

Clinical Report

An Xq22.3 Duplication Detected by Comparative Genomic Hybridization Microarray (*Array-CGH*) Defines a New Locus (*FGS5*) for FG Syndrome

Fernanda Sarquis Jehee,¹ Carla Rosenberg,¹ Ana Cristina Krepischi-Santos,¹ Fernando Kok,² Jeroen Knijnenburg,³ Guy Froyen,⁴ Angela M. Vianna-Morgante,¹ John M. Opitz,⁵ and Maria Rita Passos-Bueno^{1*}

¹Centro de Estudos do Genoma Humano, Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

²Depto de Neurologia Infantil, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

³Laboratory of Cytochemistry and Cytometry, Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands

⁴Human Genome Laboratory, Centre for Human Genetics, Flanders Interuniversity, Institute for Biotechnology (VIB), Leuven, Belgium

⁵Pediatrics (Medical Genetics), Human Genetics, Obstetrics and Gynecology, Pathology, University of Utah, Salt Lake City, Utah

FG syndrome is an X-linked multiple congenital anomalies (MCA) syndrome. It has been mapped to four distinct loci *FGS1-4*, through linkage analysis (Xq13, Xp22.3, and Xp11.4-p11.3) and based on the breakpoints of an X chromosome inversion (Xq11:Xq28), but so far no gene has been identified. We describe a boy with FG syndrome who has an inherited duplication at band Xq22.3 detected by comparative genomic hybridization microarray (*Array-CGH*). These duplication maps outside all four loci described so far for FG syndrome, representing therefore a new locus, which we propose to be called *FGS5*. *MID2*, a gene closely related to *MID1*, which is known to be mutated in Opitz G/BBB syndrome, maps within the duplicated segment of our patient. Since FG and Opitz G/BBB syndromes share many manifestations we considered *MID2* a candidate gene for FG syndrome. We also discuss the involvement of other potential genes within the duplicated segment and its relationship with clinical symptoms of our patient, as well as the laboratory abnormalities found in his mother, a carrier of the duplication. © 2005 Wiley-Liss, Inc.

KEY WORDS: FG syndrome; X-linked inheritance; X chromosome; *FGS5*; Xq22.3; X-chromosome duplication; trigonocephaly

INTRODUCTION

FG syndrome is an X-linked MCA syndrome first described by Opitz and Kaveggia [1974]. The main phenotypic characteristics of the original cases included small size at birth, catch-up growth in childhood, relatively large head, congenital hypotonia, seizures, imperforate anus, and constipation.

Facial anomalies include high broad forehead with frontal cowlick, dolichocephaly with frontal prominence, hypertelorism, strabismus, prominent lower lip, high-arched palate, and short and abnormally modeled ears. FG children seem to have a characteristic behavior, being friendly, attention-seeking, and hyperactive with occasional tantrums due to frustrations. Female carriers show milder manifestations. For a comprehensive clinical description of FG patients see Opitz et al. [2001]. FG syndrome was mapped to four loci, but no gene has been identified to date. Zhu et al. [1991] originally mapped *FGS1* in the family described by Keller et al. [1976] to a large segment- Xp21.31-q22. A further linkage study including this family and nine others performed by Briault et al. [1997] delimited the *FGS1* locus between DXS135 at Xq12 and DXS1066 at Xq21.31, with a maximum lod score at Xq13. A second locus (*FGS2*) was found to be either at Xq11 or at Xq28 based on a report of an inv(X)(q11q28) in a boy with FG syndrome [Briault et al., 1999, 2000]. Two other loci, *FGS3* [Dessay et al., 2002] and *FGS4* [Piluso et al., 2003], were mapped through linkage analysis to Xp22.3 and Xp11, respectively.

We report on a boy with FG syndrome who has an inherited duplication found by comparative genomic hybridization microarray (*Array-CGH*) of approximately 4 Mb at Xq22.3. This duplication identifies a new locus for FG syndrome at Xq22.3.

