THE CLINICAL AND GENETIC EPIDEMIOLOGY OF LAURENCE-MOON-BARDET-BIEDL SYNDROME IN NEWFOUNDLAND

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SJ MOORE
The Clinical and Genetic Epidemiology of Laurence-Moon-Bardet-Biedl Syndrome in Newfoundland

by

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

Background

Bardet-Biedl Syndrome (BBS) and Laurence-Moon Syndrome (LMS) are differentiated by the presence of spasticity and the absence of polydactyly in LMS. The aims of this study were to determine whether BBS and LMS are the same disorder, describe the clinical and genetic epidemiology and examine genotype-phenotype relationships.

Methods

A population-based, prospective study was conducted. 46 patients from 26 families were enrolled. Patients and relatives were genotyped at BBS loci. Longitudinal and cross-sectional clinical data were analysed.

Results

There were at least six BBS genes in the cohort. Characteristic craniofacial dysmorphic features were identified. Neurological manifestations were prevalent. 2/46 patients were diagnosed clinically as LMS but both had mutations in a BBS gene. Major clinical outcomes were similar for all genotypes.

Conclusions

LMS and BBS are the same disorder. It is associated with a characteristic cranio-facial dysmorphology and abnormalities in almost every organ system. The widespread systemic involvement and lack of a genotype-phenotype correlation implies that the BBS genes are involved in the same early developmental pathway.
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1 INTRODUCTION

1.1 Laurence-Moon Syndrome (LMS) Versus Bardet-Biedl Syndrome (BBS)

In 1866, Laurence and Moon described a family with retinal dystrophy, obesity, mental retardation, male hypogenitalism, and a "slouching gait" (Laurence and Moon, 1866). More than 30 years later, Bardet and Biedl reported similar phenotypes in individuals who also had polydactyly (Bardet, 1920; Biedl 1922).

The cases described by Laurence, Moon, Bardet and Biedl were regarded as the same syndrome until the 1980s. Different combinations of the original authors' names were used to refer to the disorder: Laurence-Moon-Bardet-Biedl syndrome (Abdel-Aziz, 1972; Blumel and Kniker, 1959; Klein and Ammann, 1969; McLoughlin and Shanklin, 1967), Laurence-Moon-Biedl syndrome (Bauman and Hogan, 1973; Burn, 1950; Ciccarelli and Vesell, 1961; Cockayne et al., 1935; Krill et al., 1961; McCullagh EP and Leiser AE, 1957; Roth 1947), Laurence-Biedl Syndrome (Solis-Cohen and Weiss, 1925) and Laurence-Moon Syndrome (Bell, 1958; Dekaban et al, 1972).

In 1981, a new classification for the cases described by Laurence, Moon, Bardet and Biedl was proposed, dividing these into two separate syndromes, LMS and
BBS (Schachat and Maumenee, 1981). The authors proposed that four of the five cardinal features, tapetoretinal degeneration, mental retardation, obesity, polydactyly and hypogenitalism, should be present for a diagnosis of BBS. They differentiated LMS from BBS by the presence of spastic paraplegia, and the absence of obesity and polydactyly in LMS. They cited 21 cases of LMS from the literature, though most of these had obesity, three had polydactyly, and only a third (7/21) had spastic paraplegia, whereas almost all had ataxia.

The new classification of LMS and BBS was adopted, and LMS (OMIM 245800) and BBS (OMIM 209900) have been regarded as separate disorders since the 1980s, differentiated by the presence of spasticity and the absence of polydactyly in LMS (Lancet 1988; OMIM; Schachat and Maumenee, 1982).

BBS is the more commonly reported of the two syndromes, with a prevalence of 1 in 125,000 to 160,000 in Europe, (Beales et al, 1997; Klein and Amman, 1969) and 1 in 65,000 in an Arab population (Farag and Teebi, 1988). The cardinal features are retinal dystrophy, obesity, dystrophic extremities, renal structural abnormalities and male hypogenitalism (Green et al, 1989). Other features which are more variable include renal failure, learning difficulties, diabetes and hypertension (Beales et al, 1999; Green et al, 1989). The diagnosis may be delayed because of the age-related penetrance of many of the clinical
manifestations (Beales et al, 1999). There is considerable inter- and intra-familial variation in the phenotype. (Beales et al, 1997; Riise et al, 1997).

More recently, some authors have suggested that LMS and BBS may represent allelic forms of the same condition (Beales et al, 1999). While several disease genes have been identified for BBS, the molecular basis of LMS is not known.

1.2 Molecular Genetics of BBS

BBS is genetically heterogeneous, with at least eight loci. Five genes have been identified: BBS1 (11q13) (Mykytyn et al, 2002), BBS2 (16q21) (Nishimura et al, 2001). BBS4 (15q22) (Mykytyn et al, 2001), BBS6 (20p12) (Katsanis et al, 2000; Slavotinek et al, 2000) and BBS7 (4q27) (Badano et al, 2003a) and an additional two loci, BBS3 (3p12) (Sheffield et al, 1994; Young et al, 1998) and BBS5 (2q31) (Young et al, 1999a) have been mapped. In most populations, BBS1 is the most prevalent genotype, accounting for approximately 30-40% of patients (Beales et al, 2001). A common missense mutation, M390R, in BBS1 accounts for up to 80% of all BBS1 mutations (Beales et al, 2003; Mykytyn et al, 2003).

Despite the identification of five BBS genes, the molecular mechanism responsible for the syndrome remains obscure. The BBS6 gene (MKKS )
encodes a type 2 chaperonin that is probably involved in protein folding (Stone et al. 2000). The BBS4 gene product shares sequence similarity with O-GlcNAC-transferase (OGT) (Mykytyn et al. 2001) but whether it is enzymatically active is uncertain. The BBS1, BBS2, and BBS7 proteins share structural features (Badano et al, 2003) but no functions have been assigned to them.

The inheritance of BBS has traditionally been considered as autosomal recessive (Mykytyn et al, 2003). However, following the cloning of BBS genes, sequence analysis has shown that in some families, three mutations in two BBS genes are necessary for pathogenesis (Badano et al, 2003a; Badano et al, 2003b; Beales et al, 2003; Katsanis et al, 2001a; Katsanis et al, 2001b; Katsanis et al, 2002). The discovery of triallelic inheritance in BBS has generated the hypothesis that BBS may, at least in some cases, follow oligogenic inheritance, in which specific alleles at more than one locus cause and/or modify the severity of the phenotype (Badano and Katsanis, 2002).

Further understanding of the relationship between genotype and phenotype may help to clarify how the different BBS genes and their products interact.
1.3 Clinical Manifestations of BBS: Critique of the Literature

1.3.1 Visual Loss

The studies involving larger cohorts of BBS patients will be reviewed.

Riise et al reported the ocular findings in 44 Scandinavian BBS patients, with a maximal follow-up time of 9 years (Riise, 1987). Patients were ascertained through the Swedish and Danish Retinitis Pigmentosa registers, the Swedish register of the visually impaired child and the Norwegian Laurence-Moon-Bardet-Biedl Association. The criteria for inclusion were retinal dystrophy (a mandatory finding) plus at least two of the traditional cardinal signs, which were not specified. The ascertainment and inclusion criteria bias this sample towards patients with visual impairment, so the conclusions drawn about visual function may be pessimistic. One of the strengths of this study is that all patients were examined using the same protocol, which is clearly described, in the same centre during a one day period, which should help to minimise inter-observer bias. The first reported visual symptom was night blindness, at an average age of 4 years (range 0 to 16 years). The mean age for first visual problems during the day was 6.4 years, however this is likely to be influenced by investigation bias since routine testing of acuity in schoolchildren takes place at 7 years, causing the peak of first daytime visual impairment at this age, which the authors show. There is a deterioration in visual acuity and fields with age for all patients, maximal from 10 to 20 years of age with a wide variation in rate.
A subsequent study on the intrafamilial variation in BBS reported the wider phenotype in a subgroup of these patients, for 25 patients from 11 families, all of whom had at least one sibling with BBS (Riise et al, 1997). There was substantial inter- and intrafamilial variation, with a variation of up to 14 years for the age at first sign of daytime visual impairment between siblings.

