 region (OMIM #611913, #614671) [Jacquemont et al., 2011].

Microdeletion 22q13.3 (Phelan-McDermid syndrome) was revealed in one patient of our cohort (Fig. 2B). To our knowledge, neither Phelan-McDermid syndrome has been discussed as a possible differential diagnosis of LFS [Van and Fryns, 2006], nor has LFS been proposed as differential diagnosis for microdeletion 22q13.3. However, another *MED12*-related disorder – FG syndrome – was considered to have a certain overlap with Phelan-McDermid syndrome [Phelan and Rogers, 2005]. Analyzing the clinical features present in >75% of patients with Phelan-McDermid syndrome (hypotonia, global developmental delay, autism/autistic-like behavior, and especially accelerated growth) as well as less frequent features, like high-arched palate and seizures [Phelan and Rogers, 2005], it becomes clear that the condition shows certain overlap with LFS which should be kept in mind during the diagnostic work-up.

We also applied X-exome sequencing to identify the causes of the syndromic ID in four individuals. Although we identified five variants that were not known as polymorphisms, two of which being predicted to be potentially pathogenic (Table IV), segregation analyses involving unaffected male relatives made three var-

iants from the list of candidates unlikely to be associated with the phenotype. The two variants that we detected in *NKAP* and *LAS1L* deserve further attention as potential causes of XLID. Furthermore, we found two variants that were recorded as single nucleotide variants in dbSNP but have low allele frequency as it seems and they were only detected in female individuals. One of the affected genes was *CCDC22*. A missense variant in *CCDC22* has recently been named as cause of ID with features of Ritscher-Schinzel/3C syndrome in male individuals [Kolanczyk et al., 2014]. While this might explain the ID, our patient does not fit well with the described clinical presentation. *CCDC22* mutations have been described in patients with Dandy-Walker malformation and heart defects (ASD/VSD), therefore classified as having Ritscher-Schinzel/3C syndrome. Patient 3938 carrying *CCDC22* variant presented with microcephaly, tall stature with marfanoid habitus and mitral valve prolapsed and did not show any brain malformations. Whether the missense variant in *SYTL4* has a potential impact on the clinical presentation of the affected individual needs further evaluation.

It has been estimated that 25–30% of male ID cases relate to the X-chromosome [Lisik and Sieron, 2008]. More recent publications presented lower estimates of 16%, 10%, and 5–10% [Stevenson and Schwartz, 2009; Lubs et al., 2012; Rauch et al., 2012]. The study of Callier et al. comprised 27 female and 73 male individuals. It seems that the phenotypic appearance of marfanoid habitus and ID is more frequently diagnosed in males than in females. However, as far as we are aware no unbiased data exists estimating whether ID with marfanoid habitus is more frequent in males than in females.

Callier et al. concluded from this observation that the causative mutation of their patients is likely to be located on the X-chromosome. However, among the 20 aberrations that they regarded as causative only one (*MED12* variant of unclear pathogenicity) was located on the X-chromosome. In 128 individuals with ID and marfanoid habitus (Callier et al. and this work) only CNVs at 16p11.2 appeared to be recurrent.

In conclusion, our data suggest that the search for a suspected X-linked cause may fail—even in a cohort of only male individuals, if there is no clear X-linked segregation.

In keeping with others we think that the diagnosis of LFS should be restricted to cases of ID, marfanoid habitus, and distinct facial features (long narrow face with high prominent forehead, high nasal bridge, high palate and micrognathia) that present with a mutation in one of the LFS-related genes or obvious X-linked segregation [Schwartz et al., 2007]. Till now only one gene—*MED12*—has been associated with LFS phenotype, as it was defined in the original reports. Expanding LFS to the broad spectrum of ID with marfanoid habitus leads to inaccurate genetic counseling and results, as shown by our study, in a low diagnostic yield of the genetic testing. The presence of marfanoid habitus in a male patient with ID might increase the alert regarding the possible X-linked inheritance. However, if detailed family history does not indicate a clearly X-linked segregation we propose a standard diagnostic procedure that starts with molecular karyotyping [Miller et al., 2010]. Indeed, a microduplication 16p11.2 is an important differential diagnosis in patients with ID and marfanoid habitus. If this is unsuccessful,

