Gene 533 (2014) 78–85

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

De novo MECP2 duplication derived from paternal germ line result in dysmorphism and developmental delay

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ARTICLE INFO

Article history:
Accepted 1 October 2013
Available online 12 October 2013

Keywords:
Xq28
MECP2 duplication
Disomy
Translocation
SNP array
FISH

ABSTRACT

Xq28 duplications encompassing the methyl CpG binding protein 2 (*MECP2*) in males exhibit a distinct phenotype, including developmental delay, facial dysmorphism, muscular hypotonia, intellectual disability, poor or absent speech, recurrent infections and early death. The vast majority of affected males inherit the *MECP2* duplication from their usually asymptomatic carrier mothers. Only a few cases with Xq28 duplication originating from *de novo* unbalanced X/Y translocation have been reported and the paternal origin of the aberration has only been validated in three males in the related literature. Here we present a karyotypically normal male with features characteristic of the *MECP2* duplication syndrome. The genome-wide SNP genotyping shows a *de novo* 2.26-Mb duplication from Xq28 to the terminus. The genotypes of the SNPs within the duplicated region indicated a paternal origin. Furthermore, the results of fluorescence *in situ* hybridization (FISH) indicated a novel Xq:Yp translocation, characterized as der(Y)t(Y;X)(p11.32;q28), which suggests an aberrant that occurred during spermatogenesis. The phenotype is compared to the previously reported cases with Xq28 duplication originated from an unbalanced X/Y translocation, and there was no specific part of the phenotype that could be contributed to the origin of parental imbalances. This report further highlights the capacity of high-molecular cytogenetic methods, such as SNP array and FISH, in the identification of submicroscopic rearrangement, structural configuration and parental origin of aberrant while in the evaluation of children with idiopathic developmental delay and intellectual disability.

1. Introduction

The methyl CpG binding protein 2 (MECP2) (OMIM 300005) interacts specifically with methylated CpG dinucleotides and mediates transcriptional repression of target gene (Nan et al., 1998). The loss-of-function mutations in MECP2 result in Rett syndrome (Amir et al., 1999; Rett, 1966), a progressive neurological disorder characterized by its almost exclusive occurrence in females. Whereas *MECP2* mutations in males lead to a broader range of neurodevelopmental phenotype from mild intellectual disability to fatal infantile encephalopathy (Villard, 2007). Moreover, a submicroscopic duplication of *MECP2* on Xq28 was first identified by quantitative polymerase chain reaction assays in a boy with features of severe intellectual disability and Rett syndrome (Meins et al., 2005). Recently, the broader implementation of high resolution human genome analyses allows for the identification of more cases of Xq28 duplication encompassing *MECP2* in neurodevelopmentally delayed males (Ramocki et al., 2010).

Interestingly, Xq28 duplications of different sizes encompassing the *MECP2* are associated with distinct clinical phenotype in males, and have been validated in 1–2% males with unexplained X-linked intellectual disability (Honda et al., 2010; Lugtenberg et al., 2009). It is recognized that increased dosage of *MECP2* is primarily responsible for this phenotype (Van Esch et al., 2005). Affected males exhibit a distinct

Abbreviation: MECP2, methyl CpG binding protein 2; FISH, fluorescence *in situ* hybridization; SNP, single-nucleotide polymorphism; CGH, comparative genome hybridization; CNVs, copy number variations; FoSTeS, Fork Stalling and Template Switching; IFN-α, interferon alpha.

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http://dx.doi.org/10.1016/j.gene.2013.10.001

phenotype, including developmental delay, facial dysmorphism, muscular hypotonia, intellectual disability, poor or absent speech, recurrent infections and early death (Van Esch, 2012). Currently, no correlation between the duplication size and severity of phenotype can be made; however, the male patients harboring a triplication of *MECP2* had the most severe phenotype implicating a significant impact of dosage on the phenotypic severity (del Gaudio et al., 2006; Tang et al., 2012). To date, the vast majority of affected males inherit the *MECP2* duplication from their usually asymptomatic carrier mothers, while only a few reports of *de novo* aberration have been identified (Clayton-Smith et al., 2000; del Gaudio et al., 2006; Meins et al., 2005; Ramocki et al., 2010; Sanlaville et al., 2005; Smyk et al., 2008; Van Esch et al., 2005). Nonetheless, a few cases with Xq28 duplication were the result of unbalanced X/Y translocation where the aberrations in three affected males were validated as paternal origin resulting from the recombinogenic pairing of distal Xq and Yq during male meiosis (Lahn et al., 1994). Herein, we present a male with intellectual disability and a *de novo* duplication encompassing *MECP2* derived from paternal origin using a single-nucleotide polymorphism (SNP) array analysis and fluorescence *in situ* hybridization (FISH).

2. Materials and methods

2.1. SNP array assay and data analysis

The study was designed to perform genome-wide SNP genotyping in the trio of samples. Genomic DNA was extracted from the peripheral blood of the patient and the parents by DNA Isolation Kit from Purgene, Gentra System, Inc. (Minneapolis, MN, USA). The DNA samples were genotyped by using Affymetrix Genome-Wide Human SNP Array 6.0 according to the manufacturer's instruction. Genotyping assay was done by the National Center for Genome Medicine at Academia Sinica, Taipei, Taiwan. After washing, the hybridization intensities were captured by GeneChip Scanner 3000 (Affymetrix, CA, US) (Lugtenberg et al., 2009). The intensity data were analyzed by using Affymetrix Genotyping Console 3.0 (Affymetrix, Santa Clara, CA, US). Segments with deletion and duplication larger than 200 kb were selected for further study. The parental origin of the duplicated DNA segment was determined by inconsistencies of Mendelian inheritance for X chromosome.

