METABOLIC AND RENAL EVENTS IN AUTOSOMAL RECESSIVE BARDET-BIEDL SYNDROME (BBS) AND IN FIRST DEGREE RELATIVES WITH AND WITHOUT A BBS MUTATION

MICHAEL P.K. WEBB

Metabolic and Renal Events in Autosomal Recessive Bardet-Biedl Syndrome (BBS) and in First Degree Relatives with and without a BBS Mutation

By Michael P.K. Webb

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

Faculty of Medicine

Memorial University of Newfoundland

St John's, Newfoundland

February 2009

ABSTRACT

Bardet-Biedl Syndrome (BBS) is an autosomal recessive, genetically heterogenous, ciliopathic condition, characterized by dystrophic extremities, retinal dystrophy, obesity, renal abnormalities and male hypogonadism. It is possible that inheritance of a single BBS mutation may predispose to complex diseases such as obesity, hypertension and diabetes, particularly as these disorders occur frequently in BBS. To determine the incidence of metabolic and renal events 46 BBS cases, 96 heterozygote BBS mutation carriers, and 37 relatives without a BBS mutation were studied. Cases have been followed prospectively for up to 28 years, but relatives were assessed for the first time.

The molecular basis of BBS was identified in all families in whom DNA was obtained: 9 mutations in 6 different BBS genes were discovered in 21 families. Body mass index in adult cases was 38 ± 12, in carriers 28 ± 6 and in non carriers 29 ± 3. Hypertension had developed in 72% of cases, in 54% of carriers and 49% of non carriers. Median time to onset of hypertension treatment was 34, 63 and 67 years respectively. Diabetes had developed in 50% of cases, 17% of carriers, and 24% of non carriers, with median time to diabetes being 43, 75 years and not achieved respectively. Stage 3 chronic kidney disease had developed in 47% of cases, 11% of carriers, 15% of non carriers, with median age to diagnosis being 58, 86 and 81 years respectively.

Metabolic and renal events occurred frequently and at an early age in BBS. There were no significant differences in the risk of these events comparing carriers of a BBS

mutation to non carriers. Inheritance of a BBS mutation does not predispose to obesity, diabetes, hypertension or renal impairment.

Acknowledgements

I would like to thank the Newfoundland BBS families for their enduring participation and commitment to BBS research. I would also like to thank my supervisor Dr. Pat Parfrey; his efforts and tutelage have had a profound effect on my graduate studies, and on my life. Dr. Ban Younghusband and Dr. Jane Green have formed a fine co-supervisory committee, providing countless revisions and support. Thanks are also due to Dr. Betty Dicks for all of her work with me on this project both at home and on the road. A special thanks goes to my mother, sister and the rest of my family, my friends, and the Parfrey family, for all that they have done to make the submission of this document a reality.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

BBS: Bardet-Biedl Syndrome
LMS: Laurence-Moon Syndrome
LMBBS: Laurence-Moon Bardet-Biedl Syndrome
BBS1-12: Distinct Bardet-Biedl Syndrome Genotypes
BMI: Body Mass Index
IQ: Intelligence Quotient
CNIB: Canadian National Institute of the Blind
ADP: Adenosine Diphosphate
GTP: Guanosine Triphosphate
IFT: Intraflagellar Transport/Trafficking
MKKS: McKusick Kaufman Syndrome
TTC8: Tetratricopeptide Repeat Protein 8
TRIM32: Tripartite Motif Protein 32
AS: Alstrom Syndrome
ATP: Adenosine Triphosphate
NHANES: The National Health and Nutrition Examination Study
DNA: Deoxyribonucleic Acid
HbA1c: Hemoglobin A1c
MDRD: Modification of Diet in Renal Disease Measure of Renal Function
PCR: Polymerase Chain Reaction
HIC: Human Investigation Committee
CKD: Chronic Kidney Disease
SD: Standard Deviation
BP: Blood Pressure
Mm Hg: Millimeters of Mercury
95% CI: 95% Confidence Interval
ESRD: End Stage Renal Disease

1 Introduction:

1.1 Newfoundland's Founder Population:

The Island of Newfoundland is one of the world's richest resources for study of genetic disease. Arrythmogenic right ventricular cardiomyopathy, hereditary cancers such as colon cancer, polycystic kidney disease, and numerous other inherited conditions all have a high prevalence in Canada's easternmost province. Newfoundland's population may be useful in the study of autosomal recessive, autosomal dominant and complex genetic disease. A high co-efficient of kinship, essentially the marriage of cousins, has resulted in a predisposition to some autosomal recessive conditions like Bardet Biedl Syndrome [1]. The settling of Newfoundland has led to several distinct genetic isolates, within which autosomal recessive conditions are more likely to arise. Ninety percent of Newfoundland's current population has arisen from approximately 30,000 founders [1]. Geographic isolation, segregation by religion, and founder effects predispose Newfoundlanders to genetic disease. In addition, Newfoundlanders have had large families throughout generations, who have settled in or near the core community and there has been little in or out migration. Close family connections facilitate the study of genetic conditions [1].

The island of Newfoundland was settled primarily in response to high demand for cod fish in Europe in the late 1700's and early 1800's. The bulk of the settlers who came to exploit the rich fishery in Eastern Canada came from two distinct populations. These settlers came from Southeast Ireland and Southwest England. Both of these groups were

fairly homogenous in their homeland origins, and often settled within specific outport communities of Newfoundland that were geographically isolated. The Irish settlers came in two major migrations, and the English came in a fairly steady flow for a period of the early 1800's and the 1830's [1]. After this rush to settle, the migration essentially stopped, and the population growth of Newfoundland was driven by natural reproduction. In the 1980's Newfoundland's population reached its peak at approximately 580,000 people, of whom an estimated 90% can be traced to the initial 20,000-30,000 settlers.

Geographic isolation of coastal settlements was perpetuated by dependence on the cod fishery, lack of roads and by the segregation of the Irish settlers who were mostly Catholic from the English settlers who were largely Protestant. Any expansion of these isolated communities was primarily due to the division of land amongst descendants of original settlers, and settlement of descendents in nearby coves and bays which did little to contribute to the genetic diversity of large families [1, 2]. Little has changed over time into the modern era. In 1982, 50% of the Newfoundland population lived in towns of less than 2500, and 41% in towns of less than 1000 [1, 2].

The settlement and expansion of Newfoundland, coupled with religious segregation, close family ties, a high coefficient of kinship, low gross in and out migration has created a number of genetic isolates, defined by the coastal geography [1, 2]. None the less, Newfoundland is the most generalizable of founder populations to Caucasian populations. [3].

1.2 Bardet-Biedl Syndrome:

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterized predominantly by obesity, retinal dystrophy, dystrophic extremities, male hypogenitalism, and renal malformations. Secondary features of this syndrome include diabetes mellitus, endocrine dysfunction, neurologic abnormalities, learning difficulties, and systemic abnormalities in nearly every organ [4].

The prevalence of BBS has been estimated variously as 1 in 160000 (Switzerland) [5,6], 1 in 150000 in the European population [7], and 1 in 100000 as the global prevalence [8]. Newfoundland has a very high prevalence of Bardet-Biedl syndrome, estimated at 1 in 18000 live births [9], and that figure is only surpassed by the rate seen in Kuwaiti Bedouins which is 1 in 13500 [10].

1.2.1 Early Descriptions of BBS:

The Britons, Laurence and Moon first described four mentally retarded siblings with obesity and retinal dystrophy in 1866. The three males in the case studies had small genitals and walked with an ataxic gait [11]. The syndrome was called Laurence-Moon Syndrome. Bardet and Biedl, in 1920, and 1922 respectively, reported similar cases, in French and Austrian children, and the patients of Biedl also had polydactyly [12, 13]. After these reports, the conditions Laurence-Moon Syndrome and Bardet Biedl syndrome were considered to be expressions of the same condition, called Laurence-Moon-Bardet Biedl Syndrome (LMBBS) [14]. Literature reviews performed by Klein and Ammann

(1969) and Schachat (1982) suggested that the two conditions were distinct, with LMS involving a progressive spasticity and no sign of polydactyly [6, 15]. This division was widely adopted in the scientific community, with the majority of patients previously diagnosed with LMBBS then being given the diagnosis of BBS. However, more recent molecular genetic research has shown that BBS mutations are responsible for phenotypes that appear to conform to the LMS diagnosis [9]. Of 46 BBS cases in the Newfoundland cohort, two cases met the criteria for LMS. One case, meeting the LMS diagnostic criteria, had siblings with BBS who did not meet LMS criteria. All affected individuals in this extended family were later shown to have the same *BBS5* mutations. The other instance of a phenotype consistent with LMS was seen in a family with *BBS6* mutations.

This project is the extension of several years of work on the Newfoundland population of Bardet-Biedl syndrome. The project began with ascertainment and assessment of cases of BBS through the Canadian National Institute for the Blind.

1.2.2 Clinical Manifestations of BBS

Bardet-Biedl syndrome is associated with many deleterious clinical outcomes. BBS has manifestations in almost all of the body's organs. The lives of BBS affected individuals are significantly burdened by the syndrome, and their lifespan is often much shorter than the average for their population [9].

1.2.2.1 Vision:

Bardet-Biedl patients have severely impaired vision. The retinal dystrophy found in BBS is often classed as a rod-cone dystrophy. Individuals with BBS are frequently registered blind in their teens or twenties, and many lose all vision by their twenties or thirties.

1.2.2.2 External Physical Abnormalities:

Brachydactyly, syndactyly, and polydactyly of the hands and feet are seen regularly. Facial and cranial structure is also frequently affected. Patients show a flat affect in their faces and often have narrowing of the skull around the temples. Men with BBS are subject to hypogonadism, characterized by small, buried penises and undescended testes [4, 7, 9]. Women also may be burdened with sexual structural abnormalities, some women having dystrophic vaginas [9]. It is of note that none of the men with BBS in the Newfoundland population have fathered a child, women have similarly reduced or impaired fecundity; with only two lives birth reported [9].

1.2.2.3 Obesity:

Obesity is one of the cardinal manifestations of BBS. Twenty five percent of the Newfoundland BBS population had a Body Mass Index (BMI) higher than 40 [9]. This is known as morbid obesity, and is associated with substantial morbidity.

1.2.2.4 Endocrine Disease:

Diabetes and impaired glucose tolerance are very commonly associated with BBS. A study from the late 80 s and early 90 s, showed abnormalities in gonadotrophins in women, and that reproduction was low [4].

1.2.2.5 Hypertension:

BBS patients in Newfoundland are subject to early onset of hypertension (median age 34) [9].

1.2.2.6 Renal Structure and Function:

In the Newfoundland BBS population, 100% of patients who underwent a renal ultrasound had a structural abnormality [9]. BBS patient kidneys frequently show fetal lobulation. Calyceal blunting or clubbing, renal cysts, and some degree of kidney failure are all features of the syndrome [4, 9, 16]. Depressed renal function in those with BBS could predispose to hypertension.

1.2.2.7 Neurological Disease:

Traditionally BBS was thought to be associated with mental retardation. However, with IQ testing appropriate for those with severe vision loss, only a third had below normal IQ [4]. Learning disabilities, however, are common. BBS patients also have a host of other neurological deficits. Ataxic gait, poor coordination, abnormal cranial nerve function,

impaired eye movement, difficulty forming words and sounds are all common [9]. There is also an increased prevalence of mental disorders in the BBS population [4, 9].

1.2.3 Establishment of a Clinical Phenotype for BBS:

To classify and diagnose BBS a clinical phenotype was defined that took into account the cardinal and secondary manifestations of BBS [9, 17]. This undertaking created a concrete list a characteristics and outcomes most associated with BBS. This clinical phenotype excluded mental retardation, which was previously listed as a clinical manifestation of the syndrome. In recent years, it has been suggested that the lack of intellectual stimulation in BBS affected persons contributed to their developmental delay [4, 9]. Their blank and expressionless faces, caused by poor coordination of facial muscles, together with blindness, obesity, and dystrophic extremities also gave the impression of retardation.

Newfoundland Bardet-Biedl syndrome patients were first ascertained through a review of the records of the Canadian National Institute of the Blind (CNIB), and subsequently through referral by Ophthalmologists or the Newfoundland Provincial Medical Genetics program. All patients had complete clinical investigations and detailed medical record reviews. Clinical diagnosis of BBS was made by the patient being positive for 4 of the cardinal BBS manifestations, or by being a sibling, with 3 cardinal manifestations, of a positively identified BBS patient. The description of the clinical phenotype of the Newfoundland BBS cohort was derived initially from the work of Green et al, Harnett et

al, and O'Dea et al. [4, 16, 18] . More recently, Moore et al. performed a detailed assessment of all available BBS cases in Newfoundland in 2005 [9].

1.3 BBS Molecular Genetics And Genotypes:

Since 1995, Newfoundland families have participated in research to identify the genes responsible for BBS. Similar studies of Bedouin BBS families as well as cohorts of BBS patients particularly in the United States, Great Britain, and France, have contributed to the elucidation of the BBS genes. There are now 12 confirmed BBS genes, *BBS12* having recently been discovered [19]. Two major genes, *BBS1* and *BBS10*, each account for ~20% of the mutational load in families of European descent, whereas ten other genes each account for approximately 5%, and some of these were found mutated in only a few families or even a single family (the latter in the case of *BBS11*) [19, 20]. The 12 known BBS genes account for ~70% of affected families, suggesting that additional BBS genes, for the remaining 30% remain to be identified. A further complication is the finding that, in some cases, inheritance departs from classic autosomal recessive inheritance and may involve three mutated alleles in two genes, defined as oligogenic inheritance. It is also possible that severity can be modulated by an allele of a modifier gene [19, 21].

1.3.1 BBS1:

Mutations in *BBS1* are the most common cause of BBS, and account for approximately 20% of the cases world wide [21, 22]. The gene's chromosomal locus is 11q13 [23].

BBS1 because of mutations that result in dysfunction in sensory capabilities of the ciliated cells [23, 24]. The *BBS1* mutation in Newfoundland families is M390R.

1.3.2 BBS2:

Mutations in *BBS2* account for 8% of BBS cases [21]. Like *BBS1*, mutations in *BBS2* result in dysfunction of cellular sensory machinery. The chromosomal locus for *BBS2* is 16q21 [23, 25]. *BBS2* mutations are thought to have negative effects in regulation of development and growth, which may result in the failure of BBS2 cases to develop fully at early stages of growth. Mice with *BBS2* mutations display obesity, retinal degeneration, renal cysts, male infertility, and olfactory deficiencies [23, 26]. The *BBS2* mutation identified in Newfoundland families is Y24X.

1.3.3 BBS3:

BBS3 accounts for a small proportion (0.5%) of world wide BBS cases [21] . The BBS3 protein product is also known as ADP-ribosylation factor-like protein 6 and its gene is

found at chromosome location 3p12-q13 [23]. This intracellular protein is responsible for a series of molecular signals in the cell via the GTP and the RAS proteins, which are key to intracellular trafficking [26, 27]. *BBS3* mutations result in changes in amino acid residues near the GTP binding site and are likely to prevent GTP binding and therefore signal conduction [27]. The mutation identified in Newfoundland is G169A.

1.3.4 BBS4:

This gene contributes to less than three percent of BBS cases [28]. BBS4 may be an adaptor protein that facilitates the loading of cargo onto the dynein-dynactin molecular motor in preparation for microtubule-dependent intracellular transport in the cilium or the cytosol (figure 1) [29]. Mutations in *BBS 4* have been linked with impaired olfaction. The gene's chromosomal locus is 15q22.3-q23 [30]. Mutations in *BBS4* in mice led to obesity, retinal degeneration, sperm defects, olfactory deficiencies and improperly formed olfaction structures [31, 32]. *BBS4* mutations have not been identified in Newfoundland.

1.3.5 BBS5:

Like *BBS3*, mutations in *BBS5*, also account for about 0.5% of BBS cases [21]. Localization of the BBS5 protein to basal bodies suggests that it is involved in ciliary function (figure 1). In *C. elegans*, *BBS5* silencing results in an unciliated model of the species. *BBS 5* is located at 2q31 and the mutation seen in the Newfoundland BBS5 families is IVS6+3A>G

1.3.6 BBS6:

Mutations in the *BBS6* gene are thought to be the cause of 5% of BBS cases. However, this mutation is much more common in Newfoundland [21]. The *BBS6* gene is known as MKKS/BBS6 and is located at locus- 20p12. BBS6 is proposed to be an atypical member of the superfamily of type II chaperonins, which are mediators for the proper

folding of proteins [21, 33]. *BBS6* may code for a chaperonin which is thought to be key in proper mechanoreception and photoreception [34]. Defective BBS6 protein has been found in models that have cytokinesis defects, and in mice that are obese, have retinal degeneration, sperm flagellation defects and olfactory deficiencies [29, 35]. There are three *BBS6* mutations seen in the Newfoundland families, F94fsX103, D143fsX157, and L227P.

