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ARTICLE

Autozygosity mapping of Bardet–Biedl syndrome to 12q21.2 and confirmation of FLJ23560 as BBS10

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Bardet–Biedl syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder characterized by variable obesity, pigmentary retinopathy, polydactyly, mental retardation, hypogonadism and renal failure. In order to identify novel BBS loci we undertook autozygosity mapping studies using high-density SNP microarrays in consanguineous kindreds. We mapped a BBS locus to a 10.1 Mb region at 12q15–q21.2 in a large Omani BBS family (peak lod score 8.3 at $\theta = 0.0$ for marker D12S1722) that contained the recently described *BBS10* locus. Mutation analysis of candidate genes within the target interval, including the *BBS10* gene, revealed a homozygous frameshift mutation in *FLJ23560* and mutations were also detected in four smaller consanguineous families with regions of autozygosity at 12q21.2. These findings (a) confirm a previous report that *FLJ23560* (*BBS10*) mutations are a significant cause of BBS, and (b) further demonstrate the utility of high-density SNP array mapping in consanguineous families for the mapping and identification of recessive disease genes.
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Introduction

Bardet–Biedl Syndrome (BBS: OMIM 209900) is an autosomal recessively inherited disorder with variable expression. Frequent manifestations include obesity, renal dysplasia, obesity, cognitive impairment, postaxial polydactyly, pigmentary retinal degeneration and hypogonadism.[1] The prevalence of BBS in North America and Europe has been estimated to range from 1:140 000 to 1:160 000 live

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births,[2–4] but in Kuwait, Newfoundland and Oman, BBS is more frequent (eg estimated incidences of 1:13 500, 1:17 500 and 1:30 000, respectively).[1,5,6] The genetics of BBS are complex and at the start of 2006 nine susceptibility genes had been mapped previously: *BBS1* at 11q13 (MIM209901); *BBS2* at 16q21 (MIM606151); *BBS3* (ARL6) at 3p12–q13 (MIM608845); *BBS4* at 15q22.3–q23 (MIM600374); *BBS5* at 2q31 (MIM603650), MKKS *BBS6* at 20p12 (MIM604896); *BBS7* at 4q27 (MIM607590); *BBS8* at 14q32.1 (MIM608132) and *BBS9* (PTHB1) (MIM607968) at 7p14.[7–20] However, mutations in BBS1-9 only accounted for only a small portion of BBS patients, indicating the existence of additional BBS genes. Although the identification of additional BBS genes in the presence of marked locus heterogeneity is challenging, genetic linkage studies in consanguineous families provide a powerful strategy for identifying novel recessive disease genes. In order to map novel BBS genes we undertook genetic linkage studies in a large Omani BBS kindred. We mapped a novel locus to chromosome 12q21.2 that overlapped the recently described *BBS10* locus[21] and, furthermore, confirmed mutations in the *BBS10* gene, *FLJ23560*. We also excluded linkage to the recently described *BBS11* locus on chromosome 9q33.1. (Chiang *et al*[22]) in this kindred.

Patients and methods

Clinical ascertainment and phenotype

A consanguineous Omani family (F1, see Figure 1) presented with characteristic features of BBS to the Paediatric Ophthalmology service at Sultan Qaboos University Hospital. The detailed clinical features of four affected family members are summarized in Table 1. After institutional approval and informed consent from the patients, parents and unaffected relatives, blood was collected for DNA extraction from 14 family members (marked with a* in Figure 1) and, following DNA extraction, microsatellite and SNP-microarray genotyping studies were instigated.

