Gene 508 (2012) 92–95

Contents lists available at SciVerse ScienceDirect

Gene

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

15q11.2 microdeletion and *FMR1* premutation in a family with intellectual disabilities and autism

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ARTICLE INFO

Article history:
Accepted 15 July 2012
Available online 25 July 2012

Keywords:
15q11.2 deletion
Autism
Intellectual disabilities
CYFIP1
FMR1

ABSTRACT

Genomic rearrangements of chromosome 15q11–q13 are responsible for diverse phenotypes including intellectual disabilities and autism. 15q11.2 deletion, implicating common PWS/AS breakpoints BP1–BP2, has been described in patients with delayed motor and speech development and behavioural problems. Here we report the clinical and molecular characterisation of a maternally inherited BP1–BP2 deletion in two siblings with intellectual, motor and speech delay, autistic syndrome disorder and several dysmorphic features. One of the patients was also a carrier of an *FMR1* allele in the low premutation range. The four genes within the deletion were under-expressed in all deletion carriers but *FMR1 mRNA* levels remained normal. Our results suggest that BP1-BP2 deletion could be considered as a risk factor for neuropsychological phenotypes and that it presents with variable clinical expressivity.

1. Introduction

Genomic rearrangements of chromosome 15q11–q13 are responsible for diverse phenotypes including intellectual disabilities (ID), autism and two well recognised microdeletion syndromes: Prader–Willi syndrome (PWS; OMIM#176270) and Angelman syndrome (AS; OMIM#105830). This region is characterised by complex combinations of low copy repeats (BP1–BP5) that mediate various deletions and duplications via non-allelic homologous recombination (Locke et al., 2004; Makoff and Flomen, 2007; Pujana et al., 2002). The distal breakpoint (BP3) in PWS/AS appears to be similar in all cases while proximal breakpoints (BP1 and BP2) differ depending on the cases. Based on these breakpoints, two common classes of deletions are described in individuals with PWS/AS: type I deletion between BP1 and BP3, involving the largest region and type II deletions, between BP2 and BP3. Individuals with type I deletion generally have more behavioural and psychological problems than individuals with the type II deletion (Bittel et al., 2006; Butler et al., 2004). The microdeletion in 15q11.2 between BP1 and BP2 has been described in patients with delayed motor and speech development, dysmorphisms and behavioural problems (attention deficit hyperactivity disorder (ADHD), autistic spectrum disorder (ASD), obsessive–compulsive behaviour) (Burnside et al., 2011; Depienne et al., 2009; Doornbos et al., 2009; Murthy et al., 2007; Sempere Perez et al., 2011). BP1–BP2 deletions have also been associated with schizophrenia (Stefansson et al., 2008), which suggests that these variants might increase risk for various neuropsychiatric phenotypes, albeit with low penetrance and/or variable expressivity.

The BP1–BP2 deletion involves four highly conserved genes, *TUBGCP5*, *NIPA1*, *NIPA2*, and *CYFIP1*; the latter three widely expressed in the central nervous system. Copy number gains and losses in this region have also been identified in control individuals with an estimated frequency of 1–3% (http://projects.tcag.ca/variation/). This suggests that the 15q11.2 syndrome presents with reduced penetrance and/or variable expressivity.

Here we present a three generation family in which the 15q11.2 deletion is presented with *FMR1* premutation in some individuals.

2. Material and methods

2.1. Patients

Patients were a 12-year old boy and a 9-year old girl with ID and several dysmorphic features. They were born from the same mother but different fathers. Family history was negative for intellectual disabilities. They were referred to our laboratory in order to determine the genetic cause of ID.

2.2. Molecular analysis

Following our molecular diagnostic protocol for ID patients we performed fragile X syndrome and subtelomeric rearrangement testing. Confirmatory analyses included aCGH, qPCR and expression studies.

Abbreviations: ID, intellectual delay; PWS, Prader–Willi syndrome; AS, Angelman syndrome; ADHD, attention deficit hyperactivity disorder; ASD, autistic spectrum disorder; array CGH, array-based comparative genomic hybridisation; CNV, copy number variants.

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http://dx.doi.org/10.1016/j.gene.2012.07.023

2.2.1. FMR1 CGG repeat number

FMR1 CGG-repeat number was determined by PCR following manufacturer's instructions (CG-Rich PCR, Roche).

2.2.2. Subtelomeric rearrangements

Screening for subtelomeric rearrangements was assessed according to manufacturer's recommendations using Salsa PO36B and SALSA P070 (MRC-Holland, Amsterdam, The Netherlands). Both kits contain one probe for each subtelomeric region and one probe on the q-arm near the centromere for each acrocentric chromosome.

2.2.3. Quantitative PCR (qPCR)

Copy number changes were confirmed by qPCR analysis by using the Power Master Mix PCR SYBR Green on an automated ABI 7300 PCR System (Applied Biosystems, CA, USA). Relative quantification was determined by standard curve method for quantification against a control amplicon of the *GUSB* gene following manufacturer instructions (Applied Biosystems, CA, USA).

2.2.4. Array-based comparative genomic hybridisation (array CGH) analysis

Array CGH was performed by using a 244 K oligo array (Agilent Technologies Inc., Santa Clara, CA, USA). Procedures were carried out according to the manufacturer's protocols. Briefly, 400 ng of patient DNA and of a sex-matched control was labelled by random priming (Genomic DNA Labelling Kit Plus; Agilent Technologies). Patient and control DNAs labelled with Cy3-dUTP and Cy5-dUTP, respectively, were purified by filtration (Microcon YM-30 filters; Millipore, Billerica, Massachusetts, USA). Patient and control DNAs were pooled and hybridised with 50 ng of human Cot I DNA at 65 °C with rotation for 40 h. Washing was performed according to the Agilent protocol. Arrays were analysed using a scanner (Agilent G2565BA Microarray Scanner System, Agilent Technologies) and Agilent Feature Extraction V.9.1 software. Results were presented by Agilent CGH Analytics V.3.4 software. DNA sequence information refers to the public UCSC database (Human Genome Browser, March 2006 Assembly (hg18)).

