MOLECULAR GENETICS OF BARDET-BIEDL SYNDROME (BBS) IN THE NEWFOUNDLAND POPULATION

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by

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Abstract

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disorder characterized by congenital dysmorphic extremities, infantile onset obesity, progressive retinal degeneration, renal abnormalities, and male hypogonadism. There are at least five genetic loci, four previously mapped (3p, 11q, 15q and 16q), that give the same pleiotropic BBS phenotype. The limited number of cases and the genetic heterogeneity of BBS have hindered efforts to positionally clone the BBS genes. Newfoundland, a genetic isolate, is enriched for BBS with a prevalence (1/17,500) that is ten times higher than the world estimate. The availability of DNA from 17 of the 22 identified BBS families provided an opportunity to study the molecular genetics of BBS in Newfoundland.

Seventeen families were genotyped and linkage and haplotype analyses were conducted at each of the four mapped loci (*BBS1-BBS4*). Three families were assigned to the *BBS1* locus. The finding of linkage disequilibrium resulted in the assignment of three additional *BBS1* families and the refinement of the *BBS1* disease interval on chromosome 11q. One large family was linked to the relatively rare *BBS3* locus and was used to confirm this locus and refine its map position on chromosome 3p. Six families were excluded from all previously mapped loci. A genomewide scan was used to successfully map the fifth locus, *BBS5*, to chromosome 2q31. In summary, of the 22 Newfoundland BBS families, six (27%) have been unambiguously assigned to *BBS1*, one to *BBS3* (5%) and one to *BBS5* (5%), suggesting that the relatively high prevalence of BBS in Newfoundland is the result of a minimum of three BBS genes and a *BBS1* founder.

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Introduction and Overview

1.1 Introduction

The Human Genome Project (HGP) is an international research program designed primarily to determine the complete nucleotide sequence of the human genome. By the spring of the year 2000, a first draft of the 3 billion base pair sequence will be released to the public domain (Francis Collins, Director of NIH, in a public address to the 49th annual meeting of "The American Society of Human Genetics", San Francisco, USA). The sequence will be accessible to anyone with a computer and a connection to the Internet. However, annotation of the sequence, including the identity of the estimated ~100,000 genes, representing a mere 10% of the 3 billion bases, remains the most formidable task. Of the ~100,000 genes, only 10% or so are believed to cause disease phenotypes. Mutations to the majority of genes are believed to cause either embryonic lethals or, conversely, subtle effects (modifier genes) or have no effect because of redundancy in the genome. Comparative genomics holds great promise for the identification of not only genes but entire biochemical pathways. However, to study the majority of human diseases with a major genetic component, we must begin with the identity of a human gene because there are no known animal models or prior biochemical insight. In this way, the annotation of human sequences in public databases will continue to rely on the exploitation of the most private of mutations. So, for the present generation of geneticists on the brink of the "New Millennium", the rule still is "cherish your exceptions".

Bardet-Biedl syndrome (BBS) is a rare, recessive disorder with an estimated worldwide prevalence of 1 /125,000-160,000. Mutations at any one of at least five genes result in a relatively unified clinical presentation: congenital dystrophic extremities, infantile onset obesity, progressive retinal degeneration, renal abnormalities and male hypogonadism. These major features of BBS are associated to varying degrees with other features including mental retardation and diabetes mellitus. The spectrum of clinical manifestations associated with the disruption of a single gene suggests that the putative BBS genes are part of a unifying developmental network that continues to play a functional role in the development and/or maintenance of adult retina, kidney, gonad and adipose tissues. In this way, it is anticipated that the cloning of the BBS genes will advance the study of more common diseases like obesity and diabetes.

As always, the study of rare diseases is limited by the paucity of reported cases and, in the case of BBS, complicated by a growing list of mapped loci. Generally speaking, the availability of study patients can be improved and the genetic complexity reduced if a genetically isolated population is identified with an increased incidence of disease. **This study was undertaken to advance the efforts underway to map and clone the BBS genes, by the investigation of patients and their families affected by BBS in Newfoundland, a genetically isolated population with ten times the worldwide prevalence of BBS.**

1.2 Bardet-Biedl Syndrome

1.2.1 Nosology

In 1866, Laurence and Moon (and Hutchinson in 1882 ,1900) reported the features of mental retardation, pigmentary retinopathy, hypogenitalism and spastic paraplegia in four siblings of the same family (see OMIM 245800). A similar syndrome, characterized by mental retardation, pigmentary retinopathy, and hypogenitalism, in the presence of congenital obesity and polydactyly, was subsequently described by Bardet (1920) and Biedl (1922) (see OMIM 209900). Since three of the four phenotypic features of Laurence-Moon (LM; OMIM 245800) overlap with those of Bardet-Biedl syndrome (BBS; OMIM 209900), Solis-Cohen and Weiss (1925) argued that LM and BBS were variants of a single disease entity that should be referred to as the Laurence-Moon-Biedl (LMB) syndrome. Schachat and Maumenee (1982) reviewed the nosography of these and related syndromes and argued that BBS and LM are distinct. However, the issue is far from settled. The LMB (or LMBBS) designation is readily found in the recent medical literature and Beales et al. (1999) recently described the finding of neurological features in patients meeting accepted diagnostic criteria for BBS (Schachat and Maumenee 1982). Until the genes are cloned, we cannot know which diagnostic scheme is genetically relevant. For the purpose of this study, we considered BBS to be a distinct disease from LM.

1.2.2 Cardinal Features of BBS

Schachat and Maumenee (1982) have suggested that the diagnostic criteria for BBS should include four of the five following major features: mental retardation, obesity, hypogenitalism, polydactyly and pigmentary retinopathy. From extensive clinical investigations of BBS cases in Newfoundland, we consider that mental retardation is not usually present, if assessed quantitatively, with tests that account for the visual defect in these patients. Hypogenitalism is a consistent feature in men but difficult to assess in women. Brachydactyly (short, broad hands and/or feet) and syndactyly (webbed fingers and/or toes) are more frequent than polydactyly (extra fingers and/or toes) in patients from Newfoundland. Renal changes, although not routinely assessed by others, are prevalent and characteristic of BBS. Therefore, we have suggested that the cardinal features of BBS are: obesity, male hypogenitalism, dystrophic extremities, retinal dystrophy and renal disease. We considered the presence of either brachydactyly, syndactyly or polydactyly in combination with retinal dystrophy and obesity (in the absence of neurological complications), to constitute the minimal diagnostic criteria required to define a BBS case (Harnett et al. 1988; Green et al. 1989; O'Dea et al. 1996; reviewed by Parfrey et al. 1997). The following general description of the phenotypic features of BBS is based on our observations in conjunction with other clinical reviews (Bell, 1958; Klein and Ammann 1969; Schachat and Maumenee 1982; Riise et al. 1997; Beales et al. 1999).

Retinal Dystrophy

Severe retinal dystrophy is a consistent feature and the most reliable diagnostic criterion of BBS. An early sign of the retinopathy associated with BBS is often the onset of night blindness in children. After that, the visual loss is rapid (usually begins by seven years) and involves central and/or peripheral vision loss that progresses to an incapacitating blindness with a visual acuity at or below the ability to count fingers. The retinal dystrophy in BBS patients was initially referred to as retinitis pigmentosa (RP). RP is characterized by constriction of the visual fields, night blindness and fundus changes, including dense bone-spicule pigmentation, pale optic discs and attenuated vessels. Most BBS patients are more likely to show sparse pigmentation with or without vascular changes, mild to severe optic disk pallor and severely attenuated vessels. The fundus appearance associated with BBS is different from isolated typical RP, the age of onset is earlier, and the progression to blindness is more rapid in BBS patients than for most RP patients. Therefore, the retinal dystrophy associated with BBS is referred to as an "atypical" form of RP.

In Newfoundland, fifty percent of patients were registered as legally blind by 18 years of age with the age of registered blindness in patients ranging from 5 to 29 years of age. Ophthalmologic investigations of patients reveal markedly restricted visual fields, severe abnormalities of color vision, raised dark-adaptation thresholds and extinguished or minimal rod and cone responses on electroretinography. Since the majority of patients were ascertained through eye clinics, it is likely that the spectrum of eye disease in this

patient group is biased towards BBS cases with severe eye manifestations.

Dystrophic Extremities

Postaxial polydactyly, involving from one to four extremities, is usually the first clinical sign of BBS and has traditionally been considered a cardinal feature of the syndrome. However, polydactyly is not a universal finding: it is absent in at least 30% of BBS patients, and, we have found it to be less frequent than brachydactyly and syndactyly. Since syndactyly in BBS patients may be subtle and brachydactyly is best assessed with metacarpophalangeal pattern profile (MCPP) analysis, we suggest that any one or combination of polydactyly, syndactyly and/or brachydactyly be referred to as dysmorphic extremities and replace polydactyly as a cardinal feature of BBS.

Obesity

Obesity is a characteristic feature of BBS and, along with dystrophic extremities, useful for the diagnosis of young children. Eighty-eight percent of patients from Newfoundland were above the 90th percentile of weight for height. The age of onset is typically between 2-3 years. The obesity associated with BBS is progressive with age but responsive to caloric restriction. The adipose tissue is diffuse in young children but predominantly distributed to the trunk and the proximal sections of the limbs of adults.

Renal Anomalies

Renal disease, although recognized frequently as associated with BBS, has not traditionally been considered a cardinal feature of the disorder. Evaluation of renal disease has suffered from problems of ascertainment bias and lack of appropriate renal imaging studies. However, all patients identified through ophthalmology records in Newfoundland had some abnormality of renal structure, function or both. The radiological appearance of calyceal clubbing, or blunting, non-communicating calyceal cysts, or diverticula, and fetal lobulation suggest a defect in maturation of the kidneys. Renal impairment (as defined by a creatinine clearance of less than 1.2 mL/sec or a serum creatinine >120 μmolL) was detected as early as 2 years and occurred in 25% of BBS patients examined. By age 48, 25% of Bardet-Biedl cases had chronic renal insufficiency (Harnett et al. 1988; O'Dea et al. 1996). The consistency of renal findings among BBS patients from Newfoundland (unselected for kidney disease), and elsewhere (if imaging tests are done), suggest it is a cardinal feature of BBS. Renal imaging to test for the persistence of fetal lobulation (ultrasound) and calyceal morphology (intravenous pyelography) should be performed on suspected BBS cases. The significance of the renal findings are underscored by the fact that renal impairment is an important cause of morbidity and mortality in BBS patients.

Male Hypogenitalism

Although adults with BBS have secondary sexual characteristics, male hypogenitalism

is nearly always present, and presents as small testes and a very small penis. A valuable diagnostic criterion in males, poor sexual development is difficult to assess in females. The majority of women with BBS report irregular menstruation and relative infertility and recent reports emphasize the importance of examination of young female patients in light of the findings of vaginal atresia and hydrometrocolpos in females (David et al. 1999; Oguzkurt et al. 1999). However, there are reports of women with BBS who have given birth to children, including two patients from Newfoundland.

1.2.3 Minor Features of BBS

Other clinical manifestations associated with BBS include mental retardation, hypertension and diabetes mellitus (usually non-insulin dependent). As well, patients often present with other ocular findings associated with retinal dystrophies, such as myopia, astigmatism, nystagmus, glaucoma, and cataracts. Failure to meet developmental milestones and behavioral affects such as inappropriate mannerisms and emotional immaturity are frequently reported in BBS patients. Since some of these traits may be confused with mental retardation, there should be formal testing for mental capacity that takes into account the visual deficit.

1.2.4 Clinical Heterogeneity of BBS

Pleiotropic genes, or genes that affect a wide array of tissues and organs, frequently show variable severity of the phenotype (expressivity). This is certainly the case with BBS, in which the number and severity of major and minor manifestations of BBS are quite variable, even among patients within a family (reviewed in **Chapter 3**). A particularly illustrative example of the complex nature of the BBS phenotype was recently reported in monozygotic twins: one twin was born with polydactyly of three limbs while the other had no polydactyly (Beales et al. 1999). This variability, along with the delay in onset of age-dependent features, may make it difficult to make an accurate and/or timely diagnosis because of the inability to exclude syndromes with overlapping phenotypes (table 1.1), particularly in young patients (Beales et al. 1999). Several other syndromes of unknown etiology, beside LM, have overlapping features with BBS. Notable among these are Biemond syndrome II (OMIM 210350) and Alstrom syndrome (OMIM 203800) that can be distinguished from BBS by the presence of iris coloboma and deafness, respectively (table 1.1). David et al. (1999) recently reported nine BBS patients who were mis-diagnosed in infancy with McKusick-Kaufman syndrome (MKKS; a recessive condition characterized by postaxial polydactyly, vaginal atresia with hydrometrocolpos and congenital heart defect; OMIM 236700). This may indicate a general undereporting of the incidence of BBS.

An early, accurate diagnosis of BBS is very important for disease management in

patients. The provision of special education with low-vision aids, dietary intervention to prevent or treat obesity and diabetes mellitus, treatment of hypertension and chronic renal failure, and counseling around issues of contraception, genetic risk, and end-stage renal therapy options, can do much to improve the quality of life for these individuals and their families.

Table 1.1

Differential Diagnosis of BBS and Syndromes with Overlapping Phenotypes

Cardinal Features of BBS	BBS	LM	Biemond II	Alstrom
Renal abnormalities	✓			
Dysmorphic Extremities	✓		✓	
Retinal dystrophy	✓	✓		✓
Obesity	✓		✓	✓
Male Hypogenitalism	✓	✓	✓	

Features of Overlapping Syndromes	BBS	LM	Biemond II	Alstrom
Mental Retardation	✓	✓	✓	
Diabetes mellitus	✓			✓
Spastic Paraplegia		✓		
Iris Coloboma			✓	
Deafness				✓

1.2.5 Genetic Heterogeneity of BBS

BBS is clearly an autosomal recessive disorder. At the start of this study, four distinct genetic loci (*BBS1*, 11q13; *BBS2*, 16q21; *BBS3*, 3p13-14; *BBS4*, 15q21-23) had been mapped and a fifth locus was anticipated due to the frequent finding of unlinked families (reviewed in **Chapter 2**). In contrast to genetically homogeneous disorders, the genetic heterogeneous nature of BBS makes it difficult to assign a single family to any given locus. Although problematic for genetic analysis, the underlying genetic heterogeneity of BBS raises the possibility that locus-specific phenotypes account for the variable expressivity of the disorder. This hypothesis has received little attention because most BBS families cannot be competently assigned to a specific BBS locus. A cursory exploration of this is made in **Chapter 3,** in which the phenotypes of affected members in two *BBS3*-linked families, are examined for *BBS3*-specific patterns of expression. It is not expected that all of the phenotypic variation will be explained by locus-specific expression, because it cannot account for the widely observed intrafamilial variation (reviewed in **Chapter 3**). Even though BBS is a "single gene" disorder, other factors, such as other genes (modifiers), environmental cues, stochastic influences, etc., obviously modulate the phenotype. The study of the complex determinants of "phenotype" is a growing branch of genetics (phenomics) and will be relevant to the investigation of BBS once the genes are identified.

1.3 Gene Discovery

1.3.1 Introduction

Over the past 20 years, the era of "genetic engineering" has resulted in the ability to clone and sequence genes. Initially, the only genetic diseases that were amenable to analysis at the gene level were those in which prior information about the biochemical defect was known. Disease genes that were identified from prior functional knowledge include the genes for beta-thalassemia (Orkin et al. 1979) and phenylketonuria (DiLella et al. 1986). This functional approach to gene mapping was fruitful but restrictive: the majority of the more than 10,000 genetic diseases cataloged in McKusick's "Mendelian Inheritance in Man" (http://www.ncbi.nlm.gov/omim) give no clues as to the biochemical nature of the causative genes.

A major advance in gene discovery was the strategy of positional cloning, a systematic approach to the identification of disease genes without functional information. The discovery of genetic markers (Botstein et al. 1980), advances in cloning and sequencing techniques (Burke et al. 1987), and the application of pre-existing theories and algorithms for linkage analysis (Elston and Stewart 1971; Ott 1974), allowed the identification of markers in the genome that cosegregate with disease in families. The chromosomal position of markers linked to disease loci predict the position of the disease gene (Collins 1991). The first step in positional cloning is linkage analysis to map the gene of interest

to a particular chromosomal region. The ultimate goal is the identification of a disease-causing gene in the region. So far, more than 100 genes have been identified by positional cloning (http://genome.nhgri.nih.gov).

1.3.2 Markers and Maps

Most genetic diseases are caused by variations in DNA sequence. However, the vast majority of variation observed in the human genome is not deleterious. These innocuous sequence variations, or polymorphisms, can be detected at the DNA level and used as markers for finding disease genes. The most prevalent type of polymorphic sequences in the human genome are variations that occur at random in the genome to single nucleotides (single nucleotide polymorphisms; SNPs) at approximately one in every 100-300 base pairs (www.ncbi.nlm.nih.gov/snp/index.html). Also abundant in the genome are microsatellites, consisting of repeats of simple sequence embedded in DNA with unique sequence. The variation is in the length of the sequence which, in turn, depends on the number of repeated units (e.g., di- ,tri-, and tetra-nucleotide repeats) (Beckmann and Weber, 1992; Hearne et al. 1992). The most useful markers for linkage analysis are highly polymorphic (multiple alleles of near equal frequencies) and located at known positions with respect to other genes and markers on specific chromosomes.

1.3.3 Linkage and Haplotype (Recombination) Analysis

The primary goal of linkage analysis is to determine if two or more genetic loci (e.g., a marker and a disease locus) are cosegregating in a pedigree. The principles of linkage analysis are based on the behavior of chromosomes in gametogenesis. During meiosis, homologous chromosomes pair up and then separate into different gametes. During this process, sister chromatids may break and recombine by a process known as crossing over, resulting in the exchange of genetic material (recombination). Mendel's law of independent assortment states that if two loci are located on different chromosomes, they will segregate independently of each other 50% of the time. However, due to recombination, the frequency of cosegregation of two loci on the same chromosome is usually less than 100%. The number of recombinants per meiosis (theta, θ) is dependent on the degree of physical separation between two loci (the value of θ equals the portion of offspring that are recombinant). As crossovers occur more or less randomly along the chromosome, they rarely occur between loci which are physically close together.

Linkage analysis is a statistical assessment of the likelihood that genetic loci (e.g., a marker and a disease locus) are inherited nonindependently from each other within families. The LOD score, *Z*, introduced by Morton (1955), is the logarithm of the odds that the loci are linked ($\theta < 0.5$) rather than unlinked ($\theta = 0.5$). LOD scores are calculated by looking at each meiosis in turn and comparing the likelihood of the observed genotypes on the alternative hypothesis of linkage or no linkage ($\theta = 0.5$). LOD scores are

calculated for a range of θ values in order to estimate the maximum Z score. The power of the analysis can be increased by using multiple pedigrees and summing the LOD scores across families. A LOD of 3 or more indicates a 1000:1 odds that the loci are linked versus not linked (for a given value of θ). A LOD score of -2 is taken as strong evidence that the loci are not closely linked. Two loci which show 1% recombination are defined as being 1 centimorgan (cM) apart on a genetic map. A genetic distance of 1 cM, on average, represents approximately 1 megabase (Mb) of DNA sequence.

Linkage is confirmed by typing other markers in the vicinity of the linked marker and/or by typing additional families. The alleles that are transmitted from parent to child are arranged in linear order (haplotype), corresponding to the map position of the markers. A haplotype that is transmitted, unaltered, from parent to child is said to be nonrecombinant (parental). Identifying markers that have recombined with the disease trait are used to define the minimal region that must contain the disease gene (referred to as the disease interval or critical region).

1.3.4 Homozygosity and Linkage Disequilibrium Mapping

There are special circumstances, both at the population and subpopulation level, where matings between related individuals are frequent. Consanguineous unions may be tolerated, or even encouraged, as a result of cultural tradition (e.g., among the Bedouin) (Sheffield et al. 1998). Alternatively, individuals may be related through a common

ancestor due to genetic isolation as a result of historical, geographical, or cultural isolation. The relatively small number of founders that are characteristic of these populations facilitate the search for human disease genes. There are various populations like this around the world, one of the most well known being the Finnish population (reviewed by de la Chapelle 1993). Rare genetic diseases that occur in founder populations are more likely to be caused by homozygosity of a single founder mutation than by two independent rare events (different mutations). More to the point, markers tightly linked to the disease gene that have not recombined can be identified in affected individuals as homozygous alleles. Individuals with a rare genetic disease from a founder population can essentially be considered as members of one very large kindred, without knowing how they are related. This allows refinement of the disease interval by linkage disequilibrium (LD) mapping to identify historical recombinations of the founder haplotype. Homozygosity mapping in consanguineous kindreds and LD mapping in founder populations are powerful methods for both locating and fine mapping of disease genes.

