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RESEARCH ARTICLE

A mosaic intragenic microduplication of *LAMA1* and a constitutional 18p11.32 microduplication in a patient with *keratosis pilaris* and intellectual disability

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The application of array-based comparative genomic hybridization and next-generation sequencing has identified many chromosomal microdeletions and microduplications in patients with different pathological phenotypes. Different copy number variations are described within the short arm of chromosome 18 in patients with skin diseases. In particular, full or partial monosomy 18p has also been associated with *keratosis pilaris*. Here, for the first time, we report a young male patient with intellectual disability, diabetes mellitus (type I), and *keratosis pilaris*, who exhibited a de novo 45-kb microduplication of exons 4–22 of *LAMA1*, located at 18p11.31, and a 432-kb 18p11.32 microduplication of paternal origin containing the genes *METTL4*, *NDC80*, and *CBX3P2* and exons 1–15 of the *SMCHD1* gene. The microduplication of *LAMA1* was identified in skin fibroblasts but not in lymphocytes, whereas the larger microduplication was present in both tissues. We propose *LAMA1* as a novel candidate gene for *keratosis pilaris*. Although inherited from a healthy father, the 18p11.32 microduplication, which included relevant genes, could also contribute to phenotype manifestation.

KEYWORDS

18p11.32 microduplication, developmental delay, diabetes mellitus, *EMILIN2*, intellectual disability, *keratosis pilaris*, *LAMA1*, *LPIN2*, *METTL4*, mosaic CNVs, *NDC80*, *SMCHD1*

1 | INTRODUCTION

Keratosis pilaris is the most common form of follicular keratosis, a group of hereditary disorders of hair follicle keratinization characterized by follicular inflammation and subsequent atrophy (Liakou, Esteves de Carvalho, & Nazarenko, 2014). The disease occurs with a frequency of 2–20% among children and is found in a mild form in approximately half of the population. It may be present alone or as part of a syndromic phenotype (e.g., Noonan, Greither, and Down syndromes) but not as a dominant feature. *Keratosis pilaris* is often described in association with other dry skin conditions, such as ichthyosis vulgaris, xerosis, and atopic dermatitis.

Different chromosomal alterations, including deletions of the chromosomal regions 12q21-q22 (*BTG1*) (Al-Maawali et al., 2014) and 17q21.31 (*KANSL1*) (Wright, Donnai, Johnson, & Clayton-Smith, 2011), and the genes *LAMA1* (18p11.31) (Zouboulis, Stratakis, Gollnick, & Orfanos, 2001) and *LRP1* (12q13.3) (Klar et al., 2015) have been considered to be associated with *keratosis pilaris*, suggesting genetic heterogeneity for the disease. Five reports indicate an association of full or partial 18p monosomy with *keratosis pilaris* (Carvalho, Carvalho, Kiss, Paskulin, & Götze, 2011; Fiorentini, Bardazzi, Bianchi, & Patrizi, 1999; Horsley et al., 1998; Nazarenko et al., 1999; Zouboulis et al., 1994).

Here, for the first time, we report a patient with intellectual disability, diabetes mellitus (type I), and *keratosis pilaris* who exhibited a de novo intragenic microduplication of *LAMA1* exons 4–22, located at 18p11.31 and detected in skin fibroblasts, and a constitutional 18p11.32 microduplication of paternal origin containing the genes *METTL4*, *NDC80*, and *CBX3P2* and exons 1–15 of *SMCHD1*.

2 | CLINICAL REPORT

The patient (Figure 1a), a 14-year-old boy, was referred to the clinical geneticist for the first time because of intellectual disability. He is a single child of nonconsanguineous, healthy parents. His pedigree was unremarkable. Informed consent for the publication of clinical pictures was obtained from his parents.

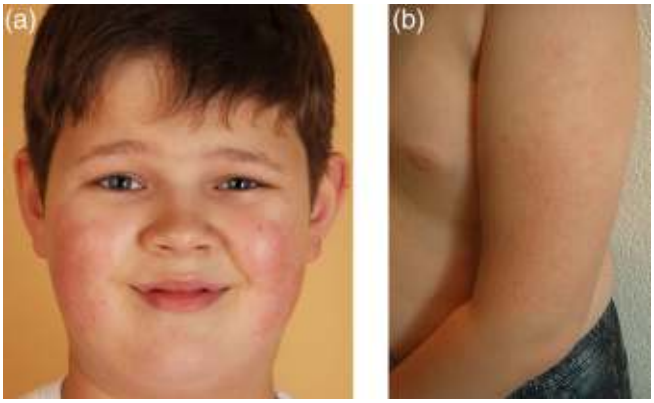

FIGURE 1 The patient at the age of 14 years (note the wide nasal bridge, narrow mouth, short neck, low anterior hairline, and follicular keratosis on the cheeks [a] and upper arm [b]) [Color figure can be viewed at wileyonlinelibrary.com]

The patient was born at the thirty-eighth week of gestation. His birth weight was 3,200 g (25th percentile), birth length was 54 cm (90–95th percentile), head circumference was 33 cm (5–10th percentile), and chest circumference was 31 cm (25th percentile). His Apgar score was 8. He was able to sit at the age of 6 months and walk independently at the age of 10 months. The boy did not speak until the age of 2 years.

The patient was a calm child. Constipation and hepatomegaly appeared with age. His father was treated for inflammatory bowel disease. When the father was 7 and 9 years old, capillary purpura was diagnosed and successfully treated without relapse.

