Familial 1.3-Mb 11p15.5p15.4 Duplication in Three Generations Causing Silver-Russell and Beckwith-Wiedemann Syndromes

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Abstract

Silver-Russell syndrome (SRS) and Beckwith-Wiedemann syndrome (BWS) are 2 opposite growth-affecting disorders. The common molecular cause for both syndromes is an abnormal regulation of genes in chromosomal region 11p15, where 2 imprinting control regions (ICR) control fetal and postnatal growth. Also, many submicroscopic chromosomal disturbances like duplications in 11p15 have been described among SRS and BWS patients. Duplications involving both ICRs cause SRS or BWS, depending on which parent the aberration is inherited from. We describe to our knowledge the smallest familial pure 1.3-Mb duplication in chromosomal region 11p15.5p15.4 that involves both ICRs and is present in 3 generations causing an SRS or BWS phenotype.

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Silver-Russell syndrome (SRS, OMIM: 180860) is a clinical disorder that primarily includes pre- and postnatal growth disturbances with normal head circumference,

along with other distinctive characteristics like asymmetry of body and limbs, craniofacial features, and 5th finger (F5) clinodactyly. Beckwith-Wiedemann syndrome (BWS, OMIM: 130650) is also a growth-affecting disorder which causes overgrowth with many additional clinical features like macroglossia, organomegaly, and increased risk of childhood tumors [Weksberg et al., 2010]. The most common molecular cause for both syndromes is an abnormal regulation of genes in chromosomal region 11p15 [Gicquel et al., 2005], where 2 imprinting control regions (ICR1 and ICR2) control fetal and postnatal growth. ICR1 contains the maternally expressed *H19* gene and the paternally expressed *IGF2* gene, whereas ICR2 contains the maternally expressed *KCNQ1* and *CDKN1C* genes and the paternally expressed *KCNQ1OT1* gene. Normally, genes that are expressed in 1 allele are imprinted (methylated) and silenced in the other allele. Imprinting disturbances lead to abnormal expression of these genes and the clinical phenotypes of SRS or BWS. Approximately 40% of SRS patients show hypomethylation of ICR1, whereas up to 50% of BWS patients have ICR2 hypomethylation [Eggermann et al., 2008]. In addition, in SRS and BWS patients, numerous submicroscopic chromosomal disturbances have been described, among them duplications, deletions, inversions and/or translocations affecting chromosome 11p15 [Begemann et al., 2012; Fokstuen and Kotzot, 2014]. Large duplica-

Fig. 1. Pedigree of the family with duplication of chromosome 11p15 that involves ICR1 and ICR2. Maternal inheritance of the duplication causes SRS, whereas paternal inheritance causes BWS.

Fig. 2. The proband (III-3) at the age of 7 months (**a**) and at the age of 4.5 years (**b**). Note a relative macrocephaly, facial asymmetry, prominent forehead, and thin upper lip with downturned corners of the mouth. **c** Hands of the proband at the age of 4.5 years showing 5th finger clinodactyly.

tions that affect both ICRs in 11p15 are known to be associated with growth retardation/SRS or overgrowth/BWS depending on the parental origin of the imbalance [Begemann et al., 2012]. These duplications are mostly associated with translocations.

To our knowledge, we describe here the smallest familial pure 1.3-Mb duplication in chromosomal region 11p15.5p15.4 that involves both ICRs and is present in 3 generations causing an SRS or BWS phenotype.

Case Report

The proband (fig. 1, III:3), aged 5 years, was prematurely born at the 31st gestational week via cesarean section due to intrauterine growth restriction, oligohydramnion, and fetal distress. Her prenatal course is also shortly described by Muru et al. [2013]. The fetal karyotype was normal (46,XX), and no visible rearrangements were seen. Her birth weight was 890 g (−2.5 SD), birth length 34 cm (−3.5 SD), and head circumference 28 cm (−1 SD). SRS was clinically diagnosed as she also had body asymmetry, distinctive facial features (triangular face, prominent forehead, thin upper lip with downturned corners of the mouth) as well as dysmorphic ears, hypertelorism, blue sclerae, clinodactyly, and sandal groove of the toes (fig. 2a). Despite prematurity, her early postnatal period was normal. After birth, she had many respiratory infections, and in the second year of life, she was diagnosed with allergic asthma. As an infant, she had problems with gastroesophageal reflux and still has poor appetite. Her psychomotor development has been appropriate to age, although she has slight muscular hypotonia. She started walking independently when she was 17 months old, and she has good language skills. Because of astigmatism, she wears glasses.

