Risk factors for severe renal disease in Bardet-Biedl syndrome.

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Abstract

Bardet-Biedl syndrome is a rare autosomal recessive multi-system disease characterised by retinal dystrophy, renal malformation, obesity, intellectual disability, polydactyly and hypogonadism. Nineteen disease causing genes (*BBS1-19*) have been identified of which mutations in *BBS1* are most common in North America and Europe.

A hallmark of the disease, renal malformation is heterogeneous and is a cause of morbidity and mortality through the development of chronic kidney disease. We studied the prevalence and severity of chronic kidney disease in the largest reported cohort of patients with Bardet-Biedl syndrome-related renal disease, further elucidating the phenotype and identifying risk indicators.

Results from 350 patients attending the UK national Bardet-Biedl syndrome clinics were analysed. Thirty one per cent of children and 42% of adults had chronic kidney disease. Eight percent of adults had stage 4-5 chronic kidney disease. In childhood, renal disease was primarily detected within the first year of life and stage 4-5 chronic kidney disease was present in 6%. Fifty one percent of patients had structural renal abnormalities on ultrasonography and 35% were hypertensive. These risk factors also correlated statistically with chronic kidney disease stages 3b-5.

Genotype and mutation type were statistically significant risk indicators. Mutations in *BBS1* or two missense mutations were associated with less severe or lack of chronic kidney disease in comparison to mutations in *BBS10* or two truncating mutations.

This study describes the largest reported cohort of patients with renal disease in Bardet-Biedl syndrome and identifies risk factors to be considered in genetic counselling.

Introduction

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathy characterised by rod cone dystrophy, renal malformations, learning difficulties, obesity, post-axial polydactyly and hypogonadism.[1] Nineteen disease causing genes have been identified (*BBS1-BBS19*) in the last two decades. BBS genes code for proteins that localise to the cilia or the basal body and are thought to be involved in cilia development and maintenance[2]. Mutations in BBS genes lead to defective cilia. Sequencing of known disease causing genes confirms a clinical diagnosis of BBS in around 80% of patients[2]. Variable expressivity is a hallmark of BBS and both inter- and intrafamilial phenotypic variation is observed.[2]

Structural renal and urinary tract anomalies and renal dysfunction is a cause of considerable morbidity and reported to affect 53-82% of patients with BBS.[2-6] The primary renal phenotype is highly variable ranging from cystic tubular disease, dysplastic renal disease and focal segmental glomerulosclerosis to concentrating defects.[3-7] Lower urinary tract dysfunction is observed in many patients and may have upper renal tract sequelae.[8] It is thought that ciliary dysfunction leads to disturbance of the non-canonical Wnt signalling pathway which may contribute to the development of cystic kidney disease classically associated with BBS.[2] Secondary renal disease may occur as a consequence of hypertension and diabetes which are frequently observed in this population.

The high frequency of renal disease in BBS is a cause of great anxiety among patients due to the devastating effect this can have on quality of life, morbidity and mortality.[4-6] This study examines renal disease in the largest reported cohort of BBS

patients and identifies indicators of disease which are directly relevant to patient management and clinical stratification.

Results

Overview of the BBS population

Three hundred and fifty patients attended the adult and paediatric national BBS clinics in Birmingham and London over a four year period (2010-14). The patient population ranged in age from birth to 60 years old, with a peak frequency in the six to ten year old category, and with few older adults.

Diagnosis was based on clinical phenotyping. All patients underwent genetic testing. On sequencing 13 disease related genes, molecular confirmation of the diagnosis was achieved in 80% of all pedigrees (216 of 270) and in 77% of all patients (265 of 350). A full list of genotypes can be found in supplemental table 1. The gender distribution was 54% male and 46% female. Sixty nine per cent of patients were Caucasian, 28% South East Asian and the remaining 3% had a mixture of other backgrounds (African, Chinese and Ashkenazi Jewish).

The distribution of genotypes is demonstrated in figure 1. For the purpose of statistical analysis mutation type was classified according to severity. One hundred and twenty five patients had two missense mutations, eighty two had two truncating mutations (nonsense, frameshift, splice site or a combination), 39 had a combination of missense and truncating mutations and the remaining nineteen patients had other mutation combinations, including exon deletions and start codon aberrations.