At the age of 8 years, the boy's weight was 35 kg (95th percentile), height was 135 cm (90th percentile), and head circumference was 52 cm (50th percentile). He exhibited a wide nasal bridge, a narrow mouth, macrodontia, macrotia, a short neck, hypertelorism of the nipples, and pes planus. Atopic dermatitis and follicular keratosis were first noted on the cheeks and upper arms (Figure 1a,b). When the patient was tired, he complained about pain in his eyes and blinked frequently. His IQ was 45. The boy attended a special school but did not enjoy any subject. While answering questions, he threw up his hands. Aggression, signs of autism, and attention deficit hyperactivity disorder were observed. MRI findings were normal.

At the age of 14 years, the boy's weight was 48 kg (25–50th percentile), and his height was 149 cm (3–5th percentile). His body mass index was 21.62 kg/m^2 (75th percentile). Abnormal fat distribution and type I diabetes mellitus were diagnosed at this age. Clinical examination additionally revealed a low anterior hairline. Follicular keratosis was observed on the cheeks, the outer sides of the upper arms, and the hips and nates. The disease has progressed, as recorded in clinical examinations of the patient from 8 to 14 years, worsening in winter and improving in summer time.

3 | METHODS

3.1 | Cytogenetic analyses

Conventional cytogenetic analysis was performed based on GTG-banded metaphases from peripheral blood lymphocytes from the patient and his parents at a 400-band resolution. A primary culture of the patient's skin fibroblasts was obtained from two full-thickness skin biopsies. The biopsies were washed with Hanks solution containing antibiotics and antifungal agents twice and then treated with 0.2% collagenase in culture medium for 3 hr at 37 °C. The suspension was subsequently cultured in AmnioMAX culture medium. A confluent monolayer formed in 1 day.

3.2 | Array-based comparative genomic hybridization

Array-based comparative genomic hybridization (aCGH) was performed using the SurePrint G3 Human CGH Microarray Kit (4 × 180K) and the SurePrint G3 Human CGH Microarray Kit (2 × 400K) (Agilent Technologies, Santa Clara, CA), according to the manufacturer's recommendations. The patient's and reference DNA

(#5190-3796, Human Reference DNA, Agilent Technologies) were labeled and hybridized using enzymatic labeling and hybridization protocols (v. 7.3 Agilent Technologies). Array images were acquired with an Agilent SureScan Microarray Scanner (Agilent Technologies). Data were analyzed using CytoGenomics Software (v. 3.0) (Agilent Technologies), the publicly available Database of Genomic Variants (DGV), and the Database of Chromosomal Imbalance and Phenotype in Humans employing Ensembl Resources (DECIPHER). Annotations of genes located within the region of genomic imbalance were retrieved from the NCBI Gene Database, OMIM, and the literature.

3.3 | Confirmation of CNV using quantitative real-time PCR

Target sequences within and outside of the duplicated chromosomal regions and specific amplification primers for quantitative real-time PCR assays were selected using Primer3 software (Tables S1, Supporting Information). The presence of 18p11.31 and 18p11.32 microduplications was tested in the genomic DNA from peripheral blood lymphocytes from the patient and his parents as well as in cultured skin fibroblasts from the patient using the AriaMx Real-Time PCR System (Agilent Technologies). The reference genomic DNA was obtained from peripheral blood lymphocytes and skin fibroblasts of a healthy donor. The reference gene was *HEXB*, encoding the beta subunit of hexosaminidase and located at 5q13 (Tables S1).

3.4 | Genomic DNA preparation and whole-exome sequencing

Genomic DNA was extracted from primary culture of skin fibroblasts obtained from the patient using phenol-chloroform extraction. In addition, two genomic DNA samples were obtained from different tissues of a healthy male adult using the same method (hereafter referred as Control Samples 1A and 1B). Paired-end sequencing libraries were prepared using the SureSelect Human All Exon V6 r2 Kit according to the manufacturer's instructions (Agilent Technologies). All three libraries were sequenced on an Illumina NextSeq system generating 58–69 million paired 2 × 151 bp reads per sample.

3.5 | Whole-exome sequencing data analysis

Whole-exome sequencing data were aligned to the hg19 human genome assembly using Bowtie2 software (Langmead & Salzberg, 2012) with default options. To call variants, we used bcftools mpileup and call function with filter applied -e "%QUAL<20 || DP > 1000 || DP < 5." The resulting vcf file was submitted to Ensemble GRCh37 VEP application (McLaren et al., 2016) to annotate variants. The parameters of VEP were all default except: (a) «RefSeq transcripts» were used as the transcript source, (b) all available databases were used to identify known variants, and (c) the "exclude common variants" filter was applied.

To call copy number variations (CNVs) from whole-exome sequencing data, we used two packages, Control-FREEC (Boeva et al., 2012) and EXCAVATOR2 (D'Aurizio et al., 2016), both with default parameters. We used data from two samples from a healthy donor as controls in this analysis, which resulted in two sets of predicted CNVs. Although these sets were highly overlapping, they varied in some cases. As a target region, we used S07604514 Agilent bed track, which corresponded to that of the enrichment kit utilized during library preparation.