The population of Newfoundland has features that make homozygosity and linkage disequilibrium mapping feasible. The colonization of the island occurred primarily by a natural increase from northern European settlers of English and Irish extraction who participated in the mercantile fishing industry in the 1700 and 1800s. Mating segregation between Irish Catholics and English Protestants, low immigration, and geographical isolation of fishing communities have resulted in genetic isolation of the population.

1.3.5 Positional Cloning of Disease Genes

Define the Disease and Collect Families

A general strategy for positional cloning of a single gene trait is diagramed in fig. 1.1. The first priority (step 1) is to carefully define the phenotype of the disease. Strict criteria will limit the cases available for study but a broad definition may result in the inclusion of cases that are genetically heterogeneous. For instance, in this study, LM patients were excluded in case LM is a distinct entity encoded by non-BBS genes. Once the pedigrees are obtained (step 2), the genetic model or mode of inheritance (step 3) must be determined, including the penetrance (the probability that a genotype will yield the predicted phenotype). Until the BBS genes are cloned, the frequency of unaffected gene carriers can only be estimated. Penetrance values between 0.9 and 1.0 are routinely employed in linkage studies of BBS based on the consistency of limb dysmorphology, retinal dystrophy and obesity in affected child and adult members of BBS families and the fact that the observed frequency of affected offspring in families of BBS cases approaches that expected of an autosomal recessive disease that is fully penetrant. Steps 1-3 were completed prior to the present study (Harnett et al. 1988; Green et al. 1989; O'Dea et al. 1996).

Genotype Families and Analyze Data

Blood samples are collected for DNA extraction from patients and available first and

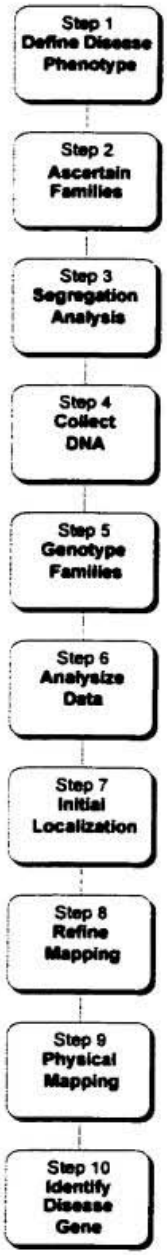

Figure 1.1 Step-wise approach to positional cloning (adapted from "Protocols in Human Genetics")

second degree relatives (step 4). If candidate loci are to be tested, polymorphic markers within the disease interval of each candidate locus will be genotyped. If no candidate loci for the disease are known, the markers selected will consist of a panel of ~200-300 evenly spaced, highly polymorphic markers for a genomewide scan (step 5). Linkage analysis is performed under the genetic model of the disease (step 6). LOD scores > 3 constitute an initial localization (step 7). Additional markers and/or families are genotyped to confirm the linkage. Haplotypes are extended by typing additional markers in the vicinity of the linked marker and all informative crossovers involving disease haplotypes need to be identified (step 8) as this will determine the amount of effort it takes to find the disease gene. Homozygosity and LD mapping, if applicable, are employed. The flow of events depicted in fig. 1.1 is two-way and any number of loops can be created. For example, the recruitment of patients and family members is often an on-going process. As well, once the critical region has been refined, it is prudent to look for candidate genes before proceeding with physical mapping.

Identify the Disease Gene

Identifying the disease gene (step 10) involves finding functional mutations in a gene that cosegregate with disease in affected families. A necessary but loathsome juncture between refinement of the genetic interval and gene identification is physical mapping (step 9). The goal of physical mapping is to identify all genes within the disease interval. This involves obtaining sequence data within the genetic boundaries of the disease and

identifying all gene sequences. Genes may be recognized by their sequence signatures, like CpG islands, conserved sequences, open reading frames, or by searching for expressed sequence tagged sites (ESTs) that map to the region, or by comparison with syntenic regions in the mouse. A physical map consists of cloned DNA fragments assembled into overlapping contiguous segments (contigs) constructed across the region. The amount of time and effort required to identify the disease gene will depend not only on the physical distance between boundary markers (thus the importance of step 8), but also on the complexity of the sequence. A high intrinsic gene density (e.g., 11q13) or the presence of reiterated sequences (e.g., pseudogenes within the disease interval of the *PKD1* gene (The International Polycystic Kidney Disease Consortium, 1995)), may complicate efforts to identify the disease gene. A ~1-Mb critical region is usually considered small enough to start the physical mapping stage.

As a direct benefit from the rapid progress of the HGP, the necessity for every lab to develop a physical map of their "region of interest" is diminishing as more of the human genome is sequenced. At the moment, the completeness of the genetic and physical map and the amount of sequencing data vary across the genome. The complete sequence of chromosome 22 is published (Dunham et al. 1999) but there is a notable lack of sequencing information available for chromosomes 2 or 3 (www.ncbi.nlm.nih.gov). Soon, it should be possible to tap into the various genomic databases and pick out candidate genes without having to do any physical mapping.

Once the disease gene is identified, there are several possible scenarios that will dictate

the rate of progress in elucidating the pathophysiological processes of the disorder. If the sequence represents a novel gene, i.e., there is no available information about its function (e.g., BRCA1 and BRCA2; reviewed by Welcsh et al. 1998), then there is much work to be done. However, if the disease gene shares some coding sequence with other known genes, (e.g., a protein motif with known function), gene function may be predicted as was the case in the identification of transmembrane domains in the gene for cystic fibrosis (see OMIM 602421). The best case scenario is that the gene is homologous to other genes or class of genes that are well represented in other model organisms, such as the nematode, fly or mouse. Information obtained from a positive "hit" in a model organism may instantaneously identify not only the disease gene, but its location in the cell, its function, biochemical pathway and evolutionary history and provide animal models for study (e.g., MSH2 gene; see OMIM 120435). The number of positive "hits" should increase exponentially as data accumulates in the public databases. Once the disease gene is identified, the type and scope of studies available are limitless and often begin with the identification or making of a transgenic animal disease model.

1.4 Outline of Investigation

Within this thesis, the results of a genetic survey of BBS families originating from Newfoundland are discussed. At the onset of this project, four BBS loci (*BBS1-BBS4*) had been mapped but the assignment of the *BBS3* locus (3p13-p12) was still provisional. Recent surveys reported that the majority of families with BBS were linked to the *BBS1* locus on chromosome 11q13 and predicted the existence of a fifth BBS gene in the genome.

Linkage and haplotype analyses were performed on 17 Newfoundland families affected with BBS at the four known loci (**Chapter 2**). The finding of linkage of one extended family from the Conception Bay area to the rare *BBS3* locus confirmed the original linkage report and provided the opportunity to examine the *BBS3* phenotype. Intrafamilial recombination analysis was used to refine the location of the *BBS3* gene (**Chapter 3**). An unlinked, consanguineous family from White Bay (**Chapter 2**) was informative enough to use in a successful genomewide search for a new BBS locus (**Chapter 4**). The finding of several families from the south and southwest coast of the island with evidence of linkage to *BBS1* (**Chapter 2**) prompted a search for an underlying founder effect (**Chapter 5**). The impact of these studies on the direction of future BBS research, with emphasis on the most feasible approach to clone the first BBS gene, is discussed (**Chapter 6**).

2

Genetic Heterogeneity of Bardet-Biedl Syndrome in a Distinct Canadian Population: Evidence for a Fifth Locus

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Summary

Bardet-Biedl syndrome (BBS; OMIM 209900) is a rare, autosomal recessive disease characterized by dysmorphic extremities, obesity, retinal dystrophy, hypogenitalism in males and renal structural abnormalities. So far, four distinct BBS loci (*BBS1*:11q; *BBS2*:16q; *BBS3*:3p; *BBS4*:15q) have been mapped. The island population of Newfoundland, considered a genetic isolate due to the nature of its founding and subsequent isolation, is enriched for BBS with ten times the prevalence of other Caucasian populations of northern European ancestry. A population-based genetic survey was performed on 17 Newfoundland BBS families. Twelve multiplex and five singleton families containing 36 affected individuals were tested for linkage at each of the four known BBS loci. Of the 17 families, ten were informative for linkage and could be unambiguously assigned. Of these, three families (30%) were linked to *BBS1* and one family (10%) was linked to *BBS3*. Most notably, six of the informative families (60%) were excluded from all known BBS loci. These results suggest that the high prevalence of BBS in the Newfoundland population is due to multiple BBS genes and that a significant portion of families are linked to one or more novel BBS loci.

Introduction

Bardet-Biedl syndrome is characterized by retinal dystrophy, obesity, dystrophic extremities, and hypogenitalism in males (Schachat and Maumenee 1982; Green et al. 1989; Parfrey et al. 1997). Structural anomalies of the kidneys, such as persistent fetal lobulation and calyceal abnormalities are also characteristic of Newfoundland BBS patients, with renal impairment being a frequent and important cause of death (Harnett et al. 1988; O'Dea et al. 1996). Four BBS loci, each on a different chromosome, have been identified: *BBS1* on chromosome 11q (Leppert et al. 1994); *BBS2* on chromosome 16q (Kwitek-Black et al. 1993); *BBS3* on chromosome 3p (Sheffield et al. 1994) and *BBS4* on chromosome 15q (Carmi et al. 1995). There is also evidence for a fifth locus in recent reports of families that are excluded at all four BBS loci (Beales et al. 1997; Bruford et al. 1997).

BBS is a relatively rare disorder with a prevalence ranging from 1/160,000 in Switzerland (Klein and Amman 1969) to 1/125,000 in the United Kingdom (Beales et al. 1997). Even though the island of Newfoundland was settled predominantly by northern Europeans, the prevalence of BBS is ten times higher than these estimates (1/17,500; Green et al. 1989) and more closely resembles that found among the Bedouin of Kuwait (1/13,500) where consanguinity is frequent (Farag and Teebi 1989). In order to investigate the high prevalence of BBS in the Newfoundland population and, in particular, to determine if it is the result of a single founder effect, we genotyped

members of 17 families affected with BBS using polymorphic microsatellite markers spanning the critical regions of each of the four known BBS loci.

Materials and Methods

Subjects

Twenty-two families with BBS have been identified from the island of Newfoundland (Green et al. 1989; Harnett et al. 1988; O'Dea et al. 1996). Of these, 17 families were available for genotyping (fig. 2.1). These families are widely distributed in coastal communities around the island (fig. 2.2). Consanguinity between the parents of affected individuals was documented in five families (B3, B8, B9, B13, and B14), and suspected in seven families (B2, B10, B11, B12, B15, B16, and B19) based on progenitors with the same surname and originating from the same community (fig. 2.1). The natural history of the disease in patients originating from the island has been previously reported (O'Dea et al. 1996; Parfrey et al. 1997).

Clinical Investigations

A protocol for clinical investigations was approved by the Human Investigations Committee of the Faculty of Medicine, Memorial University of Newfoundland, and by the Medical Advisory Council of the Health Care Corporation of St. John's. Diagnosis of affected family members was based on the following criteria: the presence of retinal dystrophy, obesity and dysmorphic extremities, in the absence of neurological complications.

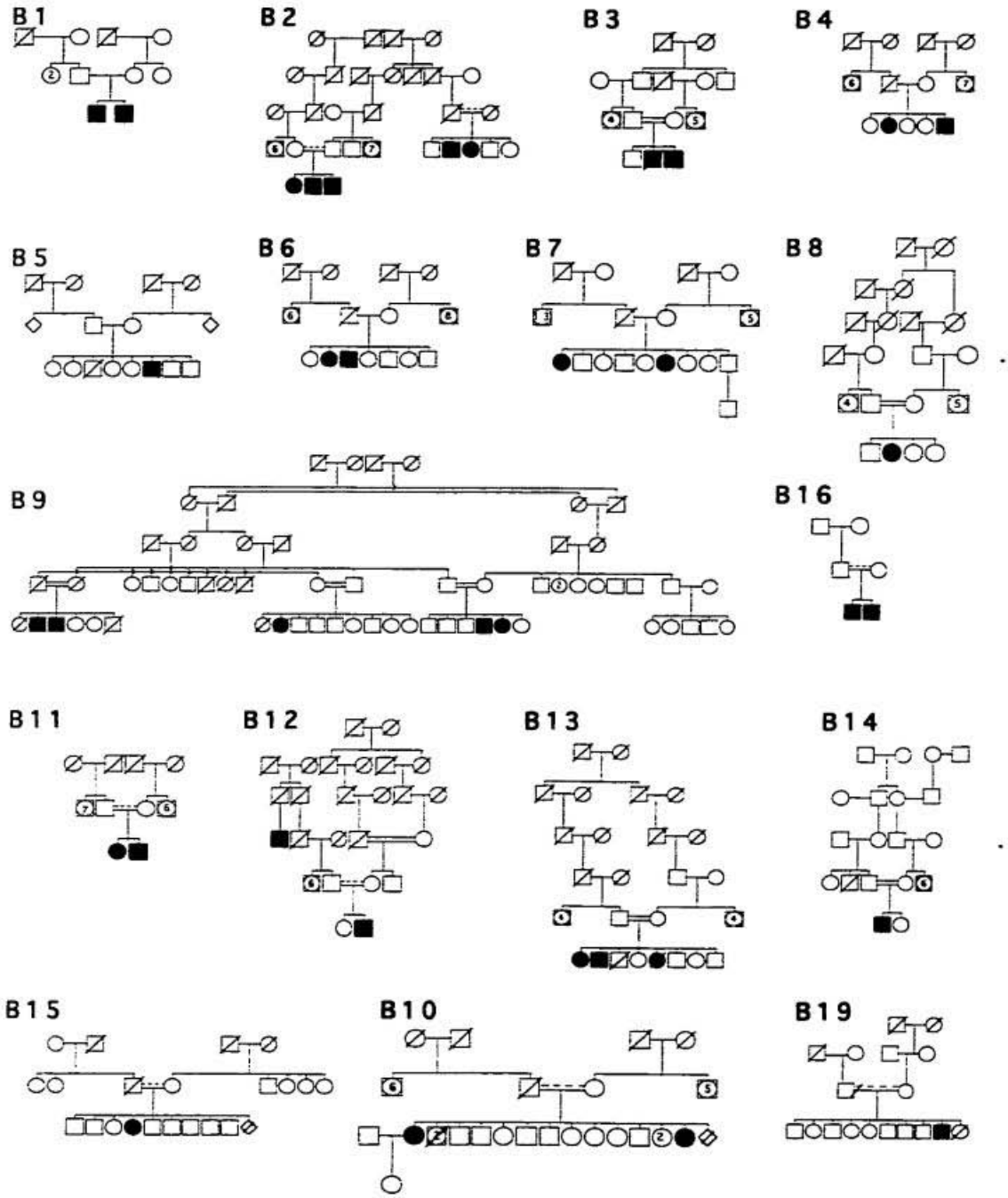


Figure 2.1 Pedigrees of 17 Newfoundland BBS families. Filled symbols indicate individuals diagnosed with Bardet-Biedl syndrome (BBS). Double marriage lines depict consanguineous unions: documented (solid line) and suspected (dashed line).

Figure 2.2 Geographical distribution of the 17 Newfoundland BBS families available for genotyping.

Genotyping

Blood was collected from 20 males and 14 females diagnosed with BBS and 111 unaffected family members. DNA was prepared from whole blood using a simple salting out method (Miller et al. 1988). PCR using 100-200 ng of template DNA was conducted with primers purchased from Research Genetics Inc. (Huntsville, Alabama, USA). One primer of each pair was end-labeled using T_4 polynucleotide kinase (Pharmacia Biotech, Uppsala, Sweden) and γ-^{32}P ATP (Amersham, Ontario, Canada). Samples were subjected to 27-33 cycles of 94°C for 30 sec (denaturation), 55-65°C for 20 sec (annealing) and 72°C for 30 sec (elongation) after an initial denaturing step of 2 min at 95°C. Amplified DNA was run on urea/polyacrylamide denaturing gels with or without formamide (Litt et al. 1993). Autorads were developed after 2-5 days exposure and the alleles were scored blind with respect to disease status.

Linkage and Haplotype Analyses

Two-point linkage analyses were performed using the MLINK subroutine of FASTLINK (V4.0P) (Lathrop and Lalouel 1984; Cottingham et al. 1993; Schäffer et al. 1994). LOD scores were calculated for the most informative microsatellite markers spanning the critical region of the four BBS loci, for each pedigree. Informative markers were recognized as those in which the parents were heterozygous for distinct genotypes. BBS was modeled as an autosomal recessive disorder with a penetrance of 1. The disease allele frequency was adjusted to 0.008, based on the disease incidence of 1/17,500 in the

Newfoundland population (Green et al. 1989). Haplotypes, representing the minimal number of recombination events, were constructed manually from a minimum of four markers spanning each of the four BBS critical regions (fig. 2.3) for all families.

Locus Assignment/Exclusion Criteria

Several criteria were used to assign or exclude a locus based on informative haplotypes within families. A locus was excluded on the basis of either, (i) shared haplotypes between affected and unaffected siblings, and/or (ii) the presence of dissimilar haplotypes among two or more affected siblings. These results were supported by significantly negative LOD scores ($\leq$ -2.00, θ=0) for informative markers. Support for linkage to a locus was given in consanguineous families if the affected individual(s) displayed homozygosity by descent (HBD) in the critical region of only one of the four BBS loci and their unaffected siblings did not (Lander and Botstein 1987). In families where there was unconfirmed or no indication of consanguinity, either homozygosity by state (HBS) or haplotype sharing (HS) of the BBS critical region in affected but not unaffected sibling(s) and positive LOD scores were interpreted as support for linkage.

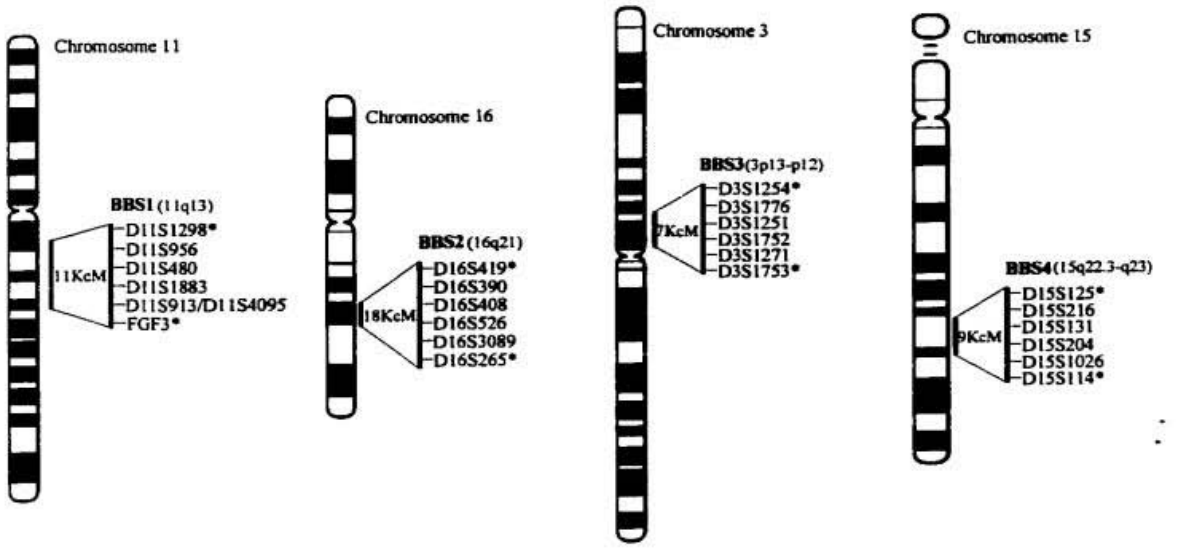


Figure 2.3 Relative map position of the polymorphic markers used to genotype the families at each of the four mapped BBS loci. Boundary markers for each BBS interval (Kwitek-Black et al. 1993; Leppert et al. 1994; Sheffield et al. 1994; Carmi et al. 1995) are denoted by *. The marker *D3S1254* was replaced by the more polymorphic marker *D3S1776*, located < 1 cM away. Genetic distances are based on the Genethon and Marsfield sex-averaged linkage maps (Genome Database). Not all markers were typed or were informative in all families.

