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Short Report

Carrier frequency of two *BBS2* mutations in the Ashkenazi population

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Bardet–Biedl syndrome (BBS) is known to be caused by numerous mutations that occur in at least 15 of the BBS genes. As the disease follows an autosomal recessive pattern of inheritance, carrier screening can be performed for at-risk couples, but the number of potential mutation sites to screen can be daunting. Ethnic studies can help to narrow this range by highlighting mutations that are present at higher percentages in certain populations. In this article, the carrier frequency for two mutations that occur in the *BBS2* gene, c.311A>C and c.1895G>C were studied in individuals of Ashkenazi Jewish descent in order to advise on including them in existing mutation panels for this population. Carrier screenings were performed on individuals from the Ashkenazi Jewish population using a combination of TaqMan genotyping assays followed by real-time polymerase chain reaction (PCR) and allelic discrimination, and allele-specific PCR confirmed by restriction analysis. The combined results indicated carrier frequencies of 0.473% (±0.0071%) for the c.311A>C mutation and 0.261% (±0.0064%) for the c.1895G>C mutation. On the basis of these frequencies, we believe that the two mutations should be considered for inclusion in screening panels for the Ashkenazi population.

Conflict of interest

All authors declare no conflict of interests.

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Bardet–Biedl syndrome (BBS) is an autosomal recessive, ciliary dysfunction disorder that results from mutations in the BBS genes. Non-allelic heterogeneity in at least 15 different genes has been identified through linkage and pedigree analyses as causative of phenotypes (1, 2), the severity of which can be increased by epistatic alleles (3). The BBS genes code for proteins that localize to the basal body of the cilium and are associated with ciliogenesis, intraflagellar transport, and other forms of transport (4). Seven of these genes code for proteins that form the BBSome and function in ciliary membrane biogenesis, and three interact to form the BBS-chaperonin complex required for BBSome assembly (5).

The high number of mutations responsible for causing BBS makes identifying carriers and performing prenatal diagnoses complicated. In addition to having to screen multiple genes for mutations to make a genetic diagnosis, drawing conclusions based on the presence of phenotypes is difficult due to the variety of clinical features with which patients present, which can include: obesity, retinal degeneration, polydactyly, kidney abnormalities and renal disease, cognitive impairment, hypertension, and diabetes, among

others (6–8). Additionally, phenotype severity cannot be used to predict which gene is mutated, although a correlation between *BBS1* mutations and ocular severity has been reported (9). Because of this, procedures such as genome-wide homozygosity mapping or DNA pooling followed by massive parallel resequencing (10) are performed to identify the mutations, although each test has limitations and can be expensive.

As founder mutations are often the cause of BBS (11), population-specific screening of ethnically prevalent mutations can be used as a cost-reducing approach by specifically testing for mutations that individuals are at an increased chance of carrying due to their ethnicity. This would potentially mean that fewer genes would need to be screened before the causative mutation was identified and it would also allow patients to be quickly diagnosed independent of family studies. For instance, specific mutations in the *BBS2* gene are increased in the Bedouin (1, 9) and Hutterite populations (11) compared to other mutations in the remaining BBS genes. Owing to an observed rise of BBS in the Ashkenazi population (four patients from three families in the past 2 years, Bonei Olam and Dor Yeshorim, personal communications), two mutations that occur in the *BBS2* gene are examined in this article for their prevalence in the Ashkenazi Jewish population. Of the four patients, two were homozygous affected for the c.311A>C (rs121908179) mutation and two were compound heterozygous for this mutation along with the c.1895G>C mutation.

The *BBS2* gene was the first BBS locus mapped (1), and mutations in this gene account for 8.10% of all BBS cases (4), making it the third most frequent BBS gene to harbor mutations. *BBS2* mutations are detrimental because *BBS2* directly interacts with *BBS7* and *BBS9* to form the BBSome core complex, which is required for complete formation of the BBSome and ciliary membrane biogenesis (5). Currently, screening for BBS is not included in mutation panels designed for individuals of Ashkenazi Jewish descent; however, the observed prevalence of the above two missense mutations suggests that they are ethnically prevalent. Therefore in order to investigate whether these two mutations should be included in Ashkenazi Jewish screening panels, carrier frequency studies were performed for both mutations on samples obtained from individuals belonging to this population.

Materials and methods

Ethics statement

The samples used in this study were obtained with written patient consent from self-identified Ashkenazi Jews enrolled in the carrier testing Dor Yeshorim program (12) to be used for research purposes. Consent form information included that patient material would be used for clinical testing and that excess material would be de-identified and used for research purposes to characterize single gene disorders in the Ashkenazi Jewish population. The control samples were obtained in conjunction with clinical testing, and institutional review board permission was not required for the carrier frequency samples because all sample identifiers were removed prior to receipt by our laboratory [45 CFR part 46.101(b)(4)].

