Renal cancer and malformations in relatives of patients with Bardet-Biedl syndrome

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Abstract

Background. Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder with five loci identified thus far. The spectrum of disease includes diverse malformations of the kidney and lower urinary tract. The incidence of BBS is approximately 1/100,000 with a predicted heterozygote frequency of 1/160, and it has been suggested that heterozygotes are at increased risk of obesity and hypertension.

Methods. We describe renal disease in relatives of 109 UK BBS patients. Using PCR with fluorescent microsatellite markers we amplified DNA derived from renal tumours of affected parents to determine whether there was loss of heterozygosity at any of four BBS loci and two other gene loci associated with clear cell renal cell carcinoma (CC-RCC).

Results. CC-RCC was diagnosed in three of 180 BBS parents and there was loss of heterozygosity at BBS1 (11q13) in the tumour tissue of one of these subjects. In addition, there was a high incidence of renal agenesis in siblings of BBS patients and two BBS families were identified with apparently dominant inheritance of renal malformations. In one family we were able to demonstrate that renal malformations segregated with the BBS2 locus (16q21).

Conclusions. Since all parents and two-thirds of siblings of BBS patients must be heterozygous for BBS mutations, our observations may implicate BBS genes in the pathogenesis of both renal cancer and malformations, both disorders of precursor cell growth and differentiation. We suggest these observations may have important implications for screening potential BBS carriers for kidney disease and may lead to a greater understanding of the aetiology of renal disease in the general population.

Keywords: Bardet-Biedl syndrome; loss of heterozygosity; renal cell carcinoma; renal malformations

Introduction

Bardet-Biedl syndrome (BBS) (MIM 209900) is an autosomal recessive condition with a wide spectrum of clinical features. The cardinal manifestations in homozygous (affected) individuals comprise, rod/cone dystrophy, obesity, polydactyly, hypogonadism, learning difficulties and renal defects [1,2]. Minor features include diabetes mellitus, hepatic fibrosis, speech deficit, dental anomalies and altered behaviour. A diagnosis is made by the presence of four major criteria [3]. Renal abnormalities have been described in up to 100% of BBS patients [2], and renal failure is the major cause of morbidity and early mortality in BBS [2]. Dysplasia, chronic glomerulonephritis, cystic tubular disease, calyceal and lower urinary tract malformations, and defects of tubular concentrating ability have all been reported [4].

BBS is a genetically heterogeneous disease with loci thus far identified on 11q13 (BBS1), 16q21 (BBS2), 3p13 (BBS3), and 15q23 (BBS4), each associated with a similar phenotype in studies to date [5–8]. Very recently, a fifth locus has been mapped to 2q31 (BBS5) in a single Newfoundland family [9].

In several recessive conditions, partial disease manifestations in heterozygote carriers have been observed. Carriers of ataxia telangiectasia have an increased risk of developing breast cancer [10], and carriers of autosomal recessive-type Alport’s syndrome may have ‘benign’ haematuria [11]. Carrier effects have been
suggested in BBS families with an increased incidence of obesity, hypertension and diabetes [12,13]. We completed a clinical survey of 109 BBS patients and their relatives living in the UK [14], and observed a high prevalence of clear cell renal cell carcinoma (CC-RCC) and renal malformations amongst the unaffected relatives. We now describe several cases and two pedigrees of renal malformations and present the results of a search for allele loss at BBS loci in three obligate carrier parents who developed CC-RCC. We suggest that individuals heterozygous for BBS mutations are at increased risk of these disorders. Our observations have implications for understanding the pathogenesis of disorders of kidney growth and differentiation, and have potentially important practical implications for the screening of BBS relatives.

Materials and methods

Subjects

BBS individuals and their first-degree relatives were interviewed by questionnaire through the Guy’s Hospital Bardet-Biedl database and the Laurence-Moon–Bardet-Biedl Society members’ list, and have been reported in a separate manuscript [14]; this study constitutes the largest single survey of living BBS patients. The questionnaire addressed prior knowledge of significant family medical history. Medical notes were sought, with patients’ consent, for verification of diagnosis, treatment and renal disease. In the cases of reported renal malformations, radiological reports, ultrasound scans and intravenous pyelograms were obtained; however, no systematic renal ultrasound imaging was performed on the relatives of the BBS probands. One hundred and twenty-five probands from 105 families were sent questionnaires with an explanatory letter inviting participation, and 111 returned them completed. Only two patients did not fulfill inclusion criteria. Where appropriate, patients and relatives were seen in the clinic, at home, or were contacted by telephone to obtain further details. All histological analyses were performed by renal pathologists.