We also manually analyzed duplication breakpoints. The tandem duplication breakpoint may be enriched by read pairs with abnormal insert size and/or wrong mate orientation (FF or RR), depending on the structure of duplication (head-to-tail, head-to-head, or tail-to-tail). To check these options, we: (a) observed all reads aligned near duplication breakpoints in the IGV browser (http://software.broadinstitute.org/software/igv/) and visualized reads with abnormal insert size/pair orientation; (b) extracted all reads with insert size >10 kb using awk and manually analyzed these reads (~30 read pairs in total); and (c) extracted all read pairs where both reads mapped to the same strand using the SAMtools view command with flags -F 12 -F 48 for FF orientation or -F 12 -F 48 for RR orientation. Next, we calculated coverage tracks of FF, RR, or all reads for chromosome 18 using the SAMtools depth command, binned the coverage tracks using 10 kb windows and compared the obtained tracks to find potential regions enriched with FF or RR reads. All calculations were performed on Novosibirsk State University High-Throughput Computing Cluster (http://www.nusc.ru/) and Computational Node of Institute of Cytology and Genetics.

3.6 | Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed with PCR-based probes for the *SMCHD1*, *METTL4*, and *LAMA1* genes in cultured skin fibroblasts from the proband following the standard protocol. DNA probes for FISH were generated using a long-range PCR kit (BioLabMix, Novosibirsk, Russia) (Tables S1). The generated PCR fragments were then labeled with TAMRA-dUTP and fluorescein-dUTP (BioSan, Novosibirsk, Russia) via nick translation.

3.7 | Expression analysis

Total RNA was extracted from primary cultures of skin fibroblasts obtained from the patient and three healthy donors using the RNeasy Plus Mini Kit (QIAGEN, Hilden, Germany). The RNA was reverse transcribed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA).

All amplification reactions were performed using the AriaMx Real-Time PCR System (Agilent Technologies) and BioMaster HS-qPCR (BioLabMix). The primers and TaqMan probes employed in these assays are listed in Tables S1. The thermal cycling conditions consisted of an initial denaturation step at 95 °C for 7 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 30 s. The experiments were performed in triplicate for each data point. Relative quantification was performed using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001). The *ACTB* and *GAPDH* genes were employed as internal controls.

The experimental studies were performed in the Medical Genomics Core Facility of the Research Institute of Medical Genetics, Tomsk NMRC RAS.

4 | RESULTS

Metaphase analysis of G-banded chromosomes from peripheral blood lymphocytes showed a normal karyotype for the patient and his parents. Microarray analysis using an Agilent 180K microarray revealed an 18p11.32 microduplication of 428.7 kb in size: arr[hg19]18p11.32 (2,275,610-2,704,317)×3 (Figure 2a,b). The microduplication involved the full *METTL4* and *NDC80* genes, the pseudogene *CBX3P2*, and exons 1–13 of *SMCHD1*, which was disrupted in intron 13. The microduplication was confirmed via quantitative real-time PCR analysis, and it was shown to be inherited from the healthy father (Figure 2c).

FISH with probes for the *SMCHD1* and *METTL4* genes revealed that the duplicated region was located on the short arm of chromosome 18 (Figure 3). The expression of the duplicated *METTL4* and exons 6/7 of *SMCHD1* (inside the duplication) genes was increased 1.5- to 3-fold in the patient compared with that of healthy individuals, whereas *NDC80* gene expression was within the normal range (Figure 4). The expression of exons 15/16 of *SMCHD1* (the breakpoint is inside the transcript) was similar to control values. These three genes are not directly associated with skin disorders; however, we postulate that they may regulate the expression of two other candidate genes for *keratosis pilaris*: *EMILIN2* and *LPIN2*. We found that *EMILIN2* was down-regulated (2-5-fold), whereas the level of *LPIN2* expression was unaffected (Figure 4). Similar microduplications and microdeletions were present in DGV and DECIPHER (nos. 266406 and 272853). Unfortunately, the clinical descriptions of the DECIPHER patients are not available.

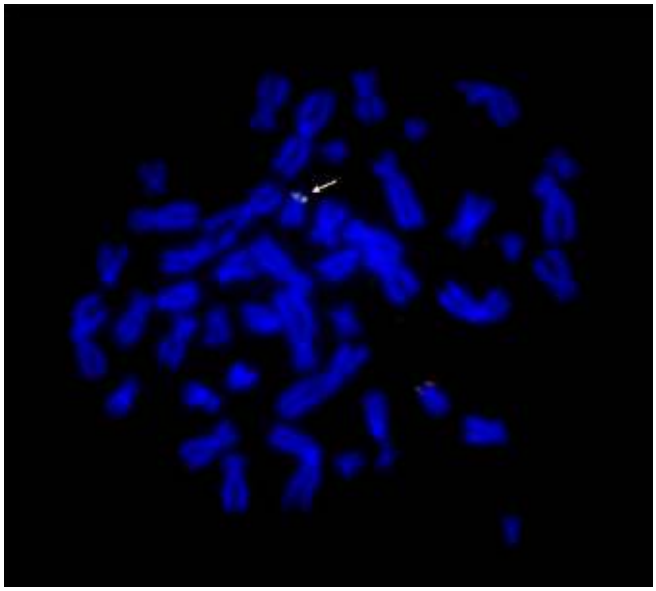

FIGURE 3 Fluorescence in situ hybridization analysis using PCR-based probes for *SMCHD1* (red) and *METTL4* (green) in cultured skin fibroblasts of the proband. Chromosome 18 with microduplication is indicated by an arrow [Color figure can be viewed at wileyonlinelibrary.com]

