Gene 519 (2013) 177–181

Contents lists available at SciVerse ScienceDirect

Gene

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

Short Communication

A novel homozygous 10 nucleotide deletion in *BBS10* causes Bardet–Biedl syndrome in a Pakistani family

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ARTICLE INFO

Article history:
Accepted 27 January 2013
Available online 09 February 2013

Keywords:
BBS10
Fahr's disease
Retinitis pigmentosa
Intellectual disability
Bilateral basal ganglia calcification

ABSTRACT

Bardet–Biedl Syndrome is a multisystem autosomal recessive disorder characterized by central obesity, polydactyly, hypogonadism, learning difficulties, rod-cone dystrophy and renal dysplasia. Bardet–Biedl Syndrome has a prevalence rate ranging from 1 in 100,000 to 1 in 160,000 births although there are communities where Bardet–Biedl Syndrome is found at a higher frequency due to consanguinity. We report here a Pakistani consanguineous family with two affected sons with typical clinical features of Bardet–Biedl Syndrome, in addition to abnormal liver functioning and bilateral basal ganglia calcification, the latter feature being typical of Fahr's disease. Homozygous regions obtained from SNP array depicted three known genes *BBS10*, *BBS14* and *BBS2*. Bidirectional sequencing of all coding exons by traditional sequencing of all these three genes showed a homozygous deletion of 10 nucleotides (c.1958_1967del), in *BBS10* in both affected brothers. The segregation analysis revealed that the parents, paternal grandfather, maternal grandmother and an unaffected sister were heterozygous for the deletion. Such a large deletion in *BBS10* has not been reported previously in any population and is likely to be contributing to the phenotype of Bardet–Biedl Syndrome in this family.

1. Introduction

Bardet–Biedl Syndrome (BBS [OMIM 209900]) is a rare genetically heterogeneous autosomal recessive developmental disorder characterized by primary features like retinitis pigmentosa, postaxial polydactyly, obesity, intellectual disability (ID), hypogonadism and renal dysfunctions. The secondary features may include hepatic fibrosis, language deficits, behavioral traits, facial dysmorphism, dental anomalies, developmental delay and cardiac abnormalities (Beales et al., 1999). BBS was first described in 1920 by Bardet and Biedl (Green et al., 1989). The genetics of BBS are complex and there are a large number of variations in genotype and phenotype of the disease (White et al., 2007). BBS is a ciliopathy; therefore the mutated genes resulting in the disease are mostly involved in the synthesis of ciliary proteins (Gerth et al., 2008). Many *in vitro* and *in vivo* studies of the BBS genes and their products have shown that BBS occurs due to the defects of the primary cilium and basal body functions, which cause aberration of both transport and paracrine signals such as planar cell polarity pathways (Seo et al., 2010). To date 17 genes have been identified to cause BBS including *BBS1* (11q13) (Mykytyn et al., 2002), *BBS2* (16q21) (Kweitek-Black et al., 1993; Nishimura et al., 2001), *BBS3* (3p12-q13) (Chiang et al., 2004), *BBS4* (15q22.3) (Chiang et al., 2004), *BBS5* (2q31) (Mykytyn et al., 2001), *BBS6* (20p12) (Hjortshoj et al., 2008), *BBS7* (4q27) (Badano et al., 2003), *BBS8* (14q32.11) (Ansley et al., 2003), *BBS9* (7p14) (Nishimura et al., 2005), *BBS10* (12q21.2) (Laurier et al., 2006), *BBS11* (9q33.1) (Chiang et al., 2006), *BBS12* (4q27) (Stoetzel et al., 2007), *BBS13* (17q23) (Chen et al., 2011), *BBS14* (12q21.3) (Iqbal et al., 2010), *BBS15* (2p15) (Kim et al., 2010), *BBS16* (1q43) (Schaefer et al., 2011) and *BBS17* (*LZTFL1*) (3p21.31) (Marion et al., 2012). Mutations in these genes have been shown to explain BBS in 70% of the families studied (Chen et al., 2011). Genome wide SNP array analysis is a powerful tool used in molecular diagnostic studies, which is helpful in resolving genetically heterogeneous and complex diseases such as BBS (Pereiro et al., 2010).

Abbreviations: BBS, Bardet–Biedl syndrome; ID, intellectual disability; SNP, single nucleotide polymorphism; CF, counting finger; CT, computed tomography; SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxaloacetic transaminase; CBC, complete blood count; DNA, deoxyribonucleic acid; CNAG, copy number analyzer for gene chip; JSI, seqpilot software; CNV, copy number variation; TRIC, the homotrimeric cation channel; TCP1, tailless complex polypeptide 1; CCT2-CCT8, chaperonin containing TCP-1; MKKS, McKusick–Kaufman syndrome; CEP290, centrosomal protein 290 kDa gene, GSK3, glycogen synthase kinase 3; Ser, serine; Ile, isoleucine; fsx, frame shift; Cys, cysteine; Del, deletion.

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http://dx.doi.org/10.1016/j.gene.2013.01.047

In the current study we report two affected brothers from a consanguineous Pakistani Punjabi family (MRQ19); both the brothers were homozygous for c.1958_1967del, which is a novel deletion in *BBS10* that is likely to be causing the BBS pathology in this family.

2. Methods

2.1. Clinical studies

A consanguineous Pakistani family MRQ19 (Fig. 1) presented with characteristic features of BBS to the Neurology Clinic of Shifa International Hospital, Islamabad. The family had two affected male siblings and one normal female sibling.

