CLINICAL REPORT

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Somatic/Gonadal Mosaicism in a Syndromic Form of Ectrodactyly, Including Eye Abnormalities, Documented Through Array-Based Comparative Genomic Hybridization

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Split hand/foot malformation (SHFM) is characterized by underdeveloped or absent central digital rays, clefts of hands and feet, and variable syndactyly of the remaining digits. SHFM is a heterogeneous condition caused by abnormalities at one of multiple loci, including SHFM1 (*SHFM1* at 7q21–q22), SHFM2 (Xq26), SHFM3 (*FBXW4/DACTYLIN* at 10q24), SHFM4 (*TP63* at 3q27), and SHFM5 (*DLX1* and *DLX 2* at 2q31). SHFM3 is unique in that it is caused by submicroscopic tandem chromosome duplications of *FBXW4/DACTYLIN*. In order to show that array -based comparative genomic hybridization should be considered an essential aspect of the genetic analysis of patients with SHFM, we report on a family with two brothers who have ectrodactyly. Interestingly, both also have ocular abnormalities. Their sister and both parents are healthy. DNA of all five family members was analyzed using oligonucleotide-based DNA microarray and quantitative PCR. The two affected brothers were found to have a small duplication of approximately 539 kb at 10q24.32. The patients' sister and father do not have the microduplication, but qPCR showed that mother's DNA carries the duplication in 20% of blood lymphocytes. In this family, two children were affected with ectrodactyly having a duplication over the SHFM3 locus. The mother, who shows no clinical features of ectrodacytyly, is a mosaic for the same duplication. Therefore, we demonstrate that somatic/gonadal mosaicism is a mechanism that gives rise to SHFM. We also suggest that ocular abnormalities may be part of the clinical description of SHFM3.

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Key words: split hand/foot malformation; ectrodactyly; somatic/gonadal mosaicism; array-based comparative genomic hybridization; eye abnormalities

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INTRODUCTION

Split hand/foot malformation (SHFM), or ectrodactyly, is characterized by underdeveloped or absent central digital rays, clefts of the hands and/or feet, and variable syndactyly of the remaining digits. The condition occurs in 1 in 8,500–25,000 newborns [de Mollerat et al., 2003; Elliott et al., 2006]. Idiosyncratic patterns of transmission have been reported in many SHFM families. Non-syndromic ectrodactyly was characterized by Zlotogora [1994] as having

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dominant transmission with nearly complete penetrance. Other investigators, however, have reported variable expressivity between and within kindreds. Several parent–child dyads with SHFM have demonstrated progression of the clinical phenotype from a mild form in an affected parent to a more severe phenotype in an affected offspring. In 1977, Emery first raised the question of whether minor hand anomalies such as split nail or extra joints are reduced expression of SHFM. Two or more affected sibs with normal parents have been noted by several authors [Freire-Maia, 1971; Spranger and Schapera, 1988; Majewski et al., 1996]. This has been used as evidence for recessive inheritance. Germinal mosaicism has also been suggested as a likely explanation of this idiosyncratic pattern of SHFM transmission in familial cases [Zaremba, 1969; David, 1972; Wong et al., 1998] and was demonstrated by Dimitrov et al. in 2009.

SHFM occurs as an isolated finding or as a part of several syndromes. It is a heterogeneous condition that can be caused by a mutation in any one of several genes and loci. These include SHFM1 (*SHFM1* at 7q21–q22), SHFM2 (Xq26), SHFM3 (*FBXW4/DACTYLIN* at 10q24), SHFM4 (*TP63* at 3q27), and SHFM5 (*DLX1* and *DLX 2* at 2q31).

In 1995, Nunes mapped SHFM3 to chromosome 10q24–q25. Later, de Mollerat et al. [2003] showed that submicroscopic chromosomal duplications within the SHFM3 locus are associated with non-syndromic SHFM in familial and sporadic cases. Approximately 0.5 Mb in size, this complex tandem rearrangement includes *LBX1* (ladybird homeobox 1), *BTRC* (beta-transducin repeat containing), *POLL* (polymerase (DNA directed), lambda) and a portion (exons 9–6) of *DACTYLIN* (*FBXW4*, F-box and WD repeat domain containing 4), thus suggesting that a disturbed function of gene(s) in the locus is the probable molecular defect underlying this limb anomaly.