Age of onset of renal disease

One hundred and fifty six paediatric patients were seen in the paediatric clinics. Of these we were able to retrospectively obtain the earliest recorded age of onset of Chronic Kidney Disease (CKD) stage[9] 2-5 in 49 paediatric patients attending the BBS clinic (figure 2). All paediatric patients with CKD4-5 were diagnosed before the age of 5. The majority of patients with any stage of CKD presented before the age of 10. Since the peak referral age to the clinic is late childhood, later recorded onset of CKD is likely to reflect significant ascertainment bias as patients may have asymptomatic renal disease and no previous renal sonography.

Observations from the national BBS clinics suggest that patients either develop CKD4-5 in childhood or maintain normal or near-normal renal function into adulthood. Figure 3 demonstrates that frequency of CKD4-5 remains similar in adults and children (8% and 6% respectively).

Prevalence of chronic renal disease

Estimated GFR (eGFR) results were available for 189 adults and 133 paediatric patients. Seventy per cent of adult patients had at least two eGFR readings. The prevalence of each stage of CKD in adults and children is demonstrated in figure 3 where CKD2-5 is present in 42% and 31% respectively. In the adult population (n=194) 107 patients were considered free of renal disease or had CKD1, and five did not have an eGFR or a renal ultrasounds scan. Forty three adult patients had a normal renal ultrasound scan and 47 had a structural abnormality. In the paediatric population (n=156) 84 patients were considered free of renal disease or had CKD1,

and 23 patients did not have an eGFR. Eighty seven paediatric patients had a renal ultrasound scan, 43 of which revealed a structural abnormality. Nine patients had neither a documented eGFR or ultrasound scan. All patients were requested to provide a sample for urinalysis. Ninety per cent of children and 96% of adults respectively were able to comply. Urinalysis was normal in all those considered free of renal disease. Tables 1a and 1b demonstrate the number of patients seen in the adult and paediatric clinics and the prevalence of CKD indicators. Tables 2a and 2b demonstrate the prevalence of CKD stages 2-5 in the adult and paediatric populations presenting to the Bardet-Biedl syndrome clinics.

The age at which patients reached CKD5 is known for 20 patients in this cohort. Of these patients 70% (n=14) had reached CKD5 by the age of 20. The age of onset of CKD5 was delineated retrospectively and Kaplan-Meier survival to CKD5 is demonstrated in figure 4.

Genotype and mutation type analyses in adults with BBS revealed statistically significant correlations with the presence of severe renal disease defined as CKD stage 3b to 5 (eGFR<45 ml/min/1.73m^2). Univariable logistical regression analysis indicated that mutations in *BBS2*, *BBS10* and *BBS12* were more likely to be associated with severe renal disease than mutations in *BBS1* (p values: 0.02, 0.0003, 0.03 respectively) (table 3). Univariable logistical regression analysis of mutation type revealed that truncating mutations and truncating/missense mutations were statistically associated with severe renal disease in comparison to two missense mutations (p values= 0.000003, 0.01 respectively) (table 3).

Proportional frequencies of CKD stages 2, 3, 4 and 5 in adults were compared for the commonest genotypes *BBS1* and *BBS10* as outlined in figure 5. Patients with

BBS1 mutations are more likely to be disease free or have early stage CKD. Mutations in *BBS10* were more frequently represented with increasing stage of CKD. Of note, patients attending our clinics with mutations in *BBS1* are statistically significantly older than those with mutations in *BBS10* (p=0.0011).

Previous research indicates that the recurring missense mutation M390R in *BBS1* may be hypomorphic.[10;11] We assessed this by comparing adult patients who were homozygous for M390R to patients with other mutation types in *BBS1* (supplemental figure 1). This did not reveal an obvious hypomorphic effect of homozygous *BBS1* M390R mutations. It may reflect the relatively small group of patients, or that the majority of patients with mutations in *BBS1* who are not homozygous for M390R are heterozygous for this mutation. Since BBS is an autosomal recessive disease, it is possible that the the hypomorphic effect of M390R predominates even in those who are heterozygous. Of note, only one patient homozygous for *BBS1* M390R had progressed beyond stage 3 CKD. The patient presented following renal transplant aged 23 to our service and the cause of renal failure was unclear although presumed to be a result of BBS.