CLINICAL REPORT

The proband (Fig. 1a) was the first born to a young and non-consanguineous couple. He was born at 32 weeks of gestation due to bleeding and premature labor. Birth weight was 1,800 g and length was 38 cm. He was released from hospital after one uneventful week. He was severely hypotonic and mentally retarded never being able to sit without support. The main craniofacial finding was trigonocephaly due to premature metopic closure. He also had bilateral epicanthic folds, up-slanted palpebral fissures, short nose with depressed bridge and upturned nares, long philtrum, diastema of upper central incisors, strabismus, and hypospadias. He had severe constipation and required enemas on five different occasions. His body temperature was habitually elevated, ranging from 37°C to 38°C. There were no reports of recurrent infections. He was described as an agitated and active boy and was said to have a happy demeanor. He was known to be heterozygote for sickle cell disease, but no comprehensive laboratory investigation was ever performed. He died at the age of 4 years,

*Correspondence to: Maria Rita Passos-Bueno, Depto Genética e Biologia Evolutiva-IB/USP, Rua do Matão, 277, sala 200 CEP 05508-900, São Paulo, SP-Brazil. E-mail: passos@ib.usp.br

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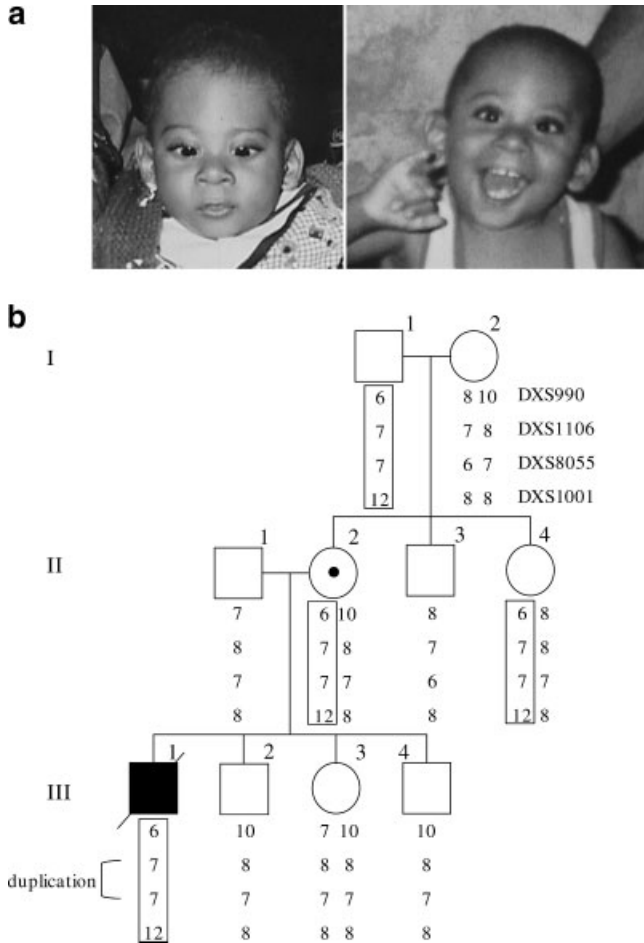


Fig. 1. Facial appearance of proband (a) and pedigree (b) with segregation of polymorphic markers flanking the duplicated segment.

supposedly due to a generalized infection and multiple organ failure. Autopsy was not performed.

The patient's mother is 155 cm tall and weighs 40 kg. She has a diastema of the upper central incisors but is, otherwise, phenotypically normal. She suffers from severe anemia, for which she has been hospitalized several times. During her fourth pregnancy a diagnosis of nephrolithiasis was made. Recent laboratory evaluation disclosed a normal value for uric acid (4.0 mg/dL); thyroid stimulating hormone, TSH (1.5 mUI/L); and free thyroxine, T4 (1.0 ng/dL). Her level of thyroxine-binding globulin (TBG) was elevated (39.0 mg/L; reference value: 10.0–29.0 mg/L) and total T4 was slightly above normal (12.6 mg/dL; reference value: 4.5–12.0 mg/dL). The proband's parents are both heterozygotes for sickle-cell disease. The three sibs of the proband were healthy.

This patient was included in an *array-CGH* screen for chromosome imbalances of individuals with syndromal trigonocephaly, without gross chromosome alterations on G-banding analyses or microdeletions at 9p22-p24 and 11q23-q24 [Jehee et al., 2005].