In 2006, Lyle evaluated the expression of 13 candidate genes within and flanking the duplicated region and showed that *BTRC* (present in three copies) and *SUFU* (suppressor of fused homolog (*Drosophila*)) (present in two copies) are over-expressed in SHFM3 patients compared to controls. They suggested that SHFM3 may be caused by over-expression of *BTRC* and *SUFU*, both of which are involved in the beta-catenin signaling pathway.

In 2006, Everman found that SHFM3 abnormalities are seen in approximately 30% of SHFM cases and appear to be a more frequent cause of non-syndromic SHFM than mutations in the *TP63* (tumor protein p63) gene. Most individuals with the tandem microduplication had an isolated form of typical SHFM, however, one patient had a medulloblastoma. Another patient had a submucous cleft palate [Everman et al., 2006]. Buttiens and Fryns in 1987 and Keymolen et al. in 2000 published on three patients with distal limb deficiencies-oral involvement-renal defect syndrome. This syndrome was later classified as distal limb deficiencies, micrognathia syndrome (DLDMS; OMIM 246560). In 2009, Dimitrov et al. showed that four individuals with distal limb deficiencies, micrognathia, hearing problems, and renal hypoplasia had the same 10q24 microduplication and three out of six affected individuals in the four published families had myopia. The observed rearrangement in all these patients was similar in size to those previously detected in non-syndromic SHFM3, but extended distally toward *FGF8* (fibroblast growth factor 8 (androgen induced)). Two of the reported cases by Dimitrov et al. in 2009 were sibs, parents were normal. The authors demonstrated somatic/gonadal mosaicism in the apparently healthy mother.

We report on two brothers with syndromic ectrodactyly plus eye abnormalities who were found to have a duplication at 10q24.32. We show that their mother carries the duplication in a mosaic state. Therefore, we demonstrate that somatic/gonadal mosaicism may occur in families with a 10q24.32 duplication. For the individuals presented here, consent was obtained using forms approved by the Ethics Committee of Pontifícia Universidade Católica do Parana.

PATIENTS AND METHODS

Patient 1

RFU, a 28-year-old Caucasian man, is the first son of non-consanguineous and healthy parents. He has long and narrow face, mild facial asymmetry, bilateral congenital cataracts, micrognathia, high palate, and hypertension. His mental development is normal and no other health problems are present besides the severe truncation defects of all four limbs. Limb defects were characterized by classical SHFM—normal long bones and bilateral split hand and foot (Fig. 1A–C). The skeletal defects and facial appearance resemble those of previously published patients with DLDMS by Buttiens and Fryns [1987], Keymolen et al. [2000], and Dimitrov et al. [2009].

Patient 2

MVU, a 27-year-old Caucasian man, is the second son of non-consanguineous and healthy parents. He has mild myopia, sensorineural hearing loss, normal mental development, and no other health problems besides upper and lower limbs malformations. He has normal long bones, monodactyly of the right hand, bidactyly of the left hand, and monodactyly of both feet (Fig. 1D–J).

Their mother has only micrognathia, and no other anomalies.

METHODS

Microarray-based comparative genomic hybridization (aCGH) was performed on DNA extracted from the peripheral blood of all five relatives using a BAC microarray (the SignatureChip Whole Genome™; Signature Genomics, Spokane, WA) as previously described [Buttiens and Fryns, 1987; Ballif et al., 2008; Dimitrov et al., 2009; Keymolen et al., 2000]. Results were visualized using Signature Genomic Laboratories' laboratory-developed computer software program (Genoglyphix; Signature Genomic Laboratories).