Family history

The control samples came from three different Ashkenazi families. There was a male (born to consanguineous parents) homozygous for the c.311A>C mutation who had retinitis pigmentosa, limb defects, polydactyly, and diabetes; a female (born to consanguineous parents) homozygous for the c.311A>C mutation with retinitis pigmentosa and polydactyly; and two siblings compound heterozygous for the c.311A>C and c.1895G>C mutations that had retinitis pigmentosa, polydactyly, and cognitive impairment. All patients were diagnosed with BBS prior to receipt by our laboratory and were received as de-identified samples to be used as controls for validation of the TaqMan assays and restriction enzymes.

TaqMan carrier frequency study

TaqMan genotyping assays were designed for both the c.311A>C and c.1895G>C mutations using File Builder software [Life Technologies (LTI), Carlsbad, CA], with VIC- and FAM-labeled probes for the detection of the normal and mutant alleles, respectively. The primers for the c.311A>C mutation were: (F-GCCTCTCTGTAGAACAAATCCGAAT) and (R-CTTGGCTATGATGCCCTTTTAGTG), and the probes were (VIC-CTTTTGGCTTATGATGTCTAC) and (FAM-TTGGCTTATGCTGTCTAC). The primers for the c.1895G>C mutation were: (F-CCTGTACTAACCATGACAAATACTCACA) and (R-AGTTTGCTGGTCGGAGCTG), and the probes were (VIC-AGGATGCTCGTCTGATG) and (FAM-AGGATGCTCCTCTGATG). Samples were normalized to 5 ng/ul and validated on controls through duplex real-time polymerase chain reaction (PCR) followed by allelic discrimination on the ABI PRISM® 7900 HT Sequence Detection System (7900s) using SDS v2.3 software (LTI). The gDNA samples used in the carrier frequency study were not normalized prior to plating, but fell within the suggested range of 1–20 ng. The samples were run on the GeneAmp® PCR System 9700 (LTI) at the following setting: holds at 50°C for 2 min and 95°C for 10 min, and then 40 cycles at 95°C for 15 s and 60°C for 1 min. Allelic discrimination was performed on the 7900s and the data was analyzed using TAQMAN GENOTYPER v1.2 software (LTI). The Central Limit Theorem was used to calculate the confidence intervals. Samples genotyped as heterozygous carriers were confirmed through Sanger Sequencing (GENEWIZ, South Plainfield, NJ), with results analyzed using SEQSCAPE Software v2.6 (LTI).

Restriction analysis carrier frequency study

Allele-specific PCR was performed using specific primers, with the c.311A>C mutation using (F-ACAC

AGACTAATCTTTTGGCTTCTGC) and (R-GAGATA AGCTCGAGTGTCACCAA), and the c.1895G>C mutation using (F-GTCGGAGCTGAGGATGCTCC) and (R-TGAGAGTTGCTATTCCATACTGCTCAC). Restriction analysis was then performed on all samples to confirm the genotypes. The *Eco*RV and *Bse*RI restriction enzymes were used to detect the c.311A>C and c.1895G>C mutations, respectively. The c.311A>C mutation used (F-GTCAGTGCATC CAGGGTCTTCCAG) and (R-AGAACAAATCCGAA TTATTGTAGACA) primers that yielded a 184-basepair (bp) product (wild type = 159 + 25 bp, mutation = 184 bp). The c.1895G>C mutation used (F-GTGGATGAATATCATTCAGTG) and (R-TGAGA GTTGCTATTCCATACTG) primers that yielded a 182-bp product (wild type = 182 bp, mutation = 100 + 82 bp).The Central Limit Theorem was used to calculate the confidence intervals.

Results

The initial validation of the TaqMan assays on a small scale yielded 100% genotyping accuracy. Figure 1a,d shows the successful genotyping of the two mutations on control samples specific for each mutation. Unique clusters indicative of different genotypes were formed based on the signal intensity ratio of the two probes being used (VIC and FAM), allowing for the successful assignment of genotypes.

The carrier frequency experiments were then performed for the c.311A>C and c.1895G>C mutations on sample sizes of 19,010 and 18,025 individuals, respectively, using a combination of TaqMan allelic discrimination and allele-specific PCR. References consisting of the samples used in the initial validation were included in the TaqMan analysis and the results were analyzed using TAQMAN GENOTYPER v1.2 software, as seen in Fig. 1b,e , or were alternatively analyzed using allele-specific PCR (Fig. 2a). The combined carrier frequencies from the two studies indicated an overall carrier frequency of 0.473% (±0.0071%) for the c.311A>C mutation (18,920 wild type samples and 90 heterozygous carriers), and 0.261% (±0.0064%) for the c.1895G>C mutation (17,978 wild type samples and 47 heterozygous carriers). All samples identified as heterozygous through TaqMan allelic discrimination had their genotypes confirmed through Sanger Sequencing (Fig. 1c,f), with results concurrent for all samples. All samples genotyped using allele-specific PCR were confirmed using restriction analysis (Fig. 2b).