109 BBS patients identified through the Laurence-Moon-Bardet-Biedl Society (UK) and the Guy's Hospital (London, UK) genetic clinic BBS register were surveyed using a standardised questionnaire (Beales et al, 1999). This was described as a population survey by the authors, though the ascertainment may have been biased towards those with more severe problems, who were referred to the genetic clinic or sought support from the LMBB Society. However, compared with some of the other studies which have recruited patients through ophthalmology clinics (Leys et al, 1988; Fulton et al, 1993; Riise et al, 1996; Riise et al, 1997; Riise et al, 1996; Jacobson et al, 1990), there may have been less of a bias towards visual problems. In this relatively large group of 109 BBS patients, visual impairment occurred in 98% before 20 years of age. 93% of patients had signs of rod-cone retinal dystrophy diagnosed by an ophthalmologist, the remaining 7 patients were all less than 8 years of age.
Several reports on different aspects of clinical features in Newfoundland BBS patients have been produced. One of the strengths of this study is the attempt made at complete ascertainment by using different routes to identify patients, including a Genetics Ophthalmology clinic, Canadian National Institute for the Blind (CNIB) registry, family studies and requesting charts of patients with diagnoses of BBS or Laurence-Moon-Biedl syndrome from other hospitals. All 28 patients examined had severe retinal dystrophy, but only two had typical retinitis pigmentosa, which was previously thought to be a characteristic finding in BBS. Visual acuity declined rapidly with age, all patients except one child of 12 years were registered blind (Green et al, 1989).

O’Dea et al subsequently reported longitudinal data for the cohort reported by Green et al, and compared the natural history of 38 BBS patients with 58 unaffected siblings (O’Dea et al, 1996). The median age at registering blind was 18 years, and all were legally blind by 30 years of age.

The findings of these studies are consistent, and show that the visual loss in BBS is severe and early onset. Visual function declines with age, particularly during the second decade of life, though the rate of decline varies widely. Most patients are legally blind by 30 years of age.
1.3.2 Renal Function

There are very few studies which have used a standardised protocol and comprehensive testing to investigate renal structure and function in BBS. Most of the studies had the aim either of describing the phenotype of BBS, or the visual function, and for several of these studies (Beales et al, 1999; Leys et al, 1988; Riise et al, 1997; Farad and Teebi, 1988) the renal data was reported without specifying how many patients were investigated, so the prevalence of renal abnormalities may be underestimates.

One study, conducted in Newfoundland, undertook to describe specifically the renal features of BBS (Harnett et al, 1988). The 20 patients in this study were investigated more thoroughly than in other studies, following a defined protocol. Clinical end-points such as hypertension were defined. All patients had some abnormality in renal structure, function or both. Fifty percent (10/20) of patients had hypertension and 15% (3/20) had chronic renal failure. Urine concentrating ability and acidification were compared to controls. There was a significant difference in urine concentrating ability between cases and controls. Nineteen patients had a renal ultrasound scan and intravenous pyelogram; 95% (18/19) patients had calyceal clubbing or blunting, which were unrelated to demonstrable vesico-ureteric reflux, and (17/19) had persistent fetal lobulation. These data lead to the conclusion that renal abnormalities are a cardinal manifestation of BBS (Green et al. 1989).
The strengths of this study include the method of ascertainment which should not have a major bias in terms of renal abnormalities, and the use of a thorough protocol which was performed in the same centre.

Longitudinal data from the Newfoundland cohort was subsequently reported for 38 patients, with a mean age of 35 years and a follow-up of 7 years (O’Dea et al, 1996). Clinical endpoints such as chronic renal failure (CRF) and hypertension were defined, and a standardised protocol was used. Hypertension was present in 66% (25/38) patients with a median age of onset of 34 years, compared with 11% of siblings (who did not have BBS). CRF occurred in 25% (9/36), and by 48 years, 25% of patients had CRF. Twenty-nine patients had a renal ultrasound scan. Fetal lobulation was present in 96% of patients, and abnormal calyces in 96%.

The major weaknesses of other studies reporting the prevalence of renal abnormalities is the paucity of renal investigations and lack of definitions. In a survey of 109 patients with BBS, only 52% (57/109) had renal imaging, by ultrasound scan in 35%, isotope renography in 40% and intravenous pyelography (IVP) in 14% (Beales et al, 1999). Five percent (6/109) of patients were reported as having chronic renal failure (defined by raised plasma urea and creatinine), but it was not clear how many were investigated. Calyceal clubbing or blunting was reported in only 10% (6/57) and fetal lobulation in 12% (7/57), however these would be difficult to detect using isotope renography, and there
would be inter-observer inconsistencies due to several different radiologists interpreting the imaging studies. Both these factors would likely lead to under-reporting of renal structural anomalies.

Similarly, no investigations of renal function were performed in a study of 44 Scandinavian BBS patients (Riise et al, 1997). Information about renal disease was obtained from a questionnaire and review of medical records. Sixteen percent (4/25) had either a congenital renal anomaly, which was not specified, or reduced function of the kidneys (on the basis of raised serum creatinine). Because investigation for renal anomalies was not part of the protocol, this likely resulted in underestimates.

1.3.3 Mental Retardation

Mental retardation was regarded as a cardinal feature of LMS and BBS since the earliest reports, based on observations rather than formal psychological testing (Laurence and Moon, 1866; Klein and Amman, 1969; Schachat and Maumenee, 1982). However, when formal intelligence testing was undertaken using tests appropriate for the visually impaired, the majority of patients were found to have an IQ in the low-normal range. In the Newfoundland cohort, only 13/32 (41%) of patients were mentally retarded (IQ<70) (Green et al, 1989).

In a survey of 109 BBS patients, the prevalence of learning difficulties was 62%, however the term was not defined (Beales et al, 1999). It was stated that
the learning difficulties were mostly mild to moderate, but it is not clear whether they result from difficulties due to impaired vision or intellectual function, nor is it clear how these patients were tested since only a minority were seen by the author, the rest of the data being ascertained from questionnaires. Ascertainment through a BBS/LMS support group may have caused a bias, since parents of children with learning difficulties may be more likely to join a support group. Hence the prevalence for behaviour problems and learning difficulties of 33% and 62% respectively in BBS from this survey may be an over-estimate.

Twenty patients with BBS from Arab families who were referred to the Kuwait genetic clinic for diagnosis and/or counselling were all reported to have had mental retardation with an IQ between 25 and 65, but the protocol and testing used in this study were not given (Farad and Teebi, 1988). In contrast, the majority of 44 Scandinavian BBS patients studied were described as having normal intelligence, though none were tested formally (Riise et al, 1996).

1.3.4 Diabetes Mellitus

In most studies of BBS patients, the number investigated for the presence of diabetes mellitus (DM) was not specified. A prevalence of 6% for DM in a survey of 109 BBS patients, and 12% in 25 BBS patients was reported, (Beales et al, 1999: Riise et al, 1997), however these are likely to be underestimates, since subclinical diabetes mellitus would not be detected without investigation.
In the Newfoundland study, earlier protocols involved glucose tolerance testing for the majority of patients (Green et al, 1989; O'Dea et al, 1996). Lifetable analysis showed that 50% of patients were diabetic by 50 years, though this was based on limited longitudinal data. The data suggested that diabetes mellitus is a feature in at least a third of BBS patients during the clinical course of the disease, but longer follow-up would give a more accurate figure.