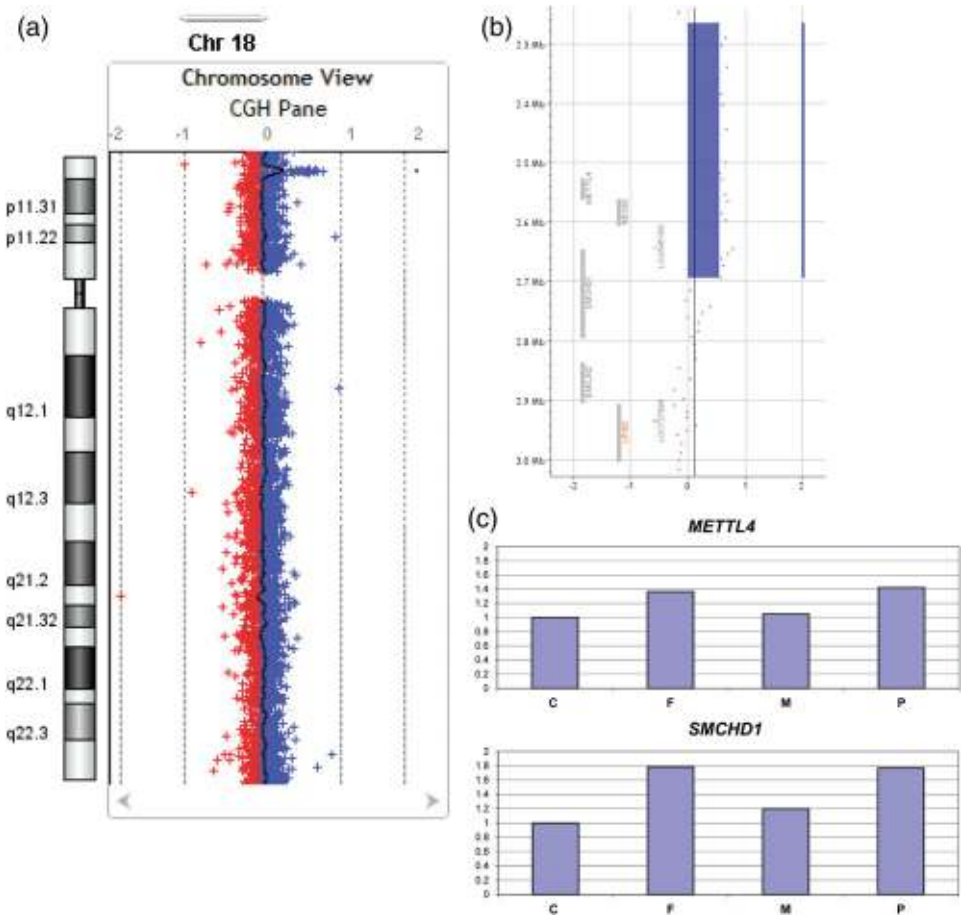

FIGURE 2 An array-based comparative genomic hybridization (aCGH) image of chromosome 18 in the lymphocytes of the patient (a); an aCGH image of the duplicated 18p11.32 region, including the involved genes (b); and the results of quantitative real-time PCR analysis: C, control; F, father; M, mother; and P, proband (c) [Color figure can be viewed at wileyonlinelibrary.com]

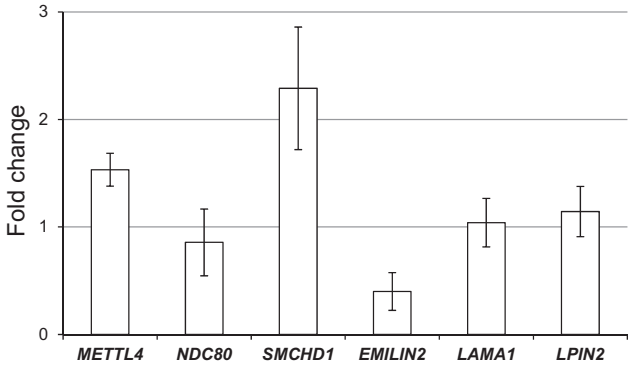

FIGURE 4 Expression analysis of the *METTL4*, *NDC80*, *SMCHD1*, *EMILIN2*, *LAMA1*, and *LPIN2* genes in cultured skin fibroblasts

Due to the development of follicular keratosis, skin fibroblast DNA was further investigated using an Agilent 400K microarray. A 45-kb 18p11.31 microduplication of *LAMA1* exons 4–22 was identified (arr[hg19]18p11.31(7,015,750-7,060,714)×3) (Figure 5a), together with a 432-kb 18p11.32 duplication (arr[hg19]18p11.32(2,275,610-2,707,594)×3) including *METTL4*, *NDC80*, *CBX3P2*, and exons 1–15 of the *SMCHD1* gene. The difference in size of the 18p11.32 duplications determined in lymphocytes and skin fibroblasts of the patients was due to the median aCGH probe spacing in Microarray Kits 4 × 180K and 2 × 400K, which were ~13 and ~5.3 kb, respectively (Agilent Technologies). The borders of microduplication were further refined by exome sequencing. According to the control samples used, they were

1. seq[GRCh37] dup(18)(p11.32): chr18:g. 2239760_2707794dup (EXCAVATOR2, Control Sample 1A, 468,034 kb in size) or

2. seq[GRCh37] dup(18)(p11.32): chr18:g. 2199760_2707794dup (EXCAVATOR2, Control Sample 1B, 508,034 kb in size).

