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SHORT REPORT

# Arrayed primer extension technology simplifies mutation detection in Bardet–Biedl and Alström syndrome

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**Bardet–Biedl syndrome (BBS; OMIM no. 209 900) and Alström syndrome (ALMS; OMIM no. 203 800) are rare, multisystem genetic disorders showing both a highly variable phenotype and considerable phenotypic overlap; they are included in the emerging group of diseases called ciliopathies. The genetic heterogeneity of BBS with 14 causal genes described to date, serves to further complicate mutational analysis. The development of the BBS–ALMS array which detects known mutations in these genes has allowed us to detect at least one mutation in 40.5% of BBS families and in 26.7% of ALMS families validating this as an efficient and cost-effective first pass screening modality. Furthermore, using this method, we found two BBS families segregating three BBS alleles further supporting oligogenicity or modifier roles for additional mutations. We did not observe more than two mutations in any ALMS family.**
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## INTRODUCTION

Bardet–Biedl syndrome (BBS; OMIM no. 209 900) and Alström syndrome (ALMS; OMIM no. 203 800) are rare, multisystem genetic disorders showing both a highly variable phenotype and considerable phenotypic overlap. BBS, the more prevalent of the two, is genetically heterogeneous and characterised by rod–cone dystrophy, obesity, postaxial polydactyly, cognitive impairment, hypogonadism and renal abnormalities.[1] A variety of secondary features have also been reported in BBS patients, including developmental delay, speech disorders, dental anomalies, diabetes mellitus and behavioural problems.

ALMS is a recessively inherited disorder with a complex and variable presentation, including progressive cone–rod dystrophy beginning at birth and leading to blindness, sensorineural hearing loss, obesity and type 2 diabetes in childhood. Most patients develop cardiomyopathy in infancy or adolescence. Pulmonary, hepatic and renal/urological dysfunctions are frequently observed, and systemic fibrosis develops by the end stage of the disease.[2]

In both BBS and ALMS, the combination of the late onset of some features, such as renal disease, and the existence of other genetic syndromes with similar cardinal manifestations often leads to confusion amongst clinicians and possible misdiagnosis.

To date, one *ALMS* and 12 *BBS* genes have been identified.[3–5] In addition, mutations in BBS patients have also been identified in *MKS1* and *CEP290*, genes known to cause other ciliopathic phenotypes such as Meckel syndrome and nephronophthisis.[6] Owing to the large number of *BBS* genes, direct sequencing of all coding exons of these (135 exons for *BBS1-12* and 23 exons for *ALMS1*) is not practical or cost-effective. For this reason, in collaboration with Asper Biotech (http://www.asperbio.com), we developed a *BBS–ALMS1* mutation array that can be used to screen a patient sample for known mutations in 10 *BBS* genes and *ALMS1*. In this study, we report the analysis of 340 patient samples using the *BBS–ALMS1* array confirming its value as a first-pass diagnostic screening test.

## MATERIALS AND METHODS

### Subjects

DNA samples from 340 patients (205 BBS and 135 ALMS) have been analysed using the *BBS–ALMS1* mutation array. Genomic DNA was prepared from blood lymphocytes by standard procedures. The DNA samples were derived from at least 24 different countries. All patients had either a confirmed or suspected diagnosis of BBS or ALMS. The research adhered to the declaration of Helsinki and was approved by the Institutional Review Boards of all participating institutions.

### *BBS–ALMS1* mutation array

The *BBS–ALMS1* mutation array uses arrayed primer extension technology first described in 1996 (Shumaker *et al*[7]). This methodology allows the detection of single base substitutions, deletions and insertions. Basically, 5′-modified sequence-specific oligonucleotides are arrayed on a glass slide. In general, these oligonucleotides are designed with their 3′ end immediately adjacent to the variable site. PCR-amplified and fragmented target nucleic acids are annealed to oligonucleotides on the slide, followed by sequence-specific extension of the 3′ ends of primers with dye-labelled nucleotides analogues by DNA polymerase (Supplementary data (so2), http://www.asperbio.com). The latest version of the

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array contains 253 sequence variants from *BBS1-7*, *BBS9*, *BBS10*, *BBS12* and *ALMS1* (Supplementary Table 1). In addition to published mutations, intragenic SNPs have also been included on the array. Data from these SNPs could potentially be used to demonstrate linkage to a particular locus and allow a more structured approach to identification of novel *BBS* and *ALMS1* mutations in these samples. Despite this facility, we have not yet formally evaluated the benefits of SNP-based haplotyping in our cohort.