1.3.5 Obesity

Varying definitions of obesity used in different studies of BBS patients may account for the difference in prevalences. The prevalence of obesity in a cohort of 109 BBS patients was reported as 72%, using a definition of Body Mass Index (BMI) greater than 29 kg/m2 (Beales et al, 1999), whereas the current and previous studies of the Newfoundland BBS patients used a definition of BMI greater than 27 kg/m2 (O'Dea et al, 1996), giving a higher prevalence. However, all studies show that a majority of patients with BBS develop obesity, using any of the definitions. These studies include children from the age of 1 year, so it appears that obesity may develop in early childhood, although there are no longitudinal data on this.

1.3.6 Life Expectancy

8/38 (21%) of the Newfoundland BBS patients had died by the end of the previous assessment, at ages ranging from 1 to 63 years (O'Dea et al, 1996).
Renal failure was present in 75% at the time of death, and was the primary cause of death in 38% (3/8). Survival analysis showed that 25% of BBS patients were dead by 44 years of age, which was significantly younger than their unaffected siblings. The data were too scanty to draw a conclusion, but suggested an adverse prognosis for life expectancy in BBS.

A review of 14 death certificates of BBS patients revealed that renal disease was a factor in 50% (7/14) (Riise, 1996). The mean age of death was 46.4 years for the 7 women, and 43 years for the 7 men. The number of patients was small, ascertainment was incomplete and the data were obtained from a retrospective rather than prospective cohort, so the ages of death seen in this study should not be regarded as accurate predictions for the life expectancy of other groups of BBS patients. Despite these flaws, the data suggest that BBS is associated with an adverse prognosis for life-expectancy, and that renal disease contributes to the death of some patients.

1.4 Genotype-Phenotype Studies in BBS

No population-based study with large numbers of patients defined by genotype has been reported previously. Studies which have compared the BBS phenotype with genotype have noted only minor phenotypic differences (Beales et al, 1997; Carmi et al, 1995; Riise et al, 2002).
The clinical features were compared between 12 Bedouin BBS3 patients, seven BBS4 patients and nine BBS2 patients (Carmi et al. 1995). They concluded that BBS3 is associated with 4 limb polydactyly, BBS4 with early onset morbid obesity and BBS2 with a lesser degree of obesity. However, the number of patients was small, and the suggested BBS3 phenotype was not confirmed in a Newfoundland BBS3 family (Young et al. 1998). Four of the five patients in the Newfoundland BBS3 family had polydactyly restricted to their feet, and the obesity in these patients was reversible with appropriate lifestyle changes.

No clinical differences were found between 29 European BBS families linked to BBS1, BBS2 or BBS4, or unlinked (Bruford et al. 1997). In another study of 18 BBS families (14 European and 4 Middle-Eastern), minor phenotypic differences were observed between families linked to different loci (Beales et al. 1997). BBS1 affected offspring were taller than their parents, whereas BBS2 and BBS4 patients were significantly shorter than their parents. The authors concluded that the different BBS genes may affect growth characteristics such as height. The number of patients was small, with the BBS4 group comprising only one family of 2 affected individuals.

In a more recent report, the clinical features of 3 Norwegian BBS4 sibling pairs were described (Riise et al. 2002). The phenotype showed inter- and intra-familial variability, and there were no major differences in clinical features
from other BBS patients, though the authors suggested there may be a characteristic ocular fundal appearance in BBS4 patients.

1.5 Advantages of Newfoundland for the Study of BBS and LMS

Newfoundland has a relatively isolated population, 90% of whom are descended from migrants from south-west England and Ireland (Mannion, 1986). Genetic founders have given rise to clusters of hereditary disorders (Parfrey et al, 2002). Persistent isolation, large sibships, and a high coefficient of kinship have facilitated the study of rare autosomal recessive disorders such as BBS, which is more common than in other populations (Bear et al, 1987; Bear et al, 1988; Green et al, 1989; Harnett et al, 1988; O’Dea et al, 1996). Furthermore, the health care structure, with a single tertiary referral centre, facilitates ascertainment for population-based studies.

1.6 Objectives of Study

This study extends the analysis of the clinical course of the Newfoundland BBS and LMS patients, and for the first time compares the clinical and molecular data for this cohort. Comprehensive ascertainment has enabled all cases of BBS and LMS to be identified in the Newfoundland population. Longitudinal clinical data from more than 20 years of follow-up has been obtained.
The objectives of the current study were:

1. To determine whether LMS and BBS are the same disorder.
2. To describe the clinical and genetic epidemiology of BBS and LMS.
3. To determine whether there are genotype-phenotype relationships in BBS.
4. To describe the clinical course of BBS/LMS.
5. To extend the phenotype by examining in more detail other organ systems, in particular respiratory, gastro-intestinal, neurologic systems and craniofacial dysmorphology.
2 METHODOLOGY

2.1 Ascertainment

Patients were initially ascertained in 1979 through an Ocular Genetics Clinic and the registry of the Canadian National Institute for the Blind (Green et al., 1989). All patients with retinal dystrophy and any clinical feature compatible with BBS or LMS were enrolled in this prospective study. Subsequent ascertainment was through family studies, a search of diagnostic codes for BBS and LMS in provincial hospitals and the Provincial Genetics Program. Two patients of Newfoundland parentage living in Ontario referred themselves. Informed consent was obtained, and the study was approved by the Human Investigation Ethics Committee of Memorial University of Newfoundland.

2.2 Patients

The current study cohort consisted of 46 patients from 26 families. Twenty-six were male and 20 female, with age range of 1.5 to 67.9 years (median 44 years). All patients had at least 4 cardinal features (retinal dystrophy, obesity, renal structural abnormalities, male hypogenitalism, dystrophic extremities) or three cardinal features if there was an affected sibling. Consanguinity was documented in 27% (7/26) families, and suspected in another 15% (4/26) on the basis of shared surnames on both sides of the family.
2.3 Data Collection

Formal, protocol-driven assessments were undertaken in 1986 (Green et al, 1989; Hamett et al, 1988), 1993 (O'Dea et al, 1996) and 2001. These included tests of height and weight, blood pressure, visual function, intelligence, glucose tolerance, pituitary function, renal function and imaging, and measures of head, extremities, and genitalia.

In the current study, medical charts were reviewed for all 46 patients; 10 patients were deceased by 2001. Medical data was also obtained from interviews with patients, caregivers and family doctors. Twenty-six patients had a clinical examination, performed by the same clinician (SJM), which included an examination of the neurological system and dysmorphic features, anthropometric measurements of the head, face, hands, feet and genitalia, and a systemic examination, including height, weight and blood pressure. Twelve patients were also examined by another clinical geneticist. Quantitative dysmorphic features were compared with normal values using standardised charts (Hall et al, 1995). Slide photographs for 19/26 patients were reviewed by two other clinical geneticists to score qualitative facial dysmorphic features. Neurological signs were reviewed in seven patients by one neurologist.

Nineteen patients had a speech assessment using a standard protocol that was administered by two speech pathologists. This included diadochokinetic tests
which measure the ability to make rapid alternating speech movements, as a measure of oro-motor co-ordination. The times to repeat syllables ("puh", "tuh", "kuh" twenty times, and "puhtuhkuh" ten times) were recorded and compared with normal values (Shipley and McAfee, 1998).

