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Original article

Phenotypic discordance upon paternal or maternal transmission of duplications of the 11p15 imprinted regions

J. Bliek [a,*], S. Snijder [a], S.M. Maas [a,b], A. Polstra [a], K. van der Lip [a], M. Alders [a], A.C. Knegt [a], M.M.A.M. Mannens [a]

[a] Department of Clinical Genetics, Academic Medical Centre, Amsterdam, The Netherlands
[b] Department of Pediatrics, Academic Medical Centre, Amsterdam, The Netherlands

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ABSTRACT

We report on two families in which the parental origin of duplications of the BWS imprinted regions on chromosome 11p15 influences the phenotype.

In family A the transmission of a t(4; 11)(q35; p15.5) translocation results in duplication of BWSIC1 and BWSIC2. If this duplication is transmitted from the father, the extra chromosomal material has the paternal imprint. This results in overexpression of *IGF2* and consequently an overgrowth phenotype. If the duplication is transmitted from the mother, the extra chromosomal material has the maternal imprint, resulting in overexpression of *CDKN1C* and a growth retardation phenotype.

In family B an interstitial duplication of BWSIC1 results in an overgrowth phenotype when inherited from the father, similar to family A. However, no change in phenotype is observed if the duplication is transmitted through the mother suggesting that increased dosage of maternally expressed genes in the duplicated region has limited effect on the phenotype.

1. Introduction

Some genes in the human genome escape Mendelian inheritance; their expression depends on the parental origin of the chromosome on which the gene resides. This phenomenon is called genomic imprinting. Imprinted genes are clustered in chromosomal domains and monoallelic expression of the genes in these domains is controlled by imprinting control regions (ICRs). ICRs are often methylated in a parent-of-origin-specific manner.

Most ICRs are less than a few kilobases in size and comprise sequences that are rich in CpG dinucleotides (CpG islands). The cytosine residues in these CpG islands are methylated either on the paternal or on the maternal chromosome. Regions in ICRs containing these CpG islands are called Differentially Methylated Regions (DMRs).

Chromosome 11p15 harbours two clusters of imprinted genes involved in the regulation of prenatal growth: Beckwith–Wiedemann Syndrome Imprinting Clusters 1 and 2 (BWSIC1 and BWSIC2) [1]. In each cluster imprinted expression of genes is regulated via an imprinting control region, which contains a Differentially Methylated Region (DMR) [2,3].

The most distal cluster, BWSIC1 includes a non-coding (nc) RNA *H19* and a gene that codes for the embryonic growth factor: Insulin-like Growth Factor 2 (*IGF2*). DMR1 is situated in BWSIC1 in close proximity to *H19*. If DMR1 is unmethylated, *H19* is expressed while if it is methylated, *IGF2* is expressed. In normal individuals the paternal allele of DMR1 is methylated, whereas the maternal allele is unmethylated; *IGF2* is expressed from the paternal allele while *H19* is expressed from the maternal allele [4].

BWSIC2 is located more centromeric on chromosome 11p15. It contains a non-coding RNA *KCNQ1OT1* and, among others, a gene that codes for a negative regulator of cell proliferation Cyclin Dependent Kinase Inhibitor 1C (*CDKN1C*). DMR2 is located in the promoter of *KCNQ1OT1*. If DMR2 is unmethylated, *KCNQ1OT1* is expressed, if it is methylated, *CDKN1C* is expressed. In normal individuals the paternal allele of the DMR2 is unmethylated,

* Corresponding author at: Department of Clinical Genetics, DNA diagnostics, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. Tel.: +31205667899.
E-mail address: j.bliek@amc.uva.nl (J. Bliek).

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whereas the maternal allele is methylated; *KCNQ1OT1* is expressed from the paternal allele whereas *CDKN1C* is expressed from the maternal allele [5].

Aberrant imprinting in the 11p15 region can result in two syndromes with opposite growth disturbances as reviewed by Eggermann [6].

Children born with Beckwith–Wiedemann Syndrome (BWS, OMIM 130650) have an increased birth weight and show typical overgrowth related features such as an enlarged tongue (macroglossia), organomegaly, abdominal wall defects and hemihypertrophy. BWS is caused by epigenetic changes within the 11p15 imprinted region [7–9].

In 5% of BWS patients the normally unmethylated maternal allele of DMR1 becomes methylated, resulting in bi-allelic expression of *IGF2* (MIM 147470). Since overexpression of *Igf2* in mice results in most of the symptoms of Beckwith–Wiedemann syndrome [10], this gene is thought to be a key determinant of the overgrowth phenotype in these patients.

In 55% of BWS patients the normally methylated maternal allele of DMR2 becomes demethylated, which abolishes the expression of *CDKN1C* [11]. Since *Cdkn1c* knockout mice show many of the BWS features [12], this gene is considered to play a major role in the overgrowth phenotype of these patients.

In 20% of BWS patients both DMRs show aberrant methylation patterns, caused by the presence of two paternal copies of the 11p15 region (paternal uniparental disomy, pUPD). In 20% of BWS patients no genetic defect can be detected.