Discussion

While the carrier frequencies of the c.311A>C and c.1895G>C mutations do not meet the current 1% threshold suggested by the American College of Medical Genetics (ACMG) for adding mutations to Ashkenazi screening panels (13), the history of BBS is well understood and the disorder has a significant morbidity rate in affected patients, which

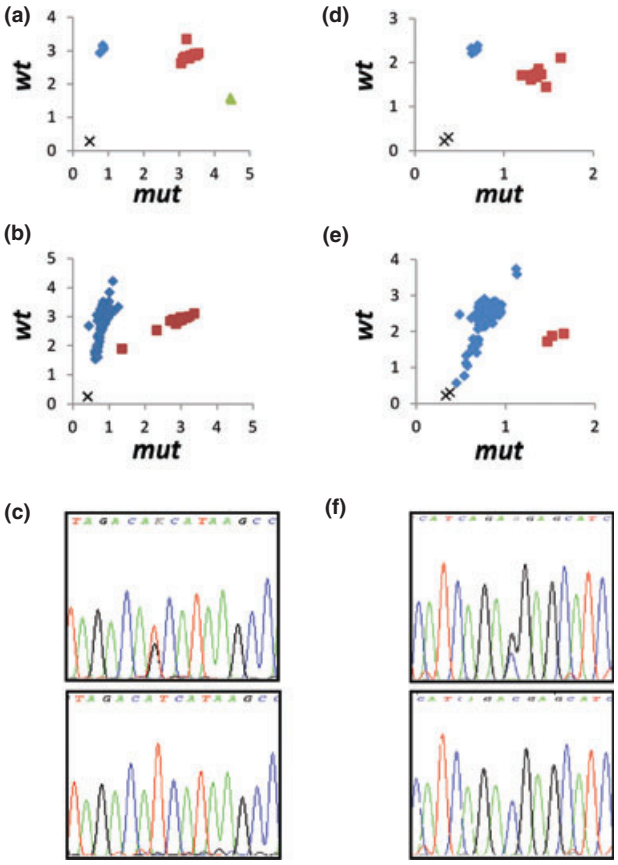

Fig. 1. Allelic discrimination and Sanger Sequencing results for the *BBS2* mutations. (**a**, **d**) The genotyping plots for the initial validation of the c.311A>C and c.1895G>C mutation assays, respectively, and (**b**, **e**) the carrier frequency results for the same mutations. For all plots, the VIC probe (wild type allele) is represented by the *Y*-axis, and the FAM probe (mutant allele) is represented by the *X*-axis. Wild type samples are represented by blue diamonds, heterozygous carrier samples by red squares, homozygous affected samples by green triangles, and no template controls (NTCs) by ×'s. Water was used as the NTCs. (**c**, **f**) The examples of the Sanger Sequencing results for a heterozygous carrier (top box) and a wild type (bottom box) samples for the c.311A>C and c.1895G>C mutations.

meet the other existing criteria. Because of the recent family studies, we believe that both mutations (c.311A>C and c.1895G>C) should be recommended for inclusion in screening panels specific for this population. The ACMG, along with the American College of Obstetricians and Gynecologists (ACOG), has acknowledged that individuals may want to be screened for additional disorders and recommends that educational material and/or genetic counselors should be made available to aid patients in making informed decisions (14, 15).

Recent advances in high-throughput screening programs (16) have made screening for additional and/or rare mutations possible and affordable, with many commercial labs already offering ethnic screening panels that extend beyond the scope of mutations specifically recommended by the ACMG and other such organizations (17). Screening for pan-ethnic BBS mutations that occur in *BBS1* and *BBS10* are already commercially