Approval for this study was granted by the Ethics Committee/Institutional Review Board of Shifa College of Medicine/Shifa International Hospital, Islamabad. Informed written consent was obtained from all the participating subjects. The proband (IV:1; Fig. 1) is the eldest male child of first cousin Pakistani parents from Punjab; he was born at full term after an uneventful pregnancy. Obesity and post axial polydactyly in both hands were noted at birth. His milestones were delayed, and he had myopia, learning disabilities, and he learned to walk at the age of 3 years and began to speak at 4½ years. At the time of the clinical examination his behavior was shy, introvert and he was anxious, at 15 years of age his face had a moon like appearance due to obesity, as his height was 156.02 cm (88th centile), and weight was 75 kg (3rd centile), but his pupillary distance was normal (58 mm). At the time of the eye exam he had complaints of night blindness for several years, his pupillary distance was normal (58 mm), whereas his visual acuity was 6/60 and he had CF < 1 m (counting finger vision). In addition, he had astigmatism and myopia. Funduscopy revealed pigmentary degeneration which confirmed retinitis pigmentosa. His brain CT (computed tomography) scan showed no intracranial hemorrhage, mass effect or midline shift. However, benign vascular calcification was noted in the bilateral basal ganglia. Subtle non-specific tiny parenchyma calcification near the vertex was seen (Fig. 2A, B and C); no other abnormal density was present in the brain parenchyma. There was neither an extra axial collection nor the sella and pineal region showed any abnormality, grey and white matter differentiation was well maintained but slight sclerosis and opacification of the right mastoid air cells was seen. Fullness was observed in nasopharynx but no osseous abnormality was noted. Enlarged adenoids were seen, while the X rays of both hands showed no fracture, dislocation, lytic or sclerotic lesion in the visualized bones. A bilateral supernumerary digit was observed having a small metacarpal, proximal and distal phalanx (Fig. 3), while the soft tissues were unremarkable. The metabolic screen and renal function tests showed normal range of different parameters. However, the liver enzymes were raised: serum glutamate pyruvate transaminase (SGPT), was 140 U/L, serum glutamic-oxaloacetic transaminase (SGOT), was 66 U/L and Alkaline phosphatase was 369 U/L. The CBC (complete blood count) and heart rate were unremarkable. In addition, the boy had hypogonadism with micropenis and undescended testis. The other affected sibling of the proband was also male; he was the third child of his parents, born after a normal pregnancy of 40 weeks. At the time of the clinical assessment he was 4 years old, he had night blindness and his vision was deteriorating gradually. He was also obese and had a small penis with undescended testis. He started speaking a few words and walking at the age of 4 years, his detailed clinical data were not available.

2.2. Molecular studies

Blood samples of the affected and healthy members of the family were collected in EDTA vacutainers (Becton Dickinson, Franklin Lakes, NJ). Genomic DNA was extracted from the blood using a standard phenol-chloroform extraction method (Sambrook and Russell, 2006). Genotyping of the affected members IV:1 and IV:3 was performed using the Affymetrix 250K (Santa Clara, CA) SNP microarray in order to perform homozygosity mapping and obtain the copy number variation (CNV) data. The Affymetrix Genotyping Console (2.0v), software was used to generate the genotype calls. To investigate CNVs, 250K SNP array data were analyzed by using Affymetrix Copy Number Analyzer for Gene Chip (CNAG) (Nannya et al., 2005). The regions of homozygosity were determined by using the online homozygosity mapper software (www.homozygositymapper.org). All three identified candidate genes were sequenced by using Sanger sequencing. The primers for the amplification of the entire coding regions and splice sites of *BBS10* (Table 1), *BBS14* and *BBS2* (not shown) were designed using Primer3 software (http://frodo.wi.mit.edu/). After amplification

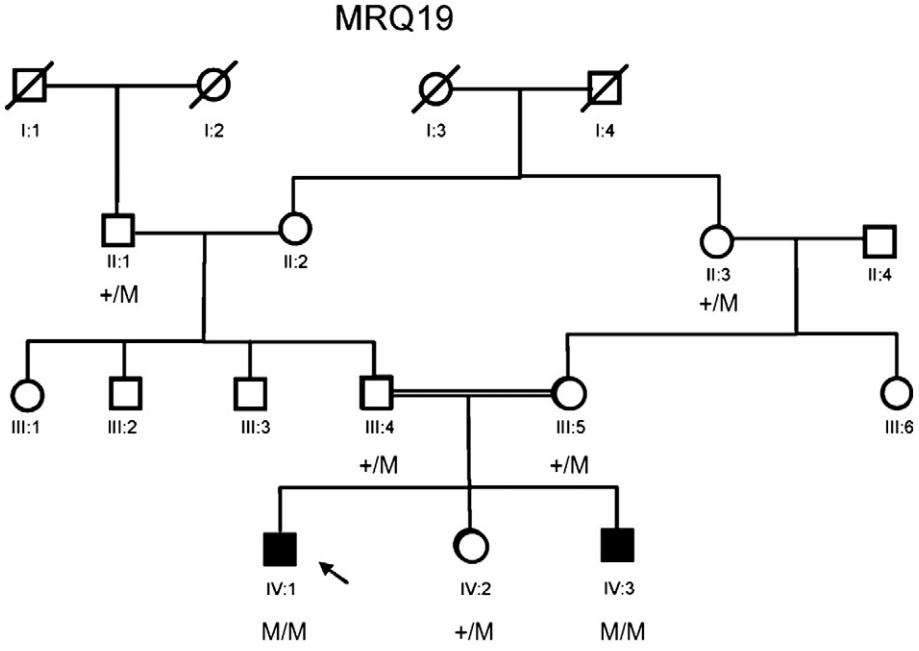

Fig. 1. Pedigree of MRQ19. Unfilled circles and squares represent unaffected females and males, respectively. Filled squares are affected males. M represents the mutant allele, + is for wild type allele. M/M is for homozygous mutant affected genotype and +/M is for carriers of the mutation.