Blood samples were taken for measurement of urea, creatinine, liver enzymes, random glucose and glycosylated hemoglobin. DNA was extracted from venous lymphocytes for 81% (21/26) families for genotyping, haplotype and linkage analysis, and mutation screening (Davidson et al. 2003; Katsanis et al. 2000; Woods et al. 1999; Young et al. 1998; Young et al. 1999a; Young et al. 1999b). Urine samples were obtained for urinalysis, microscopic analysis and culture. Psychiatric diagnoses using DSM-IV criteria were confirmed by a psychiatrist, on the basis of medical chart review. Fourteen patients had a renal ultrasound scan in 2001, and a further 18 patients had renal ultrasound scans in previous assessments. In seventy-eight percent (25/32) of cases, the renal ultrasound scan was reported by the same radiologist.

Tests of verbal intelligent quotient (VIQ) were performed for 23 adults and 1 child using the Wechsler Adult Intelligence Scale (WAIS) and Wechsler Intelligence Scale for Children (WISC-III) respectively. Twenty-two of these were reported previously (Green et al. 1989). Fourteen patients also had a performance IQ (PIQ) test. The Haptic Intelligence Scale (Shurrager and
Shurrager), which is designed for those with severe visual impairment, was used for 13, and one child with adequate vision was tested using the WISC-III. Twelve of these patients were reported previously (Green et al, 1989).

2.4 Definitions

The following definitions were used:

Bardet-Biedl Syndrome: presence of at least four cardinal features (retinal dystrophy, obesity, renal abnormalities, male hypogenitalism, dystrophic extremities) or presence of three cardinal manifestations in a sibling of an affected person with four cardinal features.

Laurence-Moon Syndrome: presence of retinal dystrophy, obesity, spasticity, and at least one of the following features (hypogenitalism or mental retardation) in the absence of polydactyly.

Registered Blind: visual acuity < 20/200 or visual field < 20 degrees in the better eye.

Obesity: Body Mass Index, BMI > 27 kg/m²

Hypertension: Sitting systolic blood pressure > 150 mm Hg or diastolic blood pressure > 90 mm Hg or taking antihypertensive medication.

Moderate Chronic Renal Failure: Estimated creatinine clearance < 60 ml/min using formula of Cockcroft and Gault (Cockcroft and Gault, 1976) or serum creatinine > 150 micromol/l in adults, or > 100 micromol/l in children < 2 years.
Mild Chronic Renal Failure: Estimated creatinine clearance 60 - 90 ml/min, or serum creatinine 121 - 150 micromol/l.

Diabetes Mellitus: Taking hypoglycemic therapy (diet/oral medication/insulin) or fulfilling the diagnostic criteria of the 1998 clinical practice guidelines for the management of diabetes in Canada (Meltzer et al, 1998).

The age of onset of hypertension, diabetes mellitus, or renal failure was considered to be the age at which the clinical end-point was first recorded in the medical chart.

2.5 Data Analysis
Kaplan-Meier survival analysis was performed for clinical endpoints of blindness, diabetes, hypertension, renal failure, cholecystectomy and death. The log rank test was used to compare ages of onset for genotypes. Differences between continuous variables were evaluated using two-tailed Student’s t-test for two groups, and ANOVA with Bonferroni adjustment for multiple comparisons for more than two groups. A result was regarded as statistically significant if the p value was below 0.05. The denominator used in the calculation of prevalence for clinical endpoints varied, depending on the number of patients available for testing.
3. RESULTS

3.1 Genetic Epidemiology of BBS in Newfoundland

Thirty-one percent (46/150; 95% confidence interval: 23.6%-38.4%) of the siblings in the 26 families were affected. The current prevalence of BBS in Newfoundland is approximately 1 in 18,000, which derives from 28 living affected people in 510,000 (population of Newfoundland). Six subjects are now living in a different province, and twelve are deceased.

Blood was obtained from 87% (40/46) of patients. 2/40 (1 family) were excluded from all known BBS loci, implying they have an as yet unidentified BBS locus, and linkage results were inconclusive in 4/40 (3 families) due to small family size. In 34 patients in whom the genotype has been identified, 42% (15/34) have a mutation in BBS6 and 22% (8/34) in BBS1. All six families affected with BBS1 are homozygous for the same mutation, whereas three mutations have been identified in the eight families with BBS6 (Table 1). Five affected members of one family showed linkage to BBS3. A complex consanguineous family with five affected members was used to locate the BBS5 locus (Young et al, 1999a). One patient is homozygous for a BBS2 mutation, and heterozygous for yet another BBS6 mutation. All patients from whom DNA is available (87%, 40/46) have been tested for all six of the BBS mutations observed in the Newfoundland population but no other cases have been found in which an affected person has mutations in more than one BBS gene.
3.2 Diagnosis of Bardet-Biedl Syndrome Versus Laurence-Moon-Syndrome

Forty-four of 46 patients identified with either BBS or LMS met the diagnostic criteria for BBS. Two met the diagnostic criteria for LMS. Both had retinal dystrophy, spastic ataxia, hypogenitalism, mild mental retardation and no polydactyly. One was from a large consanguinous pedigree which showed linkage to BBS5: spasticity was not present in another affected sibling, or two affected cousins. The other LMS patient had mutations in the BBS6 gene. Thus, although these patients were diagnosed clinically as having LMS, they had molecular genetic changes diagnostic of BBS.

3.3 Major Clinical Manifestations

3.3.1 Blindness

Ninety-one percent (42/46) of patients were registered blind (Table 2), with a median age of 18 years (Figure 1). Those not registered blind were all children and all had visual impairment by six years. The age to register blind was similar across all genotypes (Table 3).

3.3.2 Dystrophy of Extremities

Brachydactyly was present in the feet of all patients (36/36), and in the hands in 86% (31/36). Syndactyly occurred in 95% (35/37), mostly 2/3 toe syndactyly.
Polydactyly was more variable, being present in 63% (29/46) of cases (Table 2). Figure 2 shows the distribution of polydactyly by genotype. It was present in all genotype groups, except BBS5, and its extent varied widely within a genotype.

### 3.3.3 Obesity and Height

Body Mass Indices (BMI), were available for 96% (44/46) of the patients. All were obese at some time, except a child who died at 18 months (Table 2). The mean BMI of all available measurements over time was 35.5 kg/m², while the mean of the maximal BMIs was 44 kg/m². Morbid obesity (BMI greater than 40 kg/m²) was present in 25% (11/44) individuals. There was no significant difference in BMI with gender or genotype. Maximal BMI measurements with the corresponding genotypes are shown in Figure 3.

The median adult heights (N=36) were 166 cm (5th centile) for males, and 157 cm (10th - 25th centile) for females. There was no significant difference between genotypes. Eleven percent (4/36) of adult patients had tall stature (height greater than or equal to the 90th centile). Five of the six children had tall stature.

### 3.3.4 Diabetes Mellitus

Diabetes mellitus occurred in 48% (22/46) of patients (Table 2) and impaired glucose tolerance was diagnosed in a further four. The median age of onset of
diabetes mellitus was 43 years (Figure 4) and there was no significant difference among genotypes (Table 3).

3.3.5 Hypertension

Hypertension was common, with 67% (31/46) affected individuals (Table 2). The median age of onset was 34 years (Figure 5). Hypertension was associated with all genotypes except BBS2 and there was no significant difference in age of onset among genotypes (Table 3).

3.3.6 Renal Abnormalities

All 32 patients who had a renal ultrasound scan had an abnormality detected (Table 2). Fetal lobulation was seen in 84% (26/31). Calyceal blunting, clubbing or diverticula was present in 78% (25/32), and cysts in 72% (23/32). Forty percent (13/32) had loss of cortico-medullary differentiation.

Estimated creatinine clearance was available for 89% (41/46) of patients and serum creatinine in three others. Moderate chronic renal failure occurred in 20% (9/44) (Table 2) with a median age at onset of 57.6 years (Figure 6). Four patients progressed to end stage renal disease, a further seven had mild chronic renal failure, and renal impairment occurred in all genotypes except BBS2 (but this patient was only 16 years old at last follow-up).
3.3.7 Genital and Reproductive Abnormalities

These are shown in Table 4. Male hypogenitalism was present in all but one of the males examined. Other abnormalities included undescended testes, hypospadias, phimosis, recurrent urethral strictures (requiring surgery) and posterior urethral valves. None of the males had offspring.