In order to further delineate the breakpoints of the duplication, oligonucleotide-based microarray analysis was performed using a 135K-feature whole-genome microarray (SignatureChip Oligo Solution, made for Signature Genomics by Roche NimbleGen, Madison, WI.) Genomic DNA was extracted from peripheral blood using a Qiagen M48 Biorobot automated DNA extraction system. Purified genomic DNA was then labeled with Cyanine dyes Cy3 or Cy5 using a Roche NimbleGen DNA labeling kit. Array hybridization and washing were performed as specified by the manufacturer (Roche

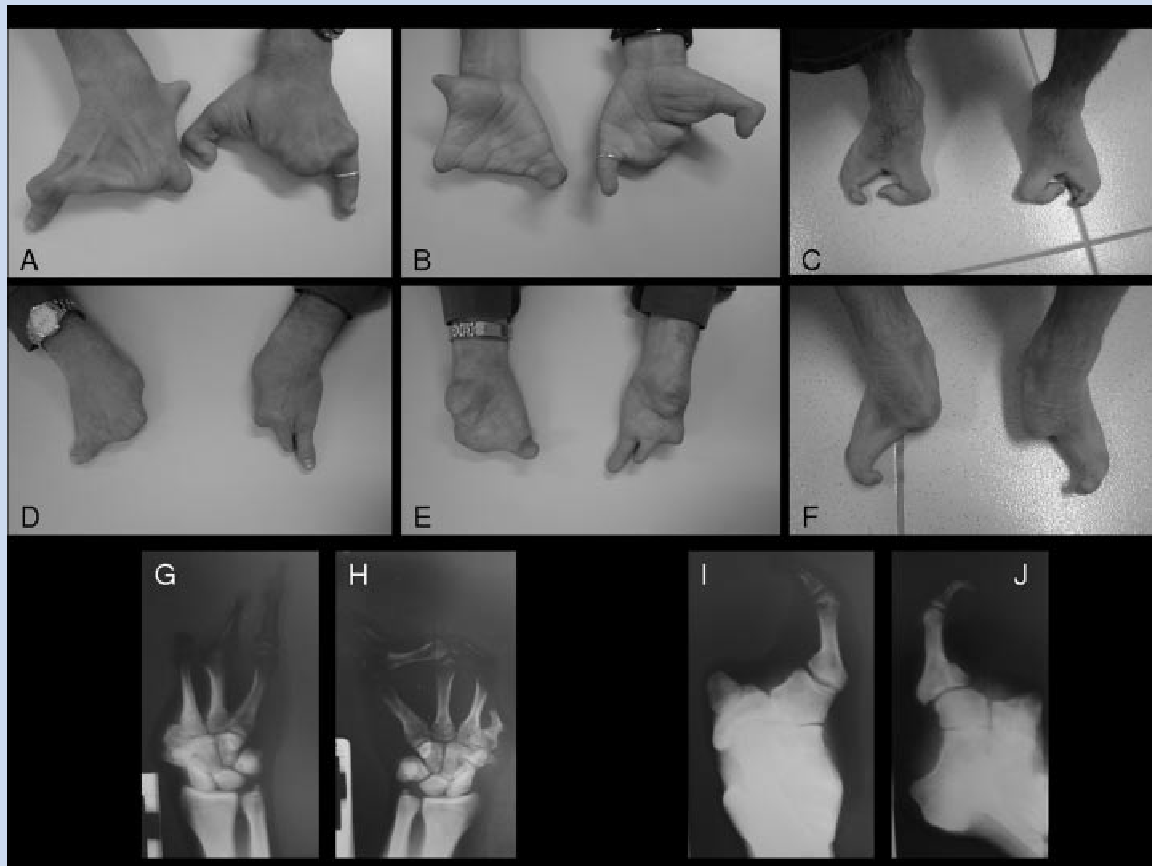

FIG. 1. Patient 1. A: Truncations of all II–IV phalanges bilaterally, rudimentary thumb, and transverse phalanx IV–V on the right hand, both 5th rays appear normal, metacarpals I–IV of both hands are shorter and malformed, no long bones deficiencies of the zeugopod. B: Smooth palmar creases with abnormal pattern, hypoplastic thenar/hypothenar. C: Classical bidactylous SHFM. Patient 2. D: Terminal truncations of the upper limbs causing monodactylous ectrodactyly/oligodactyly of both hands. E: Smooth palmar creases with abnormal pattern, hypoplastic thenar/hypothenar. F: Monodactylous ectrodactyly of both feet, only the 5th ray is present. G: X-ray, bidactyly of left hand. H: X-ray, monodactyly of right hand. I: X-ray, monodactyly of left foot. J: X-ray, monodactyly of right foot.