Results

Families Assigned to BBS1

Ten of the 17 BBS families were particularly informative and could either be assigned to a single locus or excluded from all four BBS loci. Of these, three families (B8, B10, and B19) were assigned to *BBS1* (table 2.1). The affected sib in family B8, the child of a second cousin union, was HBD for the entire *BBS1* critical region. The affected sib was not HBD at the *BBS2, BBS3* or *BBS4* locus (fig. 2.4). Furthermore, family B8 was excluded from linkage at *BBS2* and *BBS3* on the basis of haplotype sharing between affected and unaffected sibs. Even though the *BBS4* locus could not be excluded, family B8 was assigned to *BBS1* on the basis of homozygosity mapping in a known consanguineous family.

Family B19 was assigned to *BBS1* on the basis of homozygosity mapping in a suspected consanguineous family, even though the *BBS2* and *BBS4* loci could not be excluded. The affected individual in family B19 is the offspring of a suspected consanguineous union and the only family member homozygous for all markers typed within the *BBS1* critical region. The affected child was not homozygous for the *BBS2, BBS3* or *BBS4* disease intervals (fig. 2.4). The *BBS3* locus could be excluded on the basis of shared haplotypes between the affected and unaffected sibs.

Family B10 was assigned to *BBS1* on the basis of HS between the two affected individuals. Persons 9 and 22 share haplotypes exclusively at the *BBS1* locus (fig. 2.5).

Table 2.1 Summary of Linkage and Haplotype Analysis in 17 Newfoundland BBS Kindreds

Kin	*BBS1*			*BBS2*			*BBS3*			*BBS4*			BBS1	BBS2	BBS3	BBS4
N0.	Locus[a]	LOD[b]	HAP[c]	Locus[a]	LOD[b]	HAP[c]	Locus[a]	LOD[b]	HAP[c]	Locus[a]	LOD[b]	HAP[c]				
B1	913	-∞	X	390	0.60	HS	1752	-∞	X	131	0.60	HS	X	?	X	?
B2[d]	4095	-∞	X	390	-∞	X	1752	2.88	HBS	131	-∞	X	X	X	√	X
B3[e]	1883	-1.97	X	265	-0.89	X	1271	-1.56	X	216	-1.42	X	X	X	X	X
B4	913	-∞	X	390	-∞	X	1752	-∞	X	204	-∞	X	X	X	X	X
B5	1883	-∞	X	419	-∞	X	1752	-∞	X	131	-∞	X	X	X	X	X
B6	1883	-∞	X	419	-∞	X	1752	-∞	X	131	-∞	X	X	X	X	X
B7	1883	0.98	HS	265	1.10	HS	1776	-∞	X	204	-∞	X	?	?	X	X
B8[e]	1883	1.32	HBD	408	-∞	X	1752	-∞	X	131	-∞	?	√	X	X	?
B9[e]	1883	-∞	X	408	-∞	X	1251	-∞	X	216	-∞	X	X	X	X	X
B10[d]	4095	1.60	HS	408	-∞	X	1752	-∞	X	131	-∞	X	√	X	X	X
B11[d]	FGF3	-∞	X	390	0.60	HS	1776	0.60	HS	204	0.60	HS	X	?	?	?
B12[d]	913	0.13	?	265	0.13	?	1752	-∞	X	204	0.13	?	?	?	X	?
B13[e]	FGF3	-∞	X	390	-∞	X	1752	-∞	X	131	-∞	X	X	X	X	X
B14[e]	FGF3	-1.35	X	408	-0.32	HBS	1752	-0.36	?	131	-1.42	X	X	?	?	X
B15[d]	FGF3	1.00	HBS	419	0.87	?	1271	-∞	X	204	1.00	HBS	?	?	X	?
B16[d]	913	-∞	X	419	-∞	X	1251	0.56	HS	114	-∞	X	X	X	?	X
B19[d]	4095	0.87	HBS	408	0.75	?	1251	-∞	X	1026	0.87	?	√	?	X	?

Note.-[a]D number of the microsatellite marker; [b]LOD score at θ=0; [c]Results of haplotype analysis: X= Excluded, HS= Haplotype Sharing, HB(D/S)= Homozygosity by (Descent/State), ?= Inconclusive; [d]Suspected consanguinity; [e]Documented consanguinity.

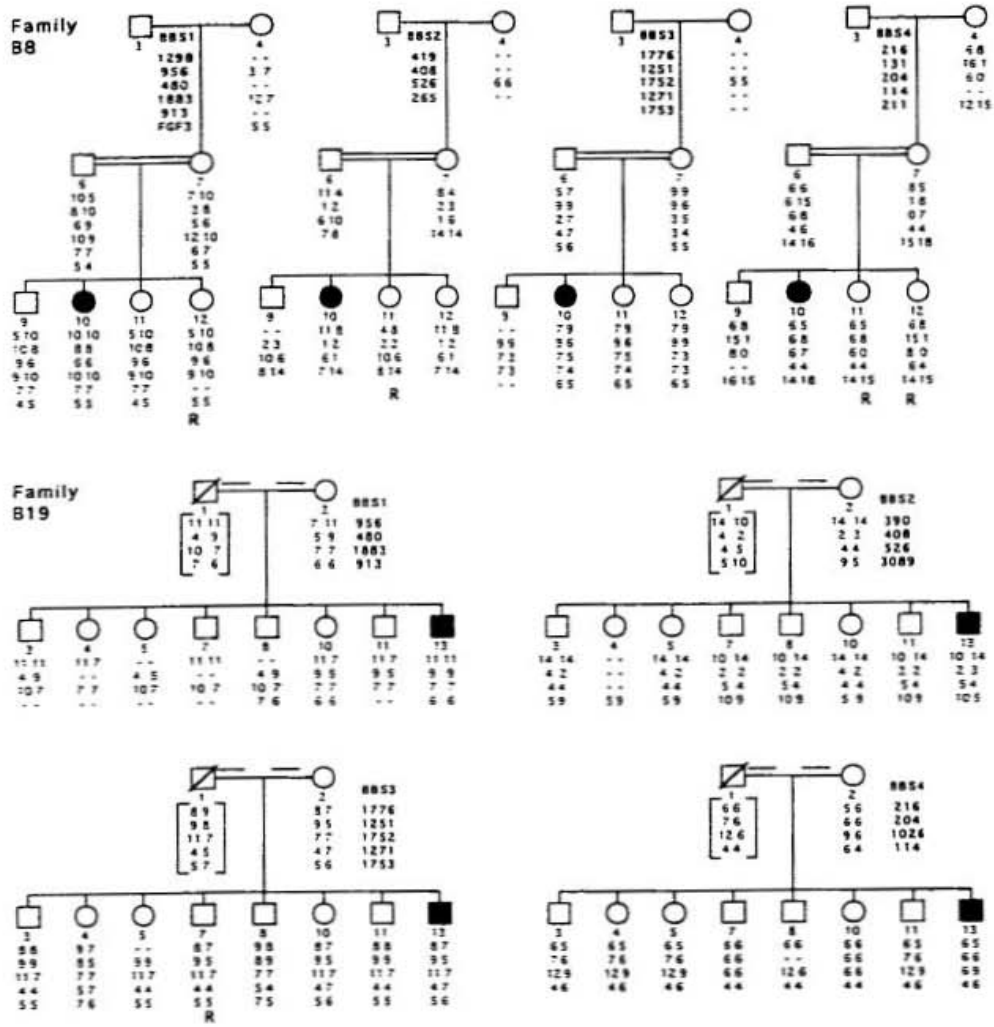


Figure 2.4 Core pedigrees of families B8 and B19, two of three families assigned to the *BBS1* locus. Haplotypes are shown for each of the four BBS loci and represent the minimum number of recombinations. Dotted lines denote suspected consanguinity; brackets indicate inferred haplotypes; an R denotes a recombinant.

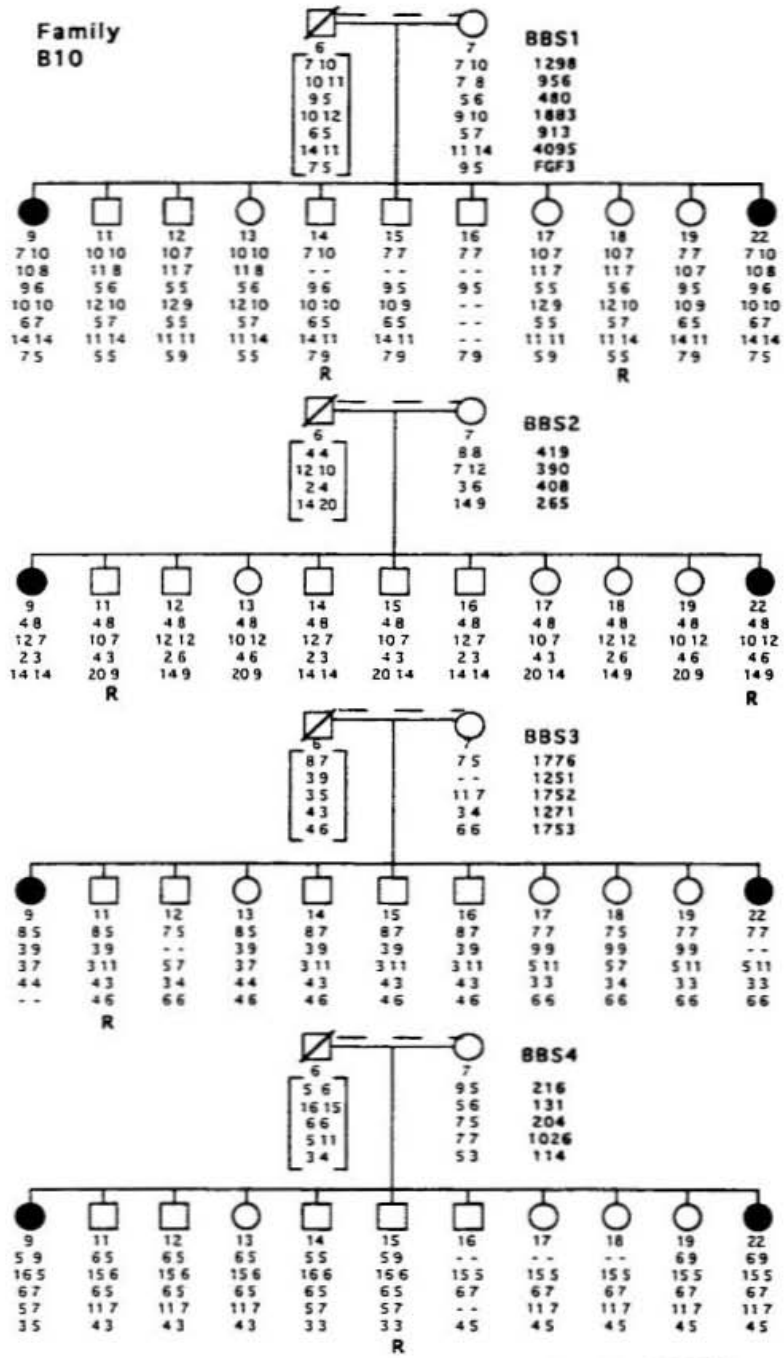


Figure 2.5 Core pedigree of family B10 assigned to the *BBS1* locus. Brackets indicate inferred haplotypes; an R denotes a recombinant. Haplotypes are shown for each of the four BBS loci and represent the minimum number of recombinations. Dotted lines denote suspected consanguinity; brackets indicate inferred haplotypes; an R denotes a recombinant.

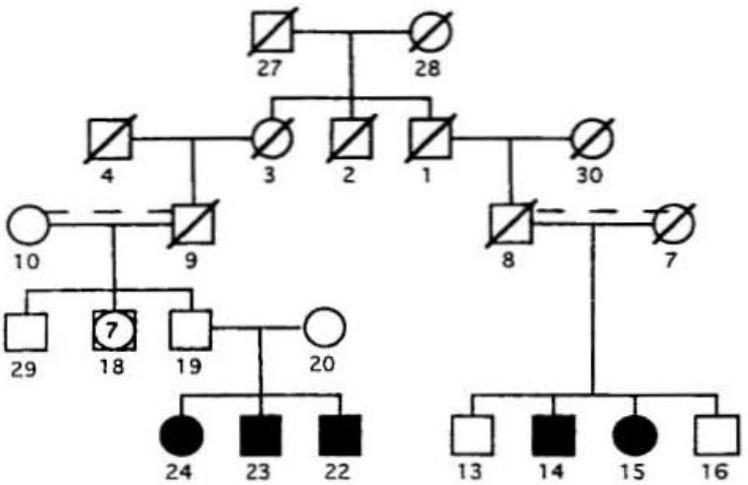
Both exclusion criteria were met at each of the other three loci in which the affected siblings inherited dissimilar haplotypes with each other but shared haplotypes with their unaffected siblings at *BBS2, BBS3* and *BBS4* (fig. 2.5). Markers mapping to the *BBS1* region gave positive LOD scores (e.g., LOD of 1.60 at θ=0 for marker *D11S4095*) and those at other loci yielded significantly negative LOD scores (table 2.1).

Family Assigned to BBS3

Family B2 is a large, multiplex family with five affected individuals in two sibships. The parents of both sibships are believed to be consanguineous. All affected individuals in this kindred were homozygous for at least four of the five markers typed at the *BBS3* locus (fig. 2.6). Furthermore, it was apparent that all affected individuals were homozygous for a single, disease-associated *BBS3* haplotype from a recent common ancestor (fig. 2.6). Linkage and haplotype analyses support the exclusion of family B2 at *BBS1, BBS2*, and *BBS4*. Therefore, family B2 was assigned to *BBS3* on the basis of homozygosity mapping in a suspected consanguineous family.

Exclusion of Families from All Known BBS Loci

Remarkably, six of the ten informative families (B3, B4, B5, B6, B9, and B13) were excluded from linkage at all four BBS loci, suggesting the presence of at least a



BBS1	1298	5 10	10 9	9 8	4 7	7 5	4 5	7 7
	480	5 9	- -	5 7	5 9	9 7	5 7	9 9
	1883	10 7	7 10	10 5	10 12	12 5	10 5	12 12
	4095	5 6	9 6	6 9	5 6	6 9	5 9	6 6
	FGF3	5 5	5 5	5 5	5 9	3 5	5 5	3 9
BBS2	419	4 9	- -	- -	9 3	9 11	3 4	3 9
	390	5 5	5 2	5 14	- -	7 12	10 14	10 14
	408	3 2	- -	3 4	3 4	4 4	2 3	2 3
	526	10 8	10 -1	10 8	6 14	6 40	14 6	14 6
	265	14 7	14 7	14 7	- -	14 7	- -	3 14
BBS3	1776	7 7	7 7	7 7	7 8	7 9	7 8	7 9
	1251	9 9	9 9	9 9	8 9	9 9	9 9	8 3
	1752	7 7	7 7	7 7	4 7	7 7	7 7	4 11
	1271	4 4	4 4	4 4	7 4	4 4	4 4	7 4
	1753	4 4	4 4	4 4	5 4	4 4	4 4	5 5
BBS4	216	- -	5 9	3 6	- -	- -	- -	- -
	131	8 15	8 15	3 16	8 16	10 16	- -	10 11
	204	4 7	4 7	6 6	6 7	7 7	6 7	7 6
	114	4 5	4 5	3 4	4 4	5 4	4 5	5 4

Figure 2.6 Core pedigree of family B2 assigned to the *BBS3* locus. Haplotypes represent the minimum number of recombinations. Dotted lines denote suspected consanguinity.

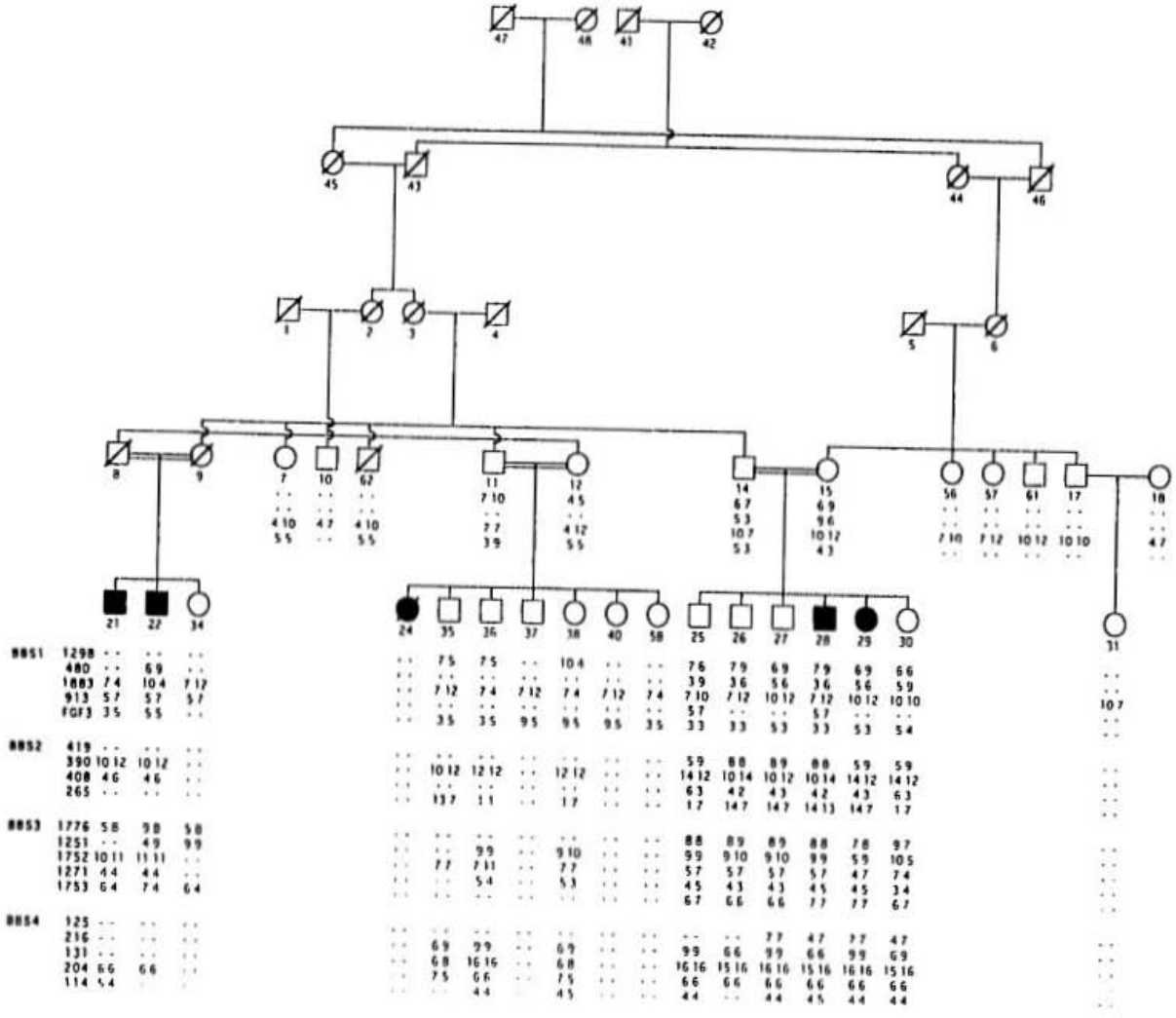


Figure 2.7 Pedigree of family B9, an unlinked family excluded at all four known BBS loci. Corresponding genotypes at the *BBS1* locus are given for generations IV and V for *BBS1* and for generation V only for *BBS2, BBS3* and *BBS4*.

fifth BBS locus in the human genome (table 2.1, fig. 2.7; Appendices 2, 3, 4, 5 and 9). The affected individuals in three of these five kindreds (B3, B9, and B13) are the product of consanguineous unions. Of these unlinked families, family B9 is remarkable in that five BBS patients are distributed among three sibships interrelated by three inbreeding and two marriage loops (fig. 2.7). This informative family was used to search for a fifth BBS locus employing the technique of homozygosity mapping (Lander and Botstein 1987) (**Chapter 4**).