1.3.7 BBS7:

Mutations in *BBS7* account for ~1.5% of cases world wide [21]. BBS7 is required for the normal localization/motility of the intraflagellar trafficking (IFT) proteins, and group of proteins required for complex shuttling of other proteins along the cilia (figure 1) [26], and dysfunction of the protein has been shown to cause ciliary defects and improper IFT in *C. Elegans* models [36]. The *BBS7* gene is located at 4q27. No Newfoundland families have been identified with a *BBS7* mutation.

1.3.8 BBS8:

Mutations in *BBS8* account for ~1% of BBS cases worldwide [21]. The *BBS8* gene is located at chromosome 14q32.1. It has been proposed that BBS8 protein, known as Tetratricopeptide repeat protein 8 (TTC 8), is required for the normal localization/motility of the IFT proteins [36]. BBS8 protein is associated with centriolar structures, ciliary structures, and interacts with a protein that is likely involved in basal body function (figure 1) [24, 37]. There are no BBS 8 mutations in Newfoundland.

BBS1-8 proteins all have some similar putative effects. They influence the structure and function of Kupffers vesicle, left/right differentiation and mutations in their *BBS* genes have been shown to result in degenerated cilia and delayed intra cellular transport. This is explained by defects in the cilia dependant intraflagellar transport chain (figure 1) [20, 38].

1.3.9 BBS9:

Parathyroid hormone-responsive gene B1 (*B1*), located at chromosome 7p14, was found to be a novel BBS gene (*BBS9*), supported by the identification of homozygous mutations in BBS patients [39]. Little is known about the function of this protein. In *BBS9* null mice, protein B1 is down regulated in the retina, and this could result in suboptimal vision [39]. There are no known Newfoundland BBS9 cases.

1.3.10 BBS10:

20% of BBS cases have mutations in *BBS10* [40]. Like *BBS6*, this gene is proposed to code for a protein that is an atypical member of the superfamily of type II chaperonins [34]. Chaperonins ensure that proteins are folded properly and may impact ciliary protein folding and function. The BBS10 protein may be an active hydrolytic enzyme. Suppressing BBS10 function in zebrafish models causes severe developmental irregularities [40]. The *BBS10* gene is located at 12q21.1, and the mutations in *BBS10* observed in Newfoundland are C91fsX95 and F198 Del/199 Del.

1.3.11 BBS11:

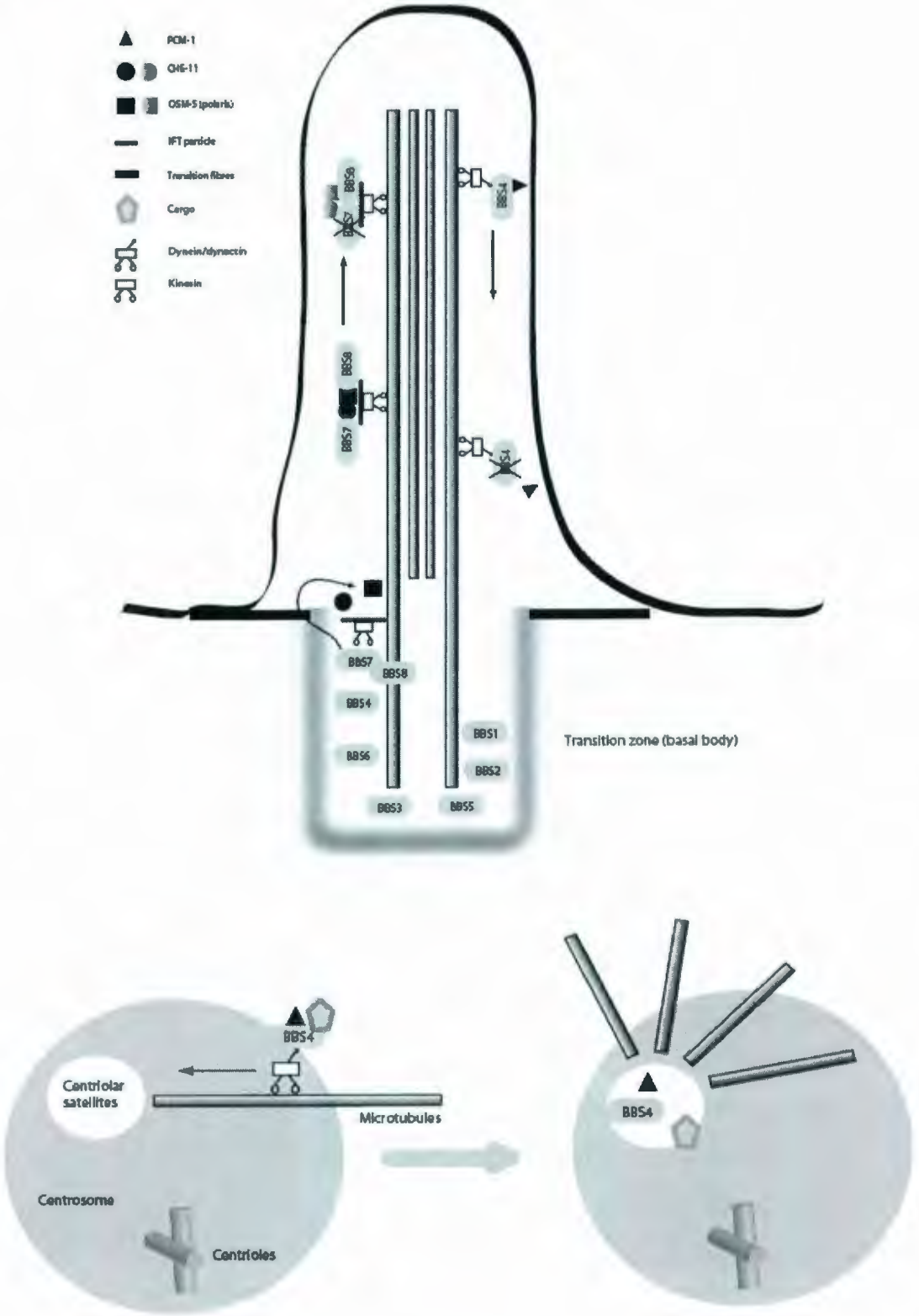
The protein product of *BBS11* is also known as "tripartite motif protein 32" (TRIM32). It is thought to be an ubiquitin ligase, involved in protein turnover by identifying proteins for disintegration by "tagging" them with ubiquitin [20]. This is proposed to cause BBS by acting on other BBS proteins either directly or indirectly. Mutations in the zebrafish model of the *BBS11* gene cause disruption of the ciliated organ, Kuppfer's vesicle and delayed intracellular transport. Mutations of *BBS11* were seen in a single consanguineous Bedouin family [20]. The *BBS11* gene is located at 9q31-q34.1. No *BBS11* mutations have been identified in Newfoundland.

1.3.12 BBS 12:

Like *BBS6* and *BBS10*, the *BBS12* gene encodes a protein which is an atypical member of the superfamily of type II chaperonins [19, 34, 40]. Mutations in the *BBS12* gene are seen in 5% of BBS families [19]. This gene is found at chromosomal location 4q27. No BBS12 mutations have been identified in Newfoundland.

Figure 1 is a schematic of the respective roles of several of the aforementioned BBS proteins. The figure displays the localization of the various BBS proteins around the cilia and basal body, further establishing BBS and a ciliopathy, and indicates that the BBS proteins are critical in the maintenance of proper functioning cilia.

Figure 1: The Putative Role of BBS Proteins



The Cause of BBS:

Figure one amalgamates data accrued from several organisms and thus represents an idealized cell system. All BBS proteins have been placed in the transition zone (basal body), centrosome, and/or ciliary axoneme. There is additional evidence for the role of BBS7 and BBS8 in facilitating the selective assembly of intraflagellar transport (IFT) proteins into IFT particles. Knock-down of either BBS7 or BBS8 (only BBS7 is illustrated) results in diminished levels of CHE-11 and OSM-5 (polaris) in the ciliary axoneme, culminating in shortening. BBS4 through its direct interaction with the $p150^{glued}$ subunit of dynactin probably behaves as an adapter assisting the loading of cargo (such as PCM-1) onto the IFT particles and subsequent transport to the centriolar satellites (in the centrosome and the basal body). Given that the primary structure of BBS6 is similar to the group II chaperonins, we may speculate that its role is to process proteins prior to IFT assembly and loading, as well as microtubule-dependent membrane trafficking [26].

1.4 Molecular Genetics of Ciliopathies:

1.4.1 BBS "Spectrum" Disorders:

There are several conditions that have a distinct likeness to Bardet-Biedl syndrome. Exploration of these conditions may prove useful in the study of BBS.

1.4.1.1 McKusick-Kaufman Syndrome (MKKS):

MKKS/BBS6 mutation is also associated with MKKS. *MKKS* was first identified as the gene for McKusick-Kaufman syndrome (MMKS) and mutations of the same gene (*BBS6*) can cause BBS. MKKS shares clinical manifestations with BBS, including post-axial polydactyly, congenital heart disease and hydrometrocolpos- a congenital abnormality of the vagina. BBS may be mistaken for MKKS in infancy or early childhood prior to the recognition of other clinical manifestations of BBS, particularly retinal dystrophy which is not present in MKKS [26].

1.4.1.2 Alstrom Syndrome:

Rod-Cone dystrophy, obesity, insulin resistance, and physical developmental delay are seen in both BBS and in Alstrom Syndrome (AS). However, Alstrom syndrome differs in the fact that cognition is generally unimpaired. There is also an absence of polydactyly, the presence of progressive hearing loss, and dilated cardiomyopathy, neither of the latter two being usually observed in BBS. AS is also transmitted through recessive inheritance [26].

1.4.1.3 Biemond 2 Syndrome:

Mental retardation, hydrocephalus, facial dystosis, hypogonadism, polydactyly, obesity and coloboma (holes in the structures of the eye), are all major manifestations of this condition. BBS shares some of these manifestations. However, little is known about the genetic basis of this condition.

1.4.2 Oligogenic Inheritance of BBS:

BBS was initially modeled as a purely recessive trait and the syndrome typically segregates in families as a classic autosomal recessive trait. Recent data suggests an oligogenic mode of disease transmission in which mutations at different BBS loci may interact genetically to cause and/or modify the phenotype [21]. Significant genetic heterogeneity and clinical variability in BBS suggest that a second site of genetic modification is possible [21]. The second site mutations could alter the penetrance or expression of the first mutations, resulting in variable severity of the condition [21, 41]. In a few cases, it has been proposed that three mutations at two gene loci may be required for BBS expression. This would be the first example of triallelic inheritance described in humans [42]. The *BBS2* and *BBS6* genes appear to be the most frequently involved in triallelic inheritance, however, it has also been stated that the *BBS8* gene is the only one of the 12 BBS genes that does not actually participate in this phenomenon [21].
It is difficult to determine the extent to which triallelism is present in BBS but it is estimated to be a small percentage of all BBS [26].

1.4.3 Molecular Biology of BBS:

BBS is caused by mutations that cause dysfunction of basal body and ciliary proteins. Thus BBS is a ciliopathy. Research on such conditions is ongoing in a wide spectrum of diseases such as BBS, Polycystic Kidney disease, Alstrom Syndrome, Meckel Syndrome, Joubert Syndrome and many others [19]. Research of this type has recently come into vogue after experimental procedures on flagellated *C. Elegans* and *Chlamydomonas* indicated that mutations in cilia related genes caused abnormalities. It was suggested that the BBS phenotype is the result of ciliary defects at the early stages of fetal development [24, 29, 31, 33, 43]. Properly functioning cilia are of critical importance for proper development of many organs, and improper function at later embryonic stages could lead to widespread organ disorder as seen in BBS and other ciliopathies.

1.4.4 Cilia and Flagella:

Cilia and flagella are microtubule-filled, cellular extensions whose enclosing membrane is continuous with the cell plasma membrane. Although cilia and flagella are identical in structure and composition, the two names were originally coined to indicate distinctive patterns of movement and are still used. A motile cilium contains nine sets of doublet microtubules arranged in the form of a hollow cylinder that surrounds a central pair of single microtubules [44]. The internal structure of the cilia provides ATP-hydrolysis driven mechanical movement that causes the movement of the motile cilia. All of these microtubules and their associated proteins together form the axoneme, the core of the

cilia. Non-motile cilia have few microtubules or a different alignment of the outer microtubules and this less advanced structure does not allow for movement [44-46]. One fascinating feature of cilia and flagella is that the basal body, which templates the assembly of their axonemes, contains the same organelle, the centriole, in man and higher life, and is also the defining element of another microtubule-related organelle, the centrosome [44]. Remarkably, cilia are conserved across many phyla. Centrosomes, which contain a pair of centrioles, are the organizing centers for cytoplasmic microtubules in the interphase cell and for the spindle microtubules in mitotic cells. The centriole and centrosomes are key in the division of mitotic cells, and therefore key in the propagation, growth and maintenance of human cells. This creates an interesting link between the cilia related defects discussed here and the over proliferation of cells that seem to be present in the cystic kidney and the cystic pancreas[44]. Cilia are recognized as being chiefly important as mechanoreceptors in kidney epithelium, in the photoreceptors of the retina, and in planar cell polarity required for embryonic development [19, 24, 29, 41].

1.4.5 BBS is a Ciliopathy:

BBS proteins (BBS 1-8) have been shown to localize to primary cilia in model organisms such as *C. Elegans* and *Chlamydamonas*. The proteins are likely to be involved in intraflagellar transport (IFT)- which moves proteins onto the cilia, ADP rybosylation, and chaperonin activity. As many BBS orthologues localise specifically to the organisational center of the microtubules, the centrosome and the basal body (which is required for clilia

formation), it is quite likely that these proteins could be involved in ciliogenesis, cilia maintenance, the IFT, and/or microtubule dependent intracellular transport [24, 33, 43, 47, 48]. Recent studies have led to a much fuller appreciation of the fact that not only do cilia act in sensory roles at critical stages in embryonic development, but their sensory roles are essential for the normal functioning of many tissues [44]. Defects in these proteins have been shown to cause abnormal ciliogenesis. These ciliary defects in critical epithelial cells, such as those of the kidney, liver, pancreas, and other areas, may well predispose to conditions such as renal cysts, pancreatic cysts, retinal dystrophy and more complex manifestations including obesity, hypertension, and diabetes [44, 45].

Discovery of new cilia related genes that cause human disease came from studies on the mechanism of assembly of the organelles. However, there is a need for further research to continue to develop these hypotheses [44].

The BBS phenotype is consistent with the hypothesis put forward by Ansley (2003) that suggests that the vision related manifestations of BBS can be explained by a ciliary defect [24]. Dysfunction of the nodal cilium causes reduced protein transport across certain photoreceptors in the eye, which then results in retinal dystrophy. Ansley also suggested that renal conditions of BBS had a similar etiology. He proposed that failure of mechanosensation at the primary cilium of renal tubular cells causes another related condition, cystic kidney disease [24]. Mouse models of polycystic kidney disease support this condition being a ciliopathy [24]. Ansley's proposal was that a link between ciliary dysfunction and cellular response was at the heart of the pathology of BBS.

1.4.6 BBS Gene Defects Impact Later Stages of Organ Differentiation:

It is proposed that the effects of BBS gene defects occur in neurulation and gastrulation during the later development of the embryo [29, 49]. Neurulation is the period of early fetal development when the rudimentary nervous system is formed. This process creates the neural tube, which gives rise to the central nervous system and creates the neural crest, which migrates away from the dorsal surface of the neural tube, and differentiates into a diverse set of cell types [50]. Gastrulation is the developmental phase in which the three major layers of human tissue are formed. These germ layers later form into the organs of the body. Also during this phase, the primitive body plan is established. Essentially these two developmental processes are mediated by cilia, via mechanoreception between cells. Current work by Mykytyn et al. has suggested that BBS proteins serve as mediators for communication and/or intracellular transport between the cilium and the interior of the cell, and suggests that a breakdown in this communication may cause the BBS phenotype, with incompletely developed organs [48]. The outward appearance of BBS seems to also give this hypothesis weight, as BBS cases present with organs that do not appear to be fully developed; including lobulated kidneys and poly- and brachydactyly [9]. Incomplete formation of organs at the critical stages of neurulation and gastrulation could lead to endocrine dysfunction, organ malformation, situs-inversus and other problems associated with BBS. Failure of the embryo to differentiate properly is suspected to be the cause of the cardinal manifestations of BBS.