DNA extraction

We used two molecular strategies to investigate selected kindreds and individuals. First, we sought evidence for loss of heterozygosity in three tumour samples at BBS1–4 (the loci known to be associated with BBS at the time of this study), the Von Hippel Lindau (VHL) and 3p14 regions (both regions associated with familial RCC). DNA was extracted from formalin-fixed, paraffin-embedded blocks of normal kidney and renal tumour. After micro-dissection of 20-μm sections, DNA was recovered from tumour and normal renal tissue, using a phenol–chloroform extraction method. Briefly, the sample was de-waxed in xylene for 30 min and then rehydrated by sequential washes in 100, 80, 50% ethanol and finally in distilled water. The sample then underwent digestion by resuspension in lysis buffer (150 mM NaCl, 25 mM EDTA, pH 8.0) with proteinase K (10 mg/ml) and 10% SDS. The mix is incubated at 55°C for 72 h. Purification was achieved, first by adding phenol, chloroform, isoamylalcohol (25:24:1) to the tube and then centrifuging to a pellet. The supernatant was then removed and added to an equal volume of chloroform, isoamylalcohol (24:1). Following a further centrifugation, 2.5 volumes of cold ethanol is added to precipitate out the DNA. This was then washed in 70% ethanol and resuspended in TE ready for use in the PCR.

Second, we sought linkage of renal malformation phenotype to the four BBS loci that were recognised at the time of study. For this, DNA was extracted from peripheral blood leucocytes and genotyped as previously described [15].

Primers

Genethon map derived primer sequences were used as described by Beales et al [15]. The primers were commercially synthesized (Life Technologies Inc.) and one primer of each pair was labelled in the 5’ position with a phosphoramidite dye (FAM, HEX or TET—Applied Biosystems). Finally each primer was purified using HPLC.

Polymerase chain reaction and gel electrophoresis

Each target DNA sequence was amplified by the polymerase chain reaction (PCR) incorporating the fluorescently labelled microsatellite markers flankng known loci. Forty nanograms of genomic DNA or a 1-μl aliquot of tumour-derived DNA (diluted 1:20) was amplified in 10 μl reaction volumes containing 1 μl PCR buffer (×10) (670 mM Tris–HCl pH 8.0, 166 mM NH₄SO₄, 67 mM MgCl₂, 1.7 mg/ml BSA), 5 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 2 pmol of each fluorescent primer (LTI) and 0.6 U of Taq polymerase (Bionol Corporation). The PCR was carried out in a PTC-100 thermal cycler (Genetic Research Instrumentation Ltd.). Typical reaction conditions were as follows, initial denaturation at 94°C for 10 min then 24 cycles of 94°C for 45 s, annealing temperatures 54–60°C for 45 s, extension at 72°C for 2 min followed by a final extension of 72°C for 10 min. The cycle number was optimised for each tissue-derived DNA sample to generate comparable product peaks in the determination of allele loss.

Polyacrylamide gel electrophoresis

A sample (1 μl) of PCR product was combined with 2 μl of de-ionized formamide and 0.5 μl of a fluorescent size marker (GS500—Tamra, Applied Biosystems). After 3 min denaturation at 94°C, the sample was loaded onto a 6% polyacrylamide denaturing gel (Sequagel-6, National Diagnostics) in 1× TBE buffer. The gel was run on an automated DNA fluorescent fragment analyser (Model 373A, Applied Biosystems) for 5 h at 35 W and 40°C. Alleles sizes, relative heights and areas of the peaks were assigned using the GENESCAN and GENOTYPER software (ABI).

Allele ratios and determination of LOH

The peak height of the two highest alleles (if heterozygous) in the paired normal and tumour samples were determined. In the case of a single (homozygous) peak, the area was determined for the paired samples. The ratio of alleles was calculated for each normal and tumour sample as described by Cawkwell et al [16]. The results are expressed in the range 0.00–1.00, where a ratio of 0.50 or less indicates significant allelic loss. The PCR was repeated a second time to exclude artefacts.
Germline VHL gene mutations were sought as described previously [17]. In brief, Southern analysis was first performed on blood DNA to detect gene deletions, and single strand conformation polymorphism (SSCP) analysis was performed to detect intragenic mutations. Direct sequencing of the whole coding region was performed to characterise a mutation in patients with a SSCP band shift and to exclude a mutation in those with normal SSCP analysis. These investigations are expected to identify mutations in at least 80% of germline VHL gene mutations [17].