Unassigned (Uninformative) Families

It was not possible to assign seven families (B1, B7, B11, B12, B14, B15, and B16) with confidence to a particular locus or to exclude them from all known BBS loci (table 2.1; Appendices 1, 6, 7, 8,10,11 and 12). This usually was the result of poor pedigree structure. For instance, the absence of unaffected individuals in affected sibships is particularly uninformative because one of the two exclusion criteria (i.e., shared haplotypes between affected and unaffected siblings) cannot be applied (e.g., families B1, B11, and B16). For example, the two affected individuals in family B11 share the same haplotype within the critical regions of the *BBS2, BBS3* and *BBS4* loci, giving equivocal evidence for linkage at three BBS loci (table 2.1; Appendix 7).

Discussion

A genetic survey was conducted on 17 of 22 families affected with BBS in the Newfoundland population. Ten of these 17 families were particularly informative and it was possible to either assign or exclude them at each of the four known BBS loci. Four of the families were linked to known BBS loci and six were unlinked at all four BBS loci. At the conclusion of the present study, twelve of the 22 BBS families identified in Newfoundland (five unavailable families and seven uninformative families) remain unassigned.

The most striking outcome of this study, in comparison to two similar studies of predominantly northern European families, was that the majority of informative Newfoundland families (60%) were unlinked at all four known BBS loci. Although this is not the first report of unlinked BBS families, the relative proportion among families is high. Beales et al. (1997), in a survey of 18 families, found that 28% of families were unlinked and Burford et al. (1997) reported an even smaller portion (8%) of unlinked families in a larger study of 29 families. The finding of unlinked families in previous reports and the overwhelming evidence from this study suggest the existence of at least a fifth BBS locus.

Three of the ten informative families (30%) were linked to the *BBS1* locus. The predominance of *BBS1* families among linked kindreds of northern European ancestry has been previously reported. Surveys of 47 BBS families (of predominantly northern

European descent) have found that 36%-56% of families are linked to *BBS1* (Beales et al. 1997; Burford et al. 1997). If the three uninformative families that could not be excluded from *BBS1* (table 2.1) are, in fact, *BBS1* families, the maximum potential contribution (based on 17 families) of the *BBS1* locus in the Newfoundland population is 35% . These results suggest that the burden of *BBS1* in the Newfoundland population (30%-35%) may reflect that reported in other northern European populations (Beales et al. 1997; Burford et al. 1997).

Although none of the families in this survey were assigned to either *BBS2* or *BBS4*, these loci cannot be considered absent from the population because of the remaining unassigned families. Of the seven uninformative families, six families (B1, B7, B11, B12, B14, and B15) could not be excluded from *BBS2* and four families (B1, B11, B12, and B15) could not be excluded from *BBS4* (table 2.1). The complete overlap between these two groups of unassigned families suggest that if both *BBS2* and *BBS4* loci are present in the population, the contribution of either locus will be relatively small. Beales et al. (1997) found that *BBS2*-linked and *BBS4*-linked families accounted for 17% and 5% of families, respectively. However, these loci were slightly more prevalent in the survey by Burford et al. (1997): *BBS2*- and *BBS4*-linked families accounted for 24-27% and 32-35% of families, respectively.

The finding of a large, *BBS3*-linked family in Newfoundland is significant in that families linked to *BBS3* were conspicuously absent from surveys of families of northern European extraction (Beales et al. 1997; Bruford et al. 1997). Moreover, this is the only

report of a family linked to the *BBS3* locus other than that used in the initial linkage study (Sheffield et al. 1994) and confirms the existence of a BBS locus on chromosome 3p. Refinement of the *BBS3* disease interval and a clinical description of this family in comparison to the Bedouin family (Sheffield et al. 1994) is presented in **Chapter 3**.

This study finds at least three independent BBS mutations (*BBS1*, *BBS3*, and "*BBS5*") segregating in the Newfoundland population. The genetic heterogeneity of BBS revealed in the present study is consistent with the scattered geographic distribution of BBS families on the island (fig. 2.2) and with the findings of other surveys. The results of this study dismisses the notion that the high incidence of BBS on the island is due to a single BBS founder. However, the finding of multiple BBS genes in the population does not preclude the possibility of a founder effect at one or more BBS loci. Family B9, the most informative of the unlinked families, was used in a homozygosity mapping strategy to search for a novel BBS locus (**Chapter 4**). To test the hypothesis of a founder effect at the *BBS1* locus, a linkage disequilibrium study was pursued (**Chapter 5**).

Acknowledgments

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Electronic Database Information

Online Mendelian Inheritance in Man (OMIM)

(http://www.ncbi.nlm.nih.gov/Omim)

The Genome Database (GDB)

(http://www.gdb.org)

3

A Canadian Bardet-Biedl Syndrome Family Reduces the Critical Region of *BBS3* (3p) and Presents with a Variable Phenotype

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Summary

There are at least five distinct Bardet-Biedl syndrome (BBS) loci, four of which have been mapped: 11q (*BBS1*), 16q (*BBS2*), 3p (*BBS3*) and 15q (*BBS4*). A comparative study of the three Arab-Bedouin kindreds used to map the *BBS2, BBS3* and *BBS4* loci suggests that the variability in the number and severity of clinical manifestations, particularly the pattern of polydactyly, reflects chromosome-specific subtypes of BBS (Carmi et al. 1995). We describe a Newfoundland kindred of northern European descent and confirm the initial finding of a BBS locus on chromosome 3. However, the "*BBS3* phenotype," which includes polydactyly of all 4 limbs and a progression to morbid obesity, was not observed. Rather, four of the five BBS patients in this family had polydactyly restricted to their feet. The obesity in these patients was reversible with caloric restriction and/or exercise. Mental retardation has traditionally been considered a cardinal feature of BBS, however, formal IQ testing reveals that the affected family members in this *BBS3*-linked family are of average intelligence. Homozygosity mapping was used to refine the *BBS3* region from 11- cM to a 6-cM interval flanked by the polymorphic markers *D3S1595* and *D3S1753*.

Introduction

Variability in the number and severity of Bardet-Biedl syndrome (BBS) manifestations is frequent and as much variation is observed among BBS patients within families as between families (Bergsma and Brown 1975; Green et al. 1989; Carmi et al. 1995; Riise et al. 1997). Linkage studies have identified four distinct BBS loci to date. The *BBS1* locus (11q) was inferred by pooling linkage data from 17 of 31 small North American families (Leppert et al. 1994). The *BBS2* (16q; Kwitek-Black et al. 1993), *BBS3* (3p; Sheffield et al. 1994) and *BBS4* (15q; Carmi et al. 1995) loci were mapped in unrelated, consanguineous Arab-Bedouin families. At least a fifth locus for BBS is anticipated due to the finding of unlinked families (Beales et al. 1997; Bruford et al. 1997; Chapter 2).

The hypothesis that the variable clinical expression of BBS is a result of genetic heterogeneity was proposed by Carmi et al. (1995). They compared the clinical manifestations of patients between the three Arab-Bedouin families used to map the *BBS2, BBS3* and *BBS4* loci. Chromosome-specific patterns in limb distribution of postaxial polydactyly and in the extent and age-association of obesity were observed. However, these chromosome-specific subtypes were not supported in the clinical findings of 2 recent surveys of 47 families linked to either *BBS1*, *BBS2 or BBS4*. Similar comparisons could not be made for families linked to *BBS3* because they were not found (Beales et al. 1997; Bruford et al. 1997).

We present a clinical investigation of a *BBS3*-linked family of northern European descent, the first report of a non-Bedouin *BBS3* family. We compare the phenotype of the 5 BBS patients in this family with that of the Arab-Bedouin kindred used to map the chromosome 3 locus (Carmi et al. 1995; Sheffield et al. 1994). A homozygosity mapping strategy was used to refine the critical region of *BBS3*.

Materials and Methods

Subjects

Family B2 is one of 22 BBS kindreds identified in the Newfoundland population (Green et al. 1989; Harnett et al. 1988; O'Dea et al. 1996). The ancestors of family B2 came from the southwest of England (personal communication) and founded a small fishing village on the tip of the south shore of Conception Bay in Newfoundland (fig. 2.2, **Chapter 2**). The extended pedigrees of the spouses, persons 7 and 20, have surnames in common with this family and are probably related to it but the connections have not been found (fig. 2.1, **Chapter 2**). All five adult patients surpass the minimal requirements of at least three major anomalies for a diagnosis of BBS. They all have retinal dystrophy, polydactyly, renal structural abnormalities and a history of obesity. The genitalia of two male patients were examined and showed hypogenitalism.

Linkage and Haplotype Analyses

DNA was extracted from the white cells of venous blood by a simple salting out procedure (Miller et al. 1988). Standard 10-μL PCR reactions contained 1.5 pmol of primer, 200 μm dNTPs and 0.125 units of Tfl DNA polymerase (Promega, Madison, WI, USA). The forward primer was end-labeled with $\gamma[^{32}P]$ -ATP by T4 polynucleotide kinase (Pharmacia Biotech, Uppsala, Sweden). PCR products were run on standard

denaturing 6% polyacrylamide-urea gels with or without formamide (Litt et al. 1993) and autoradiography was performed. Primers that flanked polymorphic microsatellite markers located within the critical regions of *BBS1*, *BBS2*, *BBS3* and *BBS4* were purchased from Research Genetics Inc. (Huntsville, Alabama, USA) and included *D3S1776*, *D3S1251*, *D3S1752*, *D3S1271*, *D3S1753*, *D11S1298*, *D11S480*, *FGF3*, *D11S1369*, *D15S216*, *D15S131*, *D15S204*, *D15S114*, *D15S211*, *D16S419*, *D16S390*, *D16S408*, *D16S526*, and *D16S265*. Additional markers used to extend the haplotypes for chromosome 3 are given in fig. 3.2.

Two-point linkage analyses were performed with the MLINK subroutine program of FASTLINK (V3.0P), a modified version of LINKAGE (Cottingham et al. 1993; Lathrop and Lalouel, 1984; Schäffer et al. 1994). We modeled the BBS trait as an autosomal recessive disorder and used a conservative estimate of penetrance [95%]. The disease-allele frequency was set at 0.008 to correspond with an estimated prevalence of 1/17,500 previously determined for BBS in the Newfoundland population (Green et al. 1989). All microsatellites were assumed to have nine alleles of equal frequencies in the study population. Changing the frequency of the marker or the disease alleles, or increasing the disease penetrance had little effect on the LOD score calculations. Haplotypes were assigned manually and represent the least number of recombinations.

Clinical Investigations

A protocol for clinical investigation was approved by the Human Investigations

Committee, Faculty of Medicine, Memorial University of Newfoundland, and by the Medical Advisory Council of the Health Care Corporation of St. John's. Appropriate informed consent was obtained and medical records were reviewed.

Polydactyly, if present, provides the first diagnostic finding in newborn infants with BBS. However, it has been our observation that brachydactyly is a consistent feature of BBS and may provide a more reliable diagnostic tool. To test the significance of brachydactyly in BBS we conducted a metacarpophalangeal pattern profile (MCPP) analysis on 14 BBS patients from 10 families (BBS group). Posterioanterior hand radiographs were taken and the length of each of the 19 tubular bones of the hand were measured according to the procedure of Poznanski et al. (1972). Measurements were standardized for age and sex by conversion to Z-scores (Z = [patient measurement-mean]/ S.D.) using the population means of Caucasian individuals of northern European extraction obtained by Garn et al. (1972). A mean MCPP was plotted for the BBS group using the average Z score for each of the 19 hand bones. The significance of hand dysmorphogenesis for the BBS group was tested with the pattern variability index (PVI; σz), a method that expresses the degree of hand dysmorphogenesis as a single number (Garn et al. 1987). By using Pearsonian correlation coefficients, we compared the similarity between the MCPP of each patient with the BBS group.

A physical examination was performed and measurements were made of height and weight. The presence of obesity was assessed using the body mass index (BMI) scale. A BMI >27 was considered obese (Nelson et al. 1994). Retinal function testing was

performed when vision permitted. Color vision was tested with the Ishihara test, AO H-R-R pseudoisochromatic plates and Farnsworth Panel D-15. Visual field testing with the Goldmann perimeter, and electroretinographic (ERG) studies were performed. The fundus was viewed with direct and indirect ophthalmoscopy and fluorescein angiography was carried out. Psychological testing consisted of the standard Wechsler Adult Intelligence Scale (WAIS) verbal sub-tests, and the Haptic Intelligence Scale (Stoelting Co., Chicago, Illinois), a performance test for those with severe visual impairment. Renal imaging was carried out by ultrasonography and intravenous pyelography. Serum and urine tests of kidney function were conducted.

Results

Linkage and Haplotype Analyses

Two-point linkage analyses provide strong support for linkage between BBS and chromosome 3 (*BBS3*) and exclude linkage to *BBS1, BBS2* and *BBS4* (table 3.1). Haplotype analysis with markers spanning the *BBS3* critical region show that all of the affected family members were homozygous for a portion of the *BBS3* critical region (fig. 3.1). The siblings, persons 22, 23 and 24, are homozygous for all typed markers. They have inherited two copies of the *BBS3* haplotype, pter 7-5-8-7-9-7-4-4 qter. Persons 14 and 15 are homozygous for the same alleles as their affected cousins at marker loci in the qter portion of the *BBS3* critical region. The finding of identical, homozygous haplotypes in affected cousins suggest that all four parents share a common ancestry. The minimal region of homozygosity in an affected individual was observed in person 14 and includes the markers *D3S1251, D3S1752, D3S1271* and *D3S1753*. If we assume that the BBS patients in this extended family are homozygous by descent (HBD) for the *BBS3* critical region, then these findings suggest that the *BBS3* gene is located on the qter side of marker *D3S1595* (figs. 3.1 and 3.2).

Table 3.1

Two-point LOD Scores Between the BBS Trait and Markers in the Critical Regions of the Four BBS Loci

Locus	Marker	θ=	0	0.00	0.01	0.10
BBS1	*D11S1298*		-7.31	-4.2	-2	-1.1
BBS2	*D16S390*		-12.9	-5.2	-2.6	-1.6
BBS3	*D3S1752*		2.84	2.77	2.5	2.15
BBS4	*D15S204*		-7.99	-4.7	-2.5	-1.5

Clinical Investigations

Limbs

All patients in this *BBS3*-linked family had brachydactyly and at least a single, well-differentiated, extra digit. The pattern was variable and none of the BBS patients had polydactyly of all four limbs (table 3.2). The MCPP analysis of 14 BBS cases in 10 Newfoundland families (fig. 3.3) generated mean Z scores ranging from -0.030 (metacarpal 4) to -3.386 (distal phalange 4). Measurements in patients were lower than the population standards but there was some overlap with normal values. Brachydactyly in BBS patients represents significant dysmorphogenesis as shown by an elevated PVI value of 1.294 ($p < 0.005$) (Garn et al. 1987). All 14 patients individually showed a significant positive correlation to the mean BBS pattern ($p < .05$; data not shown) including an affected member of family B2 (person14; coefficient of 0.91 at $p < .0005$).

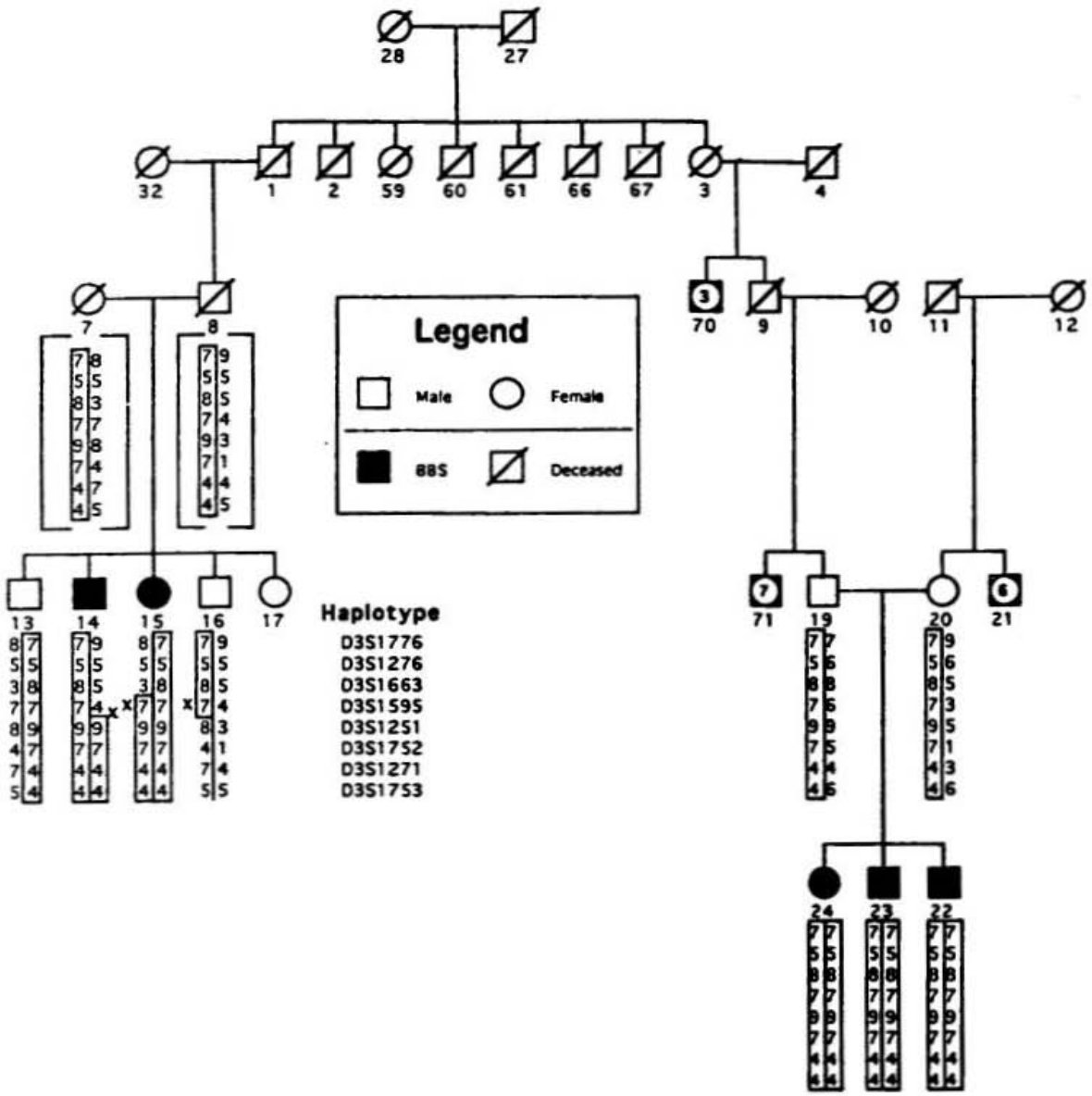

Figure 3.1 Family B2 with corresponding haplotypes at the *BBS3* critical region. The affected adults have inherited two copies of an ancestral haplotype (boxed) at the *BBS3* locus. An "x" denotes a recombinant haplotype.