METHODS

Comparative Genomic Hybridization Microarray (Array-CGH)

The 1 Mb array-CGH procedures were performed as described previously [Knijnenburg et al., 2005; Rosenberg

et al., 2005]. Briefly, slides containing triplicates of ~3,500 large insert clones spaced at ~1 Mb density over the genome were produced in the Leiden University Medical Center. The large insert clones set used to produce these arrays was provided by the Wellcome Trust Sanger Institute (UK), and information regarding the full set is available at the Wellcome Trust Sanger Institute mapping database site, Ensembl (<http://www.ensembl.org/>). DNA amplification, spotting on the slides and hybridization procedures were based on published protocols [Carter et al., 2002; Fiegler et al., 2003]. Target imbalances were determined based on log² ratios of the average of their replicates, and sequences were considered as amplified or deleted when outside the ±0.33 range.

The tiling path X chromosome-specific array consists of 1,875 genomic clones derived from the X chromosome. The production of the X-array, probe preparation, and hybridization on the array were essentially performed as described elsewhere [Van Esch et al., 2005; Bauters et al., 2005]. These protocols were mainly based on those developed at the Sanger Center [Fiegler et al., 2003]. For each hybridization, 3 µg each of Cy5 and Cy3-labeled probe with a specific activity of 40, was mixed together with 100 µg Cot-1 DNA (Invitrogen, Carlsbad, CA, USA) and hybridized for 40 h at 37°C in humid chambers. Only those spots with Cy5 as well as Cy3 signal intensities at least 1.7-fold above the local background, were further analyzed. Data normalization was performed against the mean of the spot ratios of all clones. Clones with opposite aberrant log² ratios in the color-flip experiments were regarded as aberrant [Bauters et al., 2005].

Fluorescence In Situ Hybridization (FISH)

FISH experiments were performed by standard techniques to validate the presence of the chromosome duplication identified by microarray analyses. Large insert clones mapping within and immediately adjacent to the duplicated segment were hybridized to interphase nuclei derived from cultured peripheral leukocytes from the mother.

Genotyping

Polymorphic microsatellite markers flanking the duplicated segment (DXS990, DXS1106, DXS8055, and DXS1001) from the ABI PRISM Linkage Mapping set version 2 were genotyped to determine the in-risk haplotype and its segregation in the family.

X-Inactivation Studies

To test the X-inactivation status of the patient's mother, aunt and sister, we tested the methylation status of the polymorphic CAG repeat in the androgen-receptor gene using the methylation-sensitive assay as described in Allen et al. [1992].

RESULTS AND DISCUSSION

The use of *array-CGH* enabled us to detect a microduplication of a segment of ~4 Mb at Xq22.3 in a patient previously diagnosed with non-specific syndromic trigonocephaly. This duplication comprised four probes in the 1-Mb-resolution array, RP11-230E14, RP11-539A6, RP1-75H8, and RP5-820B18. Analysis of maternal cells by interphase FISH using BAC clones within the duplicated region and immediately adjacent to it demonstrated that she is heterozygous for the same duplication as her son (Fig. 2b). The duplication is apparently inverted since we were able, in metaphase, most of the times, to distinguish two red signals (proximally duplicated probe) but only one green signal (distally duplicated probe).