NimbleGen). Arrays were scanned using an Axon 4000B scanner (Molecular Devices) and analyzed using GenePix 6.1 (Molecular Devices, Inc., Sunnyvale, CA), DNA Analytics 4.0 (Agilent Technologies, Santa Clara, CA), and NimbleScan 2.5 (Roche NimbleGen). Results were then displayed using custom oligonucleotide aCGH analysis software (Genoglyphix; Signature Genomic Laboratories).

SYBR green quantitative PCR (qPCR) was performed for confirmation of aberrations detected by microarray. qPCR is a molecular technique that allows the determination of dosage for specific sequences of interest [Buttiens and Fryns, 1987; Keymolen et al., 2000; Dimitrov et al., 2010]. For this test, primer pairs specific to target sequence within the previously reported aberration are designed and validated for specificity and accuracy. Copy gains and losses were scored relative to at least three normal controls.

RESULTS

Array CGH analysis identified a 10q24.32 duplication, with a single-copy gain of 34 oligonucleotide probes on the long arm of chromosome 10 at 10q24.31q24.32, approximately 539 kb in size (nucleotide positions 10:102,942,925–103,481,863) (Fig. 2). This chromosomal abnormality includes *LBX1*, *BTRC*, *POLL*, and *FBXW4/DACTYLIN* genes. It was present in both sibs and was absent in the healthy sister and parents.

Two independent qPCR runs showed that the mother is a carrier of a mosaic duplication, using primers to exon 11 of the *BTRC* gene. Mosaicism is estimated at 20%.

DISCUSSION

We report on two affected sibs with a microduplication at 10q24, and their mother as a mosaic carrier of the same duplication in blood lymphocytes, extending previous knowledge about this region and condition. This report reinforces the observations that somatic/gonadal mosaicism of the duplication explain the variable expression [Everman et al., 2006; Dimitrov et al., 2009].

Additionally, this study and the patients published in Dimitrov et al. [2010] show that besides a non-syndromic form, 10q24.31q24.32 microduplications also cause syndromic forms of

