Börjeson–Forssman–Lehmann syndrome: defining the distal border of the candidate gene interval in Xq26

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Börjeson–Forssman–Lehmann syndrome is an X-linked disorder with major symptoms of mental retardation, epileptic seizures, obesity, hypogonadism, large, fleshy ears, and prominent supraorbital ridge. We performed a haplotype analysis on the DNA level with 22 X-chromosomal markers in a BFLS family with four affected males and three female carriers. Our data point to a common interval of 10.5 Mbp. We defined the distal border of the BFLS candidate gene interval. Together with data of previously published reports and with data from the public databases, we have reduced the size of the BFLS interval to only 2.1 Mbp, a small region with only seven genes inside. One candidate gene for BFLS in this interval is *FGF13*.

Key words: Börjeson–Forssman–Lehmann syndrome; X-linked mental retardation, obesity, mapping; FGF13

INTRODUCTION

The term X-linked mental retardation (XLMR) comprises a group of hitherto 156 syndromal and nonspecific disorders of the human X chromosome as summarized by the online catalogue of "Mendelian Inheritance in Man" disorders (OMIM; accession date May 2002). Meanwhile, there are 112 listed among these 156 disorders where the gene locus has been determined, although the gene itself may have not been identified yet. Börjeson–Forssman–Lehmann syndrome (BFLS) belongs to the syndromic forms of X-linked mental retardation disorders [1]. BFLS has been mapped by linkage analysis to the chromosomal region Xq26-q27 with the highest LOD score between the markers DXS10 and DXS51 [2, 3]. The candidate gene region was refined later by an inclusion of additional family members [4] and by an improved genetic map of this region [5]. The highest LOD score was then calculated for the interval between the markers DXS425 and DXS105. The localization of the *SOX3* gene [6] close to the marker DXS51 stimulated the search for mutations in this *SRY*-related HMG box gene. It was found, however, that *SOX3* has no mutations in patients with BFLS [4]. In the mouse genome, a 17-map-unit interval has been defined by interspecies hybrid crossing [7]. The interval contains two loci known to affect testis weight and testis size in man. The orthologous region in man includes the *FMR1* locus which is associated with macroorchidism and the BFLS locus in this region known to be associated with hypogonadism. The murine *Sox3* locus maps outside of this interval, but its putative interaction with the product of *Ihtw1* (interspecific hybrid testis weight 1) from this interval was discussed [7]. Another candidate gene from the BFLS interval in Xq26 is the guanine nucleotide exchange factor *ARHGEF6*. A sequence analysis of this gene in individuals with BFLS or another X-linked mental retardation syndrome MRX27 excluded this gene as a candidate for BFLS [8]. The fibroblast growth factor homologous factor 2 (*FHF2*), also known as *FGF13*, maps to the BFLS candidate gene region in Xq26-q27, although a different localization in Xq21 was reported after isotopic *in situ* hybridization [9]. *FGF13* is expressed in skeletal muscle and brain. Both, its expression profile and its map position within the

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newly defined BFLS interval makes it a candidate gene [10]. This newly defined interval is characterized by a duplication breakpoint in Xq26 with the proximal border close to the marker DXS155 [10].

In this report, we present data on a family with four affected individuals with BFLS. Using segregation analysis with X-chromosomal markers, we defined the telomeric border of the BFLS candidate gene region.

MATERIALS AND METHODS

Subjects

All children in our study are from non-consanguineous marriages. The index patient III:3 reported from her sister III:1 who had two spontaneous abortions and her affected brother III:4 with a mental retardation handicap. In addition three cousins of her, all males, are affected. All boys are severely mentally retarded, had delayed milestones, and none of them had ever attended school. They live today in an institution for severely mentally retarded persons. The clinical features of affected children has been already entirely described in 1998 [11]. The characteristic phenotype of the brothers and their male cousin prompted our diagnosis of BFLS. In order to make predictions about the relative risk for BFLS for the children of III:3, the haplotypes of all family members were studied.