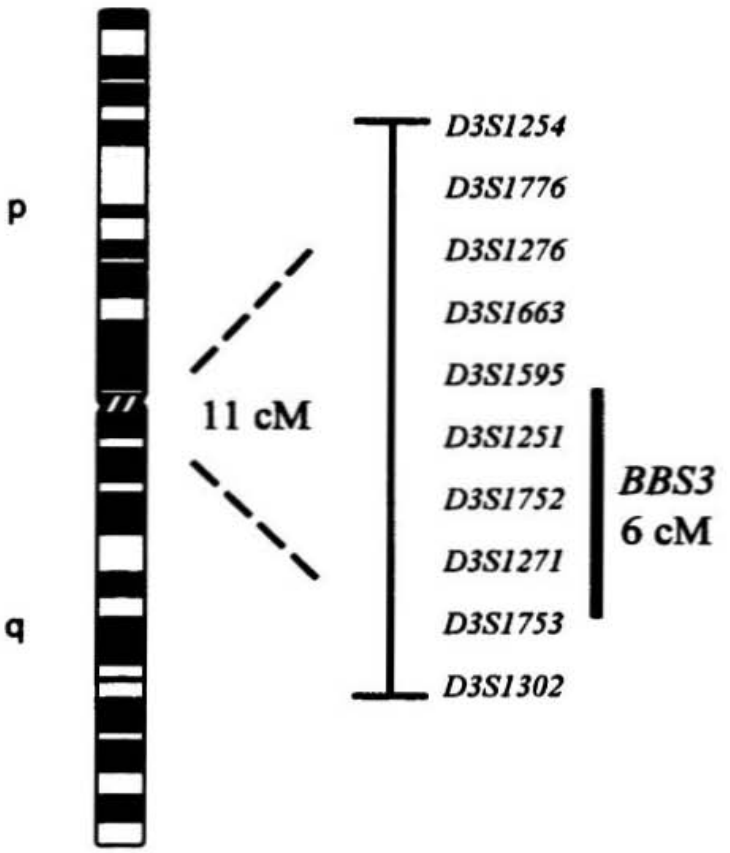

Figure 3.2 Refinement of the *BBS3* disease interval by homozygosity mapping in family B2. The loss of homozygosity at the *D3S1595* locus in person 14 (fig. 3.1) and a recombination between BBS and the marker *D3S1753* in the original Arab-Bedouin family (fig.1, person V-27, Sheffield et al. 1994) support a location for *BBS3* between markers *D3S1595* and *D3S1753*, a genetic distance of approximately 6-cM.

Table 3.2

Limb Distribution of Postaxial Polydactyly

Patient	Sex	Hand		Foot	
		L	R	L	R
14	M	-	-	x	x
15	F	-	-	x	x
22	M	-	-	-	x
23	M	-	-	x	x
24	F	x	-	-	x

Note.- x = extra digit; - = no extra digits

Obesity

Four of the five adult patients were obese (BMI>27) when we initially investigated this family (Visit 1, table 3.3). Person 14 weighed 104.3 kg with a corresponding BMI of 32.2, prior to visit 1 (personal communication). The onset of obesity was early in these patients. For example, person 23 was described in his medical records as a "grossly overweight" child at age 2 yrs when he was admitted to hospital to have his extra toes removed. Four of the BBS patients in this family have maintained or reduced their body mass by caloric restriction, medication and/or exercise for the treatment of type II diabetes. Most notably, person 14, diagnosed with diabetes at age 42 yrs, has lost 37 kg over the last 16 years. However, his cousin (person 24), who is not diabetic or physically active, has gained approximately 20 kg in the seven years since her first visit (table 3.3).

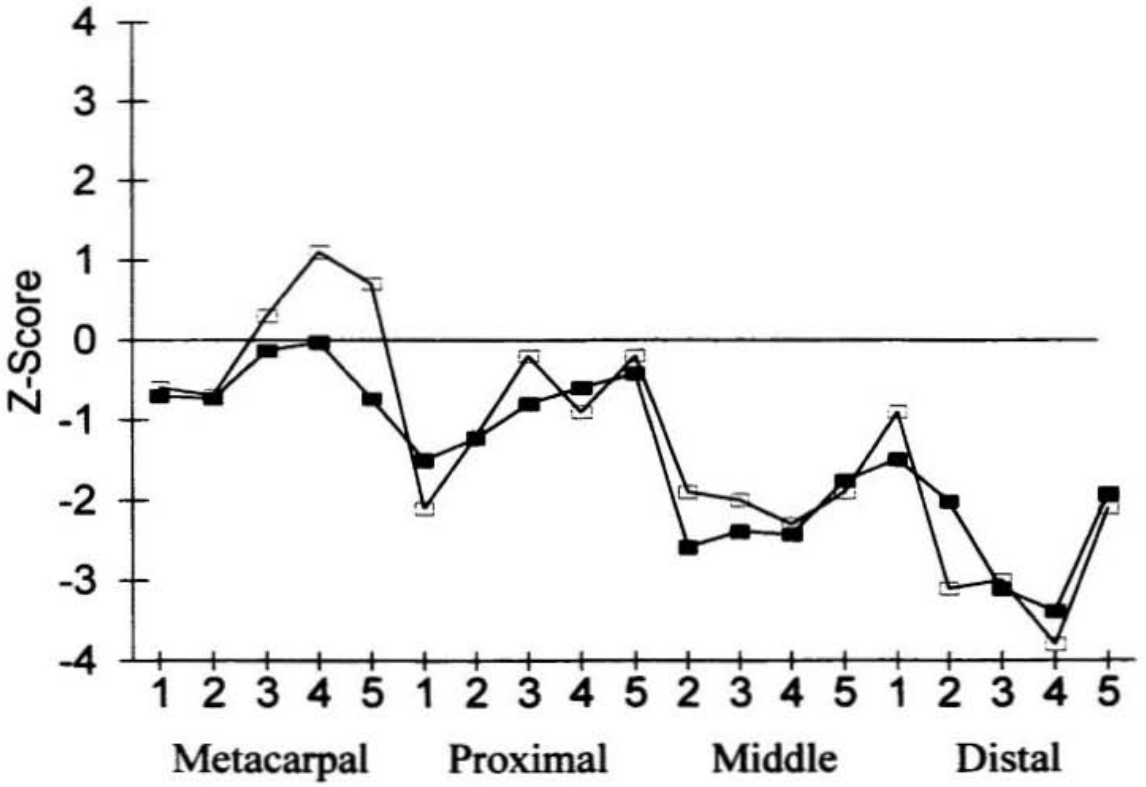

Figure 3.3 Characteristic metacarpophalangeal pattern (MCPP) profiles of BBS patients. MCPP profiles of the BBS group (n =14; mean Z-scores represented by solid squares) and patient 14 from family B2 (individual Z-scores are represented by open squares).

Table 3.3

Body Mass Index of BBS Patients

Person	Sex	Visit	Age (yrs)	Ht.(cm)	Wt(kg)	BMI
14	M	1	51	180	72.1	22.3
		2	58	180	67.3	20.8
15[a]	F	1	54	154	81.8	34.5
22	M	1	36	165	79.1	29.1
		2	44	165	79.5	29.2
23	M	1	31	176	98.8	31.9
		2	39	176	90.9	29.3
24	F	1	22	162	78	29.7
		2	29	162	98.6	37.6

Note.- BMI= body mass index (wt [kg]/ht $[m]^2$;
[a]Maintained a constant weight for the past 12 years.

Retinal Dystrophy

A review of the medical records indicates that the five affected family members experienced rapid deterioration of vision as children and four of them were legally blind as adolescents. All patients examined had advanced retinal degeneration (table 3.4). The retina, as viewed in persons 22 and 24, had the fundoscopic appearance of atypical retinitis pigmentosa, with sparse bone spicule pigmentation, central and peripheral atrophy, attenuated vessels and normal to mild optic disc palor. The retina in person 14 could not be observed due to the presence of dense cataracts.

Table 3.4

Visual Assessment of BBS Patients

Person	Sex	Age	Visual Acuity[a]	Age Onset[b]	Age Reg[c]	Nyst[d]	Cats[e]	ERG	Fundus
14	M	51	NLP	6	13	Yes	Yes	NR	NS
22	M	36	LP	10	18	No	Yes	ND	Atyp. RP
24	F	23	LP	5	11	Yes	No	ND	Atyp. RP

[a]NLP= No Light Perception; LP= Light Perception only; NR= No Response; ND= Not Done; RP= Retinitis Pigmentosa; NS= Not seen
[b]Onset of night blindness
[c]Registered blind (Visual Acuity of ≤ 20/200 or a visual field of ≤ 20 degrees)
[d]Nystagmus
[e]Cataracts

Mental Retardation

Three of the affected family members (persons 14, 22 and 24) were formally tested and had an average IQ (90-110) for both the performance and verbal components of the test. Person 24 has successfully completed public high school and her brother, person 22, has obtained an undergraduate psychology degree. Their cousin, person 14, is a retired switch board operator. Of the two untested BBS patients, we consider person 23 to be at least of average intelligence as he has successfully completed high school and one year of university courses and is employed as a customer service supervisor with a private company. Person 15 was not tested because she is being treated with lithium for bipolar disease and it was felt that the medication would prevent an accurate assessment of her mental capacity. However, she did receive her grade 5 diploma before discontinuing her

formal education.

Urogenital System

The kidneys of persons 14, 15 and 24 were examined and displayed mild to moderate bilateral fetal lobulation with none or few calyceal cysts. The bladder and urethra were normal. Results of kidney function tests on all patients were normal. Serum creatinine levels ranged from 62 to 117 μmoles per liter (normal range: 40-130 μmoles per liter) and blood urea nitrogen (BUN) levels ranged from 3.5- 6.3 mmoles per liter (normal range: 3.5-7 mmoles per liter). Urine tests were either negative or showed trace amounts of blood or protein. The genitalia of two male patients were examined and revealed hypogenitalism.

Discussion

Clinical Investigations

The variation in the number and severity of clinical manifestations of BBS is well documented (Bergsma and Brown, 1975; Carmi et al. 1995; Green et al. 1989; O'Dea et al. 1996; Riise et al. 1997). Carmi et al. (1995) presented phenotypic comparisons among three multiplex Arab-Bedouin families linked to *BBS2, BBS3* and *BBS4* and suggested that the clinical variability reflects the expression of different BBS genes. The confirmation and delineation of locus-specific BBS phenotypes could be of prognostic value and may expedite the search for family-specific (private) mutations.

Limbs

Limb abnormalities provide the first significant diagnostic finding in newborn infants of known BBS families. However, postaxial polydactyly, often cited as a major manifestation of BBS, is not a universal finding (Ammann, 1970) and is observed in only 58% of patients in the Newfoundland population (Green et al. 1989). Structural anomalies such as brachydactyly and syndactyly are present in all BBS patients from Newfoundland (Green et al. 1989; O'Dea et al. 1996). To evaluate the significance of brachydactyly in BBS, we examined the hands of patients by MCPP analysis and found a reduction in the mean tubular bone length, representing a significant deviation from the normal pattern. The group BBS profile was highly correlated with that of individual

patients. This suggests that the MCPP pattern for BBS is quite homogeneous. Since BBS is a progressive disease with multiple affects, early diagnosis of newborn infants with MCPP analysis may be useful when patients present without polydactyly.

The extent of limb involvement of postaxial polydactyly has varied from a single extra digit to an extra digit on both hands and both feet of BBS patients (Green et al. 1989; Klein and Ammann 1969). In the Arab-Bedouin kindred linked to *BBS3*, Carmi et al. (1995) observed that 11 of 12 affected family members had polydactyly of all four limbs. They suggested that polydactyly of all four limbs is a distinctive component of the *BBS3*-specific phenotype. However, none of the five patients in the Newfoundland family linked to *BBS3* had polydactyly of all four limbs and four of them had involvement of the feet only. No distinctive family pattern of polydactyly was observed in this study or has previously been observed among families in the Newfoundland population (Green et al. 1989) or in 44 other families linked to *BBS1*, *BBS2* or *BBS4* (Rudling et al. 1996). Carmi et al. (1995) noted a more random distribution of polydactyly among the *BBS2*- and *BBS4*-linked Arab-Bedouin kindreds. Our observations suggest that the pattern of polydactyly is also variable among *BBS3* patients.

Retinal Dystrophy

Retinal dystrophy is probably the most constant and invariable finding in BBS. The visual changes occur in childhood and manifest either as a loss in central visual acuity and/or decreased night and peripheral vision. The retina appears to be thin and atrophic

with a less striking scattering of black pigment than that of typical retinitis pigmentosa (Ammann, 1970; Bell 1958; Schachat and Maumenee 1982). By the second or third decade of life, the BBS patient experiences a severe loss of central and peripheral vision (Green et al. 1989; Jacobson et al. 1990; Futon et al. 1993). All BBS patients examined from Newfoundland have a severe retinopathy and 50% are legally blind by age 18 yrs (O'Dea et al. 1996). The visual defect was severe in this *BBS3* family as all patients were legally blind by age 18 yrs. The retinal appearance was that of an atypical form of retinitis pigmentosa, a familiar pattern amongst Newfoundland BBS patients (Green et al. 1989).

Mental Retardation

Mental retardation has traditionally been considered a cardinal feature of BBS, based on the delayed psychomotor development of affected children. This approach fails to take into account the diminishing educational opportunities, due to the progressive nature of the visual defect associated with retinal dystrophy, in these patients. We reported earlier that when appropriate verbal and performance IQ tests for the visually impaired were used, only 41% of Newfoundland BBS patients were considered mentally retarded (Green et al. 1989). A study by Riise et al. (1997) reports that the social performance of adults with BBS is higher than the IQ scores of childhood would predict and most patients function within the normal range of intelligence. Formal IQ testing and social/academic performance indicate that at least four of the five BBS patients in this

family are of average intelligence. Therefore, the prognosis of mental retardation for BBS patients should be reconsidered and efforts made to meet their special educational needs.

Obesity

The degree of obesity in BBS is variable within families but tends to occur early in childhood and increases with age (Bell 1958; Ammann 1970; Green et al. 1989). Observations of the Arab-Bedouin family suggest that *BBS3* patients progress toward morbid obesity (Carmi et al. 1995). All patients in this *BBS3* family are currently or have been obese as are most BBS patients in the Newfoundland population (O'Dea et al. 1996). Observations of these adult patients over the course of this study demonstrated that the obesity is reversible. In an eight year period, only one patient experienced weight gain. The maintenance or loss of weight can be attributed to efforts to control blood glucose levels due to type II diabetes, diagnosed in three of the patients in this study and 32% of BBS patients in Newfoundland. Four unrelated BBS patients in our clinic also have had successful weight reduction with caloric restriction (O'Dea et al. 1996). The responsiveness of the obesity associated with BBS to caloric restriction and aerobic activity is encouraging and warrants further evaluation.

Urogenital System

In the Newfoundland population, some abnormality of renal structure, function or both

is universal among BBS patients and was described in previous reports (Harnett et al. 1988; Green et al. 1989; O'Dea et al. 1996). The renal structural changes typically include the persistence of fetal lobulation and calyceal cysts as observed in the three affected relatives who were examined in this *BBS3* kindred. The major cause of morbidity and mortality among BBS patients is renal failure (Churchill et al. 1981; Green et al. 1989; O'Dea et al. 1996). However, the relationship, if any, of the renal structural abnormalities to renal failure in these patients is unknown.

Linkage and Haplotype Analyses

The original linkage of BBS to chromosome 3 was established in an Arab-Bedouin kindred (Sheffield et al. 1994). However, population-based surveys of 47 BBS families (of predominantly northern European descent) have failed to yield a single BBS3-linked family (Beales et al. 1997; Bruford et al. 1997). We previously surveyed 17 BBS families identified in the Newfoundland population (Chapter 2) and determined that family B2 is linked to *BBS3*, confirming the original finding of a BBS locus on chromosome 3 (Sheffield et al. 1994).

Homozygosity mapping of recessive diseases in consanguineous pedigrees can potentially refine the disease interval to a few million base pairs of DNA, a region small enough to attempt positional cloning of the putative gene (Lander and Botstein, 1987). Affected relatives will be HBD for markers closely linked to the disease gene because they inherit two copies of a single ancestral haplotype that includes the disease locus.

Haplotype analysis in family B2 reveals that only affected family members show homozygosity, presumably HBD, for markers in the *BBS3* critical region. Three affected siblings, persons 22, 23 and 24, inherited two copies of the ancestral haplotype pter 7-5-8-7-9-7-4-4 qter. Their cousins, persons 14 and15, also inherited two copies of the ancestral haplotype but one copy has been shortened through recombination (retaining the qter portion) (fig. 3.1). Since all parents of affected offspring originate from the same small, isolated fishing village on the Avalon Peninsula, it is probable that they share a common ancestry. A single, disease-associated haplotype segregating in both affected sibships in this extended family suggests the individuals affected with BBS are homozygous for a single recent founder mutation. The ancestral chromosome 3 haplotype does not appear to be prevalent in the Newfoundland population as it was not observed in any members of the other 16 families that we have genotyped.

Refinement of the BBS3 *Disease Interval*

The initial linkage of BBS to chromosome region 3p13-12 localized the putative *BBS3* gene to a 11-cM region between markers *D3S1254* and *D3S1302* (Sheffield et al. 1994). However, a close examination of the Bedouin pedigree presented in the original report depicts a recombination between *D3S1753* and the disease locus (person V-27, fig.1, Sheffield et al. 1994), indicating that the *BBS3* gene is located telomeric of the marker *D3S1753* (fig. 3.2). The results of homozygosity mapping in family B2 suggests that *BBS3* is located centromeric of the marker *D3S1595* (figs. 3.1 and 3.2). On the basis of

our observations of the Bedouin pedigree linked to *BBS3* (Sheffield et al. 1994) and the results from this study, we predict that the *BBS3* gene is located in the 6-cM region between *D3S1595* and *D3S1753*.

A mutation in any one of at least five distinct BBS genes results in a similar, multi-organ phenotype with characteristic limb abnormalities. This suggests that the BBS genes encode subunits of a multimeric protein or are members of a common biochemical pathway involved in embryogenesis. The 6-cM genetic interval containing the *BBS3* gene (fig. 3.2), corresponds to the J, K and L contigs in the centromeric region of chromosome 3 (Chromosome 3 Framework Map, The San Antonio Genome Center). This region is too big for a positional cloning approach to identify the *BBS3* gene. A search of the National Center for Biological information (NCBI) database shows that 82 cDNAs, including 17 genes, map to the region. Many sequences have appropriate temporal and spacial expression to be candidate genes for BBS3. For instance, three genes, CBLB (Cas-Br-M (murine) ectropic retroviral transforming sequence b), an inhibitor of epithelial growth factor (EGF)-induced cell growth with a distinct role in EGF signaling, TOMM70A (translocase of outer mitochondrial membrane 70 (yeast) homolog A), a thyroid hormone-regulated gene with a role in brain development, and DOC1 (down-regulated in colon cancer 1), a myosin heavy chain homolog, are all expressed in the embryo and in a wide array of adult tissues, including those perturbed in BBS, such as brain, kidney and testis. Other *BBS3*-linked families may need to be identified in order to refine the disease interval and identify the *BBS3* gene.

Acknowledgments

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Electronic Database Information

Chromosome 3 Framework Map, The San Antonio Genome Center

(http://apollo.uthscsa.edu)

National Center for Biological Information (NCBI)

(http://www.ncbi.nlm.nih.gov/)

Online Mendelian Inheritance in Man (OMIM)

(http://www.ncbi.nim.nih.gov/omim)

4

A Fifth Locus for Bardet-Biedl Syndrome Maps to Chromosome 2q31

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To the Editor:

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disorder with major clinical manifestations of retinal dystrophy, obesity, dysmorphic extremities, hypogenitalism, and renal structural and functional abnormalities. It is distinguished from Laurence-Moon syndrome (OMIM 245800), Biemond syndrome II (OMIM 210350), and Alstrom syndrome (OMIM 203800) by the absence of paraplegia, iris coloboma, and perceptive deafness, respectively. Four genetic loci for BBS have been mapped to distinct chromosomes, but the finding, in three recent population surveys, of several unlinked families with BBS provides convincing evidence for at least a fifth BBS locus (Beales et al. 1997; Bruford et al. 1997; **Chapter 2**).

BBS is a relatively rare disease, with an estimated world prevalence of 1/125,000-160,000 (Klein and Ammann 1969; Beales et al.1997). However, two genetically isolated and distinct populations have been identified that provide a resource of large inbred families with BBS. One in 13,500 individuals has BBS in the Bedouin-Arab tribes of the Negev region of Israel, where the custom of consanguineous marriages is still practiced by > 50% of the population and where two-thirds of these marriages are between first cousins (Farag and Teebi 1989; Sheffield et al. 1998). Half a world away, on the island of Newfoundland, the prevalence of BBS is 1/17,500 (Green et al. 1989). Matings between distant cousins in the Newfoundland population are frequent because of three historical factors: the geographical isolation of coastal fishing villages, the low rate

of immigration to these communities, and the religious restrictions on mate selection between the Protestant English and Catholic Irish settlers (Bear et al. 1988). Three of the four BBS loci -*BBS2* (Kwitek-Black et al. 1993), *BBS3* (Sheffield et al. 1994), and *BBS4* (Carmi et al. 1995) -were identified by homozygosity mapping in individual Bedouin families. We have used a similar methodology to map the fifth genetic locus for BBS to chromosome 2q31 in an inbred Newfoundland family of European ancestry.