Statistics

Ninety-five per cent confidence intervals (C.I.) were calculated for the relative risk of developing CC-RCC. To compare proportions, we used the binomial exact test or $\chi^2$ test. $P$-values <0.05 were taken to be significant.

Results

Cancers

Three of 180 parents, all Caucasians, aged 37 years (parent 1, male), 52 years (parent 2, male) and a 40-year-old female (parent 3) had renal adenocarcinoma, all with clear cell histology. Parent 1 was asymptomatic, and was diagnosed by ultrasound scan when asked to take part in a research programme involving his daughter, who has BBS and chronic renal failure. This parent also had evidence of malformation, with a duplex kidney and ureter. His carcinoma was completely resected and he remains disease-free 3 years after nephrectomy (Fig. 1). At diagnosis, parent 2 was asymptomatic, with metastatic disease, and died soon after. The carcinoma in parent 3 was diagnosed during a radiological assessment for potential kidney donation to her BBS daughter, who had end-stage renal failure. This parent is alive and well 3 years after nephrectomy. None of these parents were known to have the following recognized risk factors for renal carcinoma as reviewed by Vogelzang and Stadler [18]: chronic dialysis, obesity, cigarette smoking, arterial hypertension, oestrogen therapy, and occupational exposure to heavy metals and petroleum products.

The proportion of parents with renal tumours was 1.67% (3/180). The general population risk of CC-RCC rises sharply after 55 years [19], while the cumulative risk of developing CC-RCC under 55 years of age in the general population is 1 in 1041 [19]. These three parents, who were relatively young at the time of diagnosis of CC-RCC, therefore, have a significantly increased risk of CC-RCC ($P=0.0007$, bi-nominal exact test (two-sided)). Extrapolating from this, the relative cumulative risk of parents of BBS children developing CC-RCC under 55 years is 17 times (95% CI=3.6–49.9) that of the general population.

It was important to determine whether any of these parents were carrying a germline mutation in the VHL gene, which might contribute to the development of CC-RCC. One parent (parent 2) was found to have a heterozygous missense mutation in the VHL gene (C470G, Pro86Arg) although, there was no antecedent medical or family history to suggest such a diagnosis, as is recognized in this condition. However, even if this parent is removed from the analysis, there remains a significantly increased risk of CC-RCC. When this proportion of 1.11% (2/180) with RCC is compared with the general population cumulative risk of developing RCC under 55 years (1/1041), the relative risk becomes 11-fold ($P=0.017$, binomial exact test (two-sided)).

Using PCR (repeated once), we amplified tumour DNA with microsatellite markers from BBS1–4 and from 3p. Significant allelic loss was present in two BBS parents (2 and 3) with CC-RCC (Fig. 2). Parent 2 showed LOH at D11S480 (BBS1) with an allelic ratio of 0.33 and at D3S1300 (3p14) with a ratio of 0.50. Parent 3 was found to have significant LOH at D3S1263 (VHL) with a ratio of 0.27 (but no germline mutation of VHL found). Parent 1 was not found to have significant LOH at BBS1–4 and the VHL loci. In addition, we also typed parents 2 and 3 using 38 random STRPs evenly spaced across the genome (on average one per chromosome arm) (Table 1) and no significant LOH was documented.

Malformations

Of the 109 BBS subjects, five of 123 unaffected siblings (4.1%) had one or more congenital renal malformations. Two (1.6%) had unilateral renal agenesis, of whom one had a complex malformation of the solitary urinary tract comprising a duplication and a ureterocele (Table 2). According to Kiprov et al. in a survey of 9200 autopsies the incidence of unilateral renal agenesis in the general population is about one in 1000 [20]. Therefore, the occurrence of unilateral absence of functioning renal tissue in 2/123 BBS siblings represents a 20-fold increase ($P=0.007$). In addition, three (2.4%) had vesicoureteric reflux and three (2.4%) had duplication of the ureter and pelvis. However, these incidences may fall into those recognized for the general population [21,22]. In addition, it is interesting to note that of these five siblings, one grandparent had unilateral renal agenesis, with a solitary kidney in end-stage renal failure, and another parent (parent 1, above) had a duplex renal tract. These reports of renal malformation were all incidental, and neither the parents nor the siblings had been screened with a view to observing renal abnormalities. In addition, we recorded a prominent history of renal malformations in two kindreds, now described in detail.