Mutation analysis of *FLJ23560* was also undertaken in 11 additional BBS kindreds (F2–12). This included seven Pakistani BBS kindreds (F2–F8) and four consanguineous BBS families (two with two unaffected individuals and each with a single affected individual) in whom SNP-based microarray studies had demonstrated autozygous regions at (12q21.2).[20]

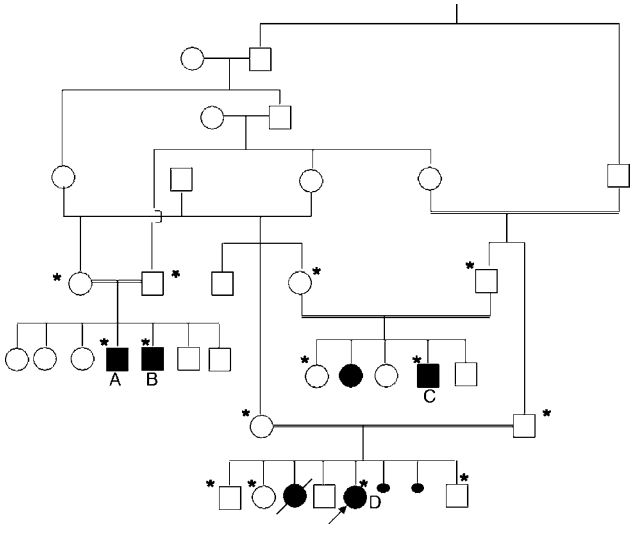

Figure 1 Large consanguineous Omani family with Bardet–Biedl syndrome. * = individuals genotyped. Affected individuals are labelled A–D (see Table 1).

Molecular studies

Microsatellite genotyping studies DNA was extracted from all patient blood samples using the Puregene extraction kit from Gentra, and stored at −80°C. Primers for microsatellite markers flanking *BBS1-9* were synthesized and genotyping performed (details available on request).

SNP genotyping studies Three affected members of the Omani family were genotyped with the Affymetrix 10k 2.0 SNP microarray (as described previously for other families[23]). SNP genotyping studies in the four small families with a BBS10 mutation have been reported previously.[20]

Mutation analysis of *CCT2*, *MDM2*, *RAB21*, *TBC1D15* and *VMDL2L3* was performed by direct sequencing using the ABI Big Dye Reaction Mix and standard methodology (details of primer sequences and methods available on request). Mutation analysis of *FLJ23560* (C12orf58) was performed as reported by Stoetzel *et al*.[21]

Results

Genetic linkage studies

After excluding linkage to *BBS1-9*, (data not shown), we initiated a genome-wide linkage scan in three affected individuals. Analysis of the data from Affymetrix 10k SNP arrays[24] revealed a common 10.1 Mb region of autozygosity at 12q15–q21.2 (between 67.2 and 77.3 Mb) shared by each of the four patients analysed (see Figure 2) and supplementary Table. Genotyping of microsatellite markers (D12S1294, D12S375, D12S1680, D12S1693, D12S1722, RH27150, SHGC-56430, D12S1052, D12S1660) confirmed linkage (peak lod score at D12S1722 of 8.3 $\theta = 0.0$) and a target interval of 10.1 Mb.

Mutation analysis of candidate genes

Thirty-three known and 24 predicted genes were contained in the 10.1 Mb candidate region identified from linkage studies in F1 (Chromosome 12: 66822895 to 77322947 Mb see Supplementary Table (http://genome.ucsc.edu/)). As BBS is a putative ciliopathy,[16,19,25] we prioritized our selection of candidate genes based on expression in the

Table 1 Clinical features of four affected individuals from the large Omani BBS Kindred (F1)