In the female LMBBS patients, gynecological disorders were common, and present in all genotypes. Irregular menses occurred in 65% (13/20), 25% (5/20) had menorrhagia, 20% (4/20) had a hysterectomy for menorrhagia (two patients) or endometriosis (two patients). Two females, both with a BBS1 genotype, each gave birth to one live born, healthy offspring.

3.4 Neurological, Psychiatric and Speech Abnormalities

A wide-based, unsteady gait and impaired limb co-ordination were present in 86% (18/21) of the patients. Twenty-one percent (5/24) had spasticity. All four limbs were affected in four patients and only the lower limbs were affected in the other.

Abnormal facial movements were observed in 75% (15/20) of patients representing all genotypes. Lower facial movements were more often affected than upper and 30% (6/20) had difficulty smiling. In most individuals the facial muscles were not weak; rather, the defects in movement appeared to be due to
impaired co-ordination or an apraxia. Facial movements were asymmetrical in 40% (8/20) of cases.

The range of eye movement was limited in 81% (17/21) of patients. Upward gaze was more often affected than horizontal or down gaze. Eight individuals had severe limitation in all directions. The abnormality was not corrected by oculocephalic manoeuvres, implying that the level of the defect is at or below the midbrain.

Thirty percent (14/46) of patients had a psychiatric disorder. Anxiety and mood disorders were the most prevalent, occurring in 20% (9/46) and 9% (4/46) of patients respectively, in all genotypes. Two-thirds (6/9) of patients with an anxiety disorder presented with somatic symptoms, which were diagnosed as psychosomatic following negative investigations. In three cases there were over ten such admissions. All except three presented between 20 and 40 years of age, two were diagnosed in childhood. Patients with mood disorders presented between 25 and 45 years of age and included three with major depression, of whom two required treatment as in-patients, and one with bipolar disorder, who had numerous hospital admissions. One child with a BBS6 genotype had a diagnosis of autistic spectrum disorder, and required special education.

Formal intelligence testing revealed a mean VIQ of 75 (range 53 to 102, N=24), and PIQ of 83 (range 44 to 105, N=14). A minority of patients had an IQ less
than 70: 33% (8/24) for VIQ and 21% (3/14) for PIQ. The BBS3 patients had a significantly higher VIQ than the BBS6 and unknown genotype groups (p=.038 and p=.015 respectively). There was no significant difference in PIQ among genotypes.

Speech assessment showed that the patients' times for syllable repetition in each of four tests were markedly prolonged compared with normal values (p<.0001). The mean time for 20 repetitions of "puh", "tuh", "kuh" and 10 repetitions of "puhtuhkuh" were 14.1, 13.1, 12.9 and 14.4 seconds, respectively, compared with normal mean values of 3.3, 3.3, 3.7 and 5.7 seconds, respectively. These data indicate a significant impairment of oro-motor co-ordination (Figure 7). These abnormalities were seen in all genotypes tested (BBS1, 3, 5, 6, and unknown).

3.5 Craniofacial Dysmorphism

Table 5 shows the prevalence of craniofacial dysmorphic features, which are illustrated in Figures 8 and 9. Dysmorphic features were present in all genotypes, and there was no clustering of specific features with genotype.

Ninety-two percent had brachycephaly, and the majority had macrocephaly, bitemporal narrowing, large ears, high arched palate, short narrow palpebral
fissures, and frontal balding (in males). Other common features included ptosis, a long shallow philtrum, thin upper lip and small downturned mouth.

3.6 Medical Problems

Table 6 shows the medical problems which occurred in at least two BBS patients, other than the major manifestations shown in Table 2. There is no evidence of clustering for any of these clinical features with genotype. The more common medical problems were present in patients with several different genotypes.

3.6.1 Hepato-biliary Disease

Cholecystectomy for cholelithiasis was performed in 37% (17/46) of patients, in 55% (11/20) of females and 23% (6/26) of males. By 30 years of age, 25% of patients had a cholecystectomy (Figure 10). There was a trend towards an earlier age to onset for females (p=0.08).

Liver enzymes were elevated in eight patients, unrelated to episodes of gall stone disease. This showed a cholestatic profile (increased alkaline phosphatase) in five, hepatic profile (increased hepatic transaminases) in three, and both cholestatic and hepatic in one.

3.6.2 Colonic Disorders

The group with colonic dysmotility included one child with a BBS6 genotype who had Hirschsprung Disease. One patient had a history of chronic constipation since early childhood. Four patients had irritable bowel syndrome.
with ages at onset between 29 and 39 years. Investigations, including barium enema, were negative.

3.6.3 Asthma

Asthma occurred in 28% (13/46) of cases. All 13 patients with asthma used inhalers or nebulisers regularly, and 8/13 (62%) required hospital admissions. One patient died of cor pulmonale secondary to chronic asthma.

3.6.4 Congenital Heart Disease

The cases of congenital heart disease involved one ventricular septal defect and one case of aortic valve stenosis. Another patient had complex anomalies, including aortic valve stenosis, coarctation of the aorta, left aortic arch and aberrant right subclavian artery.

3.6.5 Other Medical Problems

Idiopathic edema occurred in 8 patients, two of whom were male, with ages at onset between 19 and 51 years (mean 34 years). Upper and lower limbs were involved in five patients, and only the lower limbs in three. Three patients received diuretic therapy, one improved with antithrombotic stockings. None had renal or cardiac impairment at the time of diagnosis.

Chronic serous otitis media occurred in 20% (9/46). This caused conductive hearing loss in four, and five patients had t-tubes inserted.

Hyperhidrosis of the hands and feet was self-reported and noted on examination in nine cases. It was recorded in the medical chart for two patients, one of whom
was deceased at the time of the assessment. In one patient it was sufficiently severe to prevent him from reading Braille.

Epilepsy occurred in five patients, four of whom had childhood onset. Most were generalised tonic-clonic seizures. The adult onset disease involved absence seizures.

Of six patients with thyroid disease (two male, four female) three had hyperthyroidism, and three hypothyroidism.

3.7 Death

The median survival of the cohort was 63 years (see figure 11 for survival curve). Twelve patients died between the ages of 1.5 and 68 years, with a median age of 46 years. The causes of death were as follows: myocardial infarction (3), cerebrovascular disease (1), end stage renal disease (ESRD) (2), renal carcinoma (1) and septicemia due to urinary tract infection (1). Two patients died of post-operative complications, one following surgery for Hirschsprung disease, the other after colonic resection for a major lower gastrointestinal bleed, of unknown etiology. This patient also had ESRD. The other deaths were due to pulmonary embolus in a patient with morbid obesity, and aspiration pneumonia following a seizure due to a meningioma.
4. DISCUSSION

4.1 Diagnosis of Bardet-Biedl Syndrome Versus Laurence-Moon Syndrome

The ascertainment of patients throughout the province via several different routes means that all cases of BBS and LMS in the Newfoundland population should have been identified. The patients were initially identified based on retinal dystrophy, a feature that is common to BBS and LMS. They were enrolled in the study if they had other features suggestive of BBS or LMS. The clinical features differentiating LMS from BBS are the presence of a spastic, ataxic gait and the absence of polydactyly in LMS. Two patients met the diagnostic criteria for LMS, one of whom had affected relatives diagnosed clinically with BBS. Both had molecular genetic results diagnostic of BBS, implying that the underlying molecular basis for BBS and LMS is the same. Moreover, in this study 86% of the patients had a wide-based, unsteady gait, and 21% had spasticity. Furthermore, polydactyly does not always occur in patients diagnosed as having BBS, as it was absent in 27% of this cohort. There are good reasons therefore, to consider BBS and LMS part of the same syndrome and an appropriate name would be Laurence-Moon Bardet-Biedl Syndrome (LMBBS).
4.2 High Prevalence of BBS in Newfoundland