A recently completed population-based survey of 17 BBS families from Newfoundland has identified six families in which the four known BBS loci were unambiguously excluded (**Chapter 2**). Family B9, the largest of these kindreds, has five affected members who are the products of three consanguineous unions interrelated through two founding couples (fig. 2.1, **Chapter 2**). The methods used in the clinical assessment of these patients have been described elsewhere (Green et al., 1989). The five BBS patients with BBS surpass the minimal criteria of three major clinical manifestations for a BBS diagnosis, because of the presence of obesity, brachydactyly and/or syndactyly, retinal dystrophy, and male hypogenitalism, in the absence of paralysis, iris coloboma, or deafness (table 4.1). This pedigree met the requirements for the localization of *BBS5* by homozygosity mapping (Lander and Botstein 1987; Carmi et al. 1995). We anticipated that the affected individuals would be homozygous by descent for an ancestral haplotype inherited from one of the four pedigree founders.

Table 4.1

Clinical Manifestations of *BBS5* in a Newfoundland Kindred

Patient (Sex/Age)	BMI[a]	Polydactyly/ Other[b]	Visual Acuity[c]	Retinal Appearance[d]	Small Penis
21 (M /31 years)	31.7	None/Yes	LP	Advanced RP	Yes
22 (M /25 years)	34.6	None/Yes	NLP	Retinal degeneration	Not examined
24 (F /29 years)	49.4	None/Yes	NLP	Atypical RP	Not applicable
28 (M /25 years)	40.4	None/Yes	CF	RP	Yes
29 (F /21 years)	42.8	None/Yes	20/300	Macular dystrophy	Not applicable

[a]BMI=body mass index (wt [kg]/ht [m]^2). A value >27 is considered to indicate obesity (Nelson et al. 1994).
[b]Other=brachydactyly and/or syndactyly
[c]CF=count fingers; LP=light perception only; NLP=no light perception
[d]RP=Retinitis pigmentosa

A genomewide scan of pooled DNA samples was performed with microsatellite markers, the vast majority (89%) consisting of tri- and tetranucleotide repeats (Cooperative Human Linkage Consortium human screening set, Weber version 8; Research Genetics), as described elsewhere (Sheffield et al. 1994). Two control pools of DNA from 4 living parents and 11 unaffected siblings, as well as a test pool of DNA from the 4 surviving patients, were amplified. Of the first 322 markers successfully amplified, 6 showed a reduction in the number of alleles (allele shift) in the test pool, compared to the control pools. Subsequent genotyping of these markers on the extended family

proved that they were not linked to BBS, resulting in a false-positive rate of 1.9%. However, the 323rd marker, *D2S1353*, gave a 4:1 allele shift, from the control pools to the test pool. Genotyping of *D2S1353* on the pedigree showed it to be exclusively homozygous in patients with BBS. Two-point analysis showed significant linkage between BBS and *D2S1353*, with no recombination (maximum LOD score [Z_{max}] 5.675; recombination fraction [θ] 0). Genotyping of markers flanking *D2S1353* confirmed linkage to 2q31 (table 4.2) and showed an ancestral haplotype that is homozygous by descent in all affected relatives (fig. 4.1).

Table 4.2

Two-Point LOD Scores Between the BBS Trait and 2q31 Markers

Marker[a]	θ =[b]	0.000	0.010	0.050	0.100	0.200	0.300	Z_{max}	at θ
D2S442		-3.080	-2.205	-1.224	-0.699	-0.211	-0.042	0.009	0.439
D2S1399		-∞	-0.416	0.982	1.321	1.174	0.722	1.345	0.122
D2S1353		5.675	5.556	5.075	4.463	3.214	1.970	5.675	0.000
D2S1776		4.691	4.578	4.121	3.543	2.377	1.275	4.691	0.000
D2S1391		-∞	1.617	2.558	2.600	2.066	1.298	2.631	0.078

Note.-A disease model of autosomal recessive inheritance, 100% penetrance, and a gene frequency of 0.008 was invoked.

[a]Listed according to physical order (pter-qter) on chromosome 2q31.

[b]Calculated by MLINK and ILINK from the FASTLINK package (version 4.0P).

The initial assignment of the BBS phenotype in close proximity to the *HOXD* gene cluster on chromosome 2q31 suggested these nine homeobox genes of the

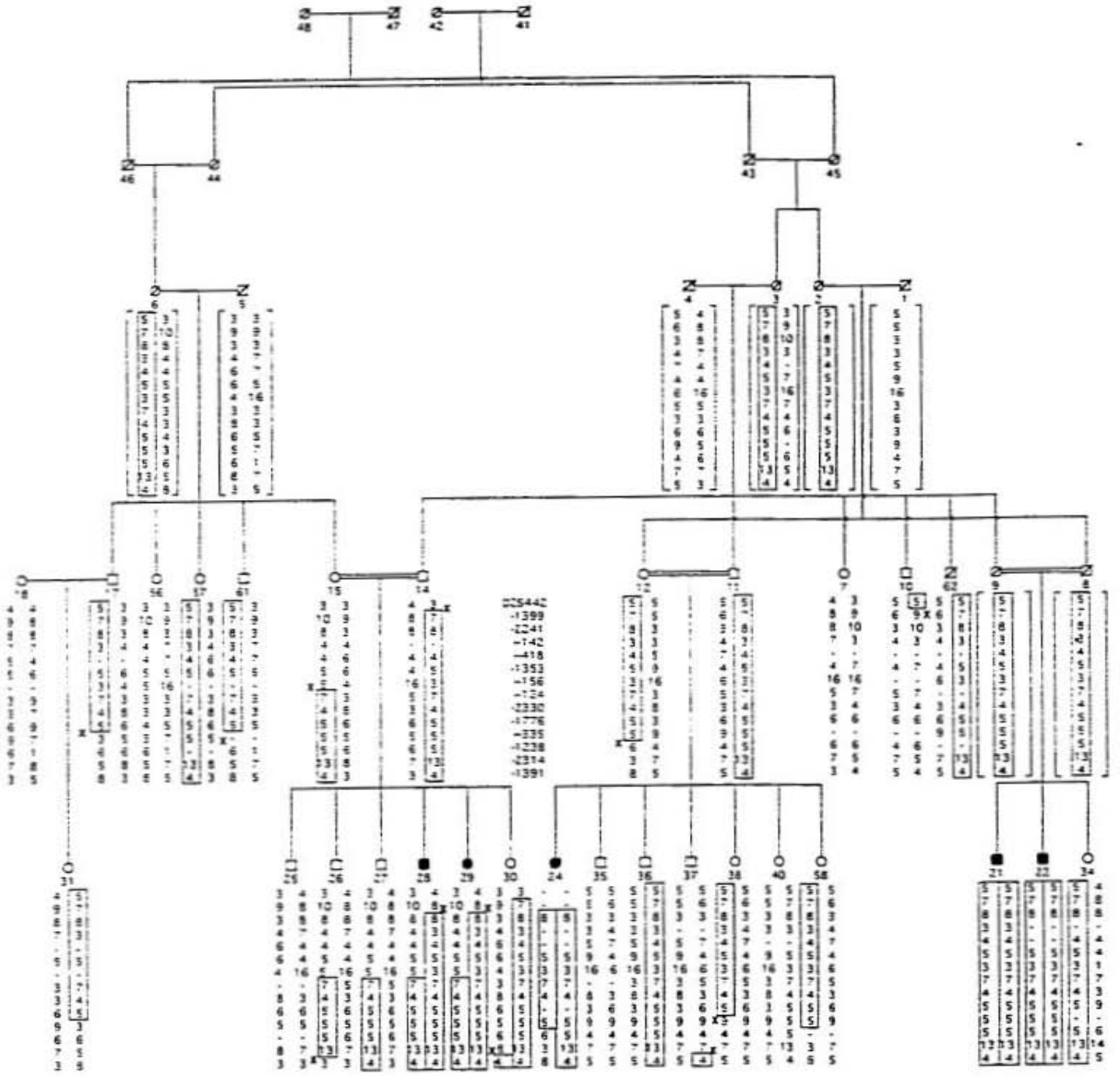

Figure 4.1 Cosegregation of BBS and an ancestral haplotype *(boxed)* on chromosome 2q31 in kindred B9. Double marriage lines indicate consanguineous unions. Haplotypes were constructed manually and represent the minimal number of recombinations (**x**). The DNA sample from person 24 was extracted from a paraffin sample and often fails to amplify, but this person appears to be nonrecombinant for both parental chromosomes. The minimal region of homozygosity in relatives with BBS includes the markers pter-*D2S124*, *D2S2330, D2S1776* and *D2S335*-qter.

Drosophila antennapedia class and other closely located genes (*EVX2* and *DLX1/DLX2*) that are involved in patterning of the embryo are candidate genes for *BBS5*. Recent findings that duplication of the *HOXD13* gene causes synpolydactyly (Akarsu et al. 1996) focused our attention on it as the most promising gene candidate, given that syndactyly and/or polydactyly are congenital manifestations of BBS. However, refined mapping of two key recombinant ancestral chromosomes in patients 15 and 12 placed *BBS5* within the13-cM interval *D2S156-D2S1238* (fig. 4.1), several centimorgans upstream from the *HOXD13* gene that is positioned at the proximal end of the *HOXD* gene cluster (fig. 4.2) (Spurr et al. 1996). Refined mapping of the recombinant ancestral chromosome excludes all genes within the *HOXD* gene cluster as candidate genes for *BBS5*.

Figure 4.2 Critical region of the novel *BBS5* locus on chromosome 2q31. Markers used to refine the position of *BBS5* are identified in terms of their "D" numbers. The marker order and distances were based on the Marshfield Chromosome 2 (sex-averaged) linkage map (Center for Medical Genetics, Marshfield Medical Research Foundation) and the Chromosome 2 Workshop Consensus Map, 1996 (Genome Database). The *HOXD*-gene cluster lies telomeric to the *BBS5* critical region.

Acknowledgments

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Electronic Database Information

Online Mendelian Inheritance in Man (OMIM)

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5

A Founder Effect in the Newfoundland Population Reduces the Bardet-Biedl Syndrome I (*BBS1*) Interval to 1-cM

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Summary

Bardet-Biedl syndrome (BBS) is a rare, autosomal recessive disorder; major phenotypic findings include dysmorphic extremities, retinal dystrophy, obesity, male hypogenitalism, and renal anomalies. In the majority of northern European BBS families with BBS, the syndrome is linked to a 26-cM region on chromosome 11q13. However, the finding, so far, of five distinct BBS loci (*BBS1*, 11q; *BBS2*, 16q; *BBS3*, 3p; *BBS4*, 15q; *BBS5*, 2q) has hampered the positional cloning of these genes. We used linkage disequilibrium (LD) mapping in an isolated founder population in Newfoundland to significantly reduce the *BBS1* critical region. Extensive haplotyping in several unrelated BBS families of English descent revealed that the affected members were homozygous for overlapping portions of a rare, disease-associated ancestral haplotype on chromosome 11q13. The LD data suggest that the *BBS1* gene lies in a 1-Mb, sequence-ready region on chromosome 11q13, which should enable its identification.

Introduction

Bardet-Biedl syndrome (BBS; OMIM 209900) is a rare, autosomal recessive disorder in which the combination of dysmorphic extremities, retinal dystrophy, obesity, male hypogenitalism and renal anomalies may be associated, to varying degrees, with mental retardation and/or diabetes mellitus (Green et al. 1989; O'Dea et al. 1996). Of the five genetic subtypes, *BBS1* appears to be the most common locus in affected individuals of northern European descent: as many as 50% of linked pedigrees are assigned to *BBS1* (Beales et al. 1997; Bruford et al. 1997; Woods et al. 1999). *BBS1* was initially mapped to chromosome 11q13 by combining the positive LOD scores achieved in 17 of 31 North American kindreds as a result of a genome scan (Leppert et al. 1994). The other BBS loci, *BBS2* (16q; Kwitek-Black et al. 1993), *BBS3* (3p; Sheffield et al. 1994), *BBS4* (15q; Carmi et al. 1995) and *BBS5* (2q; Young et al. 1999), were located by homozygosity mapping in extended inbred kindreds. In the original study of Leppert et al. (1994), the putative *BBS1* gene was tightly linked to two loci on chromosome 11q13: the gene for human muscle glycogen phosphorylase (*PYGM*) and the anonymous DNA marker *D11S913* (AFM164zf12), and was localized to a 26-cM interval between *D11S1298* and *INT2* (*FGF3*). A more precise genetic and physical location of *BBS1* is required if the *BBS1* gene is to be positionally cloned.

Refined mapping of disease genes has recently been accomplished by linkage disequilibrium (LD) mapping in founder populations (reviewed by Jorde 1995 and by

Xiong and Guo, 1997). When a disease mutation is first introduced into a population, it resides on a single disease haplotype (DH) of linked markers. As a result of meiotic recombination, the length of this DH decreases as a function of genetic distance so that, with successive generations, only the original marker alleles in the vicinity of the disease locus cosegregate on disease chromosomes. Although fine mapping of disease genes was established initially in old populations like the Finnish (Hastbacka et al. 1992), recent success in fine mapping has been accomplished in relatively young populations (Labuda et al. 1996; Groenewald et al. 1998). The island population of Newfoundland, considered a genetic isolate because of the nature of its founding and subsequent isolation, is enriched for BBS, with 10 times the incidence in other white populations of northern European ancestry (Green et al. 1989; O'Dea et al. 1996). A single founder effect has been reported in two recent studies of families from the island with either hereditary non-polyposis colorectal cancer (HNPCC) or multiple endocrine neoplasia type 1 (MEN1) (Olufemi et al. 1998; Froggatt et al. 1999). In the present study we show that, in five unrelated families from Newfoundland, the family members affected with BBS are homozygous for an ancestral DH, and we use recent and historical recombinations to the ancestral DH to map the *BBS1* gene within a 1-Mb region on chromosome 11q13.

Materials and Methods

Subjects

In a recent population survey, 17 BBS families of English ancestry were haplotyped at the *BBS1*, *BBS2*, *BBS3*, and *BBS4* loci. Of these families, three (B8, B10, and B19) were assigned to *BBS1* and three (B7, B12, and B15) could not be excluded from this locus because they yielded positive LOD scores and haplotypes consistent with linkage to *BBS1* (table 2.1, **Chapter 2**). Of these six families, parental consanguinity was documented in family B8 and suspected in families B10, B12, B15, and B19 on the basis of progenitors with the same surname originating from the same community. Extensive genotyping with markers mapping to the *BBS1* critical region was performed in the six families, representing 8 BBS patients and 44 first-and second-degree relatives. Informed consent had been obtained previously, and the clinical presentations of the adult patients have been described elsewhere (Green et al. 1989; O'Dea et al. 1996).

Genotyping

DNA was extracted from the lymphocytes of venous blood by a simple salting out procedure (Miller et al. 1988). Fifty to 100 ng of template DNA in standard 10-μL reactions containing 1.5 pmol of primer, 200 μm dNTPs, 0.125 units of Tfl DNA polymerase, and a trace amount of $\gamma[^{32}P]$ end-labeled forward primer was amplified by temperature cycling in a Perkin Elmer 9600 thermocycler. Products were run on 6%-8%

polyacrylamide sequencing gels with formamide (Litt et al. 1993) and were subjected to autoradiography. The markers *D11S1298*, *D11S956*, *D11S480*, *D11S4205*, *D11S1883*, *D11S4945*, *PYGM*, *D11S4946*, *D11S4940*, *D11S4938*, *D11S449*, *D11S4941*, *D11S913*, and *FGF3* were typed on key family members. Family B7 was excluded from the study, because several samples failed to amplify.

Family Studies

Haplotypes were constructed, for each family, to give the minimum number of recombinations. DHs were identified from alleles that were transmitted from both unaffected parents to affected offspring. Pairwise linkage analyses between BBS and six markers spanning the *BBS1* critical region were performed under an autosomal recessive model with a penetrance of 0.95 and a disease-allele frequency of .004. The disease-allele frequency for BBS was calculated from the Newfoundland population estimate of 1/17,500 (Green et al. 1989), adjusted to reflect an estimated 50% contribution of the *BBS1* locus to the overall population frequency. All markers were assumed to have nine alleles of equal frequency in the population. LOD scores were calculated by the MLINK subroutine program of FASTLINK (V3.0P) (Lathrop and Lalouel 1984; Cottingham et al. 1993; Schäffer et al. 1994).

Population Studies

The marker-allele frequencies in disease and normal (nontransmitted) chromosomes of the 10 obligate BBS carriers (parents) were compared. Normal alleles from each parent were used as population controls, to avoid the possibility of inadvertently including *BBS1* disease alleles from random carriers in the population. Allelic association was tested by means of Fisher's exact test, with one-sided probability. The DHs were compared between families in search of (i) common *BBS1* haplotypes that would indicate that the parents of two or more families were distantly related and (ii) a common ancestral DH.

Results

Preliminary Evidence of a Founder Effect Among Families with BBS1

Five of the six families in this study live along the south and southwest coasts of Newfoundland (fig. 5.1). Although a common ancestor was not identified in ancestral lineages, genetic evidence suggests that the families are interrelated. The affected individuals in family B10 are apparently heterozygous for a copy of the DH identified in family B8 and a portion of the DH transmitted by the father of family B7 (table 5.1). It was noted that these three families originate from closely-linked communities on the

Table 5.1

DHs on Chromosome 11q13 Segregating in Six BBS Families

	B7		B8		B10		B12		B15		B19	
Marker	p	m	p	m	p	m	p	m	p	m	p	m
D11S1298	7	3	10	10	7	10	7	9	11	11	-	-
D11S956	6	8	8	8	10	8	7	5	7	7	11	11
D11S480	9	5	6	6	9	6	5	9	5	5	9	9
D11S1883	10	10	10	10	10	10	10	8	10	10	7	7
D11S913	6	7	7	7	6	7	6	5	6	6	6	6
FGF3	7	12	5	5	7	5	9	9	9	9	-	-

Note.-Haplotypes are arranged with the paternal haplotype (p) on the left and the maternal haplotype (m) on the right. For markers *D11S913* and *FGF3*, the phase of the paternal haplotype in B12 is assumed.