At the age of 4.5 years, her height was 95.8 cm (−2.5 SD), weight 11.1 kg (−3.5 SD), and head circumference 51.1 cm (0 SD) (fig. 2b, c). The endocrinologist considered growth hormone therapy, but as the patient's growth velocity was appropriate (4.8 cm within 6.5 months), a decision about the treatment was postponed.

The birth weight of the proband's mother (II:2) was 4,700 g (+2.5 SD), and now her height is 182 cm (+3 SD). She has had the characteristic BWS facies since early childhood (fig. 3a, b) and also ear creases and pits. Her childhood passed without major problems. At the age of 10 years, she was diagnosed with latent diabetes, and now she has type 2 diabetes. She fulfilled 3 major diagnostic clinical criteria for BWS described by Weksberg et al. [2010]. She had 3 pregnancies, and the patient described here was her second delivery.

The brother of the patient (III:2) has a normal phenotype; his birth weight was 3,876 g, and he has a normal postnatal growth.

The maternal grandfather's (I:1) height was 180 cm (0 SD), and he died of lung cancer at the age of 84. We have the information about his phenotype only by photographs (fig. 3c). He had 2 major clinical criteria (macrosomia, positive family history) and 1 minor clinical criterion (characteristic facies) for BWS described by Weksberg et al. [2010].

Material and Methods

Microarray Analysis

DNA was extracted from peripheral blood of the proband and her mother and grandfather using a standard salting out procedure. Chromosomal microarray analysis (CMA) using Illumina HumanCytoSNP-12 BeadChips (Illumina Inc., San Diego, Calif., USA) was performed according to the manufacturer's protocol. Genotypes were called using GenomeStudio v2009.1 software (Illumina Inc.); log₂ R intensity ratio and B-allele frequency values were extracted from GenomeStudio and used in subsequent DNA copy number analysis with quantiSNP v1.1 software using parameters suggested by the developers [Colella et al., 2007].

Fig. 3. The mother (II:2) in infancy (a) and at the age of 6 years (b). c The grandfather (I:1) at the age of 70 years. Note the characteristic BWS facies.

Methylation Study

Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) was performed using SALSA® MS-MLPA® probe mixture ME030-B2 BWS/SRS (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions. PCR products were analyzed on a fluorescent capillary sequencer using the Genescan software (ABI 3130XL Genetic Analyzer, Applied Biosystems, Darmstadt, Germany). All MS-MLPA reactions were performed twice. MLPA data analysis was performed with the Coffalyser software (MRC-Holland).

Results

Microarray Analysis

In the proband, CMA revealed a ~1.3-Mb duplication on the short arm of chromosome 11 (arr[hg19] 11p15.5p15.4(1,849,354–3,116,073)×3) that involves the ICR1 and ICR2 regions. The same duplication was also found in her mother and maternal grandfather and has been inherited within 3 generations in the same size and without rearrangements. We concluded that this is a tandem duplication.

Methylation Study

The MS-MLPA analysis of the proband, her mother, and maternal grandfather revealed an increase in copy number variation in altogether 26 MLPA probes in the whole analyzed 11p15.5 region. The mean ratio of these probes was 1.38 (SD = 0.13). Probe ratios >1.3 are regarded as indicative of a heterozygous duplication (copy number change from 2 to 3 alleles). In the proband, a mild hypomethylation was detected in the ICR1 region, while in the ICR2 region hypermethylation was found. The proband's mother had ICR1 hypermethylation and ICR2 hypomethylation, and the maternal grandfather had also ICR1 hypermethylation and mild ICR2 hypomethylation.