2.2.5. mRNA quantification

Total RNA was extracted from peripheral blood using RNasy Mini Kit following manufacturer's recommendations (Qiagen, Hilden, Germany). cDNA synthesis reaction was performed on 400 ng of total RNA by using High Capacity cDNA RT kit following manufacturer's instruction (Applied Biosystems). Real time quantification was performed with the SYBRgreen method on 10 ng cDNA with cDNA specific primers for *CYFIP1, NIPA1, NIPA2* and *TUBGCP5* genes. The relative quantity of mRNA was assessed using the delta delta Ct method (Livak and Schmittgen, 2001). The β-glucoronidase (*βGUS*) gene (GenBank accession number NM_000181) was used as reference amplicon. This amplicon spans the junction between exons 11 and 12 of the *βGUS* gene. Each 25 μL reaction well contained 12.5 μL 1× SYBRgreen PCR Master Mix (Applied Biosystems), 0.2 μM of each primer and 8 μL of diluted 1/10 cDNA. PCR for determination of relative *FMR1* mRNA levels was performed with the use of TaqMan probes and Universal PCR master mix obtained from Applied Biosystems (Foster City, CA, USA). *FMR1* mRNA levels are relative to control (GUS) mRNA levels.

3. Results

3.1. Clinical findings

Patients (III.1 and III.2) presented with ID and were diagnosed with ASD. They both attended a school for children with behavioural problems. Clinical examination revealed they presented with mild motor delay and delayed speech development, microcephaly, motor dyspraxia, and minor dysmorphic features such as dysmorphic ears, broad forehead and ogival palate. The mother and a maternal uncle were reported to have learning and behavioural problems during their childhood. The maternal grandfather developed normally and attended regular education. Pedigree of the family is shown in Fig. 1.

3.2. Molecular analysis

Molecular analysis of the *FMR1* CGG-repeat region showed an allele of 58 CGG in the low premutation range in individual III.1 and two alleles within the normal range in individual III.2. The premutation was inherited from the grandmother in the maternal branch (individual I.2). Screening for subtelomeric rearrangements identified a deletion at 15q11.2 in both patients. Array CGH delineated the 15q11.2 deletion between breakpoints BP1 and BP2 of the PWS/AS region involving four genes: *TUBGCP5*, *CYFIP1*, *NIPA2*, and *NIPA1*. The deletion of the four genes was confirmed by qPCR analysis. Familial studies showed that the deletion was inherited from the mother (II.1) and was also present in two other relatives on the maternal branch (I.1 and II.3). Regarding expression studies, we found reduced quantities of mRNA of *TUBGCP5*, *CYFIP1*, *NIPA2* and *NIPA1* genes in the four individuals carrying the BP1–BP2 deletion compared with individuals without the deletion ($p<0.05$; Student's t-test). Due to the interaction of *FMR1* with *CYFIP1*, and taking into account that some of the relatives were *FMR1* premutation carriers, mRNA level of *FMR1* gene was also determined. A slightly higher expression (although insignificant) of *FMR1* gene was detected in premutated individuals compared to non-premutated individuals, but no differences were found among patients with and without the deletion ($p>0.05$; Student's t-test) (Fig. 2).

4. Discussion

Here we report the clinical and molecular characterisation of a maternally inherited BP1–BP2 deletion in two siblings with intellectual disabilities and dysmorphic features. Familial studies showed that the deletion was present in 3 other relatives of the maternal branch, two affected by learning disabilities and 1 healthy individual. Molecular analysis of the *FMR1* CGG-repeat region showed a premutated allele at the low range coexisting with the deletion in one of the affected patients. In all cases, the four genes within the deletion were under-expressed in all deletion carriers but *FMR1 mRNA* levels remained normal.

Until recently, copy number variants (CNV) were assessed as clinically irrelevant changes when inherited from an unaffected carrier while *de novo* rearrangements were commonly classified as causative. The presence of phenotypically unaffected individuals carrying same rearrangements raises the issue of whether these CNVs are benign or pathogenic variants with variable expressivity or low penetrance.

The rearranged region involves four genes, three of them widely expressed in the central nervous system (*CYFIP1*, *NIPA2* and *NIPA1*) and the other one (*TUBGCP5*) expressed in the subthalamic nuclei (Goytain et al., 2007, 2008; Napoli et al., 2008; Rainier et al., 2003). Messenger RNA levels of these four genes are positively correlated with better behavioural outcomes (Bittel et al., 2006). *Nipa1* is expressed in mouse brain tissue and in humans, this gene is implicated in autosomal dominant spastic paraplegia (MIM600363) (Rainier et al., 2003), but ASD is not a common feature in this syndrome, which suggest that this gene is less likely to contribute to ASD. The *CYFIP1* gene codes for a protein implicated in the control of neuronal network formation and maintenance, and interacts with fragile X mental retardation protein (FMRP). FMRP is involved in the regulation of mRNA translation in neuronal dendrites, which underlies synaptic plasticity and brain development. FMRP-mediated repression of translation requires interaction with CYFIP1 (Napoli et al., 2008). It has been observed that some fragile X syndrome patients with PWS like phenotype present reduced expression levels of the *CYFIP1* gene (Nowicki et al., 2007) and, based on this previous observation, this gene seems to be a good candidate gene for ASD. In addition, changes