The prevalence of 1 in 18,000 for BBS in Newfoundland is considerably higher than in most other populations. There is a particularly high prevalence of BBS6: 33% of the BBS patients in Newfoundland have a BBS6 genotype compared with 4% in other populations (Beales et al., 2001). The molecular data reveal that there are at least nine different BBS mutations in the Newfoundland population. It is not known if these mutations were brought to Newfoundland by early settlers or if the mutations arose in situ. Founder effects, consanguinity, and large sibship size are factors that likely increased the prevalence of BBS in Newfoundland but one cannot rule out the possibility that there was a heterozygous advantage (e.g., an enhanced ability to store fat) that was selected for in the past (Davidson et al., 2003). There is no evidence for assortative mating (for example, carrier relatives meeting at a clinic for BBS patients) as a cause of compound heterozygous mutations at the BBS6 locus in two families.

The presence of multiple BBS genes and multiple mutant alleles at the BBS6 locus has been referred to as the ‘Newfoundland Paradox’ (Katsanis et al., 2001). A hypothesis that has been put forward to explain this conundrum suggests that BBS is inherited in a multigenic manner rather than as a simple Mendelian autosomal recessive disorder and that the Newfoundland population is enriched for a dominant susceptibility founder locus. The combination of the dominant susceptibility locus with mutations at various BBS loci would produce a higher
prevalence of BBS than in regions where the frequencies of dominant susceptibility loci are low. This proposal has gained support from results obtained by screening families for mutations in the known BBS genes. In some families a triallelic pattern of transmission has been observed; that is, two allelic mutations in one BBS gene and a third mutation in another BBS gene are required for the disease phenotype to be manifest (Badano et al., 2003; Beales et al. 2003; Katsanis et al., 2001; Katsanis et al., 2002;).

Screening for all the known Newfoundland BBS mutations in BBS1, BBS2, and BBS6 in the 21 families from whom DNA is available revealed one case of possible triallelicism. In this family, the affected person is homozygous for a stop mutation in BBS2 (Y24X) and is heterozygous for a missense mutation in BBS6 (A242S). The A242S change was not found in 90 ethnically matched control chromosomes and has been associated previously with McKusick-Kaufman syndrome (Stone et al. 2000) and thus it appears to be a pathogenic mutation rather than a polymorphism, though the precise impact of this mutation on the family’s phenotype is difficult to determine.

It should also be noted that 31% (46/150) of siblings at risk in the Newfoundland BBS families are affected. This is considerably higher than the 12.5% that would be expected if triallelicism were the major mode of inheritance.

However, the possibility that other cases of BBS showing oligogenic inheritance
exist in this cohort cannot be excluded without comprehensive mutation screening of all known BBS genes.

4.3 Natural History

This is the only reported study of BBS that is population-based, with comprehensive ascertainment and extensive follow-up. Protocol-driven assessments have allowed standardised data collection over more than 20 years, and have enabled accurate calculation of incidence of clinical endpoints. These data confirm the severity and early-onset of the visual loss in BBS and the high prevalence of obesity which developed to morbid obesity in one quarter of the patients.

The prevalence of hypertension and diabetes was higher than previously reported (Beales et al, 1999) which may be partly due to the older age of the Newfoundland cohort. However, both features may develop at a young age (e.g., two patients had hypertension before they were two years old), so regular measurement of blood pressure in BBS patients from birth is recommended, as is screening for diabetes from mid-childhood.

The high prevalence of structural renal abnormalities in this cohort confirms the value of renal ultrasound scan in the diagnostic work-up of BBS. Functional renal abnormalities contribute to the morbidity and mortality associated with
BBS in a substantial minority, and can occur in infancy. Renal disease was the primary or contributory cause of death in five of the twelve deaths in this cohort. This confirms the findings of a study of Danish BBS patients, which found that renal disease was an important factor in half of the fourteen deaths (Riise, 1996). Regular monitoring of renal function from early childhood will enable timely intervention, and help alleviate further morbidity associated with impaired renal function.

4.4 Neurological and Psychiatric Manifestations

The neurological defects in BBS are widespread and common. There are abnormalities involving movements of the face, eyes and limbs, the gait and speech, with preservation of power, suggesting that the defect is central, and is primarily one of impaired co-ordination. One may postulate that the disorder affects the brainstem with variable involvement of the cerebellar, oculomotor and pyramidal tracts.

The high prevalence of neurological and psychiatric abnormalities in this cohort implies that the LMBBS genes cause a widespread disturbance in central nervous system development. The paucity of facial muscle movement results in a characteristic expressionless facies, which may give an impression of mental retardation, previously regarded as a cardinal feature of the syndrome (Schachat
and Maumenee, 1982). However formal IQ testing shows the majority do not have mental retardation, although IQ is in the low normal range (Green et al, 1989).

Behavioural disturbances characteristic of childhood LMBBS include anxiety, depression, somatisation, autistic and obsessive traits (Barnett et al, 2002). In this cohort, patients also frequently presented with somatisation, anxiety and mood disorders. Psychiatric disease was common, though many patients presented repeatedly to physicians, before a psychiatric diagnosis was made and appropriate therapy instituted. Psychiatric disease may be underdiagnosed in patients with LMBBS, compounded by communication difficulties resulting from associated speech defects, learning difficulties, expressionless facies and behavioural traits. Early detection and appropriate treatment would improve the care of these patients.

4.5 Phenotype-Genotype Analysis

This is the first prospective, population-based study to compare the BBS phenotype for different genotypes. There is no evidence of a correlation between genotype and phenotype from these data. Although the phenotype shows variability between and within genotype groups, the longitudinal data reveal no significant difference in the frequency and age to onset of the major clinical endpoints such as blindness, diabetes, hypertension, and chronic renal failure. In
addition, the prevalence of other manifestations such as dystrophy of the extremities and obesity show no significant difference with genotype group with one exception, polydactyly, which was absent in all five BBS5 patients. However, all were members of the same family, and a conclusion cannot be reached without further data from other BBS5 families.

These data suggest that the BBS genes are involved at the same point in an early developmental pathway, which appears to be critical in the formation of the retina, limbs, kidneys, genitalia and central nervous system, and in the development of obesity, since abnormalities in all of these systems are common in all genotypes. The high prevalence and early onset of hypertension and diabetes suggests that the BBS genes may also have a role in the pathogenesis of these conditions. Further study is required to evaluate the contribution of the BBS genes to non-syndromic obesity, hypertension and diabetes.

4.6 Craniofacial Dysmorphology

The findings in this cohort confirm that brachycephaly and macrocephaly are characteristic of the disorder (Klein and Ammann, 1969). There is a paucity of reports on the facial features of LMBBS. Two independent groups described facial features from photographs of 76 patients (Beales et al, 1999) and examination of 18 patients (Lorda-Sanchez et al, 2001), which included a long philtrum, thin upper lip, small mouth and premature male balding. The results
from this study indicate that characteristic findings in LMBBS patients, in addition to brachycephaly and macrocephaly, include large ears, short narrow palpebral fissures, bitemporal narrowing, a long shallow philtrum, thin upper lip, small downturned mouth, and male frontal balding. Increasing awareness of the facial dysmorphology may facilitate the early diagnosis of LMBBS.