Clinical features	*Patient D*	*Patient C*	*Patient B*	*Patient A*
Age at recognition of visual problems	4 years – night blindness	4 years – night blindness	6 years – vision impairment	10 years – vision impairment
Age at examination	6 years	4 years	13 years	24 years
Retinal dystrophy				
Night blindness	+	+	+	+
Visual impairment	VA = 0.3/0.15	VA NA	VA = 0.2/0.2	VA = 0.05/0.05
Fundus changes: ophthalmoscopy	Retinal vascular attenuation, mild pigmentary disturbance	Retinal vascular attenuation, mild pigmentary disturbance	Retinal vascular attenuation, mild pigmentary disturbance	Optic nerve pallor, retinal vascular attenuation, pigmentary disturbance (tigroid fundus; few bone-spicules in mid-periphery),
Visual field	Could not be tested	Could not be tested	Constricted field of vision (40°)	Constricted field of vision (20°)
Refractive error	−3/−2; −4/−2 Myopic astigmatism	+3.75/−2; +3.0/−2 Hypermetropic astigmatism	−0.5/−2; −1.5/−2.0 Myopic astigmatism	−10.75/−1.5; −8/−2 High myopic astigmatism
Weight (kg)	30 truncal obesity round facies	28 truncal obesity round facies	79 truncal obesity round facies	80 truncal obesity
Height (cm)	110.6	109	156	160
Polydactyly	All four limbs syndactyly brachydactyly	Hands brachydactyly Feet – polydactyly, syndactyly	Brachydactyly all four limbs Hands – Syndactyly (surgery)	Brachydactyly, polydactyly
Genital system (genital malformation, secondary sex characteristics)	Infantile uterus on ultrasound exam	—	Small penis; undescended testes	Small penis
Intellectual impairment or delayed development	Vineland Social Maturity Scale* Social age – 2.5 years Social quotient – 40 Moderate mental retardation	Delayed motor development and speech delay-attention deficit disorder with poor concentration*	Delayed motor development and speech delay poor academic school record	Delayed motor development and speech delay poor academic school record
Renal anomalies	Recurrent urinary tract infection+urinary incontinence No renal anomalies on ultrasound scan	No renal anomalies on ultrasound scan	No renal anomalies – (blood urea and electrolytes, ultrasound)	No renal anomalies – (blood urea and electrolytes+ultrasound)
Cardiovascular system	Normal (clinical exam)	Normal (clinical exam)	Normal (clinical exam)	Normal (clinical exam)

VA = best-corrected visual acuity; NA = not assessed.

ciliary and basal body proteomes after interrogation of the open-resource Ciliary Proteome Database[26] at www.ciliaproteome.org. Sequencing of *CCT2*, *MDM2*, *RAB21*, *TBC1D15* and *VMDL2L3* did not detect mutations, but in the light of the report of Stoetzel *et al*,[21] we then analysed *FLJ23560* (C12orf58) and detected a homozygous frame-shift mutation, c.995_999delAAGA (p.Q242fs258X), in all four affected individuals tested (Figure 3). All parents of affected individuals were heterozygous for the mutation and unaffected siblings were heterozygous or homozygous wild-type. Furthermore the, p.Q242fs258X mutation was not detected in 72 Arab control chromosomes.

Mutation analysis of seven unrelated Pakistani BBS probands from consanguineous families (three of which were unlinked to 12q15–q21.2 (data not shown) revealed normal wild-type sequence in six cases. In one family a single affected individual was homozygous for a missense substitution (p.Pro539Leu, 1880C>T) that had been reported as a polymorphic variant by Stoetzel *et al*.[21] In addition, protein structure predictions suggested that the substitution would not have a major effect on FLJ23560 protein structure (data not shown). We also detected the p.Pro539Leu substitution in three of 96 Pakistani control chromosomes.

Mutation analysis of FLJ23560 in four consanguineous BBS kindreds previously shown to have an autozygous segment at 12q21.2,[20] revealed mutations in all four probands. The four consanguineous BBS families were found to share autozygous segments at 12q21.2 that ranged in size from 4.3 to 26.3 Mb. The smallest interval