Discussion

We report the familial case of an interstitial duplication of chromosome 11p15 which involves both ICR1 and ICR2. To our knowledge, this is the first report of such a chromosomal rearrangement in 11p15 that is present in at least 3 generations. Although not described in many patients, maternal duplication of 11p15 is one possible cause of SRS with a frequency of 0.9% [Eggermann et al., 2014]. There are at least 13 studies reporting maternally inherited duplications as a cause of SRS. Most of such rearrangements involve ICR1 and ICR2 and are usually caused by unbalanced translocations, but duplication of the whole ICR2 as well as partial duplication of ICR1 can also cause SRS [Fisher et al., 2002; Eggermann et al., 2005; Schonherr et al., 2007; South et al., 2008; Blik et al., 2009; Cardarelli et al., 2010; Eggermann et al., 2010; Bonaldi et al., 2011; Demars et al., 2011; Begemann et al., 2012; Chiesa et al., 2012; Hu et al., 2013; Brown et al., 2014].

In case of maternal inheritance, duplication of both ICR1 and ICR2 in 11p15 causes the SRS phenotype, whereas a paternally inherited similar duplication causes the BWS phenotype. Such familial parent-of-origin dependent opposite clinical syndromes have been reported also in other studies, but compared to our report, their genetic causes have been different (unbalanced translocations causing duplication of 11p15 and between-arm intrachromosomal insertion of 11p15.5) [South et al., 2008; Blik et al., 2009; Cardarelli et al., 2010; Brown et al., 2014]. In our proband, interstitial duplication of 11p15 shows hypomethylation in ICR1 and hypermethylation in ICR2. Therefore, there are 2 active copies of *CDKN1C* and *H19*, which is compatible with either maternal uniparental disomy or maternal duplication in 11p15 [Chiesa et al., 2012]. Hence, the SRS phenotype in the proband

reflects an increased expression of the maternally expressed *CDKN1C*. The BWS phenotype in the mother and maternal grandfather is caused by overexpression of *IGF2*. Increased dosage of paternally expressed genes is caused by ICR1 hypermethylation.

It is very difficult to compare our patient with previously reported SRS patients with maternally inherited duplications. First, the data describe variable phenotypes even if the molecular cause was similar. Secondly, the phenotype of patients with duplications that are caused by unbalanced translocations might be influenced by accompanying chromosomal imbalances [South et al., 2008; Blik et al., 2009; Hu et al., 2013]. Chiesa et al. [2012] described a patient with a de novo maternal 1.2-Mb inverted duplication and classical SRS phenotype, who molecularly and clinically most resembles our proband. Unfortunately, we have not done FISH analysis in our proband.

For the clinical diagnosis of SRS in our proband, we used the Bartholdi scoring system [Bartholdi et al., 2009]. She met 11 positive criteria out of 15, including pre- and postnatal growth failure, body asymmetry, classical facial phenotype, and F5 clinodactyly. This relatively high score and classical findings of SRS might be explained by the fact that the interstitial duplication involves only imprinted regions. It also explains her age-appropriate psychomotor development, which is usually normal among SRS patients with primary imprinting disorders [Kotzot, 2008; Wakeling et al., 2010; Demars et al., 2011].

Contrary to the patient's classical SRS phenotype, the BWS clinical picture of her mother and grandfather has been probably quite mild and has become less evident in adulthood as described among adults with BWS [Weksberg et al., 2010]. Their childhood passed without major problems (e.g. childhood tumors). Unfortunately, we cannot determine the origin of the duplication further in this family, but the proband's maternal grandfather must have carried his duplication on a paternally inherited chromosome 11, and this might be either inherited or de novo in origin.

In conclusion, although maternal duplications involving 11p15 are rare, they must be considered when copy number variation together with an aberrant methylation pattern in ICR1 and ICR2 at 11p15 is found. This should lead to further genetic testing to detect the cause of such chromosomal rearrangement, and it might reveal new information about previous generations who have not been suspected to have a clinical syndrome.

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