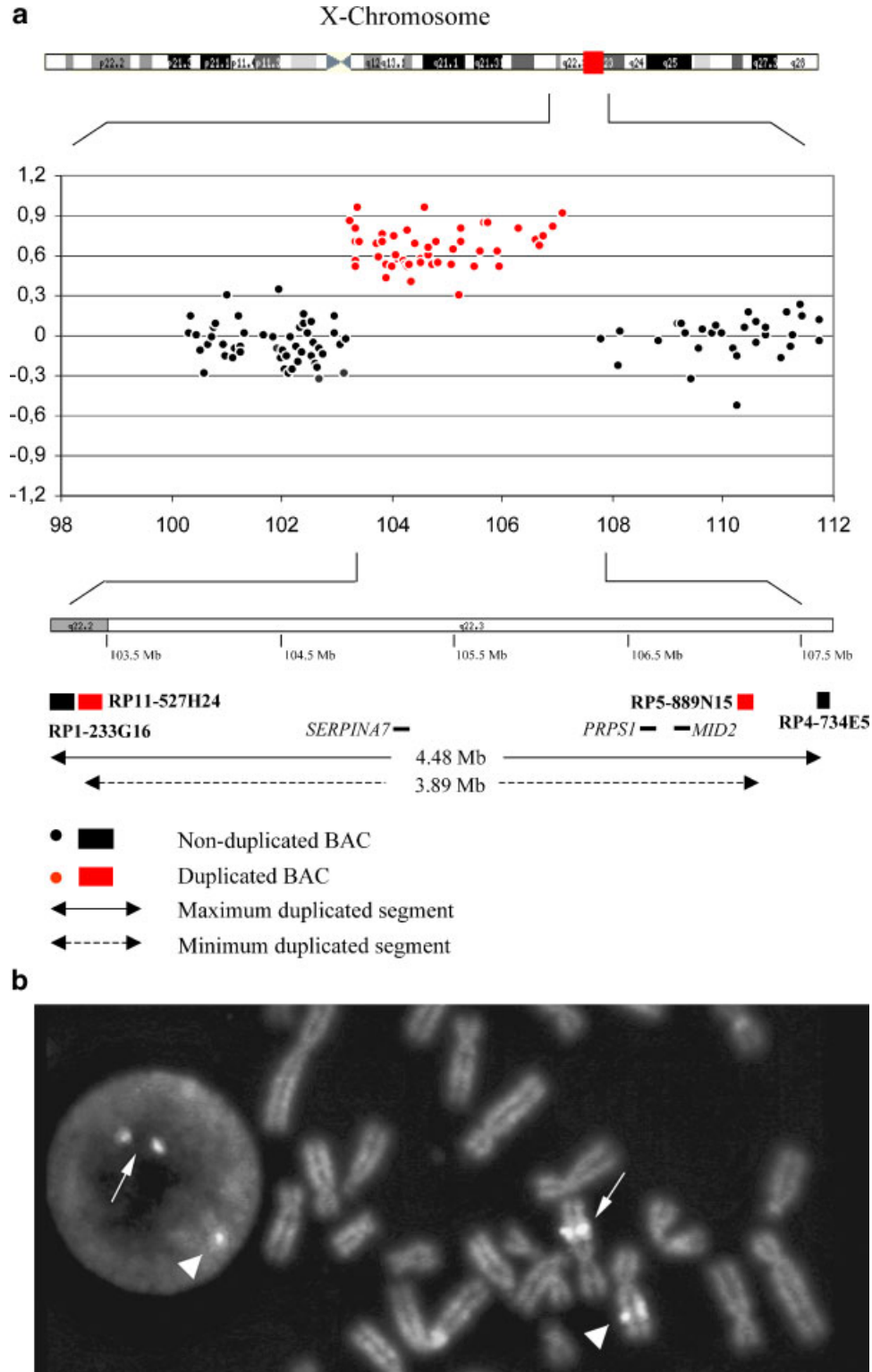


Fig. 2. Duplication at Xq22 present in the patient and his mother demonstrated by array-CGH and confirmed by FISH. **a**: Chromosome-X array-CGH profile from patient shows a duplication of 52 consecutive clones at Xq22.2-q22.3. The position of the duplication is indicated by the box on the X-ideogram. Underneath, an enlargement of the Xq22 band shows a map of the duplicated and non-duplicated flanking clones. **b**: FISH of the clone RP1-

75H8 to metaphase chromosomes of the patient's mother lymphocytes. The nucleus shows a double (white arrows) and a single signal (arrowheads), illustrating a heterozygosity for the duplication. Although the duplicated signal cannot be resolved in metaphases, a difference in signal intensities between the two X-homologues can be noted.

The possibility of a more complex rearrangement, however, cannot be disregarded. As expected, interphase results were not consistent because probes were separated by a distance larger than 1 Mb.

Segregation analysis of polymorphic microsatellite markers showed that the at-risk haplotype on the X-chromosome was inherited from the patient's non-affected maternal grandfather and was not present in the propositus' normal sibs (Fig. 1b). Array-CGH using DNA from the maternal grandfather and aunt showed that none of them carries the duplication in peripheral lymphocytes. Therefore, this duplication most probably originated de novo during the grandfather's spermatogenesis.

Analysis of the methylation status of the androgen receptor gene demonstrated that the X chromosome bearing the duplication was not preferentially inactivated in the patient's mother. Likewise, X-inactivation was not skewed in any other female relative tested.