2.2. Fluorescent in situ hybridization

The metaphase chromosomes from the peripheral blood of the patient and his parents were prepared using standard laboratory protocols. BAC clone RP11-570J18 mapped to chromosome band Xq11.1 was used as centromeric control probe. RP11-54I20 (physical position: 152,359,267–152,552,250) and RP11-405N23 (Xq28; physical position: 154,111,989–154,275,960) which map to chromosome band Xq28 were used for detecting unchanged and duplicated regions, respectively. By using nick translation, BAC DNA was labeled with Fluorescein-12-dUTP or Texas Red-5-dUTP (PerkinElmer, MA, USA). The labeled DNA probes were co-hybridized to the metaphase spreads. Metaphase chromosomes were counterstained with DAPI. At least 10 metaphase spreads were examined in each co-hybridization reaction.

2.3. Quantitative real-time polymerase chain reaction

Genomic DNA was extracted from the EDTA blood. Quantitative real-time (RT) PCR was carried out in three independent experiments, each in triplicate, on an ABI Prism 7700 Sequence detection system (Applied Biosystems, Darmstadt, Germany) by using SYBR green I as a double-strand DNA specific binding dye. The primers for measuring *NXF5*, *ABCD1*, *MECP2*, and *TMLHE* copy numbers were designed as described previously (Van Esch et al., 2005), and using *GAPDH* as an internal reference gene for each sample. Relative quantification of gene copy numbers normalized to *GAPDH* was calculated according to the delta–delta-Ct method.

2.4. Sequence analysis for polymorphic variant of MECP2

To differentiate in the affected proband regarding the maternal and the paternal allele by the presence of one (or two) neucleotide variant in *MECP2*, polymorphism analysis was carried out by analyzing the noncoding regions of the *MECP2* gene. DNA of the affected proband and his parents was extracted from the peripheral blood and analyzed for polymorphic variant in the genes *MECP2*, using appropriately designed primer sets and direct sequencing. The DNA sequencing of purified PCR was performed on an ABI 3730 DNA Automatic Sequencer (Applied Biosystems, Darmstadt, Germany) using Big Dye Termination chemistry v.3.1 (Applied Biosystems, Darmstadt, Germany). All sequences obtained were compared with a reference sequence (*MECP2*: NM_001110792.1, NP_001104262.1).

3. Results

3.1. Clinical description of the affected proband

The proband was a second child born after an uneventful pregnancy to healthy, unrelated parents. He was delivered at term by cesarian section, with birth parameters within the normal range. He has one healthy elder brother. When he was first examined at age one with a delay in psychomotor development noted in that he was babbling, had axial hypotonia, and was unable to sit independently. While assessment with the Bayley II Scales of Infant Development indicated significantly delayed performance on the mental scale, with the abilities at the 3 month level. A subsequent brain MRI showed no evidence of structure anomalies but delayed myelination was noted. At age 3, he achieved independent sitting and some crawling. At age 4, he received treatment with anticonvulsants for myoclonic seizures, where the electroencephalogram showed intermittent-wave discharges over frontal areas. He has experienced recurrent respiratory infections commencing from the age of one. At the age of 9, he had necrotizing myofascitis and sepsis necessitating intensive care for one month. He showed a hypotonic facies with opening of his mouth and excessive drooling. Dysmorphic features included brachycephaly, midfacial hypoplasia, down-slanted palpebral fissures, long philtrum, mild micrognathia, large ears, and long slender fingers with clubbing of the nails (Fig. 1). Reevaluation at the age of 9 years and 6 months, he could feed himself with a fork but had difficulty with swallowing, chronic constipation was noted, and also the absence of speech was a concern. He was unable to walk and had repetitive hand and arm movements. Patient and his parents had normal karyotypes disclosed by standard chromosome analysis.

3.2. Identification of duplication at Xq28 by SNP array and FISH

A total of 6 copy number variations (CNVs) with deletion and duplication larger than 200 kb were identified in the proband (Supplementary Table 1). All CNVs but one are common CNV reported in Database of Genomic Variants (DGV). Examination of the array data of the trio showed that all CNVs except a duplication of Xq28 to Xqter spanning approximately 2.2 Mb in the proband were inherited from the parents. Therefore, this was a *de novo* event and defined as arr Xq28(152,623,369–154,887,040) × 2 dn. (Fig. 2A). The parental origin of the duplicated Xq28 was determined by SNPs which were heterozygous and followed Mendelian inheritance in the patient, indicating the extra Xq28 segment as paternal origin. Since the paternal copy of Xq28 alone could not be replicated in the male patient, we hypothesized that this small piece of paternal X chromosome was attached to other parental chromosomes, either autosomes or Y chromosome. We performed FISH assay with BAC clone RP11-405N23 specific to chromosomal region with duplication to investigate whether