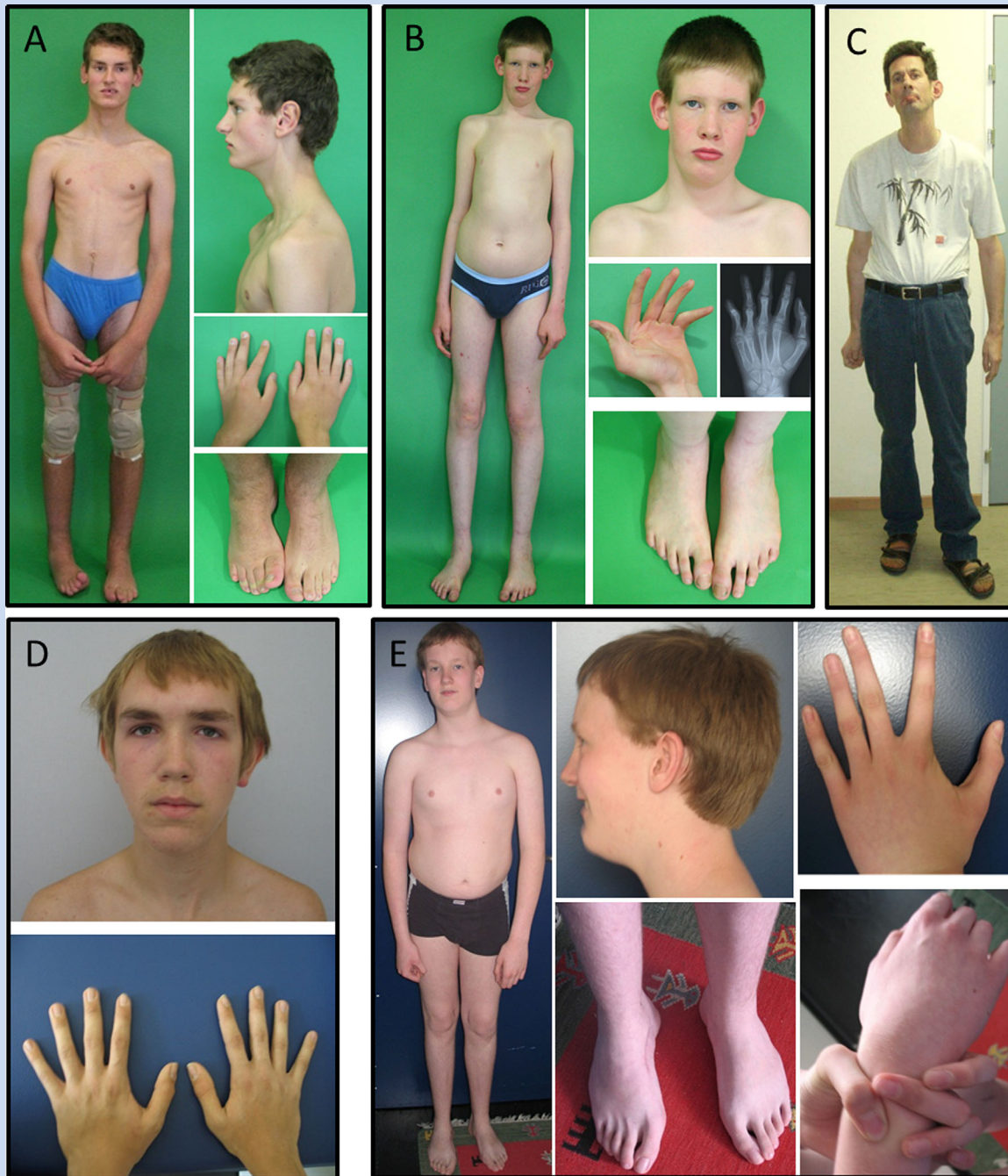


FIG. 2. Individuals with initial clinical diagnosis of LFS. **A:** Individual 5436 bears a de novo deletion on chromosome 22 that causes Phelan-McDermid syndrome [OMIM #606232, *SHANK3* haploinsufficiency]. **B:** Individual 6942 who carries two missense mutations *NKAP*. **C:** Individual 20594 has a de novo duplication on chromosome 12p13 that might be causative for his clinical presentation. **D:** Individual 14952 carries an apparently recurrent duplication on chromosome 16p11.2 that might account for a new microduplication syndrome [Suppl. Fig. 2]. **E:** Individual 14960 has a maternally inherited duplication on chromosome 2q37 that is most probably not related to his clinical presentation. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>]

next generation sequencing should be applied if realizable, carried out either as sequencing of the entire exome and applying filters that analyze only disease related genes [Neveling et al., 2013] or as targeted enrichment of only a subset of genes in which mutations might account for a specific disorder.

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mic structural variation (dbVar, www.ncbi.nlm.nih.gov/dbvar/), study nstd37. Samples and associated phenotype data were provided by ISCA Consortium member laboratories.

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