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Prenatal diagnosis of foetuses with congenital abnormalities and duplication of the *MECP2* region

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ABSTRACT

MECP2 duplication results in a well-recognised syndrome in 100% of affected male children; this syndrome is characterised by severe neurodevelopmental disabilities and recurrent infections. However, no sonographic findings have been reported for affected foetuses, and prenatal molecular diagnosis has not been possible for this disease due to lack of prenatal clinical presentation. In this study, we identified a small duplication comprising the *MECP2* and *L1CAM* genes in the Xq28 region in a patient from a family with severe X-linked mental retardation and in a prenatal foetus with brain structural abnormalities. Using high-resolution chromosome microarray analysis (CMA) to screen 108 foetuses with congenital structural abnormalities, we identified additional three foetuses with the *MECP2* duplication. Our study indicates that ventriculomegaly, hydrocephalus, agenesis of the corpus callosum, choroid plexus cysts, foetal growth restriction and hydronephrosis might be common ultrasound findings in prenatal foetuses with the MECP2 duplication and provides the first set of prenatal cases with *MECP2* duplication, the ultrasonographic phenotype described in these patients will help to recognise the foetuses with possible *MECP2* duplication and prompt the appropriate molecular testing.

1. Introduction

Gain-of-function mutations in *MECP2* (methyl-CpG-binding protein 2) result in a well-recognised syndrome in 100% of affected male children; this syndrome is characterised by recurrent respiratory infections and severe neurodevelopmental disabilities including severe mental retardation, seizures, absent or delayed speech and infantile hypotonia that progresses to spasticity (Ramocki et al., 2010; Sanlaville et al., 2009).

The female carriers of an Xq duplication are usually asymptomatic because of skewed X-chromosome inactivation (XCI) patterns that preferentially inactivate the re-arranged chromosome. However, non-specific and variable phenotypes including mild to moderate mental retardation, autistic features, recurrent infection in early childhood, constipation and late-onset neurological features have been described in several female patients (Auber et al., 2010; Grasshoff et al., 2011; Makrythanasis et al., 2010). The precise incidence of the *MECP2* duplication syndrome and *MECP2* duplication carriers in the general population has not been studied in an un-biased manner; however, several lines of evidence indicate that the MECP2 duplication syndrome might contribute to approximately 1% of the cases of X-linked mental retardation. When male patients with primary mental retardation and other clinical features of the *MECP2* duplication are screened, the probability of detecting the *MECP2* duplication increases to 15% (Ramocki et al., 2010). *MECP2* triplication has also been observed in several patients; this causes a similar but more severe phenotype than the *MECP2* duplication. Patients with the *MECP2* triplication have additional symptoms including infantile hydronephrosis and nephrolithiasis, severe childhood constipation, macrocephaly and large ears (del Gaudio et al., 2006; Tang et al., 2012).

Although more than 100 cases of *MECP2* duplication have been described, all of the reported individuals were postnatal patients who had recognisable clinical features. One exception was the prenatal foetus in the last trimester described by Wax et al. (2013); that foetus had non-specific prefrontal and prenasal skin thickening and mild ventriculomegaly (11 mm). However, molecular diagnosis of the *MECP2* triplication was not possible in that case until the neonatal period.

Chromosome microarray analysis (CMA) is used for detecting micro-deletions and micro-duplications at high resolution and provides direct information on the genomic position and the gene affected by the chromosomal aberration (Van Esch et al., 2005). Over several years, we

Abbreviations: *MECP2*, the methyl CpG binding protein 2; *XCI*, X-chromosome inactivation; *CMA*, Chromosome microarray analysis; *FGR*, foetal growth restriction; *CVS*, chorionic villus sampling; *AF*, amniocentesis; *RT-PCR*, real-time polymerase chain reaction; *VM*, ventriculomegaly; *ACC*, agenesis of the corpus callosum.

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performed high-resolution CMA using Affymetrix CytoScanHD and 750 K arrays to investigate foetuses that had structural abnormalities, which were detected by ultrasound screening (Liao et al., 2013). Using these analyses, we identified a small duplication comprising the *MECP2* and *L1CAM* genes in the Xq28 region in a patient from a family with severe X-linked mental retardation and in a prenatal foetus with brain structural abnormalities. Furthermore, we used high-resolution CMA to screen other 108 foetuses with congenital structural abnormalities and identified additional three foetuses with the *MECP2* duplication.