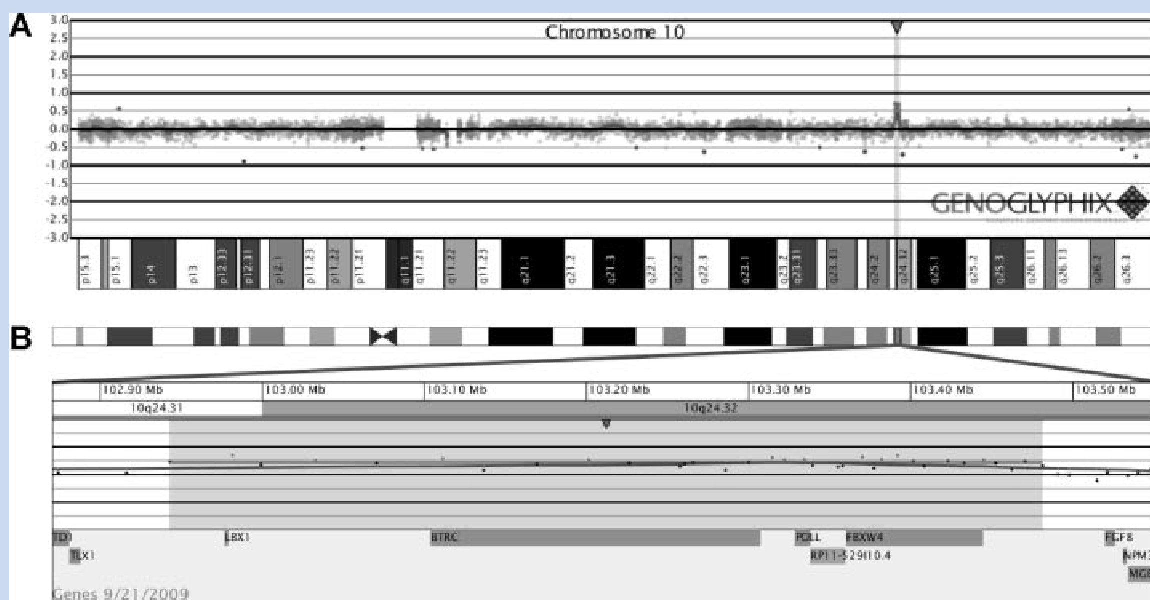

FIG. 2. Microarray characterization of 10q24.31q24.32 duplication. A: Microarray plot showing single-copy gain of 34 oligonucleotide probes from the long arm of chromosome 10 at 10q24.31q24.32, approximately 539 kb in size (chr10:102,942,925–103,481,863, hg18 coordinates). Probes are ordered on the *X*-axis according to physical mapping positions, with the most distal p-arm probes to the left and the most distal q-arm probes to the right. B: Zoomed-in microarray plot of single-copy gain shown in (A). Probes are arranged as in (A), with the most proximal 10q24.31 probes to the left and the most distal 10q24.32 probes to the right.

SHFM. Both of our patients and three of six published patients by Dimitrov et al. have eye defects (myopia and congenital cataract). Thus we suggest that the eye abnormalities may have been underestimated in previous reports on SHFM3. The detected 10q24 genomic imbalance in our syndromic patients is of a similar size to the duplication in the previously reported individuals with an isolated form of SHFM, thus extending the clinical spectrum of SHFM3. One of the patients presented in the current study (RFU) has congenital cataracts. *PITX3* (paired-like homeodomain 3) is a gene that could be related to the observed congenital cataract in our patients, as it is known to be implicated in the genetics of the posterior polar congenital cataract [Finzi et al., 2005; Sakazume et al., 2007; Summers et al., 2008]. *PITX3* is located approximately 509 kb distal to the region duplicated in our patients. We speculate that a positional effect may disturb the function of *PITX3* causing the observed eye defect in our patient. More studies are needed to identify if there are transcription factor binding sites and/or other long range regulatory elements within the SHFM3 locus that could control the spatiotemporal expression of *PITX3* gene. Karsch [1936] and Neugebauer [1962] reported split hand with congenital nystagmus, fundal changes, and cataracts. This is now known as the Karsch–Neugebauer syndrome (OMIM 183800). Interestingly, in most of the cases reported of this disorder, there is apparent gonadal mosaicism, similar to the family described in the current report [Neugebauer, 1962; Pilarski et al., 1985; Wong et al., 1998]. Based on these previous observations and in the family we report, we speculate that the Karsch–Neugebauer syndrome is indeed SHFM3 with involvement of the eye through disruption of the expression of *PITX3* and/or its regulatory elements.

There are discordant endophenotypes within the family we present. The phenotypic variability between these two sibs who are carriers of an apparently similar microduplication may be explained by existence of modifying factors. Thus, in addition to locus and allelic heterogeneity, other epigenetic events (gene modifiers, stochastic effects, and environmental factors) may be implicated in the complex genetics and clinical picture of SHFM. Our findings show the importance of aCGH in the detection of the etiology of complex, clinically heterogeneous entities. In addition, we suggest the presence of somatic/gonadal mosaicism in familial cases with pseudo-autosomal recessive inheritance. This should be considered in the genetic counseling of affected families.

Although the current data indicate that there is no obvious correlation between the size of the 10q24 duplication and the phenotype, further studies may contribute to the understanding of the complex genetics of this intriguing developmental disorder. Fine delineation of the aberration breakpoints may help to narrow the critical region of SHFM3 and explain to what extent the duplication could influence the observed phenotypic differences. However, the abnormal phenotype could be a result of disturbed function of several genes. Therefore, the contribution of *FBXW4/DACTYLIN*, *FGF8*, *PITX3* and other genes within or in the vicinity of the duplication associated with the observed phenotypic spectrum still remains to be discovered.

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