Methods

DNA was extracted from peripheral blood lymphocytes according to a standard procedure [12] and the concentration was adjusted to 100 ng/µl. Inasmuch as the pedigree is compatible with an X-chromosomal mode of inheritance we performed a segregation analysis with 22 highly (heterozygosity ≥ 60%, if available) polymorphic microsatellite markers from the human X chromosome. The markers cover the region between Xp22.3 and Xq28 in intervals of 600 Kb to 21.7 Mb. Large intervals were chosen for a coarse coverage of the entire X chromosome, and small intervals were chosen for the fine localization of crossovers and for the definition of the candidate gene region. Primer sequences were taken from the GenomeDataBase (GDB; http://www.gdb.org). Absolute X-chromosomal physical map positions (in Mbp) of the markers were taken from NCBI's databases (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome). The marker sequences were amplified via PCR according to standard PCR protocols (95 °C / 30 s; 52–55 °C / 30 s; 72 °C / 30 s; 30–35 cycles; 1.5–2.5 mM $MgCl_2$). The PCR for the androgen receptor (AR) trinucleotide repeat was performed with Amplitaq polymerase. The PCR products were separated on denaturing 6% acrylamide gels and alkali-blotted onto a nylon membrane. A $(CA)_{11}$ oligonucleotide probe was labeled terminally with γ-^{32}P-ATP by T4 polynucleotide kinase [13] and used as a probe for the Southern hybridization. Probed filters were exposed to X-ray films after stringent post-hybridization washings. Some PCR (AR, DXS1068, GATA175D03 = DXS9902) were carried out with Cy5-labelled primers and the products were analyzed in an ALFexpress sequencing device with the "Fragment Manager" software (Amersham Biosciences). Absolute fragment sizes were determined by inclusion of a labeled size marker on the same gel. The allele sizes of the markers from the pedigree were entered in the Cyrillic Pedigree Analysis software.

RESULTS

We present a family with typical symptoms of Börjeson–Forssman–Lehmann syndrome. Phenotypic similarities with previously reported cases of BFLS, the comprehensive analysis by a syndromologist, a database search in the Possum database (release 5.2) of the Murdoch Institute (http://www.possum.net.au/), and a database search at OMIM confirmed the primary diagnosis of BFLS and excluded other obesity–mental retardation–hypogonadism syndromes. Previous studies which had mapped the candidate for BFLS into the Xq26–q27 region prompted a detailed marker analysis with 22 markers of the entire X chromosome with a higher marker density in the Xq candidate gene region. Thus, we have analyzed a region of 151 Mbp in intervals from 0.6 Mbp to 21.7 Mbp. The Xq26–q27 interval itself measures 19.5 Mbp. All individuals of the pedigree were included in the haplotype analysis with the exception of the two spontaneous abortions of subject III:1 and her spouse. The pedigree is shown in Figure. The allele sizes of all markers are given. The allele sizes of marker DXS102, which maps at position 134.522 Mbp, were not determined in individuals II:1, III:1 and III:3 for two reasons. Firstly, the paternal X-chromosome (II:1) does not contribute to the phenotype. Secondly, individual III:3 has inherited the grandpaternal X chromosome from her mother, whereas III:1 obtained the grandmaternal X-chromosome with the same crossovers as her mother. Some further crossovers had occurred during meiosis in her mother. Individuals III:1 (carrier state), III:4, III:5, III:6, and III:7 share an identical haplotype from the proximal marker DXS1047 (map position 125.046 Mbp) to the more distal marker DXS1232 (map position 135.52 Mbp). This interval is inherited from the respective maternal grandmother and implies that, both, II:2 and II:3, are carriers for the di-