2. Materials, methods and results

2.1. The first family (A11454)

The study protocol was approved by the institutional review board of the hospital, and informed consent was obtained from all the couples. A pregnant woman sought prenatal diagnosis based on her family history and abnormal sonographic findings. Ultrasound scanning after 25 weeks of pregnancy revealed foetal growth restriction (FGR) and agenesis of the corpus callosum in the foetus. The patient had been pregnant five years prior to this pregnancy, and ultrasound scanning during that pregnancy had revealed that the foetus had bilateral cerebral ventriculomegaly (12 mm) after 24 weeks of gestation. During the previous pregnancy, serological screening for Down syndrome was negative, and the patient did not consent to invasive prenatal diagnosis. The patient gave birth to a 3015 g male by vaginal delivery after full-term pregnancy, and there were no complications during labour. No abnormalities were detected upon physical examination of the newborn. However, the male patient suffered from frequent respiratory infections in the neonatal and infant periods. Furthermore, he had global developmental delay and developed severe primary mental retardation. During the physical examination at six years of age, the prominent clinical features of the patient included hypotonia, severe mental retardation with complete lack of understanding, absent speech and unsteady gait. Therefore, cordocentesis was offered to the pregnant woman after 26 weeks of the latter pregnancy. Conventional cytogenetic analysis revealed that the foetus and the elder son in the A11454 family had the normal male karyotype (46, XY). High resolution and whole genome chromosome microarray analysis (CMA) technology was further performed to investigate copy number variations (CNVs) in the family using Affymetrix CytoScan 750 K Arrays. The CytoScan 750 K Array we used includes 550,000 CNV probes and 200,691 single nucleotide polymorphism (SNP) probes, respectively, which can reliably detect CNVs throughout the whole genome with an average resolution of 100 kb. The gene-centric SNPs enable confident breakpoint estimation, 5 Mb loss of heterozygosity (LOH) determination and detection of uniparental iso-disomy (UPD) and regions identical-by-descent. As a result, CMA revealed nearly identical duplications mapping to the Xq28 chromosomal region in the foetus and the 6-year-old patient. The duplicated region included the X-linked mental retardation genes *MECP2* and *L1CAM*. Parental detection indicated that the duplication was inherited from the asymptomatic mother (Fig. 1). The CMA results obtained from the A11454 family are summarised in Table 1.

2.2. Screening for the MECP2 duplication in other foetuses with congenital malformations

Other 108 foetuses were referred to our centre because ultrasound scanning had detected that these foetuses had congenital structural malformations, but they had normal karyotypes. Using chromosome microarray analysis, we screened these foetuses for the presence of the *MECP2* duplication. The maternal age of the foetuses ranged from 22 years to over 39 years, and the gestational stages of the pregnancies ranged from 11 weeks to over 36 weeks. Foetal samples were collected using chorionic villus sampling (CVS), amniocentesis (AF) or cord blood sampling (cord blood), depending on the gestational stage of the foetus.

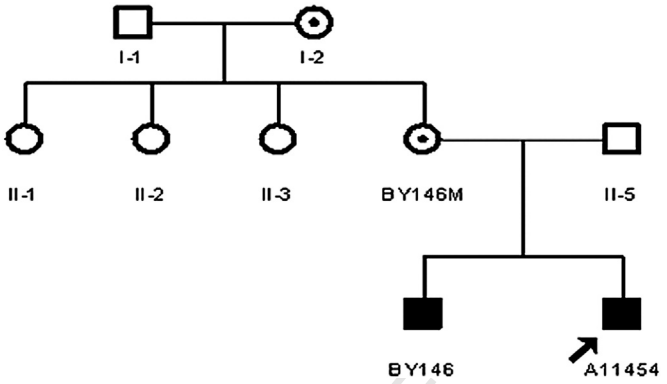

Fig. 1. The pedigree chart of the A11454 family.

The parental couples were provided pretesting genetic counselling on the benefits and limitations of CMA. Parental blood samples were required for every foetal sample for two reasons: to exclude maternal contamination and to assist in the interpretation of potential CNVs (Fig. 2). Q3 The CMA screening identified three additional foetuses with similar MECP2-region duplications; however, the duplications in all three foetuses were de novo mutations. The approximate incidence of Xq28 duplication in our cohort is 2.7% (3/108). The detailed prenatal ultrasound findings and CMA results are summarised in Table 1. All copy number variations (CNVs) identified by CMA were confirmed using real-time polymerase chain reactions (RT-PCR).

3. Discussion

MECP2 duplication is 100% penetrant in affected males and causes a well-recognised syndrome, which is characterised by variable neurodevelopmental disabilities and recurrent infections because of up-regulated or down-regulated gene transcription (Mayo et al., 2011). Molecular diagnosis are possible both pre- and post-natally. Postnatal clinical diagnosis can be made based on the presence of core clinical presentations. Prenatal clinical diagnosis was not possible due to lack of prenatal presentation reported so far. This paper provided the first set of prenatal cases with *Mecp2* duplication, the ultrasonographic phenotype described in these patients will help to recognise the foetuses with possible *Mecp2* duplication and prompt the appropriate molecular testing.

In this study, we present the first description of four foetuses carrying the sub-microscopic Xq28 duplication, which includes *MECP2*. This isolated duplication was detected prenatally using high-resolution chromosome microarray analysis, and it spanned a region of 0.8 Mb to 1 Mb. The duplication contained three genes commonly associated with neurodevelopmental disabilities, *MECP2*, *IRAK1* and *L1CAM*. Previous genotype–phenotype correlation studies in patients showed that the minimal duplicated region sufficient to cause the core phenotypes included the *MECP2* and Interleukin-1 receptor-associated kinase 1 (*IRAK1*; MIM: 300283) genes. The *MECP2* gene is the primary dosage-sensitive gene responsible for the neurological phenotypes of the Xq28 duplications described and therefore, the *MECP2* duplication syndrome is appropriately named (Carvalho et al., 2009; del Gaudio et al., 2006; Lugtenberg et al., 2009). In this study, the approximate incidence of Xq28 duplication in our cohort is 2.7% (3/108), based on the prenatal cases with ultrasound abnormalities and normal karyotypes. And the severity of the sonographic findings varied among the foetuses, which is consistent with the hypothesis that genetic modifiers contribute to or modify clinical phenotypes by disrupting the regulatory regions of neighbouring genes. In the A11454 family, the Xq28 duplication was steadily transmitted through three generations in which all male patients were 100% affected, but the female patient BY146M and her mother were phenotypically normal female carriers. This is consistent

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