We assessed for the presence of micro- and macroalbuminuria by analysing urinary albumin/creatinine ratios as a proxy for glomerular injury. Urinary albumin/creatinine ratios were available for 139 adult and paediatric patients. Seven (5%) had proteinuria (defined as urinary albumin/ creatinine >30 mg/mmol); three of whom were diabetic. Thirty two patients (28%) had microalbuminuria (defined as urinary albumin/ creatinine >3.5 mg/mmol[12]), and two of these patients were diabetic. This could be matched to an eGFR in 119 patients. There was a statistically significant correlation between severe renal disease and urinary albumin/ creatinine ratios (p=

0.006). The sample size was inadequate for correlation with genotype and mutation type.

Although under-reported, 6% of the adult BBS population report urological complications requiring specialist management. Urological abnormalities include neuropathic bladder, vesico-ureteric reflux, urinary incontinence and bladder outflow obstructions.

Presence of structural abnormalities

One hundred and seventy seven ultrasound reports from the entire cohort were available for our assessment. Eighty seven were unremarkable and 90 revealed structural defects. Abnormalities were categorised as atrophic/scarring, echogenic or loss of corticomedullary differentiation, cystic or dysplastic, other developmental abnormality or hydronephrosis as seen in figure 6. No consistent pattern was evident in the type of renal structural aberrations observed; patients with cystic disease ranged from unilateral single cysts to multiple bilateral cystic disease. Developmental abnormalities included horseshoe kidneys, ectopic kidneys, duplex and absent kidneys. Where several abnormalities were present, the predominant structural defect is reported. On assessing genotype correlations (*BBS1* versus *BBS10*) no association with the presence of structural abnormality was identified (p=0.188).

Correlating the presence of all causes of structural abnormality in adults with CKD staging revealed a strong correlation with CKD at stage 3b-5 (p=0.039). All patients with a reported renal ultrasound scan and severe renal disease had a detectable structural abnormality (n=7).

Ultrasound reports from 39 paediatric patients with known renal structural abnormalities who had both antenatal and postnatal sonography failed to identify the anatomical aberration prenatally in 14 patients (36%).

Five paediatric patients had abnormal antenatal renal ultrasound reports and normal postnatal sonography. In all cases, non-specific echogenicity was reported antenatally and no specific structural abnormalities were detected.

In this cohort, 30 patients presented with sonographic evidence of cystic kidney disease which is classically associated with BBS. Twenty four of these patients had molecular confirmation of BBS. Patients with mutations in *BBS1* and *BBS10* accounted for the majority of genotypes represented (42% and 21% respectively). Figure 7 demonstrates some of the structural renal aberrations commonly detected on ultrasonography.

Hypertension and diabetes

Thirty five per cent of adult patients (n=67) in this cohort were hypertensive. There is a statistically significant correlation between CKD3b-5 and the presence of anti-hypertensive medication (p=0.003) (table 3). There was a significant association between the presence of hypertension and albuminuria (p=0.0009). The most commonly prescribed antihypertensive medications were angiotensin-converting-enzyme inhibitors (52%) followed by diuretics (21%), calcium channel blockers (15%), beta blockers (8%) and angiotensin receptor blockers (3%).

Fifteen per cent of adult patients (n=28) were on hypoglycaemic medication. There was no statistically significant association with CKD3b-5 (p=0.471).

Discussion

To our knowledge, this is the largest reported study characterising the renal phenotype in BBS. The age distribution of our patient population is most likely a reflection of a number of factors affecting patient referral. Children are often referred to the service following the onset of visual decline which typically occurs towards the end of the first decade of life, accounting for the high frequency of children aged six to ten years old. Many children presenting in the first year of life are siblings of patients known to the service. The clinical service has been in operation since 2010 and patients over the age of 60 may not have a known diagnosis of BBS, as familiarity with the syndrome has only increased in the last two decades. The variation of genotypes presented in this study reflects the UK BBS population and is similar to that observed by others in Europe and North America.[13;14] Patients with mutations in *BBS1* generally present later to the BBS clinics than patients with mutations in *BBS10*. This may relate to a milder phenotype and later onset of retinal degeneration.