Real-time PCR confirmed the presence of the larger microduplication (data not shown) and the intragenic *LAMA1* microduplication in skin fibroblasts from the patient (Figure 5e), but the *LAMA1* microduplication was not present in lymphocytes of either the patient or his parents (Figure 5f). Duplication breakpoints were not identified by the Control-FREEC and EXCAVATOR2 packages, indicating that the breakpoint occurred in an interexonic region that was not covered by whole-exome sequencing. The number of copies of exons 2 and 58 of *LAMA1* was normal outside the microduplication, as shown by real-time PCR (Figure 5e). FISH analysis with a *LAMA1*-specific PCR-based

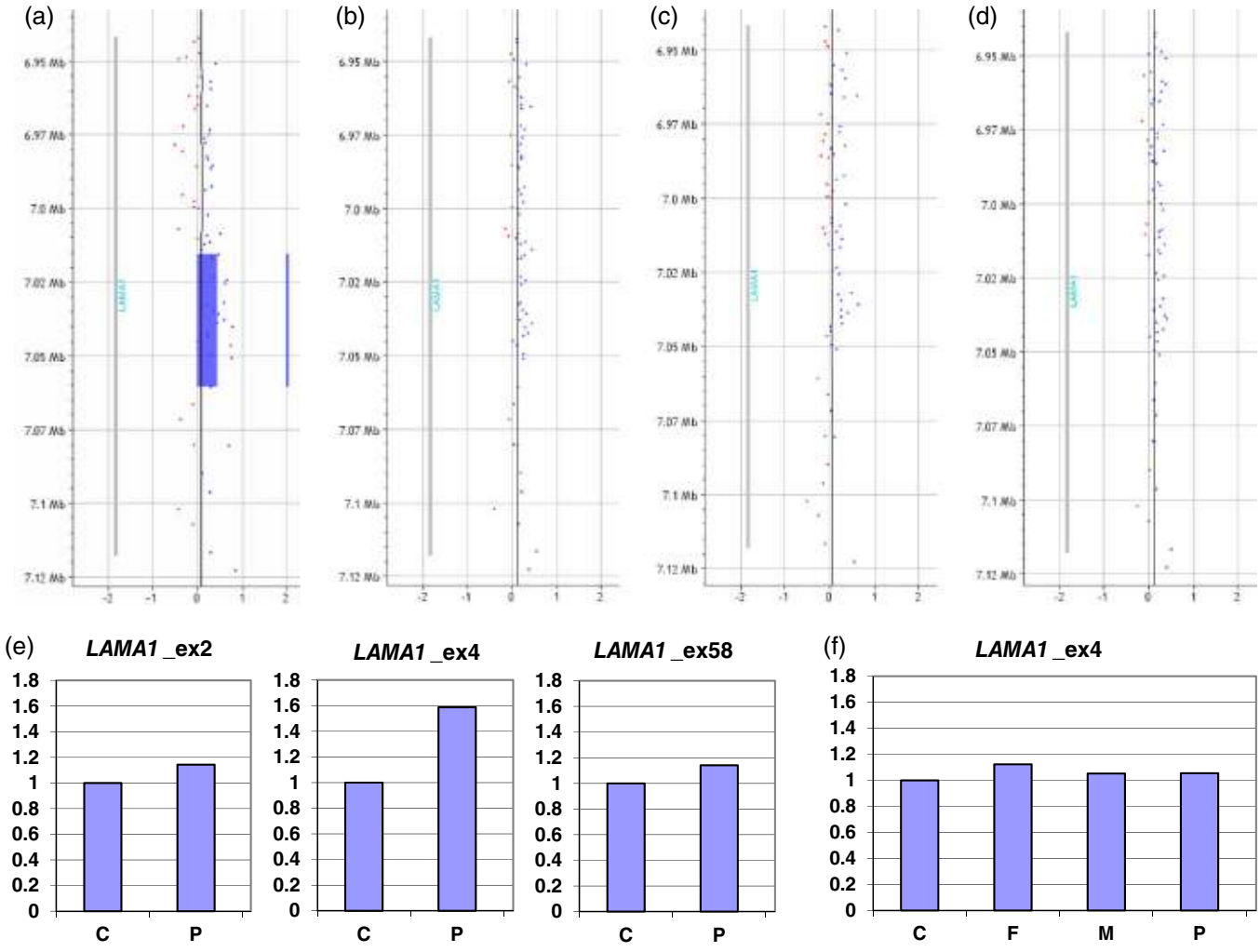

FIGURE 5 An array-based comparative genomic hybridization (aCGH) image of the microduplication of *LAMA1* in skin fibroblasts of the patient (a) and aCGH images of the normal numbers of copies of *LAMA1* in peripheral blood lymphocytes of the father (b), mother (c), and patient (d); results of quantitative real-time PCR analysis in skin fibroblasts (e) and lymphocytes (f): C, control; F, father; M, mother; and P, proband [Color figure can be viewed at wileyonlinelibrary.com]