Family A

The proband initially characterised by Runge et al. [23], was a 4-year-old BBS boy who died from renal failure secondary to renal dysplasia. His father (parent 2) died from CC-RCC, but was not known to have had any renal malformation and there is no history of
Fig. 1. (A) Ultrasound scan of daughter with BBS and end-stage renal failure showing small, dysplastic kidney. (B) Ultrasound of the father of the BBS patient (A) depicts a cystic renal tumour. (C, D) are sections stained with haematoxylin and Periodic Acid Schiff from the nephrectomy of the kidney depicted in (B). (C, D) Respectsly low (×20) and high (×63) power pictures of normal kidney tissue near the tumour depicting glomeruli and proximal tubules. (E, F) Low and high power pictures from the adjacent tumour showing tubules lined with cells with clear cytoplasm: no normal glomeruli or proximal tubules are seen.
Significant loss of heterozygosity was found at the BBS1 marker D11S480 (11q13) in parent 2, at D3S1300 (3p14) in parent 2 and D3S1263 (VHL region—3p25) in parent 3. No LOH was detected at any locus in parent 1.

Fig. 3. Significant loss of heterozygosity was found at the BBS1 marker D11S480 (11q13) in parent 2, at D3S1300 (3p14) in parent 2 and D3S1263 (VHL region—3p25) in parent 3. No LOH was detected at any locus in parent 1.

renal malformation in his family. The proband’s mother is healthy, but has a strong family history of renal malformations, which appear to be inherited in an autosomal dominant manner with variable expression (Fig. 3). These malformations comprise combinations of renal agenesis and contralateral renal dysplasia or renal tract duplications. The mother herself has normal renal architecture and function (assessed by ultrasound scan and plasma creatinine). It is unfortunate that DNA samples or post-mortem material was not available from any member of the maternal kindred as our hypothesis could be further supported by sharing of one or more BBS alleles between the mother and her relatives with renal malformations.

**Family B**

This family (Fig. 4) comprises dizygotic twins with BBS. Their unaffected sister complained of left loin pain as a teenager, and was found to have hypertension. On investigation, a small dysplastic left kidney was found and later removed, and her hypertension resolved. She also had vesicoureteric reflux into her right kidney, with recurrent pyelonephritis. Her youngest son (now 2 years old age) had critical aortic stenosis requiring valvotomy on day 5. Antenatally, he was noted to have a multicystic dysplastic left kidney, which was non-functional (confirmed postnatally by radioisotope scan) and a micturating cystogram revealed vesico-ureteric reflux in the contralateral system. Six months later, the multicystic kidney had involuted as has been reported in other individuals [24]. Haplotype analysis of this family found it to be consistent with linkage to BBS2 (16q21), which is further supported by exclusion from the other three loci [15]. To determine the carrier status of the unaffected sister, she and her family were also genotyped (Fig. 4). She, her son and another daughter have inherited the same chromosome segment, which is of grand-paternal origin, as that shared by the affected twins. It would appear that they all carry the BBS2 gene-containing region. However, the daughter has a normal kidney structure on intravenous pyelography.

**Discussion**

**Clear cell renal cell carcinoma**

Renal carcinomas account for 2% of all cancers and for 80–85% of malignant kidney tumours [25]. Known risk factors for CC-RCC include chronic dialysis, obesity, cigarette smoking, arterial hypertension, oestrogen therapy, and occupational exposure to heavy metals and petroleum products [18]. Heritable factors are increasingly recognized as being critical to the pathogenesis of many tumours, and the risk of developing CC-RCC increases on average 1.6-fold where there is an affected first-degree relative [18].

The most common form of familial clear cell RCC is VHL disease, but a non-VHL clear cell variant is also recognized [26]. Recent studies of nine kindreds with familial non-VHL clear cell RCC (HCRC) have demonstrated that most patients develop RCC < 50 years of age (EM, unpublished observations). The molecular basis of HCRC is unknown but the disease is not allelic with VHL disease or familial papillary renal cell carcinoma [26; EM, unpublished observations] and it is possible that BBS loci might be implica-
Table 1. Microsatellite markers used in study

<table>
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<tr>
<th>Markers</th>
<th>BBS1</th>
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<th>BBS3</th>
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*p = short arm, q = long arm. Markers with * denote loss of heterozygosity in renal tumours of BBS parents.