Our study suggests that the onset of primary renal disease in children predominantly occurs in infancy. For many adult patients it was difficult to accurately determine the age of onset of renal disease since patients with CKD are managed locally with an annual specialist BBS review. A striking observation is the relatively modest difference in prevalence of CKD4-5 between adults (8%) and children (6%) (figure 3). This supports our hypothesis that patients with BBS primarily either develop CKD4-5 in childhood or remain entirely or relatively free of severe renal disease. The

small proportion of adult onset severe renal disease may relate to co-morbidities associated with BBS such as urological complications, hypertension, obesity and diabetes. These are potentially modifiable risk factors which should be managed appropriately. The number of patients over the age of 30 is limited in this study and it is therefore not possible to draw statistically significant conclusions about the risk of developing renal disease in older BBS patients.

The prevalence of CKD in this cohort is lower than anticipated based on previous estimates[3]. Forty two per cent of adults have CKD stage 2-5 and only 8% of adult patients develop CKD3b-5. There appears to be both genotype and mutation type correlations with CKD with increased risk of developing CKD3b-5 for those patients who have truncating mutations and mutations in *BBS10*. This is in keeping with a previous study indicating similar findings for cardiovascular risk factors in this group.[15] However, it is difficult to ascertain if genotype and mutation type represent independent risk factors or if statistical significance of mutation types reflects the high prevalence of missense mutations in *BBS1* and truncating mutations in other genotypes. Only one of the patients in this study with homozygous *BBS1* M390R mutations developed CKD5. *BBS1* M390R is the most common mutation observed in the BBS population in Europe and Northern America and patients homozygous for M390R make up a significant proportion of the patient population (45% of the UK population are homozygous or heterozygous for this mutation, own unpublished data). This significant subgroup of patients could be counselled regarding their lower risk of progressing to CKD4-5.

Guidelines for the management of BBS recommend that every patient should have a baseline renal ultrasound examination to assess for the presence of any structural abnormalities.[16] Although some patients who have abnormal renal ultrasound scans

do not go on to develop CKD, there is a statistically significant correlation between structural abnormalities and CKD. This study validates the requirement for a baseline postnatal renal ultrasound scan following a diagnosis of BBS. Structural abnormality should alert clinicians that close monitoring is required to identify any deterioration in renal function. Anecdotal reports of structural renal abnormalities present antentally and absent on postnatal sonography could not be confirmed in this study.

One report[17] suggests that mutations in *BBS10* are associated with antenatal severe cystic kidney disease which is incompatible with life. In this study, the prevalence of *BBS10* mutations among patients presenting with postnatal sonographic evidence of cystic kidney disease (21%, n=5) was consistent with that of the overall study population (20%). The authors are not aware of a higher rate of pregnancy losses in families with *BBS10* mutations.

It has previously been suggested that proteinuria is consistently absent in BBS – associated renal disease.[18] This study demonstrates the co-existence of proteinuria and CKD and the correlation between CKD3b-5 and albuminuria, and is likely the results of renal mass reduction caused by structural abnormalities.

We have previously reported on the high prevalence of anti-hypertensive medication in this group[10]. It is not clear if the statistical correlation between CKD and anti-hypertensive medication is related to anti-hypertensive agents prescribed as a part of CKD management to decrease the rate of progression or if hypertension represents an independent statistical risk factor in this cohort.

In summary, this study maps the prevalence of renal disease in BBS and characterises the highly variable renal phenotype. We have identified risk indicators as well as potentially protective factors for renal disease. Adults who harbour

missense mutations in *BBS1* and have normal renal ultrasound scans in adulthood are less likely to develop CKD3b-5. Patients with truncating mutations in *BBS10*, hypertension and abnormal renal ultrasound scans are at significantly increased risk of CKD3b-5 compared to the general BBS population. Primary renal disease as a consequence of BBS appears to present in early childhood.