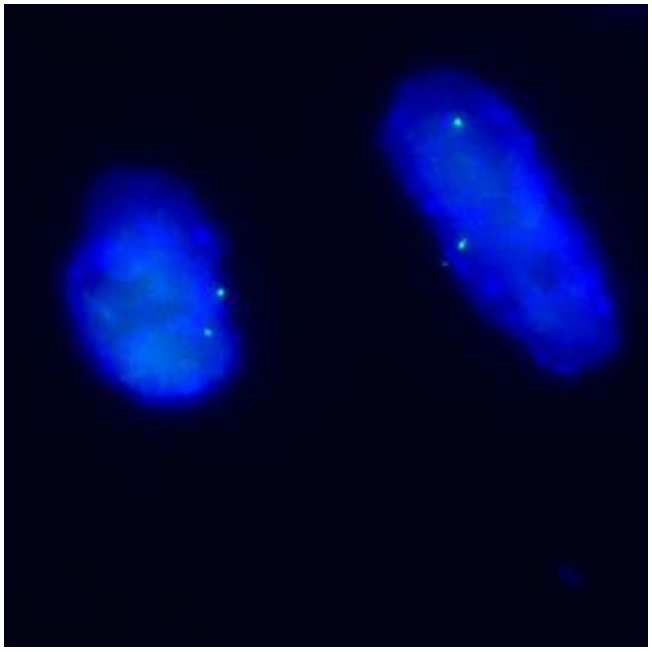

FIGURE 6 Fluorescence in situ hybridization analysis using a PCR-based probe for the *LAMA1* gene in cultured skin fibroblasts of the proband [Color figure can be viewed at wileyonlinelibrary.com]

probe in the proband's cultured skin fibroblasts demonstrated two clear signals, indicating that both copies of the duplicated region were located in close proximity on the same chromosome (Figure 6).

The level of expression of *LAMA1* exons 9–10 (inside the duplication) as well as exons 2–3 and 56–57 (both outside the duplication) in skin fibroblasts culture appeared to be normal (Figure 4). Both small and contiguous duplications and deletions involving *LAMA1* have been reported in healthy individuals in DGV and in patients in DECIPHER (nos. 323516 and 289719). The absence of intragenic *LAMA1* microduplication from lymphocytes of the patient and his parents was also confirmed by the results of an Agilent 400K microarray (Figure 5b–d).

Finally, whole-exome sequencing of DNA samples obtained from skin fibroblasts of our patient identified 333 variations with potentially high impact, among them only one ([hg19]chr22:23922314 G>A) known to be pathogenic. However, the pathogenic effect of this variant was described for the homozygous state only (Minegishi et al., 1998), whereas in our case, the identified variation was heterozygous.

5 | DISCUSSION

Here, we present a case involving a complex clinical phenotype and two microduplications at 18p11.32 (*METTL4*, *NDC80*, *CBX3P2*, and exons 1–15 of *SMCHD1*) and 18p11.31 (*LAMA1*, exons 4–22), both of which have previously been described in healthy and affected individuals.

Partial trisomy of the short arm of chromosome 18 is very rare. To date, only 28 such cases have been described in the literature, including five males with duplications at 18p (Balasubramanian, Sithambaram, & Smith, 2016; Giordano, Muratore, Babu, Meazza, & Bozzola, 2016; Jedraszak et al., 2015; Occella et al., 2013; Orendi, Uhrig, Mach, Tschepper, & Speicher, 2013). A map of microduplications and microdeletions at 18p is shown in Figure 7. A skin disorder was diagnosed only in the patient described by Occella et al. (2013), who reported a family harboring a pure 429.5-kb microduplication at 18p11.32-p11.31 that included *SMCHD1*, *EMILIN2*, *LIPIN2*, and *MYOM1*; the case involved a father and son with isolated porokeratosis of Mibelli. Porokeratosis is a heterogeneous group of disorders of epidermal keratinization, which are characterized by atrophic patches surrounded by stacks of tightly fitting parakeratotic cells. Three other members of the family (the paternal daughter, sister, and mother) also exhibited this duplication but did not show any signs of porokeratosis. Significantly, porokeratosis is more frequent in males. The authors suggested that the high diversity observed in the clinical presentations of the disease might be caused by an interaction between genetic and environmental factors or by differential environmental exposures; they also speculated that *EMILIN2* duplication underlies the porokeratosis of Mibelli described in this family.

The 18p11.32 microduplication observed in our patient included *METTL4*, *NDC80*, *CBX3P2*, and *SMCHD1*. The protein encoded by *METTL4* (Gene ID: 64863) is a probable methyltransferase whose function has not yet been fully described. *CBX3P2* (Gene ID: 645158) is a pseudogene of unknown function. *NDC80* (OMIM 607272) encodes a component of the NDC80 kinetochore complex that organizes and stabilizes microtubule–kinetochore interactions and is required for proper chromosome segregation. Zanet et al. (2010) demonstrated peribasal overinduction of the Ndc80/Hec1 complex in differentiating keratinocytes, indicating that this complex participates in preventing differentiating cells from undergoing nuclear division and chromosome segregation and in promoting increases in nuclear volume and ploidy. The protein encoded by the *SMCHD1* gene (OMIM 614982) is a chromatin repressor that is involved in the establishment and/or maintenance of CpG methylation at specific loci and binds directly to the D4Z4 repeat on chromosomes 4q35 and 10q26 (Calandra et al., 2016; Lemmers et al., 2012). Mutations of the *SMCHD1* gene leading to an insufficient amount of the SMCHD1 protein binding to the D4Z4 repeat are associated with facioscapulohumeral muscular dystrophy 2 (FSHD2) (OMIM 158901), which typically manifests in the second decade of life and is characterized by progressive weakness and atrophy of the facial and upper extremity muscles.