A syndrome with growth characteristics opposite to BWS, is Silver Russell Syndrome (SRS, OMIM 180860). Patients show intrauterine and postnatal growth retardation and a typical facial appearance. Although genetic changes in other chromosomal regions are observed [13], in one third of SRS patients the imprinted regions on chromosome 11p15 are involved. In 30% of the SRS cases demethylation of the paternal allele of DMR1 can be detected [14–17]. This is associated with decreased expression of *IGF2*. Duplications of chromosome 11p15 of maternal origin have been described in a minority of SRS patients [18,19]. In one case, a maternally transmitted duplication of 11p15 was restricted to BWSIC2 [20].

Recently a familial translocation involving chromosomes 4p and 11p has been described [21]. The unbalanced translocation results in Wolf-Hirschhorn Syndrome (WHS) caused by a deletion of chromosome 4p16.3. However, the WHS phenotype in this family is modified by the presence of a duplication of 11p15. Maternal transmission of this duplication results in SRS-like features, while paternal transmission of the duplication results in a BWS-like phenotype. The authors postulate that the phenotypic differences may be the result of methylation defects in the 11p15 imprinting domains.

In this study we describe 2 3-generation families (see Fig. 1) with an inherited duplication of chromosome 11p15. In family A, 2 carriers of the same unbalanced translocation der(4)t(4; 11)(q35; p15.5) display different clinical features, whereas in family B a small duplication within chromosome 11p15.5 results in phenotypic variation.

2. Methods

2.1. Karyotyping and fluorescent in situ hybridisation (FISH)

Routine procedures were used for G-banded chromosome analysis of cultured peripheral blood lymphocytes. For fluorescence in situ hybridisation (FISH) commercially available probes for the centromeric regions CEP4 and CEP11 (D11Z1) (Abbott Molecular, USA) and for the subtelomeres (Chromoprobe Multiprobe T System, Cytocell, UK) were applied according to manufacturer's recommendations. A similar protocol was used for non-commercial probes GS31J3 (4qter), GS908H22 (11pter), *INS*/*IGF2* (11p15.5) and *HRAS* (11p15.5).

2.2. Multiplex ligation dependent probe amplification (MLPA)

MLPA was performed using SALSA MLPA ME030-B1 BWS/SRS kit for the 11p15 region following standard procedures (MRC Holland, The Netherlands).

2.3. Array platform for comparative genomic hybridisation (CGH)

For comparative genomic hybridisation (CGH) an Oxford custom 2 × 105 k array (design ID 019015) was used (21.7 Kb overall median probe spacing, 18.9 Kb in Refseq genes, Agilent, USA). Data were analysed using DNA Analytics 4.0.76 software (Agilent, USA).

2.4. High resolution melting analysis (HRMA)

Methylation of DMR1 and DMR2 was analysed by using High Resolution Melt Analysis (HRMA) as described before [22].

2.5. Clinical reports

2.5.1. Family A

Patient AII-3 was born after an uncomplicated pregnancy with a birth weight of 1660 g (<−2,5SD) and a length of 41 cm (<−2,5SD). At birth she showed a relative large head circumference and brachydactyly of the fingers especially of the metacarpals of the fifth fingers. X-ray of the knees showed dysplasia of both patellae with lateralisation of the patellae. X-rays of the hands showed brachymesophalangy of the fifth finger of both hands and fusion of the lunate and triquetrum bones on both hands. X-rays of the feet show fusion of the navicular and cuneiform bones. Her fifth toes were removed because of complaints of pain.

Her adult height is 138 cm (−2,5SD). She is mildly retarded and works in a sheltered work environment. In 1990 she gave birth to a healthy daughter.

Patient AIII-1 was born with a high birth weight (4650 g, +2SD) at 40 weeks of gestation. She suffered of hypoglycaemic episodes persisting until the age of 6 years. She had macroglossia, a diastasis recti but no ear creases or posterior helical ear pits. She followed a special educational program because of mild mental retardation. Her adult height is 180 cm (+1½ SD) and her weight is 80 kg. Physical examination shows a long face with a sloping forehead, large mandibula with a small maxilla (Fig. 1). Furthermore there are no signs of Beckwith–Wiedemann syndrome.

2.5.2. Family B

BII-2 was born with a normal birth weight of 3400 g (0SD). He had mild learning problems. His adult height is 182 cm (+1 SD).

Patient BIII-1 was born after 39 weeks of pregnancy with a birth weight of 4160 g (+1.5 SD) During pregnancy large kidneys and macroglossia were seen and the diagnosis Beckwith–Wiedemann syndrome was suggested. After birth macroglossia was noticed. A short period of hypoglycaemia existed. There were no abdominal wall defects, no ear creases or pits, no naevus flammeus and no hemihypertrophy. Because of his macroglossia and mild hypotonia he received speech therapy. He attended a special school because of a mild mental retardation. His length is 152 cm at the age of 9 years (+2.5 SD). Physical examination show no evident dysmorphic features.

No clinical data of early childhood of BI-1 are available. Her adult height was 160 cm(−1 1/2SD).