1.5 Metabolic and Renal Conditions: Diabetes, Obesity, Chronic Renal Failure and Hypertension are Prevalent and Costly:

BBS is associated with obesity, hypertension, diabetes, and chronic renal failure. These are common conditions in Canadian society today and are detrimental to health. Further study of the mechanisms and mode of inheritance of these conditions is vital to reducing the large direct costs of treatment and burden of illness.

1.5.1 Obesity:

Obesity is a morbid state which indirectly and directly cost Canadians $4.3 billion in 2001 [51]. Between 1970-1972 and 1998, the proportion of Canadian adults considered overweight or obese increased from 40.0% to 50.7% [51].

1.5.2 Diabetes:

Diabetes is a condition in Canada that has achieved epidemic proportions. 2007 estimates are that over 2 million Canadians suffer from diabetes [52]. Diabetes is a contributing factor in the deaths of approximately 41,500 Canadians each year. Diabetes and its complications cost the Canadian healthcare system 13.2 billion dollars per year [52].

1.5.3 Hypertension:

An estimated 20% of Canadians are hypertensive [53]. This condition is the single largest predictor of heart disease in North America [54]. Seventy three percent of

hypertensive patients are under the age of 65, and the condition is associated with a massive burden of illness in North American society [54].

1.5.4 Chronic Renal Failure:

While a very small percentage of Canadians have end stage renal disease (0.1%), chronic renal failure represents a great financial burden to Canada: 1.3 billion dollars of direct medical costs and 1.9 billion dollars when mortality and morbidity indirect costs are considered [55].

1.6 Etiology of Metabolic and Renal Diseases in BBS:

Diabetes, hypertension, chronic renal failure, and obesity are all expressions of the BBS phenotype [9]. New studies on BBS raise the possibility that cilia play roles in energy metabolism, blood glucose homeostasis, and regulation of blood pressure [44, 45].

1.6.1 Renal Failure:

Cilia have long been observed on kidney epithelium, and are suggested to play a major role in the proper function of nephrons. It is suggested that ciliary dysfunction in the kidney can lead to a number of defects including cysts, calyceal clubbing and tubular defects [44, 45]. In humans ciliary dysfunction is usually the cause of cystic kidney diseases [44, 56]. The kidney epithelial cilia function in a mechanosensory system that monitors and responds to fluid flow over the surface of the renal epithelial cells [44, 46,

57]. Thus, in normal kidneys, the renal cilia are sensory transducers in a cilium-generated signaling pathway.

Cilia dysfunction caused by malfunctioning BBS proteins may also be involved in early embryonic development of the nephron or in maintenance of its normal tubular structure. Thus, in the embryo, the *BBS* genes might regulate nephron morphogenesis.

1.6.2 Hypertension:

Elevated blood pressure has a hereditary component [44]. The etiology of hypertension in BBS is likely to be complex. Predisposing factors include primary renal disease, diabetes mellitus and obesity, which occur frequently in BBS. Furthermore, abnormalities in intracellular calcium signaling in vascular cells in polycystic kidney disease may predispose to hypertension [58]. It is possible that inheritance of a BBS mutation may predispose to hypertension.

1.6.3 Diabetes:

Cilia have been identified in the exocrine, endocrine, and ductal cells of the pancreas for many years [45]. The cilia in the epithelium of the pancreas are thought to be primary, non-motile, cilia and function as mechanoreceptors and chemoreceptors. It is currently thought that these cells are responsible for regulating fluid flow and ion transport, or pressure sensation in the endocrine ducts of the pancreas. Dysfunctional cilia, like those affected by mutant BBS proteins, improperly sense flow of fluid produced in the

pancreas. Ciliary defects in mice lead to ascinar cell atrophy and duct hyperplasia in the pancreas, and compromised glucose homeostatic maintenance [45]. Evidence linking diabetes and insulin resistance has been shown in another model of recessively inherited ciliopathy, namely Alstrom Syndrome. Hearn et al. indicated that ALMS1, the protein product responsible for Alstrom syndrome, a syndrome similar to BBS, affected the function and appearance of cilia in a similar manner to BBS, and caused diabetes and insulin resistance [43, 59]. BBS patients are prone to type 2 diabetes and have high insulin levels [9]. It is likely that both obesity and primary pancreatic disease contribute to insulin resistance.

1.6.4 **Obesity**:

It is not fully understood how defective cilia may lead to obesity; however, one theory is that ciliary receptors are necessary for regulating food intake and overall energy metabolism. Several cells in humans have known extracellular receptors that are also present on cilia. For example, Integrins, cell surface receptors that mediate signaling inside the cell, have been localized to the primary cilium of certain cells, where they are proposed to influence intracellular Ca2+ levels in a flow-independent mechanism [60]. Furthermore, somatostatin receptor 3102 and the serotonin receptor 5-Ht6103 are localized to neuronal cilia in the brain [44]. This establishes a link that suggests that other receptors, possibly those involved in the sensation of fullness are found on cilia or are cilia dependant. These cilia dwelling receptors could be involved in regulating food intake and overall energy metabolism. In fact, many brain neuronal cells that are

responsive to the weight-regulating protein Leptin display somatostatin 3 receptors on their neuronal cilia [61]. Insensitivity could well cause a decreased sensation of satiety and may result in habitual overeating, leading to obesity. These theories suggest that BBS obesity could come from overeating because the patients simply do not ever feel 'full'.

Recent research in mice suggests that elements of Leptin driven appetite regulatory pathway require cilia [44]. Hedgehog pathway proteins are a family of secreted signaling molecules paramount for inductive cell interactions in embryos. Huangfu reported that the Hedgehog signaling pathway was disrupted in mouse embryos with lesions in kinesin-II and two IFT particle proteins [49]. It is possible that these IFT machinery proteins play a non-ciliary role in the pathway, but one explanation for the results is that Hedgehog pathway proteins require cilia for their function [49]. This research indicates that these hedgehog proteins rely on proper ciliary function to properly differentiate tissues and organs in embryonic development and that proper function of Leptin and its pathway are dependant on properly functioning cilia. This further substantiates a potential correlation between BBS and obesity.

It is also possible that improper type II chaperonin development, resulting from mutations of several BBS genes (BBS6, *BBS10,* and *BBS12*) may well be responsible for the improper folding of the obesity protein, Leptin [44]. Improperly folded Leptin may not correctly sit in its receptor site, and therefore could cause insensitivity to Leptin.

Cilia have been observed in the fat storing cells of the liver, and also in the thyroid gland and adrenal glands [44]. Dysfunction of the former would lead to improper distribution and storage of fat, and the latter may lead to improper regulation of thermogenesis and metabolic rate, leading to a predisposition to obesity through sluggishness, hypothyroidism, or insensitivity to Leptin.

1.7 BBS Mutation Carriers may be at Risk:

Carriers of BBS have one wild type allele as well as one mutated allele. It is proposed that being a heterozygous carrier of a BBS mutation may predispose carriers to some of the clinical manifestations of BBS. Qian et al. have shown in polycystic kidney disease mice that carrying a single mutant allele led to a variety of altered homeostatic chemical levels. It was suggested that increased intracellular calcium accumulations in these models could predispose carriers to hypertension [58]. It is possible that this phenomenon that had occurred in polycystic kidney disease mice could manifest in the carriers of BBS mutations. Beales et al. determined that there was a significant increase in renal cancers and malformations in the parents of BBS children. There were similar findings in their unaffected siblings, showing a increased risk of kidney problems of seventeen and twenty fold respectively [8]. However, others have recently discovered contradictory evidence that suggests that there is no link between BBS heterozygosity and an increased prevalence of renal cancers. [62].

In recessive conditions associated with cancer or diabetes, some disease manifestations in heterozygote carriers have been suggested [8, 63]. Carriers of an autosomal recessive syndrome, Ataxia-Telangiectasa, may have a 3 to 4 fold increased risk of developing cancers [63].

Specific to BBS, obesity, renal disease, diabetes mellitus and hypertension have been identified as risks to carriers by Croft et al. [64, 65]. In 1990, Croft and Swift obtained hospital records and personal questionnaires in a single BBS family in which they discovered that heterozygous carriers for BBS were at substantial risk of renal disease, diabetes mellitus, and hypertension. Renal disease was implicated in the death of 3 of the proband's first-degree relatives, and affected one other. Five of the proband's first-degree relatives were diabetic, and 5 of the proband's first degree relatives were hypertensive. Four of the five first-degree relatives were also classified as obese.
In 1995 Croft again examined the carriers of Bardet-Biedl Syndrome mutations, assessing records and questionnaires on thirty-four parents of BBS cases, who were obligate heterozygotes. Fathers of BBS children were predominantly overweight. In fact Croft showed 26.7% of BBS fathers to be "severely overweight" (BMI exceeding 31.2). This was a 3-fold increase above the 8.9% United States national average taken from the NHANES II study [66].

Stoetzel et al. in 2007 studied the three BBS proteins corresponding to *BBS6, 10* and *12* in wild type zebrafish. They suppressed each BBS protein individually, 6 and 10

together, 6 and 12 together, 10 and 12 together, and all three together. They found evidence that the proportion of improperly developed fetuses was dependant on the sum effect of the suppression of the BBS proteins. These findings suggest that there is a "dose dependant" effect to BBS12, 10, and 6, which also suggests that carrying a mutant allele for BBS could lead to clinical BBS manifestations.

These aforementioned studies do suggest a link between being a carrier of BBS mutations and predisposition to the clinical outcomes associated with BBS. However, the clinical studies are based only on single families or small sample sizes, and are not properly controlled. In addition, the animal studies may not extrapolate to humans.

1.8 Clinical Epidemiology of BBS in Newfoundland:

The research published by Harnett et al, Green et al, O'Dea et al provided the initial clinical information on BBS in the Newfoundland population [4, 9, 16, 18]. Moore et al. extended the BBS phenotype, determined if BBS and LMS were the same disorder, described the genetic epidemiology of BBS, and determined whether there were genotype/phenotype correlations for BBS. These reports established wide spread systemic manifestations observed across the BBS cohort, irrespective of genotype and included substantial endocrine and renal disease [9].

To assess phenotype and outcomes for the BBS cases, Moore et al. evaluated 38 of the 46 BBS patients in clinic, and reviewed the medical charts of all 46 patients. Anthropometric

measures were taken of head, face, ears, hands, feet, and of the genitals. Dysmorphic features were compared to norms created by Hall et al. 1995, and scored by clinical geneticists [67]. A neurologist examined 7 patients and 19 patients were assessed on standardized and diadochokinetic speech tests. Psychiatric evaluation and verbal IQ scores were obtained. Laboratory measures of blood urea, creatinine and random glucose were determined on whole blood drawn from the subjects. Renal ultrasound scans were performed to assess structural abnormalities.

Green et al. [4] established that retinal dystrophy leading to blindness, dystrophic extremities, obesity, renal abnormalities, and genital and reproductive abnormalities were the cardinal manifestations of BBS, and that diabetes mellitus, hypertension, and renal failure occurred frequently. Moore et al [9] extended the phenotype to neurological abnormalities, speech disorders, psychiatric abnormalities, gallstone disease, colonic disorders, asthma, congenital heart disease, other disorders such as epilepsy/thyroid disease, and early death. There were no significant differences between genotypes for any of these morbidities.

1.9 Relevance of Proposed Research:

Bardet-Biedl Syndrome cases have increased incidence of four major conditions common in the community: obesity, hypertension, diabetes and chronic renal failure [4, 9, 16, 18, 27]. Our research provides more precise estimates of risk because of the long prospective follow up of cases. What is not established, is the risk of obesity, hypertension, diabetes,

and chronic renal failure in first degree and other relatives who carry a BBS mutation. This investigation will test the hypothesis that there is greater risk of these morbid events in carriers of BBS mutations as compared to their relatives who do not carry a BBS mutation.

For relatives of those with BBS, it is important that they know if they may be predisposed to BBS related morbidities. Given the case rate of BBS in the Newfoundland population, and the likelihood that many of the BBS alleles occur in other members of the population it is possible to hypothesize that haploinsufficiency (the state of an organism having only a single normal copy of a particular gene) of a BBS protein may contribute to a high rate of chronic diseases in the Newfoundland population [27]. This research can also contribute to the investigation of the genetic nature of obesity, hypertension, diabetes, and chronic renal impairment.

This is important because the ciliary / basal body / centrosome cellular apparatus is complex and dependant on the normal function of multiple proteins. Consequently many mutations in the genes controlling this function may occur in the community.

1.9.1 Institutions:

This study was performed under the auspices of the Patient Research Center, at Memorial University, St John's, Newfoundland and was approved by the Human Investigation Committee of the Faculty of Medicine; Memorial University. The work was sponsored by the Janeway Foundation, and Genome Canada.

1.9.2 Objectives of this Study:

This study characterizes the risk of diabetes, hypertension, chronic renal failure, and obesity in the Newfoundland BBS carriers by comparing them to other relatives of BBS cases who do not have a BBS mutation. This is the first study of its type in this population.

Objectives:

1) To describe the incidence of endocrine and renal events in a large group of BBS cases followed for 28 years.

2) To characterize the risk of metabolic and renal conditions in the carriers of BBS mutations.

3) To determine the relative risk of these events in BBS cases and in carriers of BBS mutations based on their BBS genotype.

2 Methods:

2.1 Recruitment of Cases:

The cases of BBS in Newfoundland have been extensively studied since the initial recruitment of patients to the study in 1983-1985 through the Canadian National Institute for the Blind, and ongoing referrals from Ophthalmologists and the Provincial Medical Genetics program. Protocol driven assessments have taken place in 1988 [16], [4], 1993 [18] and in 2001 [9].

A fourth assessment of the cases was performed in 2008. At this time medical charts of the BBS cases were reviewed, blood pressure was measured, and blood urea, serum creatinine, random glucose, and Hemoglobin A1c tests were performed.

The current BBS case cohort consists of 46 individuals (26 males and 20 females) from 26 families. Consanguinity was documented in 27% of families (7/26) and suspected in another 15% (4/26).

2.2 Recruitment of relatives:

Twenty-one of 26 families participated in the gene discovery research program, of whom 20 families participated in the current study (Fig 2). Participants included siblings and parents of cases, together with some sibships of parents who requested to enroll in the study. Of 138 first degree relatives of cases, 78 participated in the study, 23 were dead and 37 did not participate. Of 107 siblings of parents, 55 participated, 24 were dead and

28 did not participate. Thus of 198 living eligible relatives 67% (N=133) participated in the study. DNA was obtained from 130 relatives and a further 3 were obligate carriers. Fig 3 illustrates the sibships enrolled in the study by carrier status.

Dr. Elizabeth Dicks and Michael Webb organized research clinics in the community of each family. Standardized histories, physical examinations and blood pressure were undertaken by Dr. Dicks or Mr. Webb. Physical test performed by the team entailed height and weight measurements, blood taken for serum creatinine, blood urea, blood sugar and Hemoglobin A1C, and DNA extraction.