The evidence presented here could have a direct clinical implication for BBS patients. The presence or absence of risk factors should be considered when counselling patients and may be used to stratify the clinical service. Previous recommendations advise that patients should be reviewed by a nephrologist annually unless CKD is present in which case closer monitoring is required. Based on this study the authors suggest that adults with the lowest risk of renal disease could receive community nephrology follow up and low risk children could be seen less frequently in specialist clinics. All patients with end stage renal disease require frequent specialist follow up and those with identifiable risk factors or early stable renal disease (CKD1-3) warrant annual specialist follow up. A multi-national study could facilitate the development of a statistical renal risk calculator for this unique population.

Concise methods

Patients

The following renal parameters were ascertained retrospectively for all 350 patients attending the national BBS clinics: known history of renal disease, stage of CKD if present, any abnormalities noted on renal ultrasound scanning, estimated glomerular

filtration rate (eGFR), renal function tests and relevant concomitant factors including presence of hypertension, diabetes and obesity. All subjects gave informed consent or assent. Patients were seen in the paediatric clinics if they were 16 years of age or under, or 18 years of age or under and in full time education. All other patients were seen in the adult clinics. All blood and urinary tests were completed following a six hour starvation period. The Modification of Diet in Renal Disease (MDRD) formula was applied to estimate glomerular filtration rate in all adults in keeping with its common use for patients with obesity and diabetes in the general population[19]. Estimated GFR for the paediatric population was calculated according the Schwarz-Haycock formula (height (cm) x 31/ creatinine (µmol/l)). Adults were categorised as hypertensive if they were on anti-hypertensive medication, or if they fulfilled the criteria for antihypertensive treatment in diabetic patients according to Kidney Disease Improving Global Outcomes (KDIGO) guidelines[20]. Referrals were made primarily from the British national patient support group, clinical geneticists and ophthalmologists in the United Kingdom.

Mutation analysis

Mutation analysis was undertaken through the UK national BBS gene panel which encompasses 11 BBS genes including *BBS1- BB10* and *BBS12* as well as two BBS associated genes *MKS1* and *ALMS1*.

Statistical analysis

Genotype-phenotype analysis was targeted to patients with mutations in the two most commonly affected genes: *BBS1* and *BBS10*, as well as the less common genotypes *BBS2* and *BBS12* where adequate sample sizes were available. Correlation with mutation type was also assessed. Patients with two known missense mutations were compared with two known truncating (nonsense or frameshift) mutations and a combination of missense/truncating mutations. The nonparametric Mann–Whitney U test was performed to assess differences in median age for genotypes *BBS1* and *BBS10*. For the purpose of genotype and mutation type analysis children were not included since renal failure appears to occur and progress primarily in childhood, hence CKD stage was not considered to be stable until adulthood. Multivariable regression analysis was applied to evaluate genotype-phenotype analysis and assess the effect of confounders on chronic renal disease. The relative burden of each risk factor was described in odds ratios. Statistical analyses were conducted in R (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from: http://www.R-project.org/). A 5% confidence level was considered statistically significant. All tests were two tailed.

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Statement of competing financial interests

The authors declare that they have no conflicts of interest.

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Figure legends

Figure 1. Distribution of genotypes.

Figure 2. Age at which renal disease (CKD2-5) was first noted in paediatric patients with BBS (n=49).

Figure 3. Distribution of chronic kidney disease stages in (a) adults (n=194) and (b) children (n=156).

Figure 4. Kaplan Meier survival curve: Age at which patients were first diagnosed with CKD5 (n=350).

Figure 5. Percentage distribution of chronic kidney disease stages in adults. Mutations in *BBS1* versus *BBS10*; Absolute numbers are indicated above each column.

Figure 6. Prevalence of structural abnormalities detected on sonography.

Figure 7. Renal ultrasound images demonstrating common structural abnormalities associated with Bardet-Biedl syndrome. A: Demonstrates the typical cystic dysplastic appearance associated with BBS: A small subcortical cyst, one large cyst and loss of corticomedullary differentiation. B: Subcapsular cysts and increased echogenicity. C: Nephrocalcinosis. D: Renal pelvic dilatation.

Supplemental figure 1. Percentage distribution of chronic kidney disease stages in adults. *BBS1* M390R vs other *BBS1* mutation types. Absolute numbers are indicated above each column.