SMCHD1 may regulate the expression of autosomal genes through its binding of *cis*-regulatory elements, many of which coincide with binding sites for CCCTC-binding factor (CTCF) (Chen et al., 2015). Thus, using a database for CTCF binding sites and genome organization CTCFSDB 2.0, we performed a search for the putative CTCF binding sites in the sequence 2 kb upstream of the first exon of *EMILIN2*, which might represent the promoter region of the index gene. Three motif sequences were identified: AGCCCCCTGCCGAG (EMBL_M1 motif, score 5.1549), AGAATTGCA (EMBL_M2 motif, score 11.0539), and TTCTCGGCAGGGGGCTCGGG (MIT_LM7 motif, score 3.81999). A short sequence yielding position weight matrices score > 3.0 suggested a match. Thus, there is a direct prerequisite for *EMILIN2* to be regulated by CTCF and SMCHD1. The results of expression analysis obtained for our patient support this hypothesis: we observed an increase in the expression of *SMCHD1* and a decrease in the expression of *EMILIN2* (Figure 4), and we believe that this may

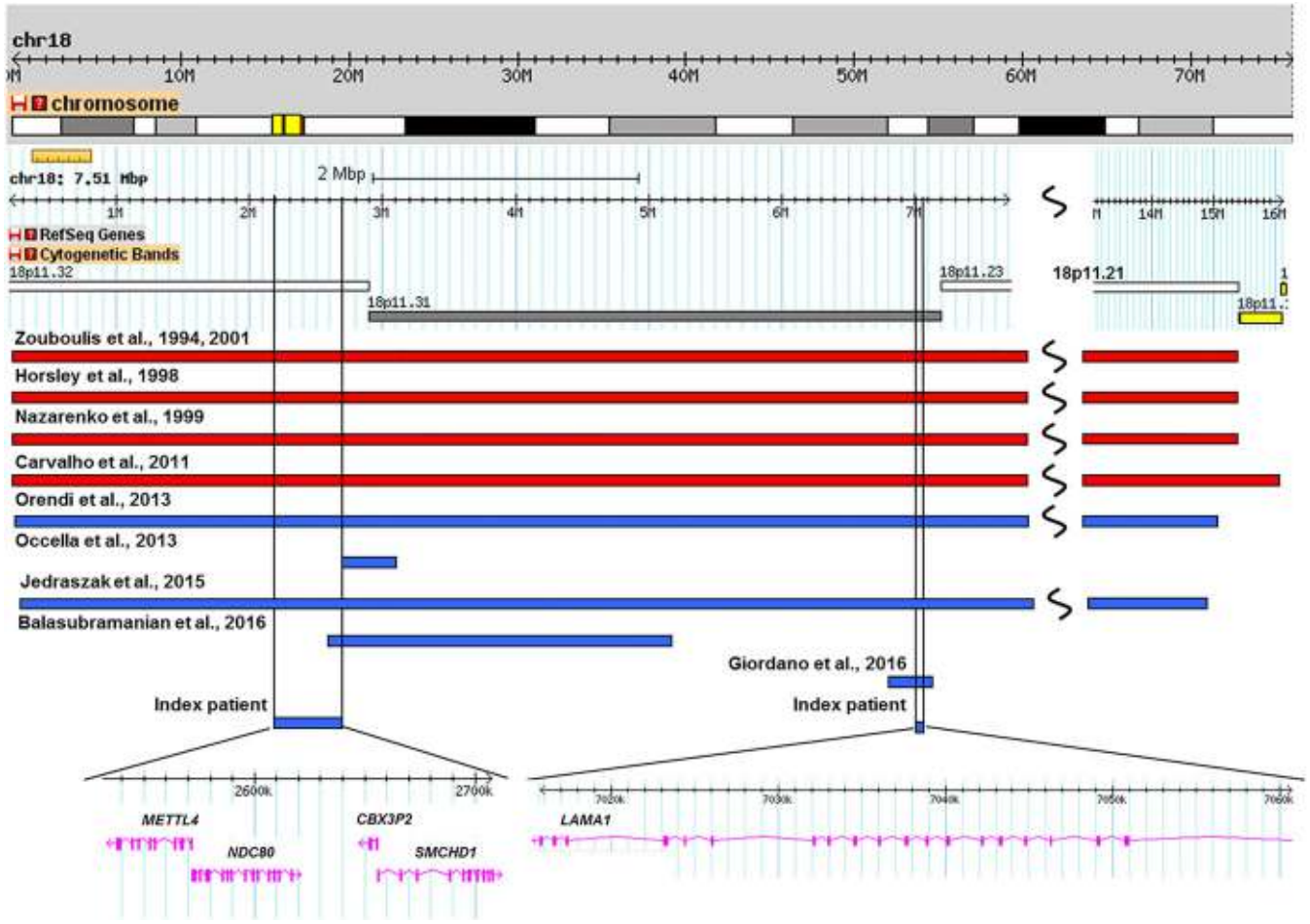

FIGURE 7 A map of microdeletions and microduplications at 18p. The microdeletions are shown in red, and the microduplications are colored blue [Color figure can be viewed at wileyonlinelibrary.com]

be one possible molecular mechanism underlying the pathogenesis of keratosis.