Figure 2: Ascertainment of BBS Family Members:

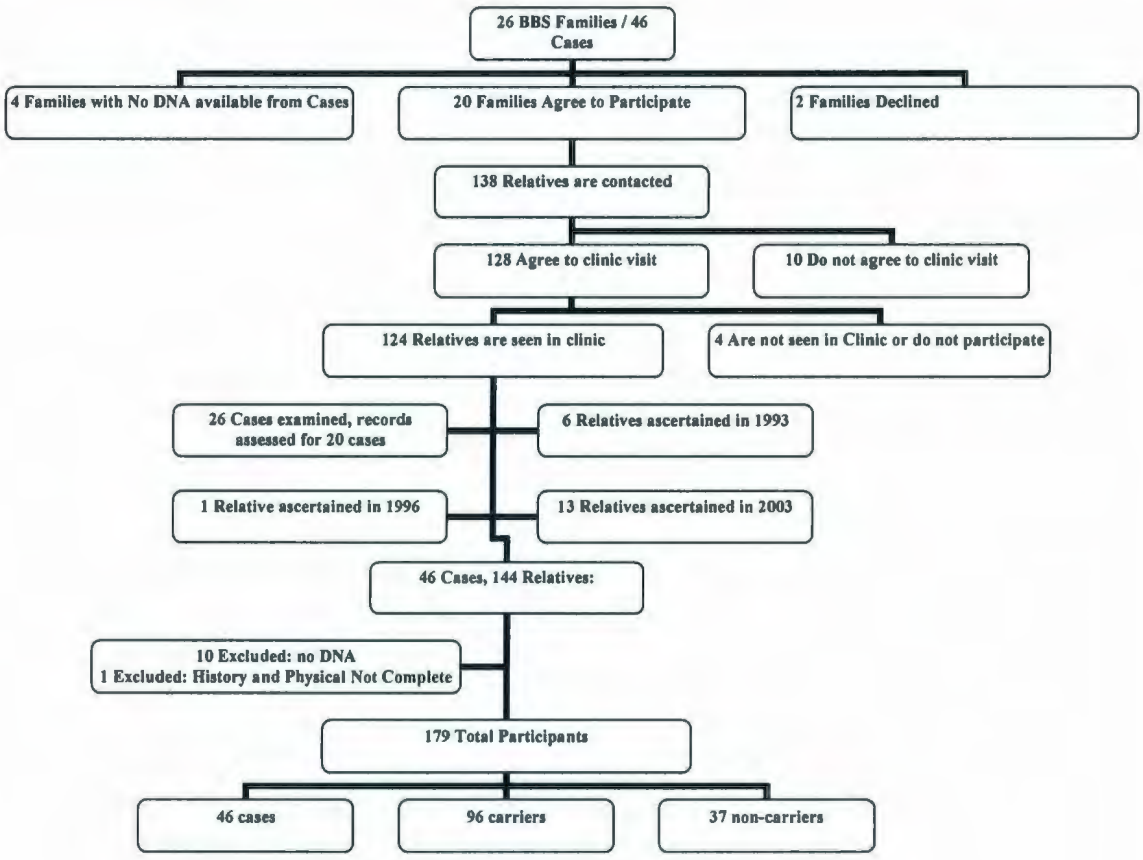
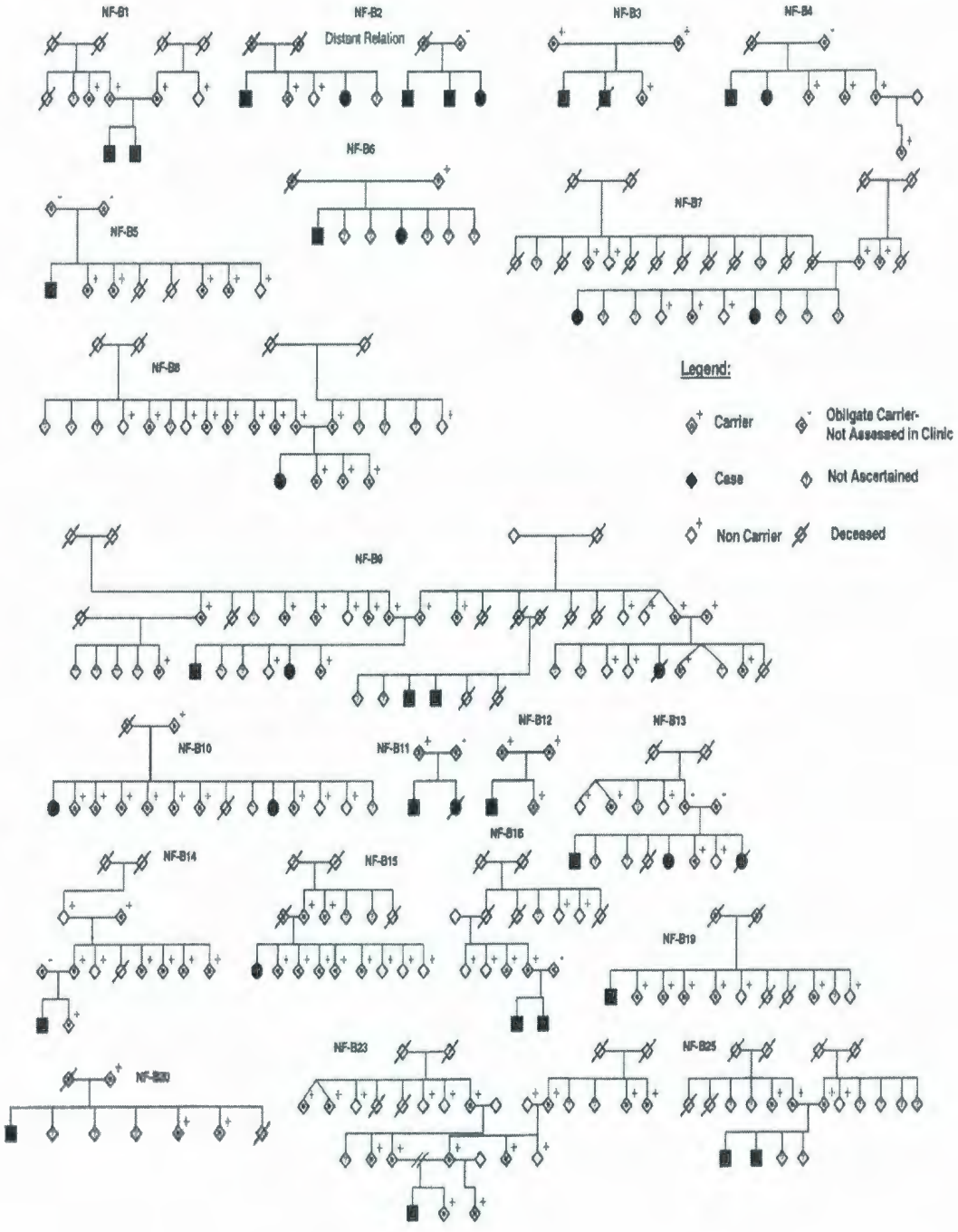


Figure 3: BBS Family Pedigrees:



2.3 Participant Assessment

Blood Pressure was taken in both a sitting and standing position, and the left arm was used for every measure. Blood pressure was measured at least twice in each position. Care was taken to ensure that the same arm was used for every study subject where possible. A standard blood pressure cuff and stethoscope were used to assess blood pressure. Patients were seated comfortably for an extended period prior to blood pressure measures being taken.

Several questions regarding blood pressure were asked in the history assessment. Patients were asked to recall if they had ever been diagnosed with hypertension. Participants were also asked if they were currently taking any medications to control blood pressure, and if they answered in the affirmative, the age they had begun therapy.

Measurements of height in centimeters, and weight in kilograms were taken using a standard tape measure, and a calibrated scale respectively. The height and weight measures were used to calculate a body mass index.

Serum creatinine assessed in the clinical laboratory of the Health Sciences Centre was used to calculate glomerular filtration rate using the MDRD (modification of diet in renal disease) equation. This estimate is reliable and valid [68, 69].

The Hemoglobin A1c test was also measured in the clinical laboratory of the Health Sciences Centre. Patients were asked if they had been diagnosed with diabetes. If so,

they were asked to recall at what age they had been told this, what kind of medication, if any, they were taking and at what age they had started treatment.

2.4 Mutation detection

A collaborative novel gene discovery program was undertaken between 1995 and 2004 [70,71,72] which lead to the discovery of BBS6 [34], BBS5 [47] and BBS3 genes [73]. In addition mutations causing BBS1, and BBS2 were identified [72, 74]. Following the discovery of BBS10, the remaining 4 Newfoundland families of unknown genetic etiology were confirmed in France to have mutations in BBS10. (Personal communication: Dr. H. Dollfus).

DNA was extracted from whole blood by a standard salting out method. DNA was amplified by the polymerase chain reaction (PCR) method and electrophoresis was performed. The PCR product was then purified. For all BBS genes except BBS3 cycle sequencing was undertaken, and sequencing products were precipitated. Sequencing was performed using the capillary based ABI 3130 XL. The resultant sequences were analysed using the Mutation Surveyor v3.2 program. To determine carrier status in the BBS3 family, a restriction enzyme assay was performed. (NEB cutter, V2.0 from New England Biolabs. http://tools.neb.com/NEBcutter2/index.php).

In 2004, the DNA from 130 family members and from 25 BBS cases was analyzed for the 9 different mutations present in Newfoundland BBS families. The mutations are as follows: BBS1: M390R; BBS2: Y24X; BBS3: G169A; BBS5: 1VS6+3A>G; BBS6:

F94fs X103, D143fs X157 and L227P; BBS10: C91fsX95 and, F198del, F199del. Three family members were obligate carriers. Of 1170 mutation analyses that were performed on relatives of BBS patients, 5 (0.48%) did not provide a conclusive result. Four carriers of a BBS mutation had these 5 inconclusive results. None of these 5 inconclusive analyses were for the mutation that was present in the relatives' family. Of 225 tests in 25 cases there were 4 (1.8%) inconclusive analyses.

2.5 Ethics:

The Human Investigation Committee (HIC) of the Faculty of Medicine, Memorial University of Newfoundland and Labrador approved this research project on April 28, 2004.

Assessment of the cases was approved by the Memorial University Human Investigation Committee and the Simon Fraser University Research Ethics Board prior to the investigations of Moore et al. [9]

2.6 Norms and Definitions:

The definitions used in this study are the same as those used in the report of Moore et al., 2005.

2.6.1 BBS:

Presence of at least four of the cardinal features (retinal dystrophy, obesity, renal abnormalities, male hypogenitalism, dystrophic extremities) or three cardinal manifestations in a sibling of an affected person with four cardinal features [10].

2.6.2 Obesity:

A body mass index, calculated from the height and weight measurements taken in the physical exam, of 30 or greater kg/m^2 [75]

2.6.3 Hypertension:

Hypertension was defined as sitting systolic blood pressure of greater than or equal to 140mm of Hg or a diastolic blood pressure of greater than or equal to 90mm of Hg in patients not taking antihypertensive drugs, or being on antihypertensive medications.

2.6.4 Chronic Renal Impairment/ End Stage Renal Disease:

Chronic renal failure stages was defined as an estimated creatinine clearance <60 ml/min using the MDRD formula [68, 69]. Patients were considered as being End Stage Renal Disease if they were on dialysis, or had received a kidney transplant.

2.6.5 Diabetes mellitus:

Patients were considered to be diabetic if they were currently undertaking hypoglycemic therapy (diet/oral medication/insulin) for diabetes mellitus, had met diagnostic criteria of

the 1998 clinical practice guidelines for the management of diabetes in Canada [76] or had a hemoglobin A1c above 6%.

2.6.6 Age of onset:

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 history, or when diagnostic tests indicated that they were hypertensive, diabetic, or in renal failure.

2.7 Analysis:

Mean BMI and blood pressure in carriers and non-carriers were compared using the two-tailed Student's t-test. Cumulative probability of having an event over time was calculated using Kaplan Meier Analysis in cases, carriers and non-carriers. Cox regression was performed for each clinical endpoint to assess the hazard in a) cases with BBS compared to non-carriers, (b) carriers of a BBS mutation compared to non-carriers. The exponent of Beta coefficient and 95% confidence intervals were calculated. Statistical significance was P value < 0.05. The denominator used in the calculation of proportions for clinical endpoints varied, depending on the number of people available for testing. Statistical analysis was performed on SPSS, by Michael Webb.

3 Results:

3.1 Cases of BBS:

Forty-six cases from 26 families were identified. Of 153 siblings 30% had BBS. DNA was obtained from members of 21 families, and the molecular genetic cause of the disease was identified in all of these families (Table 1). Nine mutations in 6 BBS genes were identified.

Four BBS1 families, homozygous for the M390R mutation, clustered on the southwest coast (Fig 3). Cases from four BBS6 families, homozygous for the F94fsX103 mutation, were from the same bay (Conception Bay). However cases from 2 BBS6 families homozygous for D143fsX157 mutations were from distinct regions. Two BBS10 families, homozygous for C91fsx95, were from communities geographically distant from each other (Seal Cove and Green Bay). Individual families with homozygous mutations in BBS2, BBS3 and BBS5, and compound heterozygotes of BBS6 and BBS10 were distributed randomly around the coast of the island.

One of 9 BBS1 cases was a heterozygous carrier of the BBS3 mutation. One of the 15 BBS6 cases was a heterozygous carrier of the BBS1 mutation. One of the BBS3 cases was a heterozygous carrier of the BBS1 mutation.

At death or last follow up, the cases were younger (40.8 years of age) than carriers (53.8 years) and non carriers (56.8 years). Fifty seven per cent of cases were male, as were 43% of carriers, and 46% of non carriers. Of 96 carriers of a BBS mutation BBS 1 accounted for 47%, BBS2 for 7%, BBS 3 for 2%, BBS 5 for 14%, BBS 6 (through its 3

different mutations) for 25%, and BBS 10 for 5% (Table 1). In one BBS1 family (NF-B10) 3 members carried both a BBS1 and BBS3 mutation, none of whom presented with any of the cardinal manifestations of BBS. These carriers were categorized according to the homozygous mutation seen in the BBS cases present in their family (BBS1) (Table 2).

3.2 Body Mass Index

The body mass index in adults with BBS was significantly higher than that of non carriers (38±12 v 29±3). (<.0001) The mean BMI of carriers and non carriers were similar (28±6 v 29±3). When analyzed by genotype no differences were observed in either the cases or carriers (Table 10).

3.3 Hypertension

In cases, 33 of 46 (72%) were diagnosed with hypertension, all of whom were treated with antihypertensive medication. The median age to onset of hypertension treatment was 34 years (95% CI = 31-38) (Table 4). Compared to non carriers, cases were 7 times more likely to develop hypertension (Table 4).

In carriers, 52 of 96 (54%) were diagnosed with hypertension, of whom 40 (77%) received antihypertensive medication. The median age to hypertension diagnosis in carriers was 57 years (95% CI = 54-61) (Table 4) and median age to hypertension treatment was 63 years (58-68 years) (Table 5).

In non carriers, 17 of 35 (49%) were diagnosed with hypertension, 15 (82%) of whom were treated. The median age to hypertension diagnosis in non carriers was 67 years (95% CI = 59-72) (Table 4) and age to hypertension treatment was 67 years (95% CI = 59-75). (Table 5) No significant difference in the incidence of hypertension between the carriers and non carriers was observed. (Figs. 5&6)

The mean systolic blood pressure in untreated carriers was 124±14 mm Hg and in non carriers 119±11 mm Hg respectively. The mean diastolic blood pressure in carriers and non carriers was 79±8 mm Hg and 77±8 mm Hg respectively.

No differences in the incidence of hypertension or treated hypertension were observed when carriers were analyzed by genotype (Table 10).

3.4 Diabetes Mellitus

In cases, 23/46 (50%) had been diagnosed with diabetes mellitus, all of whom were treated by insulin, oral hypoglycemic agents or diet. The median age of diagnosis of diabetes was 43 years (95% CI 39-48). There was an 18 fold increased risk of diabetes in cases compared to non carriers (Table 6).

No significant difference was observed in the incidence of diabetes comparing carriers and non carriers (Fig 7). In carriers 16/93 (17%) were diagnosed with diabetes, of whom 12 (75%) were receiving treatment. The median age to onset of diabetes mellitus in the carriers was 75 years. In non carriers 8/34 (24%) were diagnosed with diabetes mellitus,

of whom 7 (88%) were receiving diabetes treatment. By age 70, 45% of non carriers had developed diabetes. (Table 6)

There was no difference in the incidence of diabetes in carriers of a BBS mutation, when classified by genotype (Table 10).

3.5 Chronic Renal Failure:

In cases of BBS, 20/43 (47%) developed chronic renal failure stage 3. Median age to onset of CKD stage 3 was 58 years (95% CI 53-64) (Table 7). There was a 15 fold increased risk of CKD in cases compared to non-carriers (Table 7).

Six cases of BBS developed end-stage renal disease. By age 60 years 13% of the population had developed end-stage disease. (Figure 9)

No significant difference in the incidence of stage 3 CKD comparing carriers and non carriers was observed (Fig. 8). In carriers 8/76 (11%) were diagnosed with stage 3 CKD. The median age of onset of chronic renal failure was 86 years (95% CI 81-91) (Table 7). In non carriers 5/33 (15%) were diagnosed with stage 3 CKD, with median age to onset being 81 years (95% CI 63-99). (Table 7)

When analyzed by genotype, no differences in the incidence of chronic kidney disease in carriers were found. (Table 10)

3.6 First Renal or Metabolic Illness

There was no significant difference in incidence between the carriers of BBS and the non carriers for at least one of the renal or metabolic outcomes that they were tested for. However, there is an 11 fold hazard of composite renal and metabolic illness in BBS cases versus non carriers (Table 8).

In 55/96 (57%) of carriers there was at least one renal or metabolic illness diagnosed. The median age of the first diagnosis in composite illness was 57 years (95% C.I. 53-61) (Table 8).

Non carriers also had a large proportion of participants, 19/37 (51%), with a diagnosis of at least one renal or metabolic illness. The median age of the first diagnosis of renal or metabolic illness in this group was 64 years (95% C.I. 58-70) (Table 8).

Thirty-nine of forty-six (85%) BBS cases had a diagnosis of at least one renal or metabolic illness. The median age of the first diagnosis of renal or metabolic illness in the cases was 32 years (95% CI 30-34) (Table 8).

When analyzed by genotype, no differences were observed in either the cases or the carriers.

3.7 Survival in BBS:

Twelve BBS cases died, with median survival being 63 years (95% CI=62-64). Cumulative mortality at age 40 was 12%, at age 50 it was 20% and at age 60 it was 25% (Fig. 11 & Table 9).