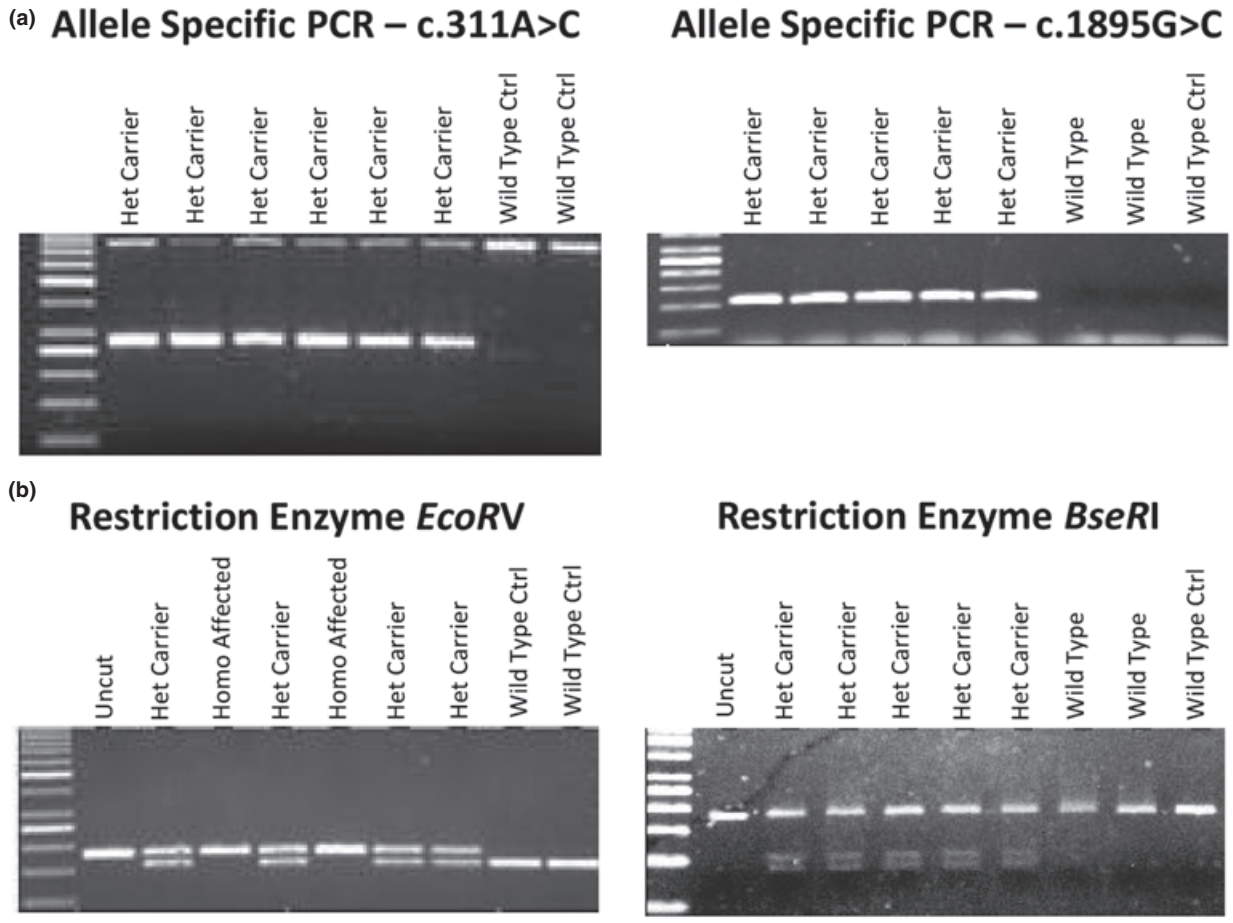

Fig. 2. Allele-specific polymerase chain reaction (PCR) and restriction analysis for the *BBS2* mutations. (**a**) Examples of the detection of the two mutations using allele-specific PCR. (**b**) Examples of the genotyping results using restriction analysis.

offered at companies such as Bio-Reference, Counsyl, and LabCorp, however Ashkenazi Jewish patients participating in such programs are not being screened for the *BBS2* mutations that frequently occur in their specific population. The inclusion of these two *BBS2* mutations in Ashkenazi screening panels could help reduce the risk of having affected children in this population. While the residual risk of inheriting BBS cannot be calculated on an individual basis as parents may be carriers for other mutations in the *BBS2* gene or the other 14 BBS genes that can cause a phenotype, screening for these two mutations should significantly reduce the risk that both parents are carrying the most prevalent BBS mutations in the Ashkenazi population.

Screening for these two *BBS2* mutations may also generate research opportunities as it has been proposed that mutations in ciliary loci on different genes can modify disease phenotypes in individuals affected with other ciliary disorders (11, 18). Specific mutations in the *PCDH15* and *TMEM216* genes causative of the ciliary disorders, Usher Syndrome and Joubert Syndrome 2, are also prevalent in the Ashkenazim (19, 20). Several commercially available Ashkenazi genotyping panels already screen for these mutations, so establishing if there is a correlation between genotype and phenotype severity in patients that harbor mutations across multiple ciliary genes could be furthered by also genotyping for the *BBS2* mutations.

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