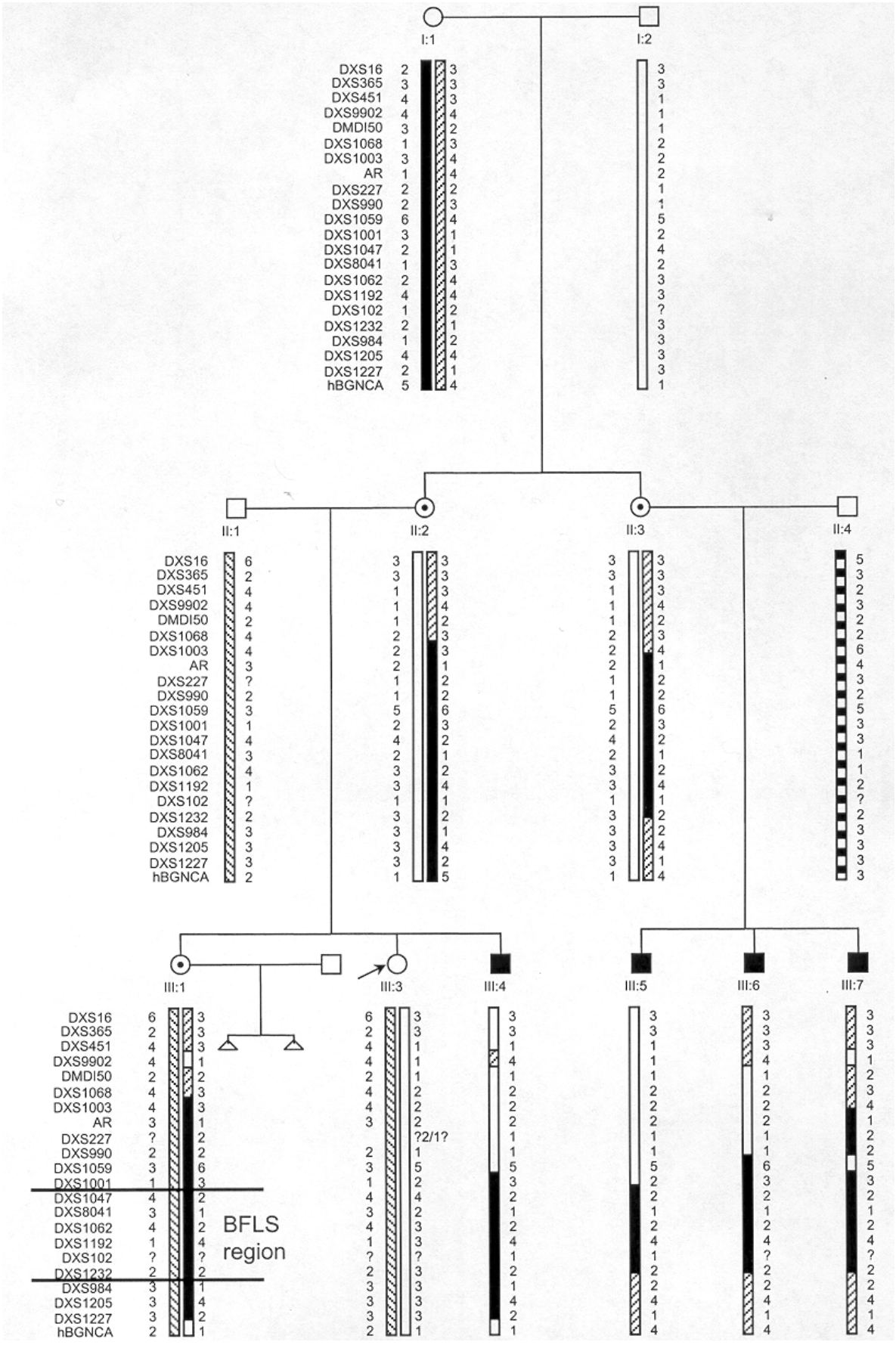

Figure. Segregation analysis with X-chromosomal markers. The common haplotype of all affected boys and the carrier female (III:6) expands from DXS1047 to DXS1232. These markers define an interval of 10.5 Mbp

sease locus. However, they are not affected, because it is an X-chromosomal recessively inherited trait and women have two alleles; one of it is silenced by Lyonization. In conclusion, there is apparently no increased risk for the offspring of III:3 because she inherited no disease-associated alleles from her mother.