Table 1: Chronic kidney disease assessment in the adult (A) and paediatric population (B).

1A: Chronic kidney disease in patients attending the adult clinic

Chronic Kidney Disease marker		**Number/ total (% of total)**
Total no. of patients		194 (100%)
eGFR	Total no. of patients who had eGFR	189/194 (97%)
	Normal or CKD1	107/ 189 (57%)
	CKD2-5 (<90ml/min/1.73m2)	82/189 (43%)
	CKD5 (<15ml/min/1.73m2)	12/189 (8%)
Renal USS		90/194 (46%)
	Normal	43/90 (48%)
	Abnormal	47/90 (52%)
No USS and no eGFR		5/194 (3%)
Urinalysis		186/194 (96%)

1B: Chronic kidney disease in patients attending the paediatric clinic

Chronic Kidney Disease marker		**Number/ total (% of total)**
Total no. of patients		156 (100%)
eGFR	Total no. of patients who had eGFR	133/156 (86%)
	Normal or CKD1(>90ml/min/1.73m2)	84/133 (63%)
	CKD2-5 (<90ml/min/1.73m2)	49/133 (37%)
	CKD5 (<15ml/min/1.73m2)	8/133 (5%)
Renal USS	Total	87/156 (55%)
	Normal	44/87 (51%)
	Abnormal	43/87 (49%)
No USS and no eGFR		9/156 (6%)
Urinalysis		140/156 (90%)

Table 2. Prevalence of chronic kidney disease in adults (A) and children (B) according to age group.

2A: Prevalence of chronic kidney disease in patients attending adult clinics. Absolute numbers (percentage)

Age group	Normal/ CKD1/ No eGFR	CKD2	CKD3	CKD4	CKD5	Total
16-20	27	0	3	0	2	32
21-25	29	5	1	1	3	39
26-30	17	5	1	1	1	25
31-35	14	4	1	1	3	23
36-40	6	7	5	0	1	19
41-45	5	8	2	1	1	17
46-50	6	5	2	0	1	14
51-55	6	8	3	0	0	17
56-60+	2	5	1	0	0	8
Total	112 (58%)	47 (24%)	19 (10%)	4(2%)	12 (6%)	194

2B: Prevalence of chronic kidney disease in patients attending paediatric clinics. Absolute numbers (percentage)

Age group	Normal/ CKD1/ no eGFR	CKD2	CKD3	CKD4	CKD5	Total
0-5	22	2	7	0	5	36
6-10	42	7	5	2	1	57
11-15	29	6	2	0	1	38
16-18	14	9	1	0	1	25
Total	107 (69%)	24 (15%)	15 (10%)	2 (1%)	8 (5%)	156

Table 3. Univariable logistical regression analysis of risk factors for severe renal disease (eGFR<45 ml/min/1.73 m^2) in adults with known common genotypes. All statistically significant findings are highlighted in bold.

Risk factors for severe renal disease (n=154)				
Risk factor	**Odds ratio**	**Confidence interval**		**P value**
		2.5%	**97.5%**	
Genetic factors				
Genotype (n=154)				
- *BBS1* mutation (n= 90)	(Reference)			
- *BBS2* mutation (n=22)	4.4	1.28	15.19	**0.016**
- *BBS9* mutation (n=6)	2.4	0.12	17.74	0.458
- *BBS10* mutation (n=26)	7.4	2.49	23.32	**0.0003**
- *BBS12* mutation (n=10)	5.9	1.08	28.39	**0.0278**
Mutation type (n=149)				
- Missense/ missense (n=76)	(Reference)			
- Truncating/ truncating (n=40)	11.4	3.9	41.8	**3.43e-05**
- Missense/truncating (n=33)	6.3	1.5	28.6	**0.0116**
Diabetes (n=137)	0.62	0.14	0.99	0.471
Hypertension (n=137)	5.43	2.21	14.29	**0.003**
BMI (n=93)	1.04	0.96	1.10	0.321
Age (n=154)	1.02	0.99	1.96	0.154

Supplemental table 1: List of known BBS genotypes, mutation types and protein changes