4.7 Medical Problems

Many medical disorders have been described in case reports of LMBBS, but there have been few comprehensive studies of the medical problems in large groups of LMBBS patients. This study extends the phenotype of LMBBS and shows that almost every organ system may be affected, in keeping with the wide-spread expression pattern demonstrated for each of the five BBS genes identified thus far (Badano et al. 2003; Mykytyn et al. 2001; Mykytyn et al. 2002; Nishimura et al. 2001; Slavotinek et al. 2000).

4.7.1 Hepato-biliary Disease

The underlying obesity may be responsible, at least in part, for some of the associated medical problems, such as gastro-esophageal reflux, fatty liver causing elevated liver enzymes, and gall stone disease. However, the prevalence of cholecystectomy found in this cohort is higher than would be expected for the BMI of the patients (Stampfer et al. 1992), suggesting an underlying metabolic or structural abnormality predisposing to gall stones. In a survey of 109 LMBBS
patients. the prevalence of cholecystectomy for gall stones was only 3% (Beales et al, 1999). This lower figure may be partly due to the method of data collection by questionnaire, which may have resulted in some under-reporting, and the young age of this cohort.

4.7.2 Colonic Disorders

Hirschsprung disease occurs in approximately 1 in 5000 live births (Passarge, 2002) and has been reported previously in association with LMBBS (Beales et al, 1999; Farag and Teebi, 1988; Islek et al, 1996; Lorda-Sanchez et al, 2000; Radetti et al, 1988; Slavotinek and Beisecker, 2000), as have other hindgut anomalies such as anal stenosis or atresia (Beales et al, 1999; Biedl, 1922; Kalangu and Wolf, 1994; Slavotinek and Beisecker, 2000). The presence of one case of Hirschsprung Disease and six cases of irritable bowel syndrome in this cohort further support the hypothesis that abnormalities of the developing hindgut are common in LMBBS.

4.7.3 Asthma

The high prevalence of asthma in this cohort confirms the findings of other reports (Beales et al, 1997; Beales et al, 1999) in which asthma was present in 25% of LMBBS patients, in association with a BBS1 genotype. In this cohort there was no evidence of a relationship between any of the clinical features and genotype, and asthma was present in all genotype groups.

4.7.4 Congenital Heart Disease
The prevalence of 6.5% for congenital heart disease (CHD) in this cohort concurs with the results of a survey of 109 LMBBS patients, 7% of whom had CHD (Beales et al. 1999). The population-based, comprehensive ascertainment of patients in this study means that this figure is likely to be a more accurate estimate of the prevalence of CHD in LMBBS than the 32% reported in three highly consanguineous Bedouin families, in which other genetic defects may have contributed (Elbedour et al. 1994). Septal defects and valvular stenosis are the most commonly reported, in addition to patent ductus arteriosus and cardiomyopathy (Beales et al. 1999; Blumel and Kniker, 1959; Elbedour et al. 1994; Farag et al. 1999; McLoughlin and Shanklin, 1967; Slavotinek and Beisecker, 2000).

4.7.5 Other Medical Problems

The skeletal abnormalities most characteristically associated with LMBBS are dystrophic extremities, particularly brachydactyly (Green et al. 1989). In a radiographic study of the extremities in 43 LMBBS patients, short, broad bones were the most common findings, and a range of other abnormalities were present (Rudling et al. 1996). However the skeletal dystrophy may be more widespread: kyphoscoliosis and talipes equinovarus have been described previously in LMBBS (Beales et al. 1999; Bell, 1958; Bowen, 1965; Dekaban, 1972; Farag, 1999; Riise, 1997) and were present in 11% and 4% respectively in this cohort.
Other medical problems in this cohort which were also reported at increased frequency in a survey of 109 LMBBS patients include hypothyroidism and chronic serous otitis media (Beales et al, 1999). Both may cause significant morbidity and be difficult to detect in a timely manner due to co-morbidity, so clinicians managing LMBBS patients should have a low threshold for initiating appropriate investigations.
5. **SUMMARY**

The hypothesis that neurological features and polydactyly can differentiate BBS from LMS (Lancet, 1988; Schachat and Maumenee, 1982; OMIM) is contradicted by the findings in this cohort of a high prevalence of neurological features and the absence of polydactyly in 37% of individuals, most of whom exhibit molecular genetic changes of BBS. Furthermore, the finding of patients with a clinical diagnosis of LMS and molecular changes diagnostic of BBS imply that these are the same disorder, Laurence-Moon-Bardet-Biedl Syndrome (LMBBS).

This study extends the phenotype of LMBBS. This widespread dystrophic disorder affects almost every organ system. In addition to retinal dystrophy, dystrophic extremities, obesity, genital abnormalities and renal anomalies, characteristic craniofacial dysmorphic features occur, and include brachycephaly, macrocephaly, large ears, short narrow palpebral fissures, a long shallow philtrum, small downturned mouth and premature male balding. Associated with this syndrome is a high prevalence of neuropsychiatric abnormalities, and a wide range of medical problems including colonic abnormalities, gall stone disease and asthma. These findings are consistent with the widespread expression of each of the five known BBS genes (Badano et al. 2003; Mykytyn et al. 2001; Mykytyn et al. 2002; Nishimura et al. 2001;
Slavotinek et al, 2000), and emphasise the need for regular, thorough clinical appraisal of patients with LMBBS.

The high prevalence of LMBBS in the Newfoundland population is associated with multiple genotypes, multiple mutations in the BBS6 gene, and rare occurrence of triallelism. A comparison of the phenotypes with genotypes indicates that the dysmorphic, neuropsychiatric and medical abnormalities do not appear to vary according to genotype. This implies that the role and function of the underlying genes probably operate within a common, ubiquitous developmental pathway, which is important in the morphogenesis of the retina, limbs, kidney, genitalia, central nervous system, and the development of obesity, hypertension and diabetes.
**TABLE 1**: Genotypes and Mutations in Patients with LMBBS identified in the Newfoundland Population

<table>
<thead>
<tr>
<th># Patients (# families)</th>
<th>Gene/Locus</th>
<th>Mutation</th>
</tr>
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<tbody>
<tr>
<td>8 (6)</td>
<td>BBS1</td>
<td>8 (6) M390R homozygous</td>
</tr>
<tr>
<td>1 (1)</td>
<td>BBS2</td>
<td>1 (1) Y24X homozygous, and A242S heterozygote for BBS6</td>
</tr>
<tr>
<td>5 (1)</td>
<td>BBS3</td>
<td>Gene not Identified</td>
</tr>
<tr>
<td>5 (1)</td>
<td>BBS5</td>
<td>Gene not Identified</td>
</tr>
<tr>
<td>15(8)</td>
<td>BBS6</td>
<td>4 (2) D143fsX157 (fs1) homozygous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 (4) F94fsX103 (fs2) homozygous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1) Fs1/fs2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (1) Fs2/L277P</td>
</tr>
<tr>
<td>2 (1)*</td>
<td></td>
<td>Excluded from BBS1 to 7</td>
</tr>
<tr>
<td>4 (3)*</td>
<td></td>
<td>Molecular Investigations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inconclusive</td>
</tr>
<tr>
<td>6 (5)b</td>
<td></td>
<td>No DNA Available</td>
</tr>
</tbody>
</table>

Fs1= frame shift 1
Fs2= frame shift 2

*All these patients are classified as “unknown” genotype
TABLE 2: Major Clinical Manifestations in LMBBS