In the family with three affected brothers, there have been some crossovers during maternal gametogenesis. All boys share an identical haplotype from the markers DXS1047 to DXS1232 (inclusive), which defines the borders of the interval for the candidate gene (see Figure). This interval measures 10.5 Mbp from positions 125.046 Mbp (DXS1047) to 135.52 Mbp (DXS1232).

DISCUSSION

We have studied a family with Börjeson–Forssman–Lehmann syndrome (BFLS) in order to provide data that allow the precise localization of the candidate gene. We present a segregation analysis for X-chromosomal marker alleles with BFLS in a three-generation family with four affected males and three carrier females. The interpretation of the marker data helped the index patient from our study in risk estimation for her family planning. Further, we present sufficient mapping data to re-define the distal (telomeric) border of the BFLS candidate gene region.

The clinical and familial findings in subjects with BFLS are infrequently diagnosed, making BFLS a rare disorder. The phenotypic range with overlapping symptoms of other obesity–mental retardation-related syndromes may be responsible that the diagnosis of BFLS might be failed to notice frequently. Therefore, it is explicable that two severely handicapped females in a family with BFLS were recognized not before 30 years after birth [14]. The phenotype of the subjects from our study agrees in most criteria with the major findings [15] of BFLS. The differential diagnosis includes Prader–Willi syndrome (PWS), Coffin–Lowry syndrome (CLS), and Bardet–Biedl syndrome (BBS1-6). The absence of postaxial polydactyly of both hands and feet which is a major constituent of Bardet–Biedl syndrome, excludes this disorder in the family of our study. CLS subjects have large, soft hands with tapering fingers, short metacarpals, and drumstick terminal phalanges, but do not show hypogonadism, thus excluding this disorder in our family. Here, we can demonstrate the invaluable data of the segregation analysis which helps to distinguish syndromes from other phenotypically similar syndromes or non-specific XLMR. With these molecular data, we could exclude CLS not by the phenotypic description alone, but also by haplotype analysis of affected and non-affected individuals of the pedigree. It is obvious that the inclusion of marker data would also help in the interpretation of cases described earlier, where one affected female with BFLS of feeble-minded parents had been presented [16]. Finally, the large fleshy ears and the prominent supraocular ridge in the affected subjects of our study clearly exclude PWS as another obesity mental retardation syndrome.

It is obvious from the haplotype analysis that the candidate gene interval is limited proximally by the marker DXS1047, which is at position 125.046 Mbp on the physical map of the X chromosome. The distal limit is defined by the marker DXS1232, positioned at 135.52 Mbp (see Figure). Thus, we present an interval of 10.5 Mbp. It is noteworthy that another marker maps within our candidate interval, namely DXS102, a marker that has already been defined in 1992 and was supposed to map "very close" to the BFLS region [17]. A duplication 46,Y,dup(X)(q26q28) has defined the proximal breakpoint of the BFLS disease region [10]. The minimal duplication breakpoint maps between the markers DXS155 (included) and SF10 (included). The non-included adjacent markers are DXS1062 (133.442 Mbp; cen) and DXS1211 (134.444 Mbp; tel). This defines a proximal border for BFLS within a range of 1 Mbp. We defined the distal border of this disorder by exclusion of the marker DXS984 (DXS1232 included; mapping to 135.52 Mbp). Thus, the BFLS candidate gene lies within an interval of only 2.1 Mbp from 133.442 to 135.52 Mbp. Several candidate genes have been suggested for BFLS and some have already been studied in more detail.