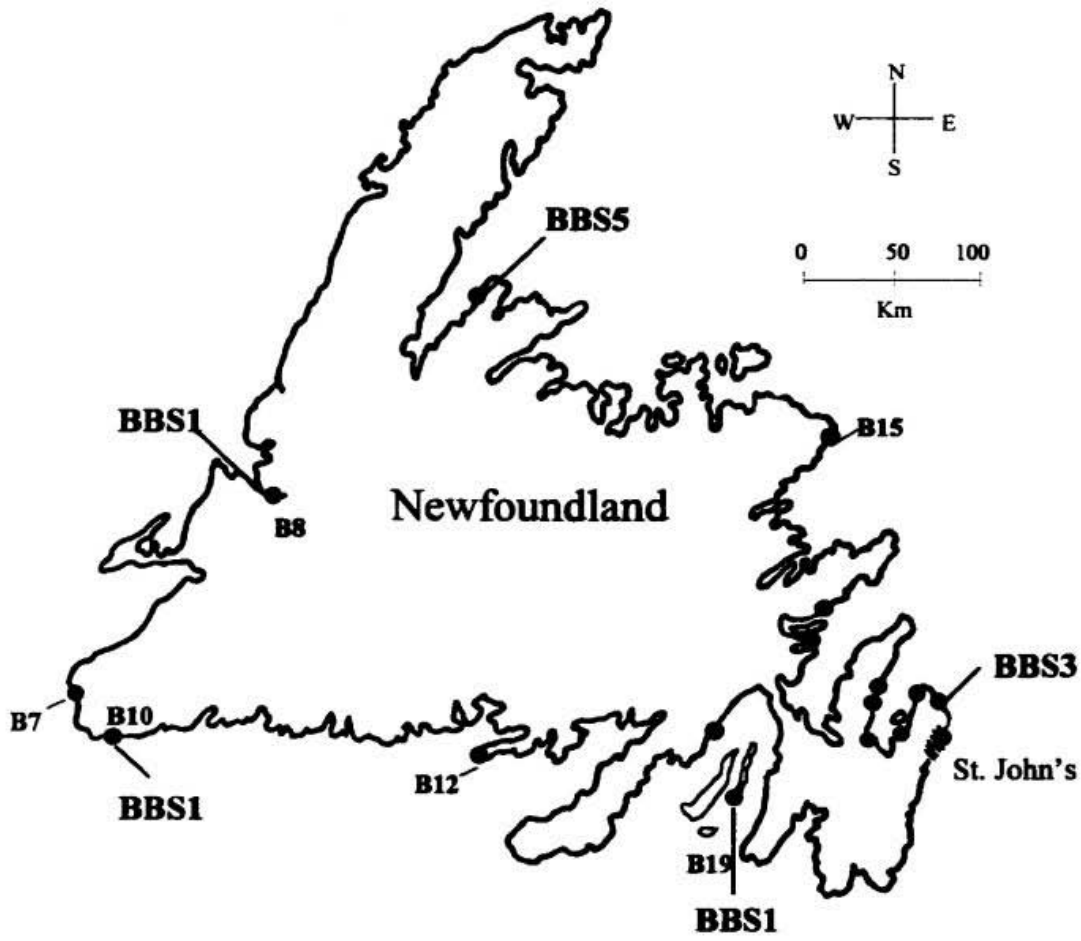

Figure 5.1 Geographical location of six BBS families with evidence of linkage to the *BBS1* locus. The families genotyped in the present study are indicated by family number. The location of *BBS1*-, *BBS3*-, and *BBS5*-linked families (as determined in **Chapters 2** and **4**) is shown.

southwest coast of the island (fig. 5.1). The data also suggest that the father of a south coast family (B12) shares a recent common ancestor with both parents of family B15, the only family not residing on the south coast of the island. Therefore, on the basis of shared DHs and geographical location, preliminary evidence suggested the presence of a founder effect among *BBS1* cases in the Newfoundland population.

Evidence of Linkage to BBS1

In a previous study, three families (B8, B10, and B19) were assigned to *BBS1* (table 2.1, Chapter 2). Family B10 consists of a large sibship in which two affected individuals share a unique genotype on chromosome 11q13, compared to their nine unaffected sibs (fig. 5.2). A maximum LOD score of 1.662 at recombination fraction (θ) 0 was obtained with fully informative markers that mapped telomeric to *D11S1883*. Family B10 was previously excluded from linkage with *BBS2*, *BBS3*, *BBS4* (Chapter 2), and *BBS5* (M..O. Woods, unpublished data) on the basis of established exclusion criteria. For a locus to be excluded, one or both of the following conditions had to be met: (i) affected individual(s) inherited the same genotype as one or more unaffected sibs, and (ii) two or more affected individuals inherited different genotypes. Family B8 is a consanguineous family in which a single affected offspring was homozygous by descent (HBD) for the entire *BBS1* locus (fig. 5.2). A maximum LOD score of 1.405 (θ=0) was obtained with fully informative markers (e.g., *D11S1883*). Homozygosity (in the affected member only) was not observed at other BBS loci: *BBS2* and *BBS3* loci were excluded. Family B19

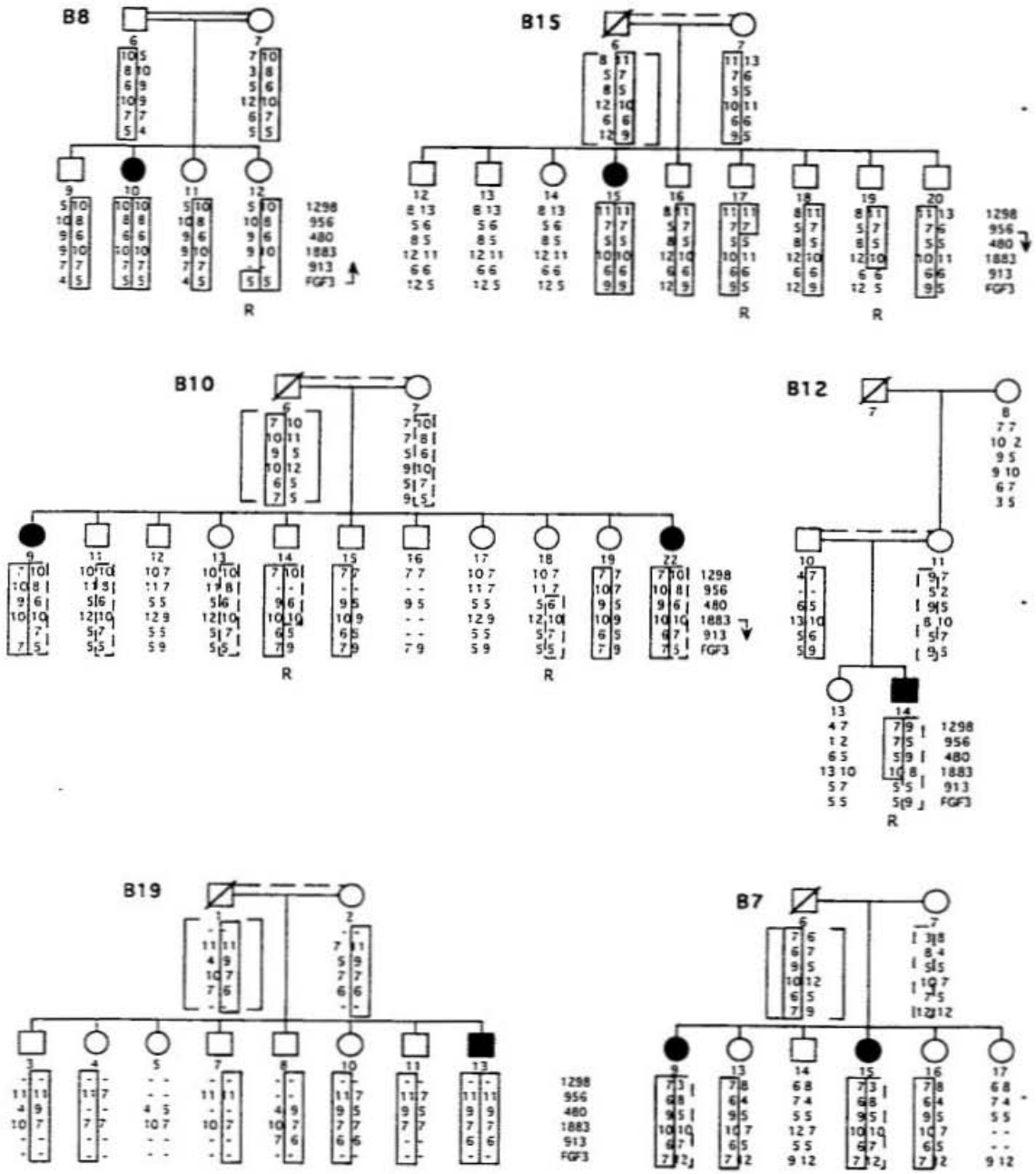

Figure 5.2 Core pedigrees of three families linked to *BBS1* (B8, B10 and B19) and three unassigned families (B7, B12 and B15) haplotyped for six polymorphic markers spanning the 26-cM *BBS1* interval on chromosome 11q13. Blackened symbols indicate individuals with diagnoses of BBS. Boxed haplotypes (solid and dashed lines) indicate DHs. Double marriage lines depict consanguineous unions, either documented (solid line) or suspected (dashed line). "R" indicates that the DH is recombinant.

consists of a large sibship with one affected individual. The affected offspring was homozygous (presumably HBD) for all markers typed within the *BBS1* interval, whereas seven unaffected sibs were either heterozygous or homozygous for a normal haplotype. Homozygosity was not observed at other BBS loci, and the *BBS3* locus could be excluded (Chapter 2).

In three families (B7, B12, and B15), BBS could not be excluded from, or confidently assigned to, the *BBS1* locus (Chapter 2). Family B15 consists of a large sibship with one affected and eight unaffected sibs available for genotyping. Homozygosity in the affected individual was observed only at the *BBS1* locus, and the *BBS3* locus could be excluded. Family B7 has two affected individuals who share a unique genotype on chromosome 11q13, compared to their four unaffected sibs (fig. 5.2). A maximum LOD score of 1.074 (θ=0) was obtained with fully informative markers, and BBS in this family was excluded from linkage to *BBS3*, *BBS4* and *BBS5* (M..O. Woods, unpublished data). Family B12 is relatively uninformative for linkage but was excluded from *BBS3* because the only affected individual (patient 14) inherited the same genotype as his unaffected sister. He is homozygous for the *BBS1* marker *D11S913*, but the phase of the paternal chromosomes cannot be determined for the *D11S913- FGF3* interval because of an apparent recombination in one of the offspring.

The pairwise LOD scores calculated for the six families at six markers covering the 26-cM *BBS1* critical region were summed. Four of six markers examined reached statistical significance when the families were considered together (LOD score > 3; table 5.2). The

Table 5.2

Sum of the Pairwise LOD Scores at Chromosome 11q13 in Six Families with BBS

	LOD Score at θ=						
Marker	0.000	0.010	0.050	0.100	0.200	0.300	0.400
D11S1298	2.227	2.263	2.169	1.856	1.124	0.052	0.142
D11S956	3.419	3.439	3.275	2.867	1.910	0.972	0.279
D11S480	2.809	2.819	2.623	2.202	1.288	0.559	0.137
D11S1883	3.657	3.663	3.430	2.952	1.868	0.894	0.241
D11S913	3.019	2.923	2.546	2.093	1.264	0.587	0.151
FGF3	3.777	3.662	3.203	2.644	1.599	0.739	0.190

finding of common DHs among *BBS1*-assigned families (B8 and B10), between *BBS1*-assigned and unassigned families (B7 and B10), and among unassigned families with evidence of linkage to *BBS1* (B12 and B15) (table 5.1) corroborates the linkage and haplotype data that these are, in fact, all *BBS1* families. Because the exact relationships among the pedigrees are unknown, the summary LOD scores given in table 5.2 are presumed to be underestimated. Obligate recombinations involving the DHs were detected in several families and used to refine the *BBS1* interval. In family B10, an unaffected individual, person 14, inherited a non-recombinant DH from his father and a recombinant DH from his mother. The presence of two DHs for the centromeric portion of the *BBS1* critical region (*D11S1298* to *D11S1883*) in an unaffected individual suggests that marker *D11S1883* is the new centromeric boundary for *BBS1* (fig. 5.2). Similarly, a

recombinant paternal haplotype inherited by an unaffected sib in family B8 (person 12) suggests that *BBS1* is located centromeric to *FGF3*. Intrafamilial recombinations on DHs reduce the *BBS1* interval from a 26-cM region to a 15-cM interval between *D11S1883* and *FGF3* (fig. 5.3).

LD and Detection of a Founder Haplotype

Extensive genotyping at the *BBS1* locus focused on markers within the new *BBS1* interval (fig. 5.3). The distribution of alleles at 14 polymorphic loci in disease and normal chromosomes is shown in table 5.3. Significant LD between specific marker-alleles on DHs was observed across the families. Strong associations were observed between alleles at five consecutive marker loci: *D11S4205*, *D11S1883*, *D11S4945*, *PYGM* and *D11S4946*. Comparison of allele frequencies between disease and normal chromosomes showed that three of these associations were statistically significant. Allele 10 at marker *D11S1883* was present on 70% (7/10) of DHs but only 20% (2/10) of normal chromosomes ($P < .05$). Similarly, allele 8 at the *PYGM* locus was present on 100% of the DHs and 50% of normal chromosomes ($P < .05$) and allele 5 at marker *D11S4946* was present on 100% of the disease chromosomes and 30% of normal chromosomes ($P < .01$). Although allele 4 at marker *D11S4205* and allele 9 at marker *D11S4945* were present on 90% and 100% of disease chromosomes, respectively, these associations were not significant, because both alleles are common in the general population; they are present on 70% of the normal chromosomes (table 5.3).

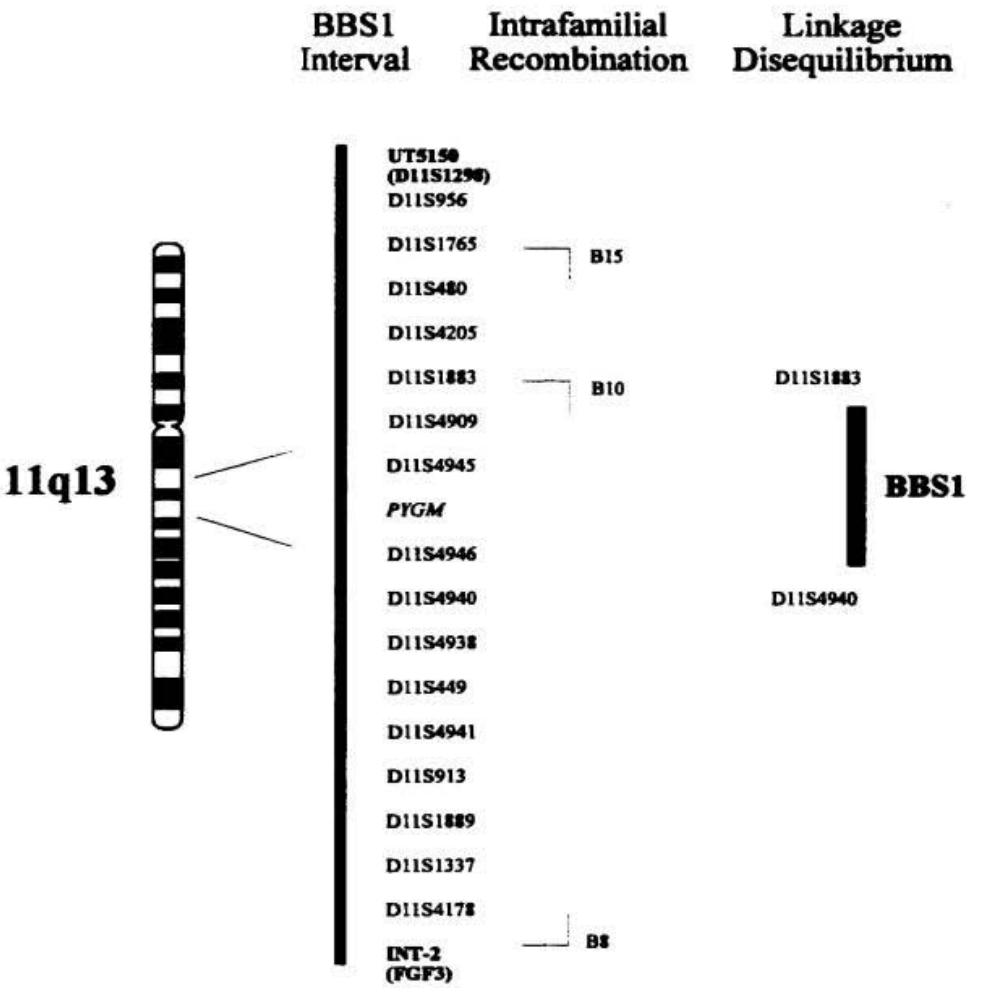

Figure 5.3 Refinement of the *BBS1* disease interval by recombination and LD mapping in Newfoundland families. The markers and their relative positions were selected from the map of the *MEN1* region on 11q13 (Manickam et al. 1997; Sixth International SCW 11 Workshop, 1998).

Table 5.3 LD at the *BBS1* Locus on Chromosome 11q13 Among Five Newfoundland Kindreds

	DHs for Kindred										Normal Haplotypes for Kindred									
	B8		B10		B12		B15		B19		B8		B10		B12		B15		B19	
Locus	p	m	p	m	p	m	p	m	p	m	p	m	p	m	p	m	p	m	p	m
1298	10	10	7	10	7	9	11	11	-	-	5	7	10	7	4	7	8	13	-	-
956	8	8	10	8	7	5	7	7	11	11	10	3	11	7	1	2	5	6	11	7
480	6	6	9	6	5	9	5	5	9	9	9	5	5	5	6	5	8	5	4	5
4205	4	4	3	4	4	4	4	4	4	4	3	4	4	5	4	4	3	4	4	4
1883	10	10	10	10	10	8	10	10	7	7	9	12	12	9	13	10	12	11	10	7
4945	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	4	10	10	9	9
PYGM	8	8	8	8	8	8	8	8	8	8	8	8	6	8	2	4	4	8	8	4
4946	5	5	5	5	5	5	5	5	5	5	4	5	5	4	4	1	1	5	3	3
4940	5	5	7	5	7	5	7	7	3	3	5	5	5	5	5	3	5	7	7	5
4938	5	5	3	5	5	3	5	5	5	5	9	3	5	3	5	9	5	5	-	9
449	7	7	3	7	3	6	5	5	3	3	5	3	4	3	4	2	5	5	3	4
4941	9	9	11	9	2	2	4	4	4	4	8	3	6	11	2	4	4	6	4	4
913	7	7	6	7	6	5	6	6	6	6	7	6	5	5	5	7	6	6	7	6
FGF3	5	5	7	5	9	9	9	9	-	-	4	5	5	9	5	5	12	5	-	-

Note.-Haplotypes arranged with paternal haplotype (p) on the left and the maternal haplotype (m) on the right. Disease-associated alleles are shaded.

A single ancestral DH was readily identified in the vicinity of marker loci with strong allelic associations. All disease chromosomes segregating in *BBS1* families contained the -9-8-5- subhaplotype at *D11S4945, PYGM,* and *D11S4946,* respectively. In contrast, the -9-8-5- subhaplotype was only found on a single normal haplotype (family B8). This suggest that, although rare, the -9-8-5- subhaplotype is not exclusive to *BBS1*-carrying chromosomes in the Newfoundland population. It was noted that the existence of a single *BBS1* founder haplotype in these families was not evident on the basis of a six-marker haplotype, due to large gaps between markers (table 5.1).

The longer - 4-10-9-8-5- haplotype (encompassing the -9-8-5- subhaplotype and the centromeric markers *D11S4205* and *D11S1883*) was identified on 60% of the DHs but none of the normal chromosomes. This longer haplotype may represent a larger portion of the ancestral DH. In this case, DHs discordant for allele10 at *D11S1883* (families B12 and B19) may have resulted from either (i) historical recombinations to the ancestral DH between *D11S1883* and *D11S4945* that were not informative at *D11S4205*, a notably uninformative marker in the population, or (ii) de novo mutations at the more polymorphic *D11S1883* locus. In any case, the evidence suggests that the -9-8-5- subhaplotype represents either the remnants of a founder *BBS1* chromosome imported from England or the background haplotype that sustained a *BBS1* mutation de novo in the germline of a single English settler.

Location of BBS1

LD mapping supports a position for the *BBS1* gene within a 1-cM region between markers *D11S1883* and *D11S4940* surrounding the *PYGM* locus. The *D11S1883* boundary is also supported on the basis of intrafamilial recombination (fig. 5.3). This 1-cM genetic interval represents a physical distance of ~ 1-Mb (Manickam et al. 1997) within a region of the genome that is gene rich and, fortuitously, sequence ready as a result of physical mapping efforts to clone the Best vitelliform macular dystrophy (*VMD-2*) and multiple endocrine neoplasia (*MEN1*) genes that map to this region (Cooper et al. 1997; Guru et al. 1997; Manickam et al. 1997).

Discussion

The island of Newfoundland is a sparsely populated region of Canada in which 50% of the population of 560,000 reside in small coastal communities. The colonization of the island occurred primarily by a natural increase from northern European settlers of predominantly English and Irish extraction who arrived before 1835. Most founders originated from the West Country of England and from southeast Ireland (Mannion 1977). Mating segregation between Irish Catholics and English Protestants, low immigration, and geographical isolation of communities have resulted in genetic isolation of the population. In a review of the historical development of genetic isolation in three Newfoundland outports, Bear et al. (1988) observed that only 1%-8% of breeding parents were immigrants to the area and 60% of births had been to parents originating from the same small community.