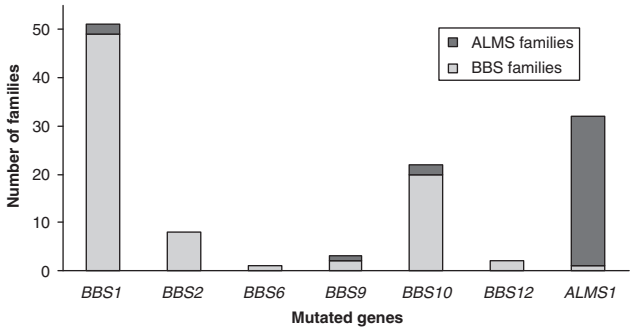


**Figure 1** Summary of results showing the contribution of each of the genes to the BBS and ALMS phenotype. No mutations were identified in *BBS3*, *BBS4*, *BBS5* or *BBS7*. Families with three mutations were assigned to the primary locus with two mutations.

## RESULTS

In designing the customised array, we performed chip validation assays obtaining an assay call rate of 99.5%, sensitivity of 99.4% and a specificity of 100%, therefore we assumed the design to be of high quality.

Of the 340 samples that were screened using the mutation array, at least one likely causative mutation was identified in 35% (119/340) of families (Figure 1). In the majority of cases (53 BBS+16 ALMS families) homozygous or compound heterozygous mutations were discovered, therefore, requiring no further analysis (Tables 1 and 2). A summary of polymorphisms that were detected is shown in Supplementary Table 2.

The *BBS1* and *BBS10* genes are the most common contributors to BBS, being involved in 84.1% (69/82) of the total BBS families with a BBS mutation detected. As it has been reported before, the most frequent disease alleles in north European origin BBS families were p.M390R, identified in all *BBS1* families, and p.C91fsX95 (Zughloul and Katsanis)[5] in 50% (10/20) of *BBS10* families (Table 1). Although the most frequently reported mutation in *ALMS1* is c.10775delC (p.T3592fs),[8] the most frequently observed *ALMS1* mutation in this series was c.11316_11319delAGAG (p.R3772fs), identified in three families.

In two BBS cases (excluding two families with the probable polymorphism p.A242S, *BBS6*), three bona fide mutations were identified (Table 1). It is, therefore, possible that these are examples

**Table 1 Summary of BBS genotypes detected with the *BBS–ALMS1* mutation array, excluding polymorphisms**

| *Genes* | *Mutation 1* | *Mutation 2* | *Mutation 3* | *Number of BBS families* | *Number of ALMS families* |
|---|---|---|---|---|---|
| *BBS1* (*n*=51) | c.1169T>G [p.M390R] | c.1169T>G [p.M390R] | c.823C>T[p.R275X] (*BBS2*) | 1 | |
| | c.1169T>G [p.M390R] | c.1169T>G [p.M390R] | | 32 | 2 |
| | c.1169T>G [p.M390R] | c.437C>T [p.R146X] | | 1 | |
| | c.1169T>G [p.M390R] | c.871C>T [p.Q291X] | | 1 | |
| | c.1169T>G [p.M390R] | c.1574G>T [p.E549X] | | 2 | |
| | c.1169T>G [p.M390R] | | | 12 | |
| *BBS2* (*n*=8) | c.823C>T [p.R275X] | c.823C>T [p.R275X] | | 1 | |
| | c.266A>G [p.Y89C] | c.266A>G [p.Y89C] | | 1[a] | |
| | c.312A>C [p.D104A] | c.1895G>C [p.R623P] | | 1 | |
| | c.175C>T [p.Q59X] | | | 2 | |
| | c.72C>T [p.Y24X] | | | 2 | |
| | c. 266A>G [p.Y89C] | | | 1 | |
| *BBS6* (*n*=1) | c.830T>C [p.L277P] | c.830T>C [p.L277P] | | 1 | |
| *BBS9* (*n*=3) | c.1861A>T [p.K622fsX647] | c.1861A>T [p.K622fsX647] | | | 1 |
| | c.1861A>T [p.K622fsX647] | | | 1 | |
| | IVS5+1 G>C | | | 1 | |
| *BBS10* (*n*=22) | c.1241T>C [p.L414S] | c.1241T>C [p.L414S] | c.1063C>T [p.Q355X] (*BBS9*) | 1 | |
| | c.273del CA [p.C91fsX95] | c.273del CA [p.C91fsX95] | | 4 | 1 |
| | c.691delT [p.V230fsX236] | c.2118_2119del TG [p.V707fsX708] | | 1 | |
| | c.273del CA [p.C91fsX95] | c.2118_2119del TG [p.V707fsX708] | | 1 | |
| | c.145C>T [p.R49W] | c.691delT [p.V230fsX236] | | 1 | |
| | c.1678delG [p.Y559fsX576] | c.1678delG [p.Y559fsX576] | | 2 | |
| | c.273del CA [p.C91fsX95] | | | 6 | 1 |
| | c.590A>G [p.Y197C] | | | 1 | |
| | c.691delT [p.V230fsX236] | | | 1 | |
| | c.986C>T [p.S329L] | | | 1 | |
| | c.1241T>C [p.L414S] | | | 1 | |
| *BBS12* (*n*=2) | c.1114delTT [p.F372fs373] | c.1114delTT [p.F372fs373] | | 1 | |
| | c.1114delTT [p.F372fs373] | | | 1 | |
| Total (*n*=87) | | | | 82 | 5 |