Two patients in DECIPHER harbor CNVs involving the *METTL4*, *NDC80*, *CBX3P2*, and *SMCHD1* genes. One proband (no. 272853) has a 571-kb microdeletion at arr[hg19]18p11.32(2,202,283-2,773,022) involving the same four genes found in our patient, while the second patient (no. 266406) has a 454-kb microduplication at arr[hg19] 18p11.32(2,152,137-2,606,609) involving only *METTL4* and *NDC80* and harbors a potentially pathogenic 475-kb microdeletion at 12q24. The phenotypes of the patients and the inheritance of the CNVs are unknown.

At the 18p11.31 locus, exons 4–22 of the *LAMA1* gene were duplicated in the fibroblasts of our patient. *LAMA1* encodes one of the α1 subunits of laminin, a member of a family of extracellular matrix glycoproteins. These proteins are major components of the basement membrane and have been implicated in a wide variety of biological processes, including cell adhesion, differentiation, migration, signaling, neurite outgrowth, and metastasis. Homozygous or compound heterozygous loss-of-function mutations in *LAMA1* have recently been associated with Poretti–Boltshauser syndrome (OMIM 615960), which is characterized by cerebellar dysplasia, cerebellar vermis hypoplasia, cerebellar cysts, high myopia, variable retinal dystrophy, eye movement abnormalities, delayed motor development, and speech delay. Cognitive function can range from normal to intellectually disabled. The patient described here did not exhibit any cerebellar anomalies according to MRI but complained of pain in his eyes and blinked frequently when tired. A role of *LAMA1* mutations in *keratosis pilaris* was suggested for the first time by Zouboulis et al. (2001); the authors used an antibody against the human laminin α1 chain and stained tissue from a patient with *keratosis pilaris*. They showed that this protein was totally absent from vessels, adnexal structures, and dermal nerves in the patient's skin and those sebaceous glands were not present in the involved skin areas.

Two patients in DECIPHER with an AT frameshift variant (c.362-363delAT) within *LAMA1* (no. 323516) and a 67.5-kb microdeletion at arr[hg19]18p11.32(6,913,067-6,980,578) partially involving *LAMA1*, *ARHGAP28*, and *LINC00668* (no. 289719) are considered suitable for comparison with our patient. Patient no. 323516 is female, and the only phenotype reported for her is an abnormality of the nervous system. The AT frameshift variant was inherited from both heterozygous parents. Patient no. 289719 exhibits intellectual disability, signs of autism, and an abnormality of the thorax. The microdeletion was paternally inherited and is constitutive in the father. The index patient has a complex clinical phenotype. Clinical features such as mild to moderate intellectual disability, delayed speech, short stature (3rd–10th percentile in patients older than 14 years), a short and broad

neck, tooth anomalies and intensive caries as well as *keratosis pilaris* are reported in most patients with 18p CNVs, including the present case (Carvalho et al., 2011; Horsley et al., 1998; Nazarenko et al., 1999; Zouboulis et al., 1994). In addition to the described phenotypes, our patient suffered from type I diabetes mellitus and abnormal fat distribution. Whether the whole phenotype of our patient is caused by the 18p CNVs that we identified is not yet clear. In addition, we excluded other possible pathogenic variations in our patient by whole-exome sequencing. Among 333 detected variations in 279 genes (information is available on request), only one ([hg19] chr22:23922314 G>A) is known as disease-causing for B cell deficiency and agammaglobulinemia. However, its pathogenic effect was reported for the homozygous state only (Minegishi et al., 1998), whereas in our case, the identified variation was heterozygous.

Importantly, there is evidence indicating that a gene conferring susceptibility to type 2 diabetes is located on chromosome 18 (Parker et al., 2001; Perry et al., 2012). Considering that the *SMCHD1* gene regulates the expression of many other genes throughout the genome, it seems possible that *SMCHD1* may affect one of the genes associated with body mass and diabetes mellitus. Regarding *keratosis pilaris*, *LAMA1* is a good candidate gene, and laminin α1 chain was absent in the skin of a patient with an 18p deletion (Zouboulis et al., 2001). Although *LAMA1* was expressed in our patient's skin fibroblasts, as demonstrated by qPCR, the presence of a large intragenic duplication may still lead to an abnormal protein product. Finally, his intellectual disability may be associated with the larger 432-kb constitutional microduplication, although this was identified in the patient's father, who did not show neurodevelopmental issues of any sorts. There are several examples of CNVs predisposing to intellectual disability, autism spectrum disorders and epilepsy that can be transmitted by a healthy parent (Girirajan et al., 2010; Kashevarova et al., 2014; Rosenfeld, Coe, Eichler, Cuckle, & Shaffer, 2013).

In conclusion, we present the first report of the combination of a mosaic 45-kb microduplication of exons 4–22 of *LAMA1* with a 432-kb constitutional microduplication at 18p11.32, encompassing the *METTL4*, *NDC80*, and *CBX3P2* genes and exons 1–15 of *SMCHD1*, both located within the critical region for skin disorders on chromosome 18, in a patient with intellectual disability, diabetes mellitus (type I) and *keratosis pilaris*. *LAMA1* is the most likely candidate gene for the skin disease; however, the pathogenic effect of regulatory genes within the larger duplication cannot be ruled out.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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SUPPORTING INFORMATION

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