To further delineate the size and breakpoints of the duplicated segment we performed high resolution X-array-CGH (Fig. 2a). Results show that the duplication contains 52 clones with the proximal breakpoint being between clones RP1-233G16 (103.17 Mb) and RP11-527H24 (103.21 Mb), and the distal breakpoint situated between clones RP5-889N15 (107.10 Mb) and RP4-734E5 (107.65 Mb).

Our patient's duplication at Xq22.3 maps outside the four known FGS loci so far reported, pointing to a fifth locus for FG syndrome (*FGS5*) on the X chromosome. Graham et al. [1998] analyzed three families with FG syndrome and found linkage to a large region between Xq12-Xq23 supporting the evidence

of a FGS locus at *FGS1*. We now know that this segment probably contains both *FGS1* and *FGS5*, and some of those families might carry alterations in *FGS5*.

Deletions distal to Xq21 are not associated with FG syndrome [Briault et al., 1997], suggesting that either there are no loci for the syndrome distal to this point, or that the mechanism responsible for the phenotype is not haploinsufficiency. Indeed, the duplication found in our patient and his mother suggests that the mechanism herein involved is an enhancement of function rather than haploinsufficiency. This could be supported by the findings of Veltman et al. [2004] who also identified duplications at each end of the inv(X) (q11q28) in the FG syndrome patient described by Briault et al. [1999]. However, we cannot exclude that the disruption of gene(s) at the breakpoint(s) of the duplication, positional effects and the double dosage of the genes in the altered segment, contributed to the phenotype in these patients.

The duplication in our patient involves most of band Xq22.3, and contains 16 genes, many without a known function (Fig. 3). Among these we identified three genes that could contribute to our patient's phenotype: *SERPINA7*, *PRPS1*, and *MID2*.

SERPINA7 encodes for TBG, the major thyroid hormone transport protein in man, and mutations at this locus have been related to either euthyroidal hyper- or hypo-thyroxinemia. Excess of TBG levels were demonstrated to be due to gene amplification in some families [Mori et al., 1999]. Our patient's mother has indeed a 35% elevation in TBG level and a 5% elevation in total T4 level. Maternal and/or neonatal hyperthyroidism is a known cause of craniosynostosis [Cohen and MacLean, 2000]. We speculate whether the elevated levels of