Abbreviations: ALMS, Alström syndrome; BBS, Bardet–Biedl syndrome.
[a]Non-European family.

**Table 2 Summary of *ALMS1* genotypes detected with the *BBS–ALMS1* mutation array, excluding polymorphisms**

| *Genes* | *Mutation 1* | *Mutation 2* | *Number of BBS families* | *Number of ALMS families* |
|---|---|---|---|---|
| *ALMS1* (*n*=32) | c.10775delC [p.T3592KfsX6] | c.11449C>T [p.Q3817X] | 1 | |
| | c.6800T>A [p.L2276X] | c.6800T>A [p.L2276X] | | 1 |
| | c.8177_8187delGCATTTCC [p.C2726FfsX5] | c.8177_8187delGCATTTCC [p.C2726FfsX5] | | 2[a] |
| | c.1769T>A [p.L590X] | c.3425C>G [p.S1142X] | | 1 |
| | c.7534C>T [p.R2512X] | c.7534C>T [p.R2512X] | | 1 |
| | c.8782C>T [p.R2928X] | c.8782C>T [p.R2928X] | | 1[a] |
| | c.9541C>T [p.R3181X] | c.10483C>T [p.Q3495X] | | 1 |
| | c.5574_5575delTG [p.V1859HfsX4] | c.7574_7587delATTGTGGATACTCC [p.C2526FfsX5] | | 1[a] |
| | c.7374_7375delAG [p.D2459X] | c.7374_7375delAG [p.D2459X] | | 1 |
| | c.8224C>T [Q2742X] | c.8224C>T [Q2742X] | | 1[a] |
| | c.10945G>T [p.E3649X] | c.10945G>T [p.E3649X] | | 1[a] |
| | c.7534C>T [p.R2512X] | c.10775delC [p.T3592KfsX6] | | 1 |
| | c.7942C>T [p.Q2648X] | | | 1[a] |
| | c.11005C>T [p.Q3669X] | | | 1 |
| | c.8164C>T [p.R2722X] | | | 1 |
| | c.8656C>T [p.R2886X] | | | 1 |
| | c.10775delC [p.T3592KfsX6] | | | 1 |
| | c.10535G>A [p.W3512X] | | | 1 |
| | c.11316_11319delAGAG [E3773WfsX18] | | | 3 |
| | c.11385delT [p.F3795LfsX38] | | | 1 |
| | c.8177_8187delGCATTTCC [p.C2726FfsX5] | | | 1 |
| | c.7534C>T [p.R2512X] | | | 1 |
| | c.11460C>G [p.Y3820X] | | | 1 |
| | c.7374_7375delAG [p.D2459X] | | | 1 |
| | c.10483C>T [p.Q3495X] | | | 1 |
| | c.11414delG [R3805fsX28][b] | | | 1 |
| | c.8782C>T [R2928X] | | | 1 |
| | c.9194T>G [L3065X] | | | 1 |
| | c.10753C>T [Q3585X] | | | 1 |
| Total (*n*=32) | | | 1 | 31 |

Abbreviations: ALMS, Alström syndrome; BBS, Bardet–Biedl syndrome.
[a]Non-European family.
[b]This *ALMS1* mutation has not been published.

of oligogenic inheritance which has previously been reported in BBS.[9] No evidence of digenic triallelism was seen in patients with ALMS.