Table 2. Range of renal malformations reported in 123 siblings of BBS patients

<table>
<thead>
<tr>
<th>Renal malformations</th>
<th>No. of siblings</th>
<th>Population prevalence</th>
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<tbody>
<tr>
<td>Unilateral renal agenesis</td>
<td>2 (1.6%)</td>
<td>0.1% (20)</td>
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<tr>
<td>Duplication of ureter and pelvis</td>
<td>3 (2.4%)</td>
<td>5% (21)</td>
</tr>
<tr>
<td>Vesicoureteric reflux</td>
<td>3 (2.4%)</td>
<td>1–2% (22)</td>
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</table>

of CC-RCC in these parents (37, 40 and 52 years) is striking (CC-RCC generally predominates in the 7th–8th decades of life [25]), and suggests a genetic aetiology. As the carrier rate for BBS in the UK is approximately 1 in 160, we would expect there to be around 350 000 BBS gene carriers (UK population ~56 million). Based on these figures, 1 in 90 of the carriers may be at risk of developing CC-RCC (3889/350 000 = 1.1%). The proportion of early onset RCC which could be accounted for by BBS heterozygotes may be as high as 6.9%.

Secondly, is there molecular evidence to implicate BBS loci in the pathogenesis of these cancers? Since BBS is a recessive syndrome, each parent is heterozygous for a BBS mutation. Many cancer susceptibility disorders result from tumour suppressor gene inactivation and loss of the wild-type allele (detected by LOH analysis) is frequent in the tumour. Our demonstration of significant LOH at a BBS locus in renal cancer from parent 2 would support the hypothesis that BBS alleles are implicated in the pathogenesis of clear cell RCC. Although parent 2 had a germline VHL mutation, the LOH at BBS1 is suggestive of a modifying effect. LOH was also seen in parent 2’s tumour tissue at 3p14 (D3S1300), a region previously implicated in CC-RCC development but not at 3p25 (D3S1263 close to VHL). BBS3 maps to 3p13. Germline mutations in the VHL gene are associated with a high risk of early-onset and multicentric CC-RCC and somatic VHL gene mutations or methylation are found in up to 70% of CC-RCC cell lines and primary tumours [27]. The 3p12–p21 interval contains several candidate CC-RCC TSG regions: 3p12 and 3p21.3 which are homozygously deleted in breast and lung cancer cell lines and 3p14 which contains the FRA3B fragile site [28] and the FHIT gene [29]. It is of interest that D3S1300 (3p14) was deleted in the parent 2 tumour, but it is not possible to know the relevance of this CC-RCC development in this case. In addition, there

Fig. 3. Family A. This pedigree illustrates dominant inheritance of two renal malformations; duplication of the kidney and renal tract and unilateral/bilateral renal agenesis in the proband’s maternal family. There was no family history of renal malformations on the paternal side. However, the proband’s father has been found to carry a germline VHL mutation.
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Fig. 4. Family B. This pedigree with the two affected non-identical twins (4.1 and 4.2) is consistent with linkage to BBS2 on 16q21 (excluded from BBS1, 2, 3 and 4). The haplotype in 4.1 shows a recombination event between D16S3034 and D16S408. Further analysis shows their unaffected sister (4.3) and two of her children (4.7, 4.8) are probable BBS2 gene carriers (having inherited the grandpaternal disease carrying chromosome). 4.7 has normal kidney structure.

was possibly LOH at BBS4 in the same tumour, but the allelic ratio of 0.65 did not attain the conventional level of significance. Although concomitant regions of LOH are well described in many tumours [30,31], the significance of, and interplay between, the different loci are unknown.

We conclude that LOH at BBS loci in CC-RCC in BBS heterozygotes is unlikely to have arisen by chance, as random chromosome-wide analysis did not reveal widespread LOH (Table 1). If BBS genes function as tumour-suppressors, then BBS homozygotes must also be at increased risk of CC-RCC. We have encountered only one case of CC-RCC in a BBS patient (PLB, personal observation) and only one case is reported in the literature [32].