Marker	Physical Location	Patient's Genotype								
		A		B		C		D		
rs725956	55,618,203	A	B	A	B	A	B	A	B	
rs1383185	59,608,447	A	B	A	B	B	B	A	B	
D12S1294	66,220,450	177	185	177	177	177	177	177	177	
rs2870793	66,484,625	A	B	A	A	A	A	A	A	
D12S375	67,231,021	175	178	175	175	176	176	175	175	
D12S1680	68,202,211	243	243	243	243	244	244	243	243	target interval
D12S1693	68,512,976	74	74	74	74	74	74	74	74	target interval
D12S1722	69,299,051	244	244	244	244	244	244	244	244	target interval
rs1512980	69,677,162	B	B	B	B	B	B	B	B	target interval
rs1960312	69,767,717	A	A	A	A	A	A	A	A	target interval
RH27150	70,367,543	106	106	106	106	106	106	106	106	target interval
rs724211	70,321,877	A	A	A	A	A	A	A	A	target interval
SHGC-56430	70,503,146	104	104	104	104	104	104	104	104	target interval
rs953960	71,921,999	A	A	A	A	A	A	A	A	target interval
rs1373221	72,264,091	B	B	B	B	B	B	B	B	target interval
D12S1052	73,895,728	158	158	158	158	158	158	158	158	target interval
D12S1660	74,748,691	197	197	197	197	197	197	197	197	target interval
rs2887451	75,418,754	B	B	B	B	B	B	A	B	target interval
rs726185	77,322,947	B	B	B	B	B	B	A	B	
rs1391072	82,563,311	B	B	B	B	B	B	A	B	
rs951142	83,494,344	B	B	B	B	A	B	A	B	
rs1873153	85,848,577	B	B	A	A	A	B	A	B	

Figure 2 Microsatellite and SNP genotyping data for four affected individuals from large Omani family with BBS. Homozygous regions are shaded grey with the region of homozygosity common to all affected individuals is indicated by black line.

of overlap defined in these families was 2.5 Mb. This 2.5 Mb region of overlap contains FLJ23560, the *BBS10* gene. Mutation analysis of FLJ23560 in these four families revealed homozygous mutations in all affected individuals. Affected individuals in two of the families were homozygous for the recently reported common *BBS10* mutation, C91fsX95. The proband of one family was homozygous for the recently reported V707fsX708 mutation, whereas the proband of the remaining family was homozygous for a novel frameshift mutation, N364fsX368.

Discussion

We initially mapped a novel BBS locus to chromosome 12q21.2 and then detected inactivating mutations in *FLJ23560* in five BBS kindreds. Thus, our study confirms that *FLJ23560* is the *BBS10* gene, as reported recently by Stoetzel *et al*.[21] The BBS genes identified to date represent a range of putative functions with some BBS gene products having been localized to basal bodies and centrosomes (BBS4, BBS5, BBS7 and BBS8.[16,27,28] Roles in ciliary function, intracellular and intraflagellar transport, and maintenance of planar cell polarity have been implicated as key pathways in the pathogenesis of BBS.[16,22,28–33,34] However, *FLJ23560* (*C12orf58*) differs from other BBS genes by encoding a group II chaperonin that is not conserved in invertebrates.[21] Although BBS6 (also known as MKKS) is also thought to encode a chaperonin,[27] BBS6 and BBS10 differ as BBS10 is restricted to vertebrates and contains a functional motif responsible for ATP hydrolysis that is found in all group II chaperonins,[35] but which is not present in BBS6.[21,27] Hence, it appears that BBS10 may represent a novel chaperonin subfamily. At present the relationship between BBS10 and ciliary body/intraflagellar transport function is not known, but if the BBS10 protein does function as a chaperonin then it may regulate the folding or stability of other ciliary or basal body proteins.

Three of the four *BBS10* mutations characterized in our cases were also detected by Stoetzel *et al*.[21] The Q242fs258X homozygous truncating mutation identified in the large Omani family (F1) was also found in a homozygous state in a Lebanese and in the heterozygous state (a second mutation was not identified) in a Middle Eastern family. Previously most Middle Eastern BBS families did not have a mutation in BB1-9, but BBS10 mutations were detected in 6/26 families of Middle Eastern ancestry.[21]

The clinical phenotype of BBS is extremely variable and genotype–phenotype correlations are hindered by marked locus heterogeneity, compound heterozygosity in non-consanguineous cases and suggestions of digenic and oligigenic inheritance in some families.[25,36,37] The identification and detailed clinical phenotyping of cohorts of BBS patients homozygous for common founder or recurrent mutations will facilitate the identification of genotype–phenotype determinants.