One patient who fulfilled diagnostic criteria for BBS was found to be compound heterozygous for a frame-shift and nonsense mutation in *ALMS1*. Four patients diagnosed as ALMS, harboured homozygous *BBS* gene mutations, and one had a heterozygous mutation in *BBS10*.

## DISCUSSION

The *BBS–ALMS1* array detected more mutations in the BBS families (40.5%; 83/205) than in ALMS (26.7%; 36/135) families. This finding could be explained by the existence of two 'common' mutations in *BBS1* and *BBS10*, identified in 71.1% (59/83) of BBS families; these mutations have been proposed previously for first pass screening.[10] In addition, most laboratories testing ALMS families, offer direct *ALMS1* sequencing of only exons 16, 10, and part of 8, which would identify the mutations most frequently found in *ALMS1*. However, other alternative approaches based on massive sequencing are not, at this time, as practical or cost-effective as the genotyping chip.

In our dataset, the array allowed us to identify two mutations in 25.8% (53/205) BBS and 11.9% (16/135) ALMS families. In the cases in whom a single heterozygous *BBS* or *ALMS1* mutation was found, the entire open reading frame of the gene containing that mutation should be screened in order to detect an additional novel causative mutation within that gene. If no mutation is found using the array, the negative results could be explained in a number of ways: (1) the patient has novel mutations in genes that are not represented on the array (*BBS8*, *BBS11*, *MKS1* or *CEP290*), (2) the specific mutation the patient carries is not included on the array, or (3) the patient has a mutation in a gene other than *BBS* or *ALMS*. A limitation of the array is that, in its present form it does not provide complete coverage of all genes in which mutations have been found in BBS and ALMS patients, nor all of the published mutations in *BBS* and *ALMS*. As positions are added on a regular basis, mutations could be picked up if the patient is re-tested at a later date, improving the success rate.

Although the mutations that are on the chip have been described in different populations, the mutation detection rate was higher in European than non-European patients (BBS: 47 *vs* 3%; ALMS: 19 *vs* 5%).

This combined syndrome array has the potential to resolve some diagnostic confusion. For example, in this dataset, misdiagnoses in that at least four patients with a clinical diagnosis of ALMS in fact harboured BBS mutations. Conversely, one patient diagnosed phenotypically with BBS had *ALMS1* compound heterozygous mutations, c.10775delC (p.T3592fsX3597) and c.11449C>T (p.E3817X), and no *BBS* mutations.

There are only a few published examples of the presence of a third mutation in a second BBS gene that is necessary for the phenotypic manifestation of BBS,[11] possibly owing to the paucity of families with multiple siblings in which the triallelic inheritance can be proven. More often reported however, is the presence of a third heterozygous mutation (at a second BBS locus) behaving as a modifier allele.[9] In this study, we were able to ascertain that two probands carried three bone fide BBS mutations, whereas the third heterozygous allele was a nonsense mutation (p.R275X in *BBS2*; p.Q355X in *BBS9*). Although there were no siblings in whom either necessity of the third allele could be tested or severity scored, it is likely that the nonsense allele would have a significant effect on the function of the protein complexes that have been described. To date, we have not seen any examples of this in ALMS.

In conclusion, we confirm the use of this array as a helpful and cost-effective first pass screening test for cases of BBS or ALMS and demonstrate it benefit in resolving phenocopy issues between these syndromes. However, the analysis by several approaches of the two more frequent mutations in BBS families (p.M390R, *BBS1*; p.C91fsX95, *BBS10*) could be an efficient preliminary step as they were found in 75.6% (60/82) of the patients in which at least one mutation was detected. However, one must be cautious as the most frequent *BBS10* mutation in our subset of BBS Spanish patients was p.Y559fsX576, supporting the value of a wide-spectrum approach, such as a genotyping array.

Nevertheless, the development of next generation sequencing technologies will eventually be used for the identification of disease-causing mutation in this group of patients; however, at this time, these approaches are not as practical or cost-effective as the genotyping array.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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