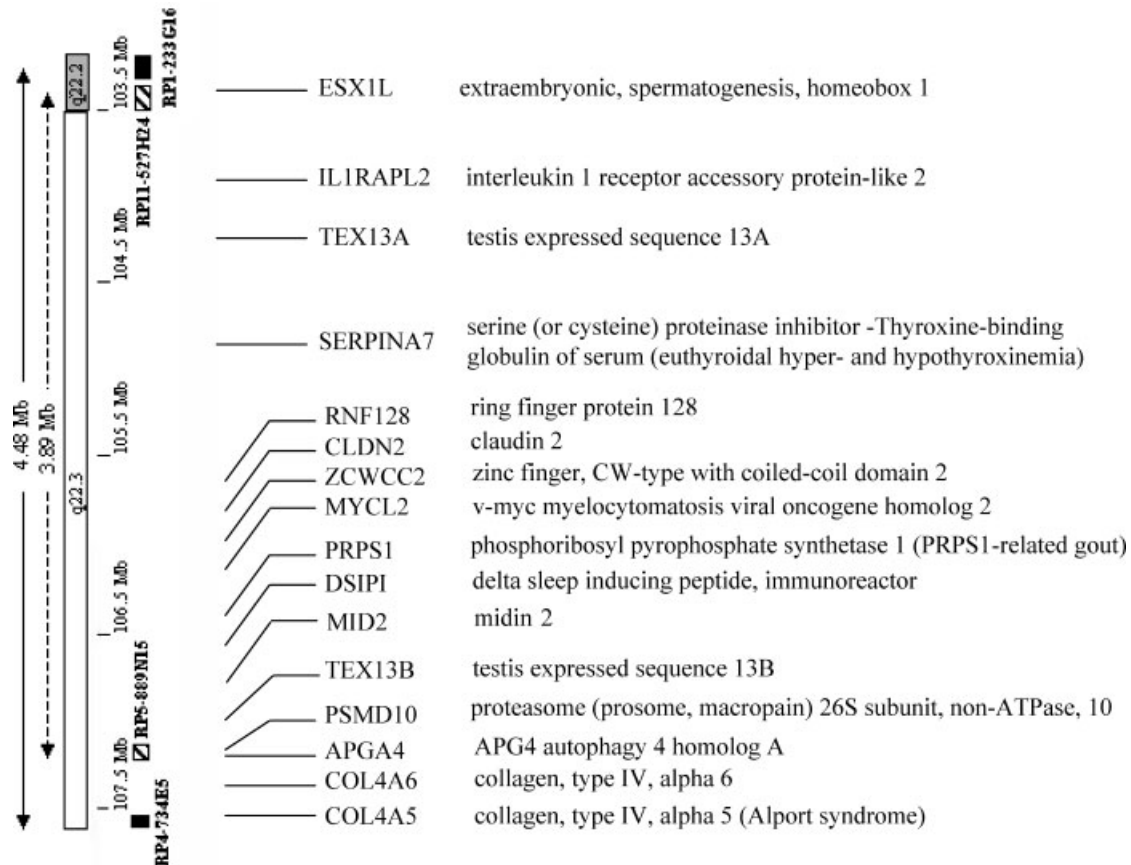


Fig. 3. Schematic ideogram of the Xq22 band showing all known genes mapped into the duplicated segment.

TBG/total T4 in the mother, although she is euthyroid, and possibly in the proband, could be a predisposing factor for the trigonocephaly in our patient. Most patients with FG syndrome do not have trigonocephaly; however, it is possible that the presence of this malformation depends not only on the genes involved in the duplication but also on the genetic background of the mother.

PRPS1, encoding a ubiquitous isoform of the phosphoribosyl pyrophosphate synthetase (PRS1), is associated with an X-linked disorder characterized by excessive purine production, nephrolithiasis, gout, and high levels of uric acid. This disorder is caused by the overexpression of PRS1 either due to gain-of-function mutations or to a mechanism not yet identified [Ahmed et al., 1999]. Clinical symptoms in severe cases can include mental retardation, seizures and minor anomalies, such as triangular face, prominent forehead and hypotelorism, characteristics shared by our patient. Patient's mother did not have elevated serum uric acid, but she presented nephrolithiasis during pregnancy, which might be caused by *PRPS1* overexpression.

MID2 encodes midin2, belongs to the B-box family and is closely related to *MID1*. Quaderi et al. [1997] showed that mutations in *MID1* were responsible for the Opitz G/BBB syndrome (MIM#300000). FG and Opitz G/BBB syndromes share many anomalies especially of midline structures. These syndromes are in many cases indistinguishable suggesting that they might be pathogenically related [Opitz et al., 1991]. The proteins encoded by *MID1* and *MID2* can homo- and heterodimerize with each other and co-localize to the microtubular cytoskeleton in the cytoplasm [Buchner et al., 1999; Short et al., 2002]. Functional redundancy and spatial and temporal overlap in the expression patterns of these two genes have been demonstrated [Buchner et al., 1999; Granata et al., 2005] but a causative relationship between *MID2* and Opitz G/BBB syndrome was not confirmed in a preliminary mutational screening [Franco, unpublished data in Short et al., 2002]. The clinical similarity between Opitz G/BBB and FG syndromes and the homologies between *MID1* and *MID2*, points to *MID2* as a positional and functional candidate gene for FG syndrome.

The segment containing *MID2* and *PRPS1* on Xq22 is highly homologous to Xp22 where *MID1* and *PRPS2* are mapped, and probably arose as a result of an intrachromosomal duplication on an ancestral X chromosome [Buchner et al., 1999]. It seems therefore, that the region duplicated in our patient is involved with a variety of duplication mechanisms, giving rise to segmental and gene duplications.

At the moment, we cannot discard the possibility that the *FGS5*-related FG syndrome is a duplication contiguous gene syndrome. Screenings of FG syndrome patients for *MID2* and *PRPS1* duplications and mutations should be performed to properly define the involvement of these genes in FG syndrome.

Taken together, we suggest that the region duplicated in our patient and his mother contains one or more genes for *FGS5* and that further studies in FG syndrome patients maybe helpful in an elucidation of this heterogeneous entity.

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