Table 1: BBS Families in Newfoundland by Genotypes

Mutation	Family Identification	Cases n=46	Carriers n=96	Non Carrier of familial mutation n=37
BBS1: M390R	B7, B8, B10, B15, B19, B23	8 Homozygous	45	18
BBS2: Y24X	B14	1 Homozygous	7	2
BBS3: G169A	B2	5 Homozygous	2	1
BBS5: IVS6+3A>G	B9	5 Homozygous	13	6
BBS6: D143fsX157	B13, B20	4 Homozygous	5	3
BBS6: F94fsX 103	B3, B4, B16, B25	8 Homozygous	13	5
BBS6: D143fsX157 / F94fsX103	B1	2 Compound Heterozygous	3	1
BBS6: F94fsX103 L227P	B5	1 Compound Heterozygous	3	1
BBS10: C91fsX95	B21, B12	4 Homozygous	5	0
BBS10: F198 del/ F199 del/ C91fsX95	B6, B11	2 Compound Heterozygous	0	0
Unknown/No DNA	B17, B18, B22, B24, B26	6	0	0
Total	**26**	**46**	**96**	**37**

Figure 4: Geographic Distribution of BBS in Newfoundland:

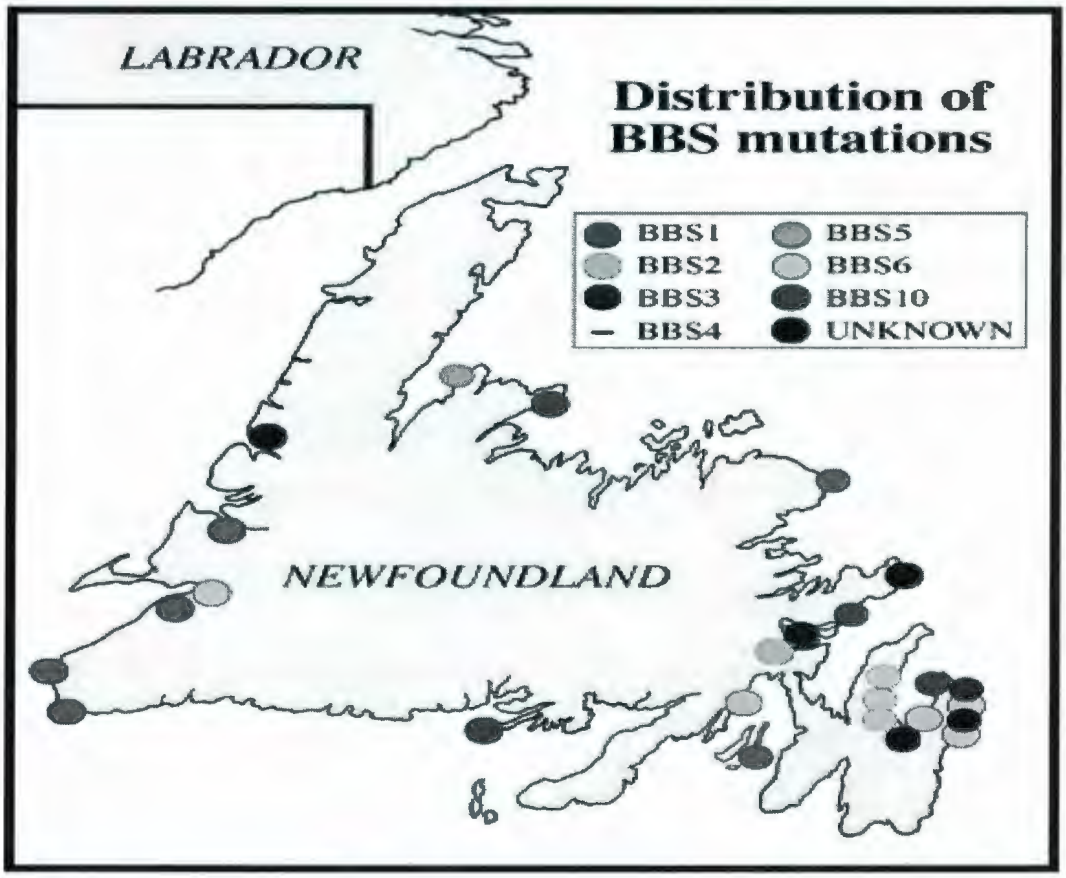


Table 2: Additional BBS Mutations That Do Not Match Family Genotype:

Family Identification	Mutation Seen in family Case	BBS Mutations observed in Relative of BBS Case
B10	BBS1	BBS 1 and 3
B10	BBS1	BBS 1 and 3
B10	BBS1	BBS 1 and 3

3.8 Analysis of Clinical Outcomes in Cases, Carriers and Non Carriers

Table 3: Measured Clinic Variables in Newfoundland Subjects

	Cases	Carriers	Non Carriers
	Mean / <S.D.> Freq. / (%)	Mean / <S.D.> Freq. / (%)	Mean / <S.D.> Freq. / (%)
Height (m) Adults age≥ 18	**n=38 1.63 / <0.10>**	**n=75 1.67 / <0.08>**	**n=30 1.68 / <0.09>**
Mass (kg) Adults age ≥ 18	**n=39 99.4 / <31.2>**	**n=73 79.4 / <17.6>**	**n=30 81.4 / <12.9>**
Adult (≥18) Body Mass Index (kg/m^2)	**n=38 37.8 / <11.7>**	**n=73 28.4 / <5.6>**	**n=30 28.7 / <2.6>**
30-35 kg/m2	**n=30**	**n=15**	**n=12**
≥35 kg/m2	**n=16**	**n=6**	**n=0**
Hypertensive Subjects	**33/46 (72%)**	**52/96 (54%)**	**17/35 (49%)**
Prescribed Medication	**33/33 (100%)**	**40/52 (77%)**	**15/17 (82%)**
Hypertensive in Clinic	**0/34 (0%)**	**12/52 (23%)**	**2/17 (18%)**
Subjects with Chronic Renal Failure	**20/43 (47%)**	**8/76 (11%)**	**5/33 (15%)**
Diabetic Subjects	**23/46 (50%)**	**16/93 (17%)**	**8/34 (24%)**
Prescribed Medication	**23/23(100%)**	**12/16 (75%)**	**7/8 (88%)**
Diabetic in Clinic	**0/23 (0%)**	**4/16 (25%)**	**1/8 (12%)**
Subjects with Diagnosis of Renal or Metabolic Illness	**39/46 (85%)**	**55/96 (57%)**	**19/37 (51%)**
Systolic B.P. (Normotensive or no previous Hypertension Diagnosis/Unmedicated Subjects) mm Hg	**N/A**	**n=43 123.7 / <13.7>**	**n=17 119.4 / <11.4>**
Diastolic B.P. (Normotensive or no previous Hypertension Diagnosis/Unmedicated Subjects) mm Hg	**N/A**	**n=43 78.6 / <8.2>**	**n=17 76.5 / <7.9>**

n= subjects for which there was available clinical data for the indicated examination.

Figure 5: Time to Hypertension in Cases, Carriers and non Carriers of Newfoundland BBS mutations:

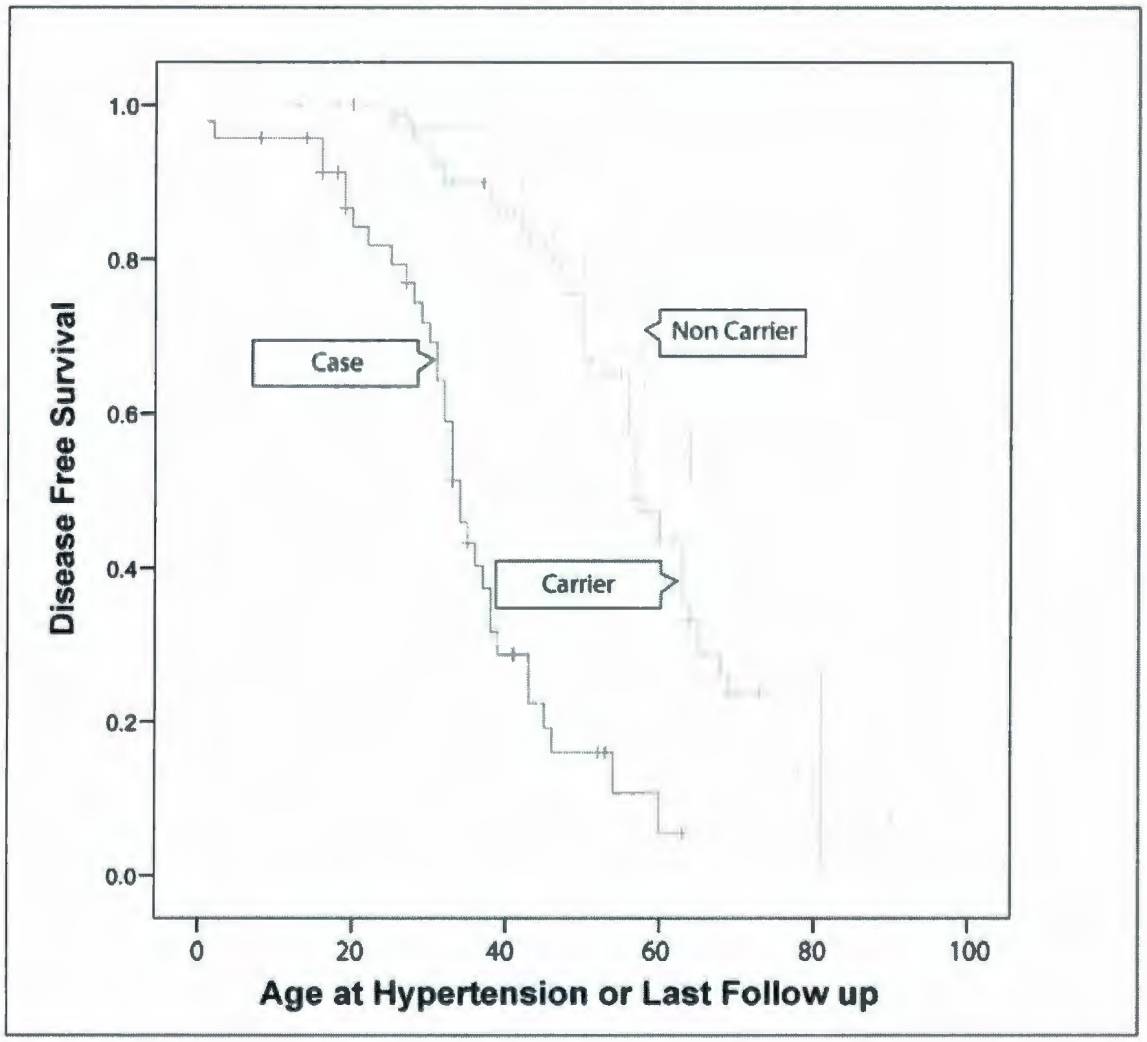


Figure 6: Time to Treated Hypertension in Cases, Carriers and non Carriers of Newfoundland BBS mutations:

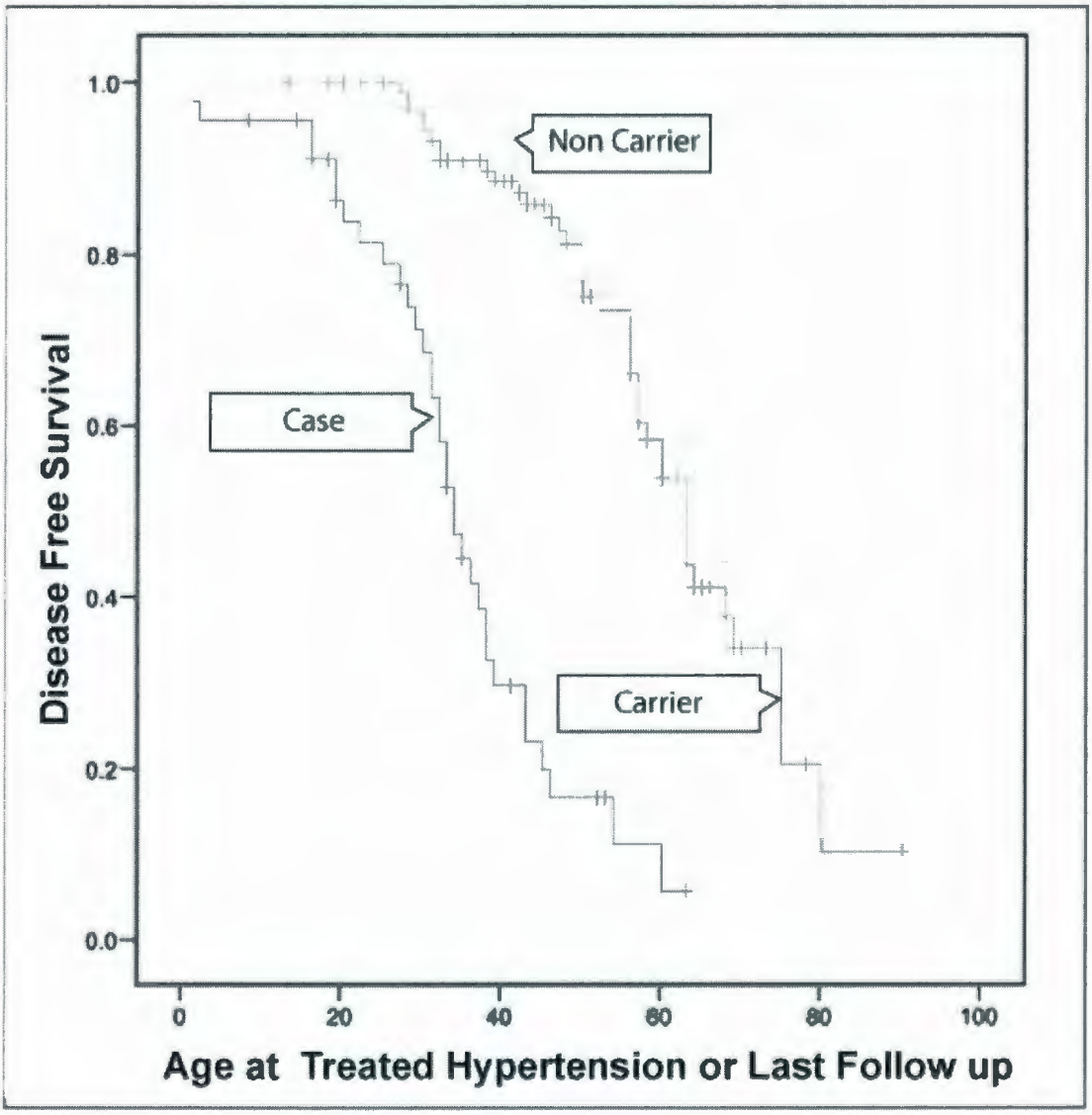


Table 4: Cumulative Events and Hazard Ratios for Hypertension comparing Cases, Carriers and non Carriers of Newfoundland BBS mutations:

Mutation Presence	Events	Cum. Events at 30	Cum. Events at 40	Cum. Events at 50	Cum. Events at 60	Cum. Events at 70	Mean Age of Event	Median Age of Event <95%CI>	Hazard Ratio <95% CI>
Carrier (n=96)	52	6.7%	13.7%	33.4%	56.4%	76.3%	58.9	57 <53.5-60.5>	1.45 <0.84-2.53>
Non Carrier (n=35)	17	2.8%	5.8%	22.6%	36.9%	56.0%	63.8	67 <58.7-72.3>	
Case (n=46)	33	30.7%	72.1%	84%	94.7%	94.7%	35.3	34 <30.5-37.5>	7.34 <3.93-13.71>

Table 5: Cumulative Events and Hazard Ratios for Treated Hypertension Comparing Cases, Carriers and non Carriers of Newfoundland BBS mutations:

Mutation Presence	Events	Cum. Events at 30	Cum. Events at 40	Cum. Events at 50	Cum. Events at 60	Cum. Events at 70	Mean Age of Event	Median Age of Event <95% CI>	Hazard Ratio <95% CI>
Carrier (n=96)	40	5.6%	14.5%	25%	46.2%	66.2%	62.6	63 <58.3-67.7>	1.24 <0.68-2.25>
Non Carrier (n=35)	15	2.8%	5.8%	25.6%	36.9%	56%	64.6	67 <58.7-75.3>	
Case (n=46)	33	30.7%	72.1%	84%	94.7%	94.7%	35.3	34 <30.5-37.5>	7.76 <4.05-14.9>

Figure 7: Time to Diabetes Mellitus in Cases, Carriers and non Carriers of Newfoundland BBS mutations:

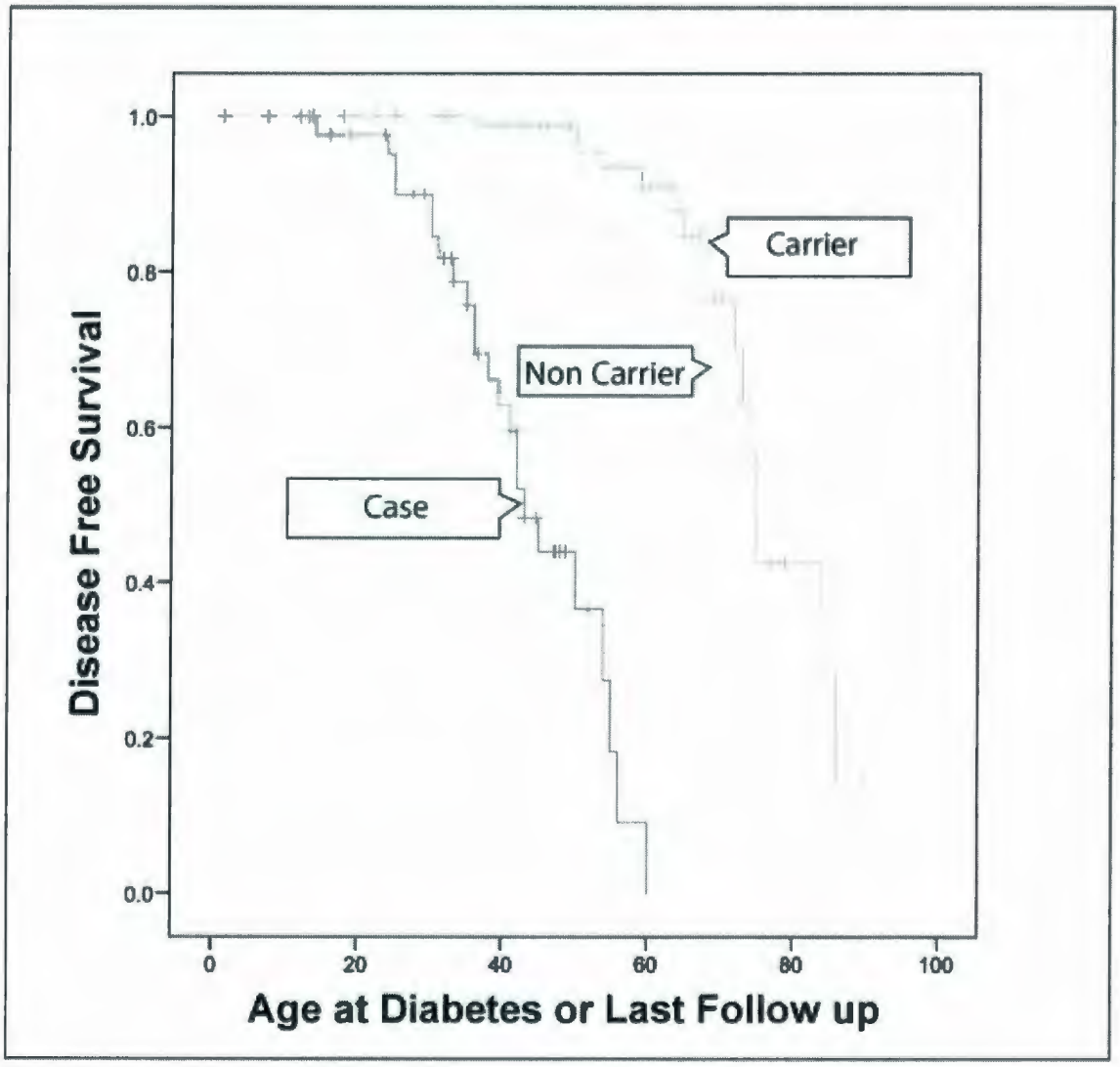


Table 6: Cumulative Events and Hazard Ratios for Diabetes Mellitus comparing Cases, Carriers and non Carriers of Newfoundland BBS mutations:

Mutation Presence	Events	Cum. Events at 30	Cum. Events at 40	Cum. Events at 50	Cum. Events at 60	Cum. Events at 70	Mean age of Event	Median age of Event <95% CI>	Hazard Ratio <95% CI>
Carrier (n=93)	16	0	1.2%	2.9%	9.0%	23.4%	76.2	75 <73.3-76.7>	0.71 <0.30-1.70>
Non Carrier (n=34)	8	0	0	7.1%	20.7 %	45.1%	72.7	<n/a>	
Case (n=46)	23	15.1 %	36.7 %	63.1 %	100 %	100%	44.0	43 <38.5-47.5>	18.16 <6.84-48.18>

Figure 8: Time to Chronic Renal Failure in Cases, Carriers and non Carriers of Newfoundland BBS mutations:

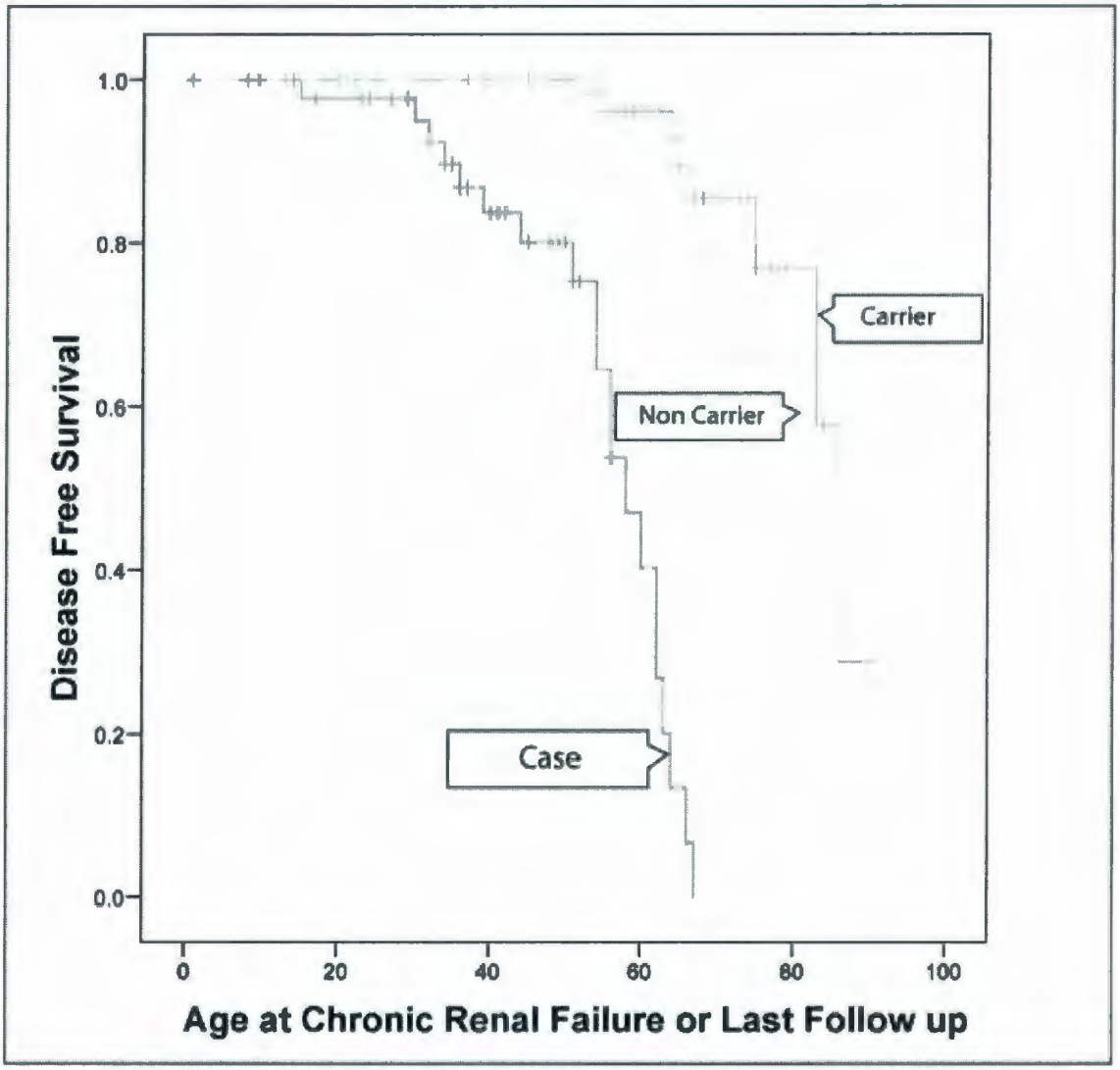


Table 7: Cumulative Events and Hazard Ratios for Chronic Renal Failure comparing Cases, Carriers and non Carriers of Newfoundland BBS mutations:

Mutation Presence	Events	Cum. Events at 30	Cum. Events at 40	Cum. Events at 50	Cum. Events at 60	Cum. Events at 70	Mean age of Event	Median age of Event <95% CI>	Hazard Ratio <95%-CI>
Carrier (n=76)	8	0	0	0	3.9%	14.5%	82.8	86 <81.4-90.6>	0.59 <0.19-1.19>
Non Carrier (n=33)	5	0	0	0	4.5%	21.2%	76.9	81 <62.6-99.4>	
Case (n=43)	20	5%	16.3%	20%	59.6%	100%	54.7	58 <52.5-63.5>	15.06 <5.06-44.84>

Figure 9: Time to End Stage Renal Disease in Cases of BBS:

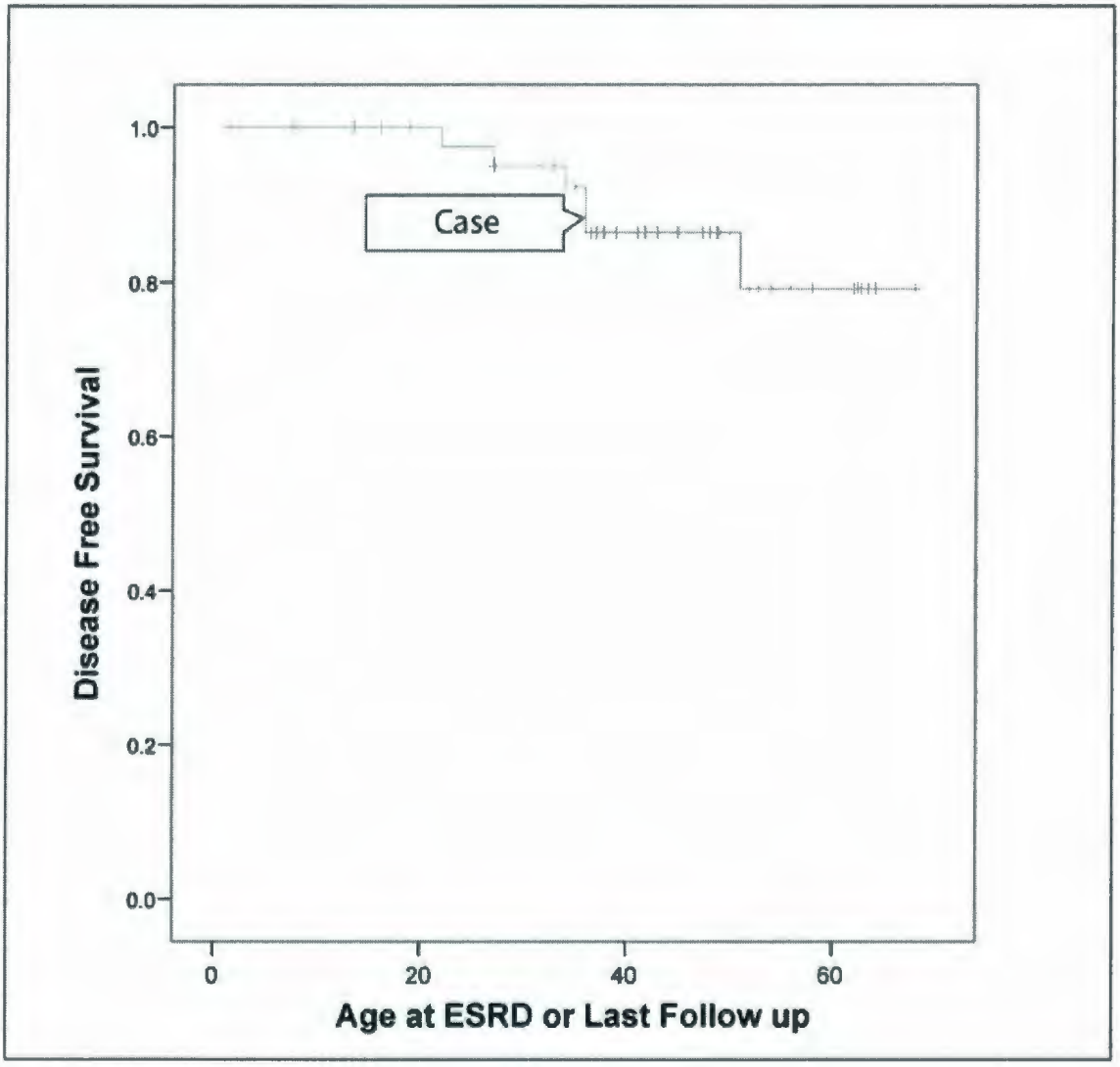


Figure 10: Time to Composite Renal and Metabolic Illness in Cases, Carriers and non Carriers of Newfoundland BBS mutations:

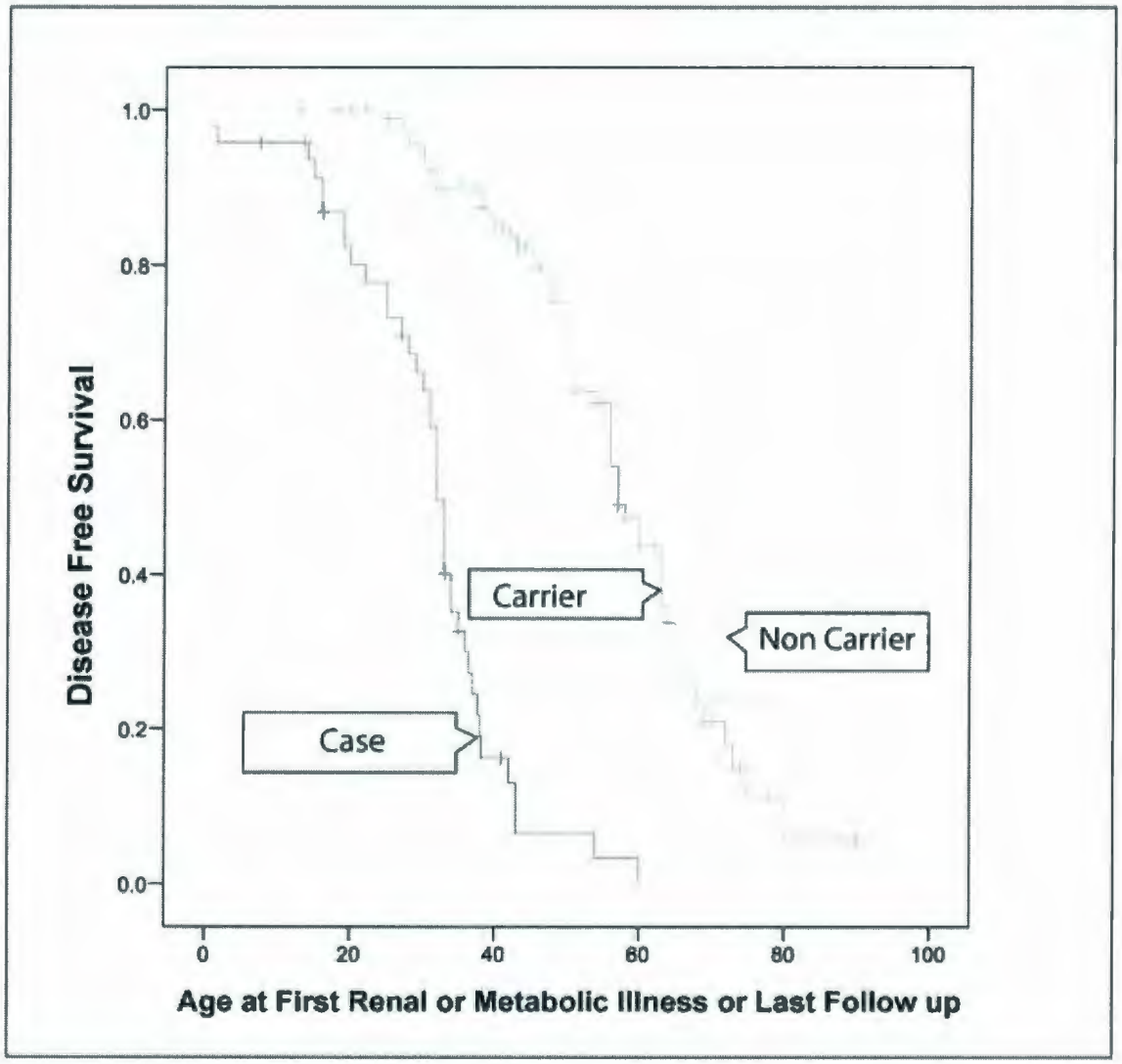


Table 8: Cumulative Events and Hazard Ratios for Composite Renal and Metabolic Illness comparing Carriers and non Carriers of Newfoundland BBS mutations:

Mutation Presence	Events	Cum. Events at 30	Cum. Events at 40	Cum. Events at 50	Cum. Events at 60	Cum. Events at 70	Mean age of Event	Median age of Event <95% CI>	Hazard Ratio <95%CI>
Carrier (n=96)	55	6.7%	15.1%	33.4%	56.3%	79.1%	58	57 <53.1-60.9>	1.38 <0.81-2.33>
Non Carrier (n=37)	19	2.8%	8.6%	21.7%	38.6%	50.8%	60	64 <58.1-69.9>	
Case (n=46)	39	31.7%	83.3%	93.5%	100%	100%	31.2	32 <30.2-33.8>	11.33 <6.11-21.0>

Figure 11: Time to Death in Cases of BBS:

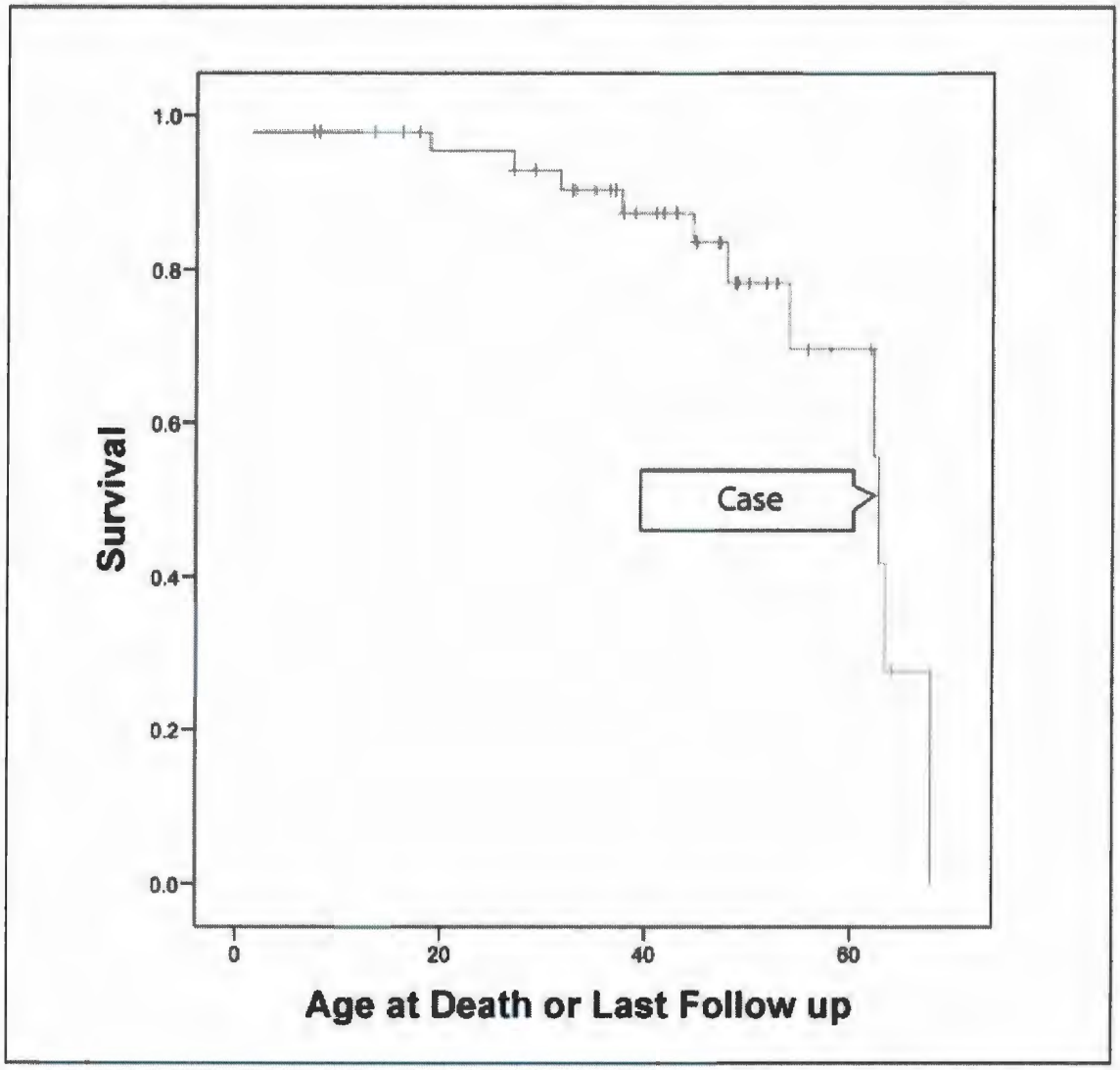


Table 9: Mortality in Cases of Newfoundland BBS

	Events	Cum. Events at 30	Cum. Events at 40	Cum. Events at 50	Cum. Events at 60	Cum. Events at 70	Mean age of Event	Median age of Event <95% CI>
Case n=46	12	6.8%	12.3%	20%	25.3%	100%	58.6	63 <61.6-64.4>

3.9: Genotype Analysis of Carriers and Cases:

Table 10: Renal and Metabolic Event Rate in Carriers and Cases by Genotype

Measure In Clinic	BBS 1		BBS 5		BBS 6		All other BBS Genotype		Unknown Mutation	Comparison Amongst Genotype: p=	
Distribution in Carriers/ Cases	**45**	**8**	**13**	**5**	**24**	**15**	**14**	**12**	**6 Cases**	**Car.**	**Case**
	Mean/S.D.		**Mean/S.D.**		**Mean/S.D.**		**Mean/S.D.**		**Mean/S.D**		
Adult Body Mass Index (kg/m^2)	n=38 29.1/ <5.4>	n=7 36.5/ <3.4>	n=12 27.5 / <4.2>	n=5 46.1/ <17.4>	n=12 27.8 / <2.6>	n=10 39.5/ <17.0>	n=11 28.1 / <9.5>	n=4 35.1/ <6.6>	n=5 33.6/ <6.2>	.812	.407
Subjects with Untreated Diabetes or no diagnosis of diabetes HbA1C (%)	n=34 5.4 / <0.4>	n/a	n=8 5.4 / <0.6>	n/a	n=12 5.2 / <0.6>	n/a	n=7 5.2 / <0.5>	n/a	n/a	.542	
Diabetes by age 70	n=3/42 7%	n=4/8 50%	n=3/13 23%	n=2/5 40%	n=2/24 8%	n=7/15 47%	n=1/14 7%	n=7/12 58%	n=3/6 50%		
Hypertension by age 70	n=27/ 45 60%	n=7/8 88%	n=5/13 38%	n=4/5 80%	n=9/24 37.5%	n=10/15 67%	n=8/14 57%	n=8/12 67%	n=4/6 67%		
Chronic Renal Failure by age 70	3/43 7%	2/8 25%	1/13 8%	4/5 80%	1/19 5%	5/14 36%	0/11 0%	5/12 42%	4/6 67%		
Systolic B.P. (Normotensive and Unmedicated Subjects) mm Hg	n=22 127.0/ <13.7>	n/a	n=7 123.4/ <18.1>	n/a	n=8 118.5/ <9.6>	n/a	n=6 119.0 <12.5>	n/a	n/a	.390	
Diastolic B.P. (Normotensive and Unmedicated subjects) mm Hg	n=22 81.3/ <8.7>	n/a	n=7 77.7 / <4.7>	n/a	n=8 74.5/ <10.6>	n/a	n=6 75.0 <7.8>	n/a	n/a	.174	

n= number of subjects for which there was available clinical data for the indicated examination

4 Discussion

4.1 Genetic Epidemiology of BBS in a Large Population:

BBS is caused by mutations in multiple different genes. The biologic complexities of the cilium/basal body/centrosome, which is dependent on multiple genes [48] implies that many pathogenic BBS mutations may occur. It is likely that some of the approximately 30,000 founders of the Newfoundland population carried different heterozygous BBS mutations to Newfoundland 8 to 10 generations ago. Their progeny experienced geographic and religious isolation which predisposed to occurrence of affected individuals with this autosomal recessive condition. In fact, 9 different BBS mutations and one variant have been discovered in the Newfoundland population. Several families with the same BBS1 or BBS6 homozygous mutations were clustered in specific genetic isolates. Individual families with homozygous mutations of different genes and other families with BBS caused by compound heterozygote mutations have been identified in random locations around the island. Thus it appears that the genetic complexity of the cilium/basal body/centrosome leading to multiple mutations, founder effects and the high inbreeding coefficient in multiple Newfoundland isolates, has caused the high incidence of autosomal recessive BBS in Newfoundland.

It is likely that BBS heterozygotes occur in the general population. In the region where BBS1 families are most common (South-West Newfoundland) 6 *BBS1* carriers were identified in a group of 400 control individuals studied (1.5%) [27]. In the current study

additional BBS mutations were identified in 3 of 130 (2.3%) relatives that differed from the mutations causing BBS in their families.

4.2 Renal and Metabolic Illness:

This study clearly demonstrates that early onset obesity, hypertension, diabetes mellitus, chronic kidney disease and early death are associated with BBS. It is possible that the high incidence of genetically complex manifestations, such as hypertension and diabetes, in the BBS cases was influenced by other genetic influences prevalent in the population. The non carriers certainly had a high incidence of hypertension, but obesity was less prevalent than in cases and the onset of hypertension, diabetes and chronic renal disease was of substantially later onset.

4.2.1 Renal Events:

It is likely that primary ciliary dysfunction in BBS predisposes to the renal and endocrine events reported here. In normal kidneys the epithelial cilia are sensory transducers in a cilium-generated signaling pathway [57]. Abnormal ciliary function leads to cysts, calyceal clubbing and tubular defects [16, 44, 46]. Defective expression of BBS proteins could also lead to permanent changes in cellular properties that lead to early onset kidney dysfunction [44].

4.2.2 Pancreatic and Diabetic Events:

Cilia have been identified in the exocrine, endocrine and ductal cells of the pancreas for many years [44]. Ciliary defects have been associated with acinar cell atrophy and duct hyperplasia, and compromised glucose homeostasis. BBS patients are prone to type 2 diabetes and have high insulin levels [4], suggesting that both obesity and primary pancreatic disease contribute to insulin resistance. Alstrom's Syndrome is another autosomal recessive ciliopathy strongly associated with diabetes and insulin resistance [59].

4.2.3 Obesity:

It is not fully understood how defective cilia predispose to obesity. Neuronal cilia-dwelling receptors could be involved in regulating food intake and overall energy metabolism. In fact, many neurons that are responsive to the weight regulating protein leptin display somatostatin 3 receptors on their cilia [61]. Consequently dysfunctional cilia associated with BBS could predispose to dysregulation of energy metabolism. Type II chaperonin proteins ensure proper folding of proteins. *BBS6*, *10* and *12* genes code for proteins with similar properties to chaperonins [19, 34, 44, 77], and mutations in these genes may result in abnormal proteins, which could possibly be responsible for improper folding of leptin, and thus predispose to the development of obesity. In addition cilia have been observed in the fat storing cells of the liver, in the thyroid and in the adrenal glands, defects in which could lead to defective storage of fat, and abnormalities in thermoregulation and metabolic rate [44].

4.2.4 Hypertension:

The etiology of hypertension in BBS is likely to be complex. Predisposing factors include primary renal disease, diabetes mellitus and obesity which occur frequently in BBS. Furthermore abnormalities in intracellular calcium signaling in vascular cells may induce dysregulation of contraction and predispose to hypertension [58]. In addition an underlying genetic predisposition to hypertension may cluster in these families.

4.3 Phenotype/Genotype Comparisons:

The fact that nine different mutations in six different BBS genes are associated with a similar phenotype suggests that all of the BBS genes are necessary for later organ differentiation. The cilium/basal body/centrosome structure appears to be critical in the proper development of kidneys, liver, pancreas, and other endocrine organs as renal and metabolic illness arising from these systems are seen in all BBS genotypes.

The heterozygote state is not associated with a predisposition to endocrine and renal events associated with BBS, as the prevalence of obesity and incidence of hypertension, diabetes and chronic kidney disease are comparable in BBS mutation carriers compared to non carriers. The observation of similar BMI in BBS mutation carriers and non-carriers is consistent with our results from a study of BBS1 genotype in obesity, in the geographic area where cases caused by *BBS1* mutations were identified [27]. The *BBS1*

mutation was found in the heterozygous state in 3 of 200 obese individuals and also in 3 of 200 matched non obese controls.

4.4 Study Limitations:

The limitations of this study include small numbers enrolled in the study, potential ascertainment bias, and definitions of clinical manifestations which may provide higher estimates of disease. However this is a population based study likely to have identified most of the cases who presented with blindness, a clinical manifestation highly prevalent in BBS. The definitions of disease are less likely to be a problem in the cases who we have been following for 28 years, but may be less reliable in relatives who have been assessed once. The virtual overlap of time-to-event curves suggests that very large numbers of relatives would be required to disprove our conclusion that endocrine/renal events are similar in carriers and non carriers.

4.5 Summary

The high prevalence of BBS in Newfoundland is likely the result of the inbreeding coefficient in multiple genetic isolates and the frequency of pathogenic BBS mutations in founders. BBS includes early onset obesity, hypertension, diabetes mellitus, and chronic kidney disease, which may be associated with ciliary dysfunction in a variety of organs. The incidence of renal and metabolic diseases was similar in carriers and non carriers of the Newfoundland BBS mutations. No statistically significant variation between either

carriers or non carriers was found in any of the outcomes sought in this study. The heterozygote BBS state is not associated with increased risk for these endocrine and renal events in this population.

References:

1. Parfrey PS, Davidson WS, Green JS: Clinical and genetic epidemiology of inherited renal disease in Newfoundland. *Kidney Int,* 61:1925-34, 2002.
2. Bear JC, Nemec TF, Kennedy JC, Marshall WH, Power AA, Kolonel VM, Burke GB: Persistent genetic isolation in outport Newfoundland. *Am J Med Genet,* 27:807-30, 1987.
3. Service S, DeYoung J, Karayiorgou M, Roos JL, Pretorious H, Bedoya G, Ospina J, Ruiz-Linares A, Macedo A, Palha JA, Heutink P, Aulchenko Y, Oostra B, van Duijn C, Jarvelin MR, Varilo T, Peddle L, Rahman P, Piras G, Monne M, Murray S, Galver L, Peltonen L, Sabatti C, Collins A, Freimer N: Magnitude and distribution of linkage disequilibrium in population isolates and implications for genome-wide association studies. *Nat Genet,* 38:556-60, 2006.
4. Green JS, Parfrey PS, Harnett JD, Farid NR, Cramer BC, Johnson G, Heath O, McManamon PJ, O'Leary E, Pryse-Phillips W: The cardinal manifestations of Bardet-Biedl syndrome, a form of Laurence-Moon-Biedl syndrome. *N Engl J Med,* 321:1002-9, 1989.
5. Ammann F, Klein D, Prader A, Hauser A: [Two big genealogical trees with Bardet-Biedl syndrome from central Switzerland. Contribution to the study of isolates]. *Arch Julius Klaus Stift Vererbungsforsch Sozialanthropol Rassenhyg,* 41:67-81, 1967.

6. Klein D, Ammann F: The syndrome of Laurence-Moon-Bardet-Biedl and allied diseases in Switzerland. Clinical, genetic and epidemiological studies. *J Neurol Sci,* 9:479-513, 1969.

7. Beales PL, Elcioglu N, Woolf AS, Parker D, Flinter FA: New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. *J Med Genet,* 36:437-46, 1999.

8. Beales PL, Reid HA, Griffiths MH, Maher ER, Flinter FA, Woolf AS: Renal cancer and malformations in relatives of patients with Bardet-Biedl syndrome. *Nephrol Dial Transplant,* 15:1977-85, 2000.

9. Moore SJ, Green JS, Fan Y, Bhogal AK, Dicks E, Fernandez BA, Stefanelli M, Murphy C, Cramer BC, Dean JC, Beales PL, Katsanis N, Bassett AS, Davidson WS, Parfrey PS: Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. *Am J Med Genet A,* 132:352-60, 2005.

10. Farag T, Teebi AS: Letters to the Editor: High Incidence of Bardet Biedl Syndrome among the Bedouin. *Clinical Genetics,* 36:463-465, 1989.

11. Laurence JZ, Moon RC: Four cases of Retinitis Pigmentosa occuring in the same family, and accompanied by general imperfections of development. *Opthalmol Rev,* 2:32-41, 1866.