One candidate gene, namely *SOX3*, had been postulated as a BFLS candidate gene. *SOX3* maps close to the marker DXS984, which still belongs to the BFLS interval as has been reported [18]. A transcript map of Xq27 shows *SOX3* on the genetic map within the BFLS candidate region of the morbid map [19]. No mutations were found in *SOX3* of patients with BFLS in another study [5]. A mental retardation syndrome with some overlapping symptoms of BFLS had been mapped by linkage analysis to the same interval as *SOX3* [20]. It must not be confused with BFLS, because the different clinical features suggest that these are distinctive conditions. With our data we provide a strong evidence which excludes *SOX3* as a candidate gene. Common alleles of marker DXS984, which maps close to *SOX3*, were excluded from the candidate gene region in our BFLS family. DXS984 maps at position 135.872 Mbp and *SOX3* at 135.826 Mbp, just 50 kbp apart. Therefore, both mutation and segregation analyses exclude *SOX3* as a BFLS candidate gene.

Considering a slightly larger 3.5 Mbp interval from 132.0 to 135.52 Mbp, only a few genes and some ESTs map into this region. The mapped genes

are *ARHGEF6*, *RBMX*, *GPR101*, *ZIC3*, *FGF13*, *F9*, and *MCF2*. The guanine nucleotide exchange factor *ARHGEF6* maps at position 132 Mbp, which is just within the limits of our interval. Exon skipping in *ARHGEF6* was reported to be responsible for the nonspecific X-linked mental retardation type 46 (MRX46; OMIM #300267). Mutations in *ARHGEF6* affect the Rho GTPase cycle [21]. No *ARHGEF6* mutations were detected in the DNA of BFLS individuals [8]. *RBMX* is an RNA-binding motif protein spermatogenesis gene with multiple Y-chromosomal copies of the *RBMY* gene family. No diseases have been reported to be associated with *GPR101*, the G protein-coupled receptor 101. This gene is expressed in the CNS solely in the caudate putamen and the hypothalamus [22]. No further data are available. Mutations in the zinc finger protein *ZIC3* are associated with visceral heterotaxy or situs abnormalities [23]. *F9* is the well known coagulation factor IX associated with hemophilia B. *MCF2*, also called *DBL*, is an oncogene, where no clinical condition could be attributed yet to the loss of the *MCF2* gene. Finally, *FGF13*, also known as *FHF2*, remains as a BFLS candidate gene. Its primary structure has been determined and shows alternate exons 1, 1A and 1B, leading to two isoforms with a different 62 amino acid N-terminus [10]. The expression of *FGF13* in the developing nervous system makes it the best and only candidate gene from this region for BFLS. A mutational analysis together with studies on the distribution of the isoforms of *FGF13* in patients with BFLS will help in pinpointing the BFLS candidate gene.

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BÖRJESON-FORSSMAN-LEHMANN SINDROMAS: KANDIDATINIO GENO DISTALINĖ RIBA, NUSTATYTA CHROMOSOMINIAME Xq26 INTERVALE

Santrauka

Börjeson-Forssman-Lehmann sindromas (BFLS) – su X chromosoma susijusi liga, kurios pagrindiniai simptomai yra protinis atsilikimas, epilepsijos priepuoliai, nutukimas, hipogonadizmas, didelės mėsingos ausys bei atsikišę antakių lankai. Buvo atlikta DNR haplotipų analizė su 22 X chromosomos žymenimis BFLS šeimoje, kurioje yra keturi sergantys vyrai ir trys heterozigotiškos moterys. Mūsų tyrimas apėmė 10,5 Mbp intervalą, leidusį nustatyti BFLS kandidatinio geno distalinę ribą. Palyginę mūsų gautus rezultatus su skelbtaisiais literatūroje, mes manome sumažinę BFLS geno intervalą iki 2,1 Mbp – nedidelio fragmento, teturinčio septynis genus. Kandidatinis sindromo genas šiame intervale yra *FGF13.*