<table>
<thead>
<tr>
<th>Clinical Manifestation</th>
<th>Number Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blindness</td>
<td>91% (42/46)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>67% (31/46)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>48% (22/46)</td>
</tr>
<tr>
<td>Male Hypogenitalism</td>
<td>92% (12/13)</td>
</tr>
<tr>
<td>Obesity</td>
<td>98% (43/44)</td>
</tr>
<tr>
<td>Renal Structural Abnormalities</td>
<td>100% (32/32)</td>
</tr>
<tr>
<td>Chronic Renal Failure</td>
<td>20% (9/44)</td>
</tr>
<tr>
<td>Polydactyly</td>
<td>63% (29/46)</td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>100% (36/36)</td>
</tr>
<tr>
<td>Syndactyly</td>
<td>95% (35/37)</td>
</tr>
<tr>
<td>Mental Retardation (VIQ&lt;70)</td>
<td>33% (8/24)</td>
</tr>
</tbody>
</table>
TABLE 3: Median Age (and 95% Confidence Intervals) to Onset of Major Clinical Endpoints in LMBBS by Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th># Patients</th>
<th>Blindness</th>
<th>Hypertension</th>
<th>Diabetes Mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBS1</td>
<td>8</td>
<td>27.0</td>
<td>27.0</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.5-47.5)</td>
<td>(9.0-45.0)</td>
<td>(41.4-44.6)</td>
</tr>
<tr>
<td>BBS2</td>
<td>1</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BBS3</td>
<td>5</td>
<td>12.0</td>
<td>34.0</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9.9-14.0)</td>
<td>(29.7-38.3)</td>
<td>(29.1-54.9)</td>
</tr>
<tr>
<td>BBS5</td>
<td>5</td>
<td>21.0</td>
<td>33.0</td>
<td>41.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18.9-23.1)</td>
<td>(15.8-50.2)</td>
<td></td>
</tr>
<tr>
<td>BBS6</td>
<td>15</td>
<td>17.0</td>
<td>33.0</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13.7-20.3)</td>
<td>(29.7-36.3)</td>
<td>(27.8-44.2)</td>
</tr>
<tr>
<td>Unknown Locus</td>
<td>12</td>
<td>18.0</td>
<td>37.0</td>
<td>54.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14.7-21.3)</td>
<td>(32.8-41.2)</td>
<td>(32.6-75.4)</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>18.0</td>
<td>34.0</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16.8-19.2)</td>
<td>(30.7-37.3)</td>
<td>(38.1-47.9)</td>
</tr>
<tr>
<td>Abnormality</td>
<td>Prevalence</td>
<td>Genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small penile length (&lt;10th centile)</td>
<td>92% (12/13)</td>
<td>BBS 1, 2, 3, 5, 6, Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undescended testes</td>
<td>11% (3/26)</td>
<td>BBS 6, Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypospadias</td>
<td>8% (2/26)</td>
<td>BBS 6, Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phimosis</td>
<td>8% (2/26)</td>
<td>BBS 1, 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent urethral strictures</td>
<td>8% (2/26)</td>
<td>BBS 5, 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior urethral valves</td>
<td>4% (1/26)</td>
<td>BBS 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal Atresia</td>
<td>10% (2/20)</td>
<td>BBS6, Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoplastic Labia Minora</td>
<td>25% (3/12)</td>
<td>BBS6, Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent Urethral Opening</td>
<td>5% (1/20)</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 5: Cranio-facial Dysmorphic Features in the Newfoundland LMBBS Patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachycephaly</td>
<td>92% (24/26)</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>58% (15/26)</td>
</tr>
<tr>
<td>Bitemporal Narrowing</td>
<td>65% (17/26)</td>
</tr>
<tr>
<td>Ear length &gt; 2SD above the mean</td>
<td>61% (16/26)</td>
</tr>
<tr>
<td>Short Palpebral Fissures</td>
<td>77% (20/26)</td>
</tr>
<tr>
<td>Narrow Palpebral Fissures</td>
<td>81% (21/26)</td>
</tr>
<tr>
<td>Ptosis</td>
<td>27% (7/26)</td>
</tr>
<tr>
<td>Shallow Philtrum</td>
<td>35% (9/26)</td>
</tr>
<tr>
<td>Long Philtrum</td>
<td>35% (9/26)</td>
</tr>
<tr>
<td>Thin Upper Lip</td>
<td>54% (14/26)</td>
</tr>
<tr>
<td>Small Mouth</td>
<td>38% (10/26)</td>
</tr>
<tr>
<td>Downturned Mouth</td>
<td>58% (15/26)</td>
</tr>
<tr>
<td>High Arched Palate</td>
<td>86% (19/22)</td>
</tr>
<tr>
<td>Frontal Balding in Adult Males</td>
<td>92% (11/12)</td>
</tr>
</tbody>
</table>
### TABLE 6: Medical Problems in the Newfoundland LMBBS Patients

<table>
<thead>
<tr>
<th>Medical Problem</th>
<th>Prevalence</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastro-intestinal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholecystectomy for gall stones</td>
<td>17/46 (37%)</td>
<td>BBS 1, 3, 5, 6, unknown</td>
</tr>
<tr>
<td>Elevated liver enzymes</td>
<td>8/33 (24%)</td>
<td>BBS 1, 2, 3, 6</td>
</tr>
<tr>
<td>Gastro-Esophageal Reflux</td>
<td>8/46 (17%)</td>
<td>BBS 1, 5, 6, unknown</td>
</tr>
<tr>
<td>Colonic Dysmotility</td>
<td>7/46 (15%)</td>
<td>BBS 1, 6, unknown</td>
</tr>
<tr>
<td>Celiac Disease</td>
<td>2/46 (4%)</td>
<td>BBS 1, unknown</td>
</tr>
<tr>
<td>Peptic Ulcer Disease</td>
<td>3/46 (6.5%)</td>
<td>BBS 5, unknown</td>
</tr>
<tr>
<td><strong>Skeletal / Connective Tissue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyphoscoliosis</td>
<td>5/46 (11%)</td>
<td>BBS 6, unknown</td>
</tr>
<tr>
<td>Talipes Equinovarus</td>
<td>2/46 (4%)</td>
<td>BBS 3, 6</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigmented nevi</td>
<td>6/25 (24%)</td>
<td>BBS 1, 5, 6, unknown</td>
</tr>
<tr>
<td>Eczema</td>
<td>5/46 (11%)</td>
<td>BBS 1, 3, 5, 6, unknown</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>3/46 (6.5%)</td>
<td>BBS 3, 6</td>
</tr>
<tr>
<td><strong>Neurological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>5/46 (11%)</td>
<td>BBS 6, unknown</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>13/46 (28%)</td>
<td>BBS 1, 2, 5, 6, unknown</td>
</tr>
<tr>
<td>Hyperhidrosis (hands and feet)</td>
<td>10/46 (22%)</td>
<td>BBS 1, 2, 5, 6, unknown</td>
</tr>
<tr>
<td>Chronic Serous Otitis Media</td>
<td>9/46 (20%)</td>
<td>BBS 2, 3, 6, unknown</td>
</tr>
<tr>
<td>Idiopathic Edema</td>
<td>8/46 (17%)</td>
<td>BBS 1, 3, unknown</td>
</tr>
<tr>
<td>Thyroid Disease</td>
<td>6/46 (13%)</td>
<td>BBS 1, 3, unknown</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>6/46 (13%)</td>
<td>BBS 1, 3, 6, unknown</td>
</tr>
<tr>
<td>Congenital Heart Disease</td>
<td>3/46 (6.5%)</td>
<td>BBS 6, unknown</td>
</tr>
</tbody>
</table>
Figure 2: Polydactyly by Genotype in LMBBS
Figure 3: Maximal Body Mass Index by Genotype in LMBBS
Figure 4: The Age of Diagnosis of Diabetes Mellitus in LMBBS
Figure 5: The Age of Diagnosis of Hypertension in LMBBS
Figure 6: The Age of Diagnosis of Chronic Renal Failure in LMBBS
Figure 7: Diadochokinetic Speech Tests in LMBBS
Figure 10: The Age of Cholecystectomy in LMBBS
Figure 11: The Age of Death in LMBBS
REFERENCES