12. Bardet G: Sur un syndrome d'obesity congenitale avec polydactyly et retinite pigmentaire (contribution a l'etude des formes cliniques de l'obesity hypophysaire). *These de Paris,* 470, 1920.

13. Biedl A: Ein Geschwister mit adiposogenitaler Dystropie. *Dtsch Med Wochenschr.,* 48:1630, 1922.

14. Solis-Cohen S, Weiss E: Dystrophia adiposogenitalis, with atypical retinitis pigmentosa and mental deficiency - The Laurence-Biedl Syndrome. A report of Four Cases in One Family. *Am J Med Sci,* 169:489-505, 1925.

15. Schachat AP, Maumenee IH: Bardet-Biedl syndrome and related disorders. *Arch Ophthalmol,* 100:285-8, 1982.

16. Harnett JD, Green JS, Cramer BC, Johnson G, Chafe L, McManamon P, Farid NR, Pryse-Phillips W, Parfrey PS: The spectrum of renal disease in Laurence-Moon-Biedl syndrome. *N Engl J Med,* 319:615-8, 1988.

17. Beales PL: Lifting the lid on Pandora's box: the Bardet-Biedl syndrome. *Curr Opin Genet Dev,* 15:315-23, 2005.

18. O'Dea D, Parfrey PS, Harnett JD, Hefferton D, Cramer BC, Green J: The importance of renal impairment in the natural history of Bardet-Biedl syndrome. *Am J Kidney Dis,* 27:776-83, 1996.

19. Stoetzel C, Muller J, Laurier V, Davis EE, Zaghloul NA, Vicaire S, Jacquelin C, Plewniak F, Leitch CC, Sarda P, Hamel C, de Ravel TJ, Lewis RA, Friederich E, Thibault C, Danse JM, Verloes A, Bonneau D, Katsanis N, Poch O, Mandel JL, Dollfus H: Identification of a novel BBS gene (BBS12) highlights the major role of a vertebrate-specific branch of chaperonin-related proteins in Bardet-Biedl syndrome. *Am J Hum Genet,* 80:1-11, 2007.

20. Chiang AP, Beck JS, Yen HJ, Tayeh MK, Scheetz TE, Swiderski RE, Nishimura DY, Braun TA, Kim KY, Huang J, Elbedour K, Carmi R, Slusarski DC, Casavant TL, Stone EM, Sheffield VC: Homozygosity mapping with SNP arrays identifies TRIM32, an E3 ubiquitin ligase, as a Bardet-Biedl syndrome gene (BBS11). *Proc Natl Acad Sci U S A,* 103:6287-92, 2006.

21. Katsanis N: The oligogenic properties of Bardet-Biedl syndrome. *Hum Mol Genet,* 13 Spec No 1:R65-71, 2004.

22. Mykytyn K, Nishimura D, Searby CC, Shastri M, Yen HJ, Beck JS, Braun T, Streb LM, Cornier AS, Cox GF, Fulton AB, Carmi R, Luleci G, Chandrasekharappa SC, Collins FS, Jacobsen SG, Heckenlively JR, Weleber RG, Stone EM, Sheffield VC: Identification of the gene (BBS1) most commonly involved in Bardet-Biedl Syndrome, a complex human obesity syndrome. *Nat Genet,* 31:435-438, 2002.

23. OMIM: Online Mendelian Inheritance in Man. www.ncbi.nlm.nih.gov/Omim.

24. Ansley S, Badano JL, Blacque OE, Hill J, Hoskins BE, Leitch CC, Kim JC, Ross AJ, Eichers ER, Teslovich TM, Mah AK, Johnsen RC, Cavender JC, Lewis RA, Leroux MR, Beales PL, Katsanis N: Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature,* 425:628-33, 2003.

25. Nishimura D, Searby CC, Carmi R, Elbedour K, Van Maldergerm L, Fulton AB, Lam BL, Powell BR, Swiderski RE, Bugge KE, Haider NB, Kwitek-Black AE, Ying L, Duhl DM, Gorman SW, Heon E, Iannaccone A, Bonneau D, Biesecker LG, Jacobsen SG, Stone EM, Sheffield VC: Positional Cloning of a novel gene on

chromasome 16q causing Bardet-Biedl Syndrome. *Hum Mol Genet,* 10:865-874, 2001.

26. Ross AJ, Beales PL: Bardet-Biedl Syndrome. *Gene Reviews, www.genetests.org,* 2007.

27. Fan Y, Rahman P, Peddle L, Hefferton D, Gladney N, Moore SJ, Green JS, Parfrey PS, Davidson WS: Bardet-Biedl syndrome 1 genotype and obesity in the Newfoundland population. *Int J Obes Relat Metab Disord,* 28:680-4, 2004.

28. Katsanis N, Eichers ER, Ansley SJ, Lewis RA, Kayserili H, Hoskins BE, Scambler PJ, Beales PL, Lupski JR: BBS4 is a minor contributor to Bardet-Biedl syndrome and may also participate in triallelic inheritance. *Am J Hum Genet,* 71:22-9, 2002.

29. Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, Leitch CC, Chapple JP, Munro PM, Fisher S, Tan PL, Phillips HM, Leroux MR, Henderson DJ, Murdoch JN, Copp AJ, Eliot MM, Lupski JR, Kemp DT, Dollfus H, Tada M, Katsanis N, Forge A, Beales PL: Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet,* 37:1135-40, 2005.

30. Mykytyn K, Braun T, Carmi R, Haider NB, Searby CC, Shastri M, Beck G, Wright AF, Iannaccone A, Elbedour K, Riise R, Baldi A, Raas-Rothschild A, Gorman SW, Duhl DM, Jacobsen SG, Casavant T, Stone EM, Sheffield VC: Identification of the gene that, when mutated, causes the human obesity syndrome BBS4. *Nat Genet,* 28:188-191, 2001.

31. Kulaga HM, Leitch CC, Eichers ER, Badano JL, Lesemann A, Hoskins BE, Lupski JR, Beales PL, Reed RR, Katsanis N: Loss of BBS proteins causes anosmia in humans and defects in olfactory cilia structure and function in the mouse. *Nat Genet,* 36:994-8, 2004.

32. Mykytyn K, Mullins RF, Andrews M, Chiang AP, Swiderski RE, Yang B, Braun T, Casavant T, Stone EM, Sheffield VC: Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc Natl Acad Sci U S A,* 101:8664-9, 2004.

33. Kim JC, Ou YY, Badano JL, Esmail MA, Leitch CC, Fiedrich E, Beales PL, Archibald JM, Katsanis N, Rattner JB, Leroux MR: MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet-Biedl syndrome, is a novel centrosomal component required for cytokinesis. *J Cell Sci,* 118:1007-20, 2005.

34. Katsanis N, Beales PL, Woods MO, Lewis RA, Green JS, Parfrey PS, Ansley SJ, Davidson WS, Lupski JR: Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. *Nat Genet,* 26:67-70, 2000.

35. Fath MA, Mullins RF, Searby C, Nishimura DY, Wei J, Rahmouni K, Davis RE, Tayeh MK, Andrews M, Yang B, Sigmund CD, Stone EM, Sheffield VC: Mkks-null mice have a phenotype resembling Bardet-Biedl syndrome. *Hum Mol Genet,* 14:1109-18, 2005.

36. Blacque OE, Reardon MJ, Li C, McCarthy J, Mahjoub MR, Ansley SJ, Badano JL, Mah AK, Beales PL, Davidson WS, Johnsen RC, Audeh M, Plasterk RH, Baillie DL, Katsanis N, Quarmby LM, Wicks SR, Leroux MR: Loss of C. elegans BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev,* 18:1630-42, 2004.

37. Kubo A, Sasaki H, Yuba-Kubo A, Tsukita S, Shiina N: Centriolar satellites: molecular characterization, ATP-dependent movement toward centrioles and possible involvement in ciliogenesis. *J Cell Biol,* 147:969-80, 1999.

38. Yen HJ, Tayeh MK, Mullins RF, Stone EM, Sheffield VC, Slusarski DC: Bardet-Biedl syndrome genes are important in retrograde intracellular trafficking and Kupffer's vesicle cilia function. *Hum Mol Genet,* 15:667-77, 2006.

39. Nishimura DY, Swiderski RE, Searby CC, Berg EM, Ferguson AL, Hennekam R, Merin S, Weleber RG, Biesecker LG, Stone EM, Sheffield VC: Comparative genomics and gene expression analysis identifies BBS9, a new Bardet-Biedl syndrome gene. *Am J Hum Genet,* 77:1021-33, 2005.

40. Stoetzel C, Laurier V, Davis EE, Muller J, Rix S, Badano JL, Leitch CC, Salem N, Chouery E, Corbani S, Jalk N, Vicaire S, Sarda P, Hamel C, Lacombe D, Holder M, Odent S, Holder S, Brooks AS, Elcioglu NH, Silva ED, Rossillion B, Sigaudy S, de Ravel TJ, Lewis RA, Leheup B, Verloes A, Amati-Bonneau P, Megarbane A, Poch O, Bonneau D, Beales PL, Mandel JL, Katsanis N, Dollfus H: BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. *Nat Genet,* 38:521-4, 2006.

41. Badano JL, Leitch CC, Ansley SJ, May-Simera H, Lawson S, Lewis RA, Beales PL, Dietz HC, Fisher S, Katsanis N: Dissection of epistasis in oligogenic Bardet-Biedl syndrome. *Nature,* 439:326-30, 2006.

42. Dollfus H, Verloes A, Bonneau D, Cossee M, Perrin-Schmitt F, Brandt C, Flament J, Mandel JL: [Update on Bardet-Biedl syndrome]. *J Fr Ophtalmol,* 28:106-12, 2005.

43. Kim JC, Badano JL, Sibold S, Esmail MA, Hill J, Hoskins BE, Leitch CC, Venner K, Ansley SJ, Ross AJ, Leroux MR, Katsanis N, Beales PL: The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat Genet,* 36:462-70, 2004.

44. Pan J, Wang Q, Snell WJ: Cilium-generated signaling and cilia-related disorders. *Lab Invest,* 85:452-63, 2005.

45. Davenport JR, Yoder BK: An incredible decade for the primary cilium: a look at a once-forgotten organelle. *Am J Physiol Renal Physiol,* 289:F1159-69, 2005.

46. Praetorius HA, Spring KR: A physiological view of the primary cilium. *Annu Rev Physiol 2004*, 2004.

47. Li JB, Gerdes JM, Haycraft CJ, Fan Y, Teslovich TM, May-Simera H, Li H, Blacque OE, Li L, Leitch CC, Lewis RA, Green JS, Parfrey PS, Leroux MR, Davidson WS, Beales PL, Guay-Woodford LM, Yoder BK, Stormo GD, Katsanis N, Dutcher SK: Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell,* 117:541-52, 2004.

48. Mykytyn K, Sheffield VC: Establishing a connection between cilia and Bardet-Biedl Syndrome. *Trends Mol Med,* 10:106-9, 2004.

49. Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV: Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature,* 426:83-7, 2003.

50. WISC:Zoology: Neurulation. *http://worms.zoology.wisc.edu/frogs/neuru/neurul_intro.html*.

51. Katzmarzyk PT, Janssen I: The economic costs associated with physical inactivity and obesity in Canada: an update. *Can J Appl Physiol,* 29:90-115, 2004.

52. Canadian Diabetic Association: The Prevalence and Cost of Diabetes in Canada.

53. Joffres MR GP, Fodor JG, Petrasovies A, Chockalingham A, Hamet P: Awareness, Treatment, and Control of Hypertension in Canada. *Am J Hypertens*:1097-1102, 1997.

54. Kannel W: Blood Pressure as a Cardiovascular Risk Factor. *Jama*:1571-1576, 1996.

55. Zelmer JL: The economic burden of end-stage renal disease in Canada. *Kidney Int*:1122-1129, 2007.

56. Davenport JR, Watts AJ, Roper VC, Croyle MJ, van Groen T, Wyss JM, Nagy TR, Kesterson RA, Yoder BK: Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. *Curr Biol,* 17:1586-94, 2007.

57. Praetorius HA, Spring KR: The renal cell primary cilium functions as a flow sensor. *Curr Opin Nephrol Hypertens,* 12:517-20, 2003.

58. Qian Q, Hunter LW, Li M, Marin-Padilla M, Prakash YS, Somlo S, Harris PC, Torres VE, Sieck GC: Pkd2 haploinsufficiency alters intracellular calcium regulation in vascular smooth muscle cells. *Hum Mol Genet,* 12:1875-80, 2003.

59. Hearn T. SC, Phillips V.J., Renforth G.L., Copin N., Hanley N.A., Wilson D.I.: Subcellular Localisation of ALMS1 Supports Involvement of Centrosome and Basal Body Dysfunction in the Pathogenesis of Obesity, Insulin Resistance, and Type 2 Diabetes. *Diabetes*:1581-1587, 2005.

60. Praetorius HA, Spring KR: Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol,* 191:69-76, 2003.

61. Stepanyan Z, Kocharyan A, Pyrski M, Hubschle T, Watson AM, Schulz S, Meyerhof W: Leptin-target neurones of the rat hypothalamus express somatostatin receptors. *J Neuroendocrinol,* 15:822-30, 2003.

62. Hjortshoj T, Gronskov K, Rosenberg T, Brodum-Nielsen K, Olsen JH: Risk for Cancer in Patients with Bardet-Biedl Syndrome and the Relatives. *Am J Med Genet A* 143:1699-1702, 2007.

63. Swift M, Morrell D, Massey RB, Chase CL: Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N Engl J Med,* 325:1831-6, 1991.

64. Croft JB, Morrell D, Chase CL, Swift M: Obesity in heterozygous carriers of the gene for the Bardet-Biedl syndrome. *Am J Med Genet,* 55:12-5, 1995.

65. Croft JB, Swift M: Obesity, hypertension, and renal disease in relatives of Bardet-Biedl syndrome sibs. *Am J Med Genet,* 36:37-42, 1990.

66. (USA) NCFHS: National Health and Nutrition Examination Survey. 1976-1980.

67. Hall JG, Froster-Iskenius UG, Allanson JE: Handbook of Normal Physical Measurements. *Oxford Medical Publications*, 1995.

68. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med,* 130:461-70, 1999.

69. Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RG, Rahman M, Deysher AE, Zhang YL, Schmid CH, Levey AS: Evaluation of the modification of diet in renal disease study equation in a large diverse population. *J Am Soc Nephrol,* 18:2749-57, 2007.

70. Woods MO, Young TL, Parfrey PS, Hefferton D, Green JS, Davidson WS: Genetic heterogeneity of Bardet-Biedl syndrome in a distinct Canadian population: evidence for a fifth locus. *Genomics,* 55:2-9, 1999.

71. Young TL, Woods MO, Parfrey PS, Green JS, O'Leary E, Hefferton D, Davidson WS: Canadian Bardet-Biedl syndrome family reduces the critical region of BBS3 (3p) and presents with a variable phenotype. *Am J Med Genet,* 78:461-7, 1998.

72. Young TL, Woods MO, Parfrey PS, Green JS, Hefferton D, Davidson WS: A founder effect in the Newfoundland population reduces the Bardet-Biedl syndrome I (BBS1) interval to 1 cM. *Am J Hum Genet,* 65:1680-7, 1999.

73. Fan Y, Esmail MA, Ansley SJ, Blacque OE, Boroevich K, Ross AJ, Moore SJ, Badano JL, May-Simera H, Compton DS, Green JS, Lewis RA, Van Haelst MM, Parfrey PS, Baillie DL, Beales PL, Katsanis N, Davidson WS, Leroux MR: Mutations in a Member of the RAS Super Family of small GTP-Binding Proteins causes Bardet-Biedl Syndrome. Nat. Genet, 36:989-93, 2004.

74. Fan Y, Green JS, Ross AJ, Beales PL, Parfrey PS, Davidson WS: Linkage disequilibrium mapping in the Newfoundland population: a re-evaluation of the refinement of the Bardet-Biedl syndrome 1 critical interval. *Hum Genet,* 116:62-71, 2005.

75. Twells L: Obesity and its Impact on a Provincial Health System. *PhD Dissertation, Memorial University*, 2008.

76. Meltzer S, Leiter L, Daneman D, Gerstein HC, Lau D, Ludwig S, Yale JF, Zinman B, Lillie D: 1998 clinical practice guidelines for the management of diabetes in Canada. Canadian Diabetes Association. *Cmaj,* 159 Suppl 8:S1-29, 1998.

77. Slavotinek A, Stone EM, Mykytyn K, Heckenlively JR, Green JS, Heon E, Musarella MA, Parfrey PS, Sheffield VC, Biesecker LG: Mutations in MKKS cause Bardet Biedl Syndrome. *Nat Genet,* 26:15-16, 2000.