The mapping of BBS10 to 12q15–q21.2 in the large Omani BBS family illustrates the power of an autozygosity mapping strategy even in the presence of locus heterogeneity. The availability of SNP microarrays for genotyping

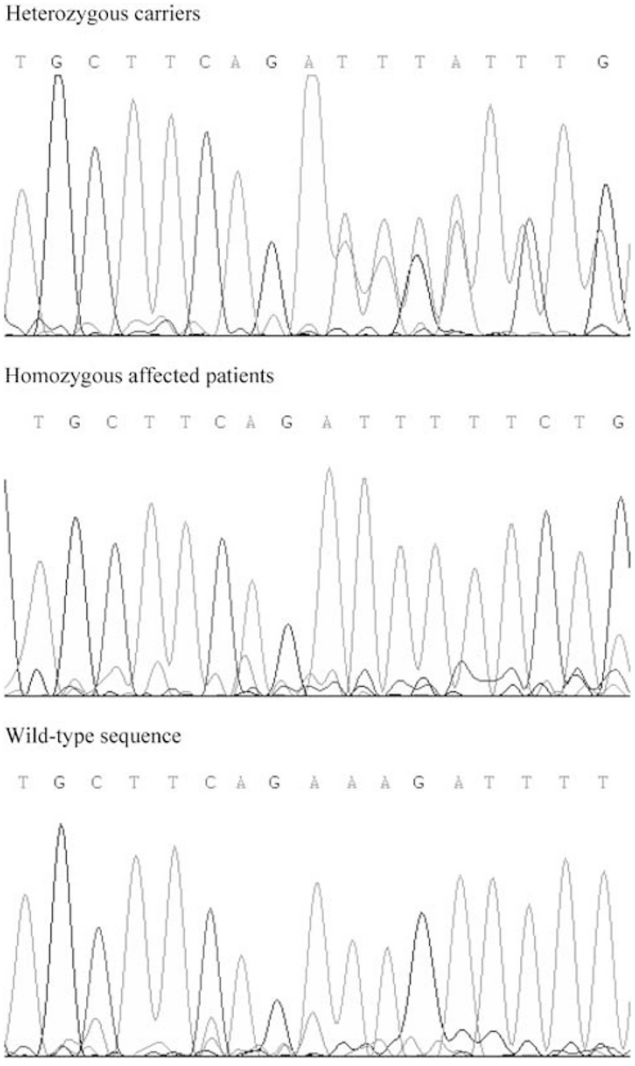

Figure 3 Sequence traces from Omani family with a p.Q242fs258X mutation in BBS10.

has further enhanced this approach. However, such families are rare and often not available. Recently, Nishimura *et al*[20] reported autozygosity mapping studies in small, consanguineous BBS pedigrees using moderately dense SNP arrays. A combination of comparative genome analysis and gene expression studies of a BBS-knockout mouse model was then used to identify BBS candidate genes and mapping of consanguineous affected sib pairs and isolated cases revealed a region at 7p14 containing the parathyroid hormone-responsive gene B1 (BBS9) in which germline mutations were identified in a consanguineous BBS family and then five nonconsanguineous kindreds.[20] In addition, among non-BBS9 linked families, four were homozygous at 12q21.1. Each of these families was found to harbour a *FLJ23560/BBS10* mutation further demonstrating the potential for homozygosity mapping with SNP arrays for gene identification studies in small, consanguineous families. Recently, Woods *et al*[38] reported that the mean size of the homozygous segment associated with recessive disease in a consanguineous family was 26 cM (range 5–70 cM). Although in many families the frequency of homozygous regions was higher than that predicted by simple models of consanguinity – which would be expected to impair autozygosity mapping studies, the longest homozygous segment was the disease-associated segment in 17% of individuals. In the large Omani family BBS10 was included in the largest autozygous region and analysis of the four small families used for autozygosity mapping in the current study revealed that the disease locus was the longest segment of autozygosity in two families and within the second longest region of autozygosity in the remaining two families. Thus, the ascertainment and sampling of both large and small consanguineous recessive disease families can have a critical role in the identification of human disease genes and the functional annotation of the human genome.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)