An alternative hypothesis may be that there are epistatic interactions between BBS loci (and perhaps other renal developmental genes) such that a loss of a normal allele at more than one locus increases the risk for CC-RCC. If this were so, then the relative risk of developing CC-RCC in both heterozygotes and homozygotes would depend on the frequency of allelic loss at the second locus (interactor). The finding of a missense VHL mutation in parent 2 as well as LOH at a VHL-associated marker in parent 3 is entirely consistent with this hypothesis. Now that a fifth BBS gene (2q31) has been mapped [9], it will also be interesting to examine this locus in these tumours.

Renal malformations

The term ‘kidney malformation’ describes diverse anomalies [33]. In renal agenesis the kidney is absent. ‘Dysplastic kidneys’ refer to poorly differentiated organs, which frequently contain cysts (‘multicystic dysplastic kidney’). Malformations affecting the lower urinary tract include duplication, vesicoureteric reflux and ureterocele. It should also be noted that multicystic dysplastic kidneys can involute pre- or postnatally [24], driven by excess apoptosis [33], to give an
appearance of ‘agenesis’. Human renal malformations may be inherited, either in isolation or as part of syndromes (e.g. renal agenesis and olfactory bulb malformation in Kallmann syndrome [33], and vesicoureteric reflux with optic nerve colobomas in the renal-coloboma syndrome [33]). The genes encoded by BBS loci probably affect precursor cell growth and differentiation in the embryonic kidney; however, as kidney disease in BBS patients varies between, and even within, kindreds, other genes or environmental factors must be involved in determining the final phenotype.

Incidences for renal malformations in the general population are unilateral renal agenesis, 1/1000; unilateral multicystic dysplastic kidney, 1/5000; bilateral agenesis and/or dysplasia, 1/5000–10 000; vesicoureteric reflux, 1/100; unilateral duplex ureter, 1/20 [20–22,36]. Therefore, the occurrence of unilateral absence of functioning renal tissue in two of 123 BBS siblings represents a significant 20-fold increase. As no formal radiological or ultrasound screening of the relatives in this study took place, these risks are likely to be under-estimated. A systematic ultrasound screen of these relatives is planned and may reveal a considerably higher incidence of renal malformations. The finding of vesicoureteric reflux in 3/123 BBS siblings is interesting, but the high incidence of this disorder in the general population precludes any definite conclusions in relation to BBS.

Our description of two families with apparently dominantly inherited kidney malformations is intriguing. In family A (Fig. 3), the proband’s father died of CC-RCC but had an otherwise unremarkable family history, whereas his mother has no renal malformation herself, but does have a strong family history of renal malformation. The mother may represent non-penetrance of a renal agenesis/dysplasia gene [35,36]. Family B (Fig. 4) clearly demonstrates that a chromosomal segment of grand-paternal origin, containing BBS2 on 16q, has been co-inherited by affected dizygotic twins, their unaffected sister (with vesicoureteric reflux and renal dysplasia), her youngest son with an involuted multicystic dysplastic kidney and vesicoureteric reflux, and her daughter whose kidneys appear normal. Again, incomplete penetrance may explain why the daughter has a normal kidney structure. Nevertheless, the inheritance pattern is compelling evidence for the operation of a common renal developmental gene in this family. The twin brothers in this family have typical renal structural anomalies (i.e. fetal lobulation and calyceal cysts) but normal renal function. Previous linkage studies have failed to distinguish any differences in renal disease in affected patients by locus [15,37,38].

Implications for health screening

Heterozygous carriers of BBS genes appear to be at increased risk of CC-RCC and urinary tract malformations. If these findings could be confirmed in further BBS families, we would suggest that all first-degree relatives of BBS cases should be screened with ultrasound scans for occult renal tract malformations, since these may be associated with hypertension or renal impairment. Furthermore, renal carcinoma would have to be excluded in all parents of BBS patients, since early detection and treatment may improve the prognosis.

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References

17. Maher ER, Webster AR, Richards FM et al. Phenotypic expres-
Renal cancer and Bardet-Biedl syndrome


**Note added in proof**

Since acceptance of this paper, we have reported mapping of a new BBS locus (BBS6) and identification of the cognate gene. Severe mutations have been found in the MKKS (McKusick-Kaufman Syndrome) gene on 20p12 in 40% of Newfoundland BBS families. The proposed mechanism of disruption by the encoded chaperonin-like assembly suggests that misfolding of nascent proteins involved in renal, retinal, limb-bud and brain development occurs [39].

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