The scattered distribution of BBS families and the recent identification of at least three distinct genetic BBS subtypes in Newfoundland (Chapter 2) is not consistent with the expectation of a single cluster of families with a recessive disease in a young founder population. However, we noted that five of the six families with evidence of linkage to *BBS1* reside on the south and southwest coasts of the island, a region that was settled predominantly by the spread of settlers by sea in an east-to-west direction (Mannion 1977). The genotyping data also suggest that these families have complex

relationships with each other in that large regions (up to 26-cM) on disease-associated haplotypes were shared among kindreds. In the absence of mutation analysis, the identification of a single founder effect would require that all patients were homozygous for specific alleles at marker loci tightly-linked to the *BBS1* gene. We have shown that all *BBS1* patients identified in the Newfoundland population are homozygous for a relatively rare haplotype spanning a 1-cM region centered around the *PYGM* gene on chromosome 11q13. The relatively high incidence of BBS and the scattered distribution of affected families in the Newfoundland population are attributable to a combination of locus heterogeneity and at least one founder effect.

The power of LD mapping is well illustrated from this study: a total of six affected individuals in five families were used to fine map the *BBS1* gene to within 1-Mb by this method. Even families not informative for linkage (e.g., B12) provided valuable information on historical recombinations. In contrast, two recent studies reporting on a total of 47 families with BBS failed to identify informative recombinations at the *BBS1* locus (Beales et al. 1997; Bruford et al. 1997). The localization of *BBS1* within a 1-Mb interval between *D11S1883* and *D11S4940* is small enough to promote the positional cloning of the *BBS1* gene.

Acknowledgments

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Electronic Database Information

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6

Summary

Bardet-Biedl syndrome is a clinically and genetically heterogeneous disorder in which mutations at any one of at least five genes cause a pleiotropic phenotype including congenital dystrophic extremities, infantile onset obesity, progressive retinal degeneration, renal abnormalities, and male hypogonadism. Despite the lapse of seven years since the first BBS locus was mapped (Kwitek-Black et al. 1993), none of the BBS genes have been cloned. The wide spectrum of clinical manifestations of BBS makes it difficult to prejudge the putative BBS gene product(s), precluding a functional candidate gene approach to the identification of the BBS genes. More significantly, the refinement of the genetic map to allow positional cloning at one or more of the BBS loci has been impeded by, (i) a limited number of BBS cases (ii) locus heterogeneity, and (iii) the lumping together of BBS with other, possibly non-allelic syndromes.

This is the first report of a population-based investigation of BBS in a well-defined population. The premise of this thesis was that a large collection of well-described BBS families, from a founder population like Newfoundland, would provide a unique opportunity to study the genetics of BBS and contribute to the cloning of BBS genes.

Genetic Heterogeneity of BBS in the Newfoundland Population

Seventeen of 22 Newfoundland families were tested for linkage at each of the previously mapped BBS loci (*BBS1-BBS4*). In total, eight families (37%) have been unambiguously assigned to a specific BBS locus, seven of them (32%) to previously mapped loci and one family to a novel locus (*BBS5*) (fig. 6.1). A total of 14 BBS families (63%) identified in the Newfoundland population remain unassigned (five families unlinked at *BBS1, BBS2, BBS3* and *BBS4*, four uninformative families and five families that were unavailable for study) (fig. 6.1).

Six families, representing 27% of the 22 BBS families, were assigned to the *BBS1* locus (fig. 6.2), three the result of linkage and haplotype analysis (**Chapter 2**) and three on the basis of linkage disequilibrium (**Chapter 5**). Like other reports of families of northern European extraction, *BBS1* is the largest linkage category so far in the population. Geographic and genetic evidence suggest that the affected individuals in these six *BBS1* families are homozygous for a founder mutation, implicating a single mutation at the *BBS1* locus in all *BBS1* cases in the population (**Chapter 5**).

The genetic survey revealed two unpredicted findings. The first was the relatively large number of families that were not linked to any of the previously mapped BBS loci (**Chapter 2**). The second surprise was the finding of a *BBS3*- linked family (**Chapter 3**), the second only *BBS3* family reported in the literature and a unique finding among patients of northern European extraction. One of the unlinked kindreds, family B9, was used to successfully locate a novel BBS locus *(BBS5)* on chromosome 2q31 (**Chapter 4**).

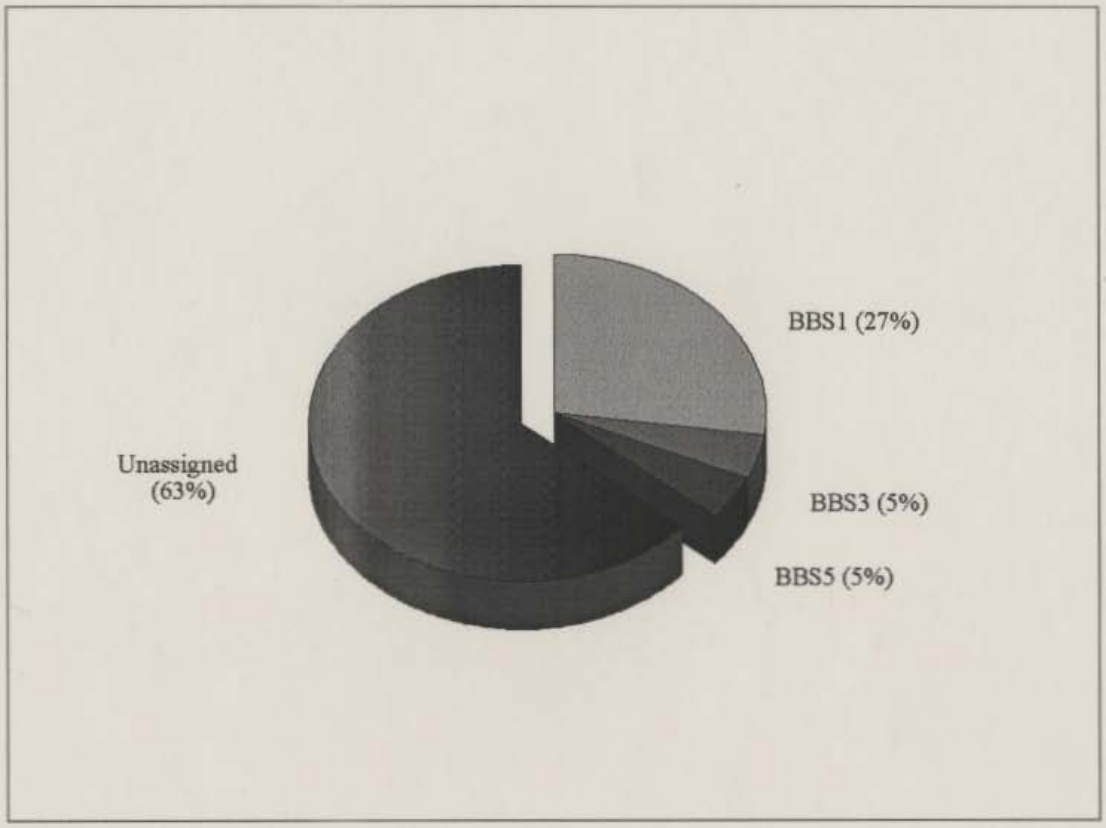

Figure 6.1 Relative proportion of assigned and unassigned BBS families in the Newfoundland population.

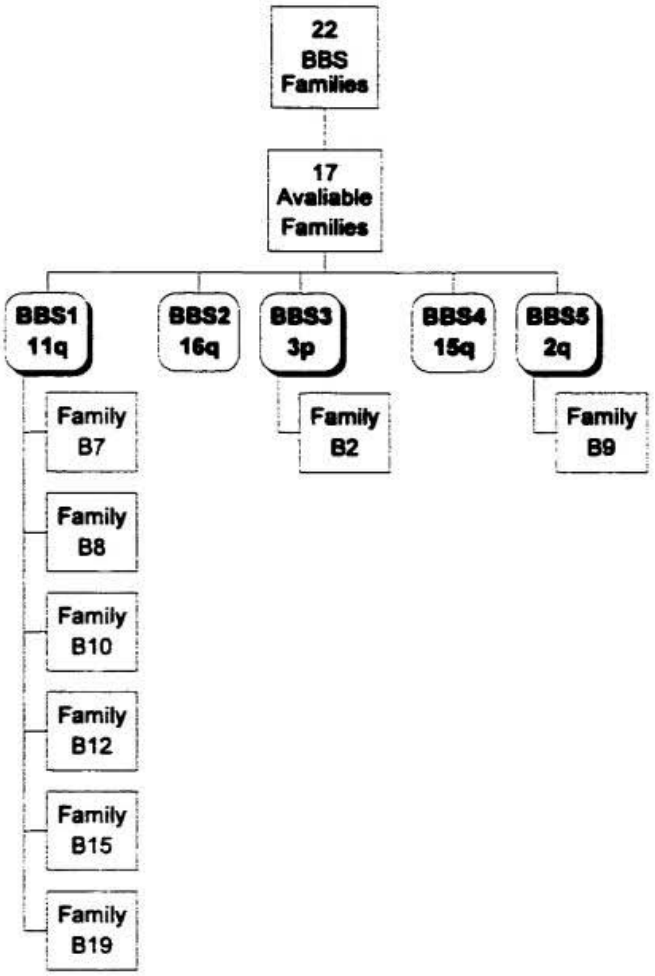

Figure 6.2 Summary of linkage assignments in Newfoundland families with BBS

The existence of either *BBS2*, *BBS4*, or other novel BBS genes in the population could not be ruled out because of the many unassigned families (fig. 6.1). **In conclusion, these findings suggest a minimum of three BBS genes (*BBS1*, *BBS3*, and *BBS5*) are present in the Newfoundland population.**

Benefit of this Study to Individuals and Families Affected by BBS

Eight Newfoundland BBS families were assigned with confidence to specific BBS loci (fig. 6.2). Although the underlying genes have not been identified, disease-associated haplotypes could be used for carrier testing in these families. This is particularly important for family members of reproductive age who choose a partner from within their community or from a neighboring community, especially for family members originating from the south and south west coasts of Newfoundland where the *BBS1* carrier rate must be relatively high due to founder effect (fig. 6.3). In identified BBS families, haplotype analysis could also be used to confirm the diagnosis in young children, a prerequisite for appropriate early interventions and patient management. For example, the provision of special education with low-vision aids, and dietary intervention to prevent or treat obesity and diabetes mellitus, are important early interventions that can maximize the potential of an person with BBS to develop into a healthy and independent adult.

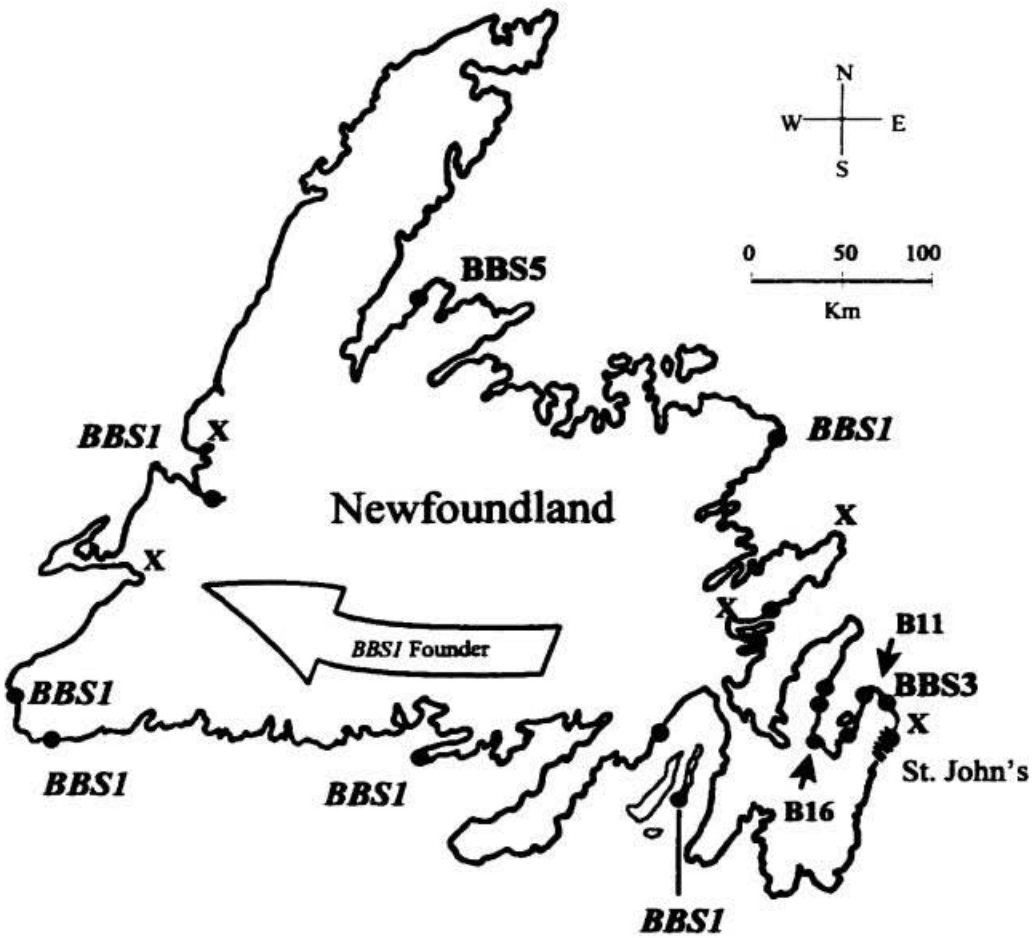

Figure 6.3 Geographical location of *BBS1*-, *BBS3*-, and *BBS5*-linked families and BBS families that remain unassigned in Newfoundland. The 5 families not available for genotyping in this study are denoted by an "x". The "BBS1 founder" arrow indicates the historical settlement pattern that occurred along the south coast.

Phenotypic Subtypes of BBS

The genetic heterogeneity of BBS in association with variable phenotypic expression begs the question of locus-specific phenotypes. The specific phenotypic manifestations of the *BBS3* locus as proposed by Carmi et al. (1995) were not supported (**Chapter 3**). However, the finding, in this study, of six families with an ancestral haplotype (**Chapter 5**) presents a rare opportunity to explore the effects of genetic background and environment on the expressivity of a single mutation, in this case at the *BBS1* locus. In general, the number of BBS families that can be assigned to a specific BBS locus, per research group, are small. Therefore, an international collaborative effort to establish diagnostic criteria and standardize clinical assessments may be the only means to truly evaluate locus-specific expression of BBS.

Future studies on Newfoundland BBS families

The conclusion of this thesis in no way completes the investigation into the genetics of BBS in Newfoundland. The occurrence of one rare (*BBS3*) and at least one novel (*BBS5*) BBS gene in the Newfoundland population is intriguing. The availability of 14 unassigned families, some of them quite informative (e.g., family B13), provides ample resources for future studies. Of the 14 unassigned families, five families have never been examined (untyped). Blood samples need to be collected on these BBS patients and their families so genetic investigations can begin (fig. 6.4, step 4). Although it is prudent to genotype all five BBS loci in each family, priority should be given to *BBS1* markers

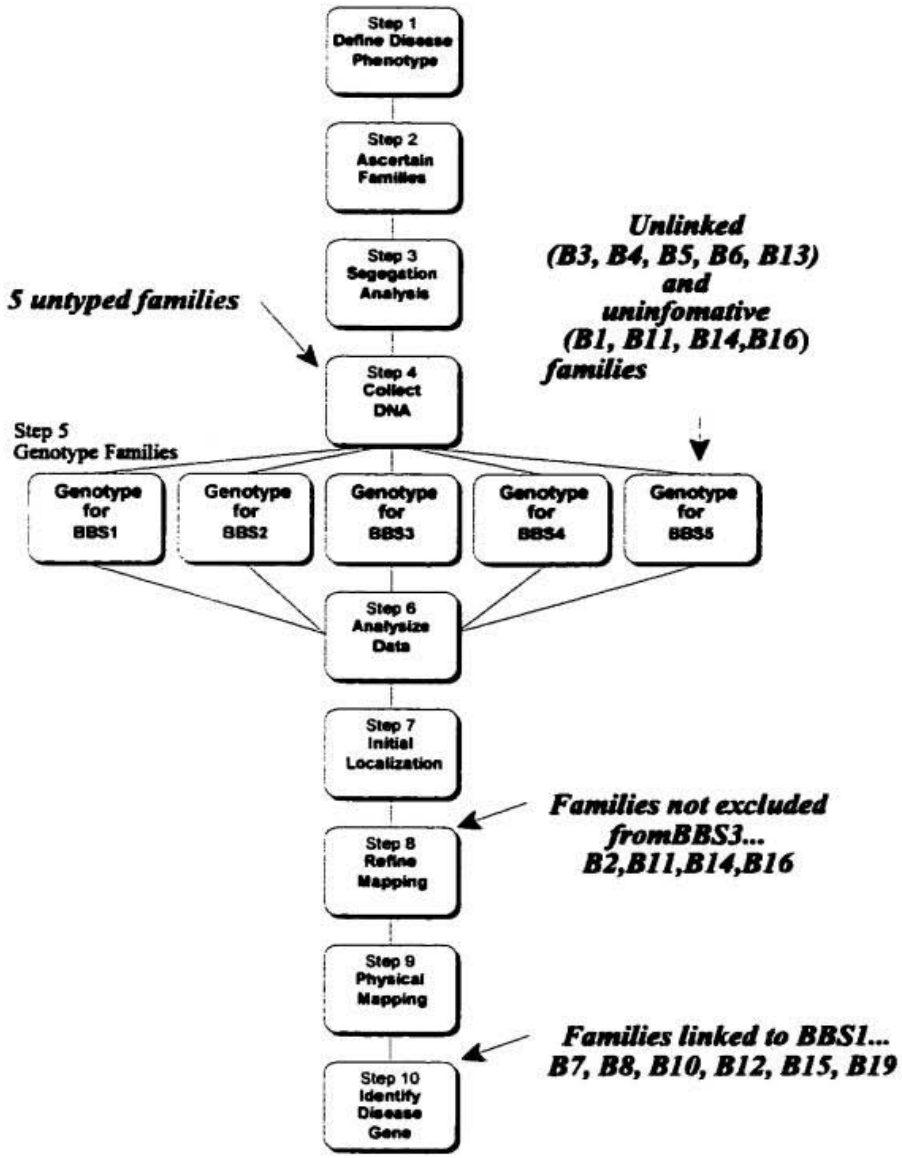

Figure 6.4 Outline of future studies involving Newfoundland BBS families and the step-wise approach to positional cloning.

because two of these families originate from the south west coast (fig. 6.3).

Another priority is that the five unlinked (and informative) families be tested for linkage at the novel *BBS5* locus (fig. 6.4, step 5). The linkage studies of *BBS5* should be augmented with a search for linkage disequilibrium using the disease-associated alleles identified in family B9 as reference alleles. The finding of founder effect among *BBS5* families in uninformative families would not only provide locus assignments for the families but may also help refine the *BBS5* disease interval.

The rare finding of a *BBS3*-linked family (B2) originating from the Conception Bay area is interesting in that several unassigned families reside in neighboring communities. This includes two of the three uninformative families (B11, B14 and B16) that could not be excluded from linkage at the *BBS3* locus and one of the untyped kindreds (fig. 6.3). These families need to be genotyped for disease-associated alleles identified in family B2 to investigate the possibility of a *BBS3* founder effect. Refine mapping has been completed on the six families linked to *BBS1*. These families are available for mutation testing of candidate genes within the *BBS1* disease interval (fig. 6.4, step 10).

Towards the Positional Cloning of BBS Genes

As the major BBS locus, it is anticipated that *BBS1* will account for the majority of BBS cases. In this regard, the most significant outcome of my thesis work is the refinement of the *BBS1* disease interval to 1-Mb, small enough to make it amenable to positional cloning. The major organ systems affected in BBS are the limbs, gonads,

kidneys, and eyes. Because of the congenital nature of several phenotypic features of BBS (limb defect, hypogenitalism and calyceal blunting), the BBS genes must normally be expressed during the first trimester when tissues and organs are being modeled. Although functional candidate genes for BBS are difficult to imagine, the search for candidate genes within the refined *BBS1* interval should focus on sequences that are expressed in the tissues involved in the BBS phenotype.

Refinement of the *BBS1* disease interval from a 26-cM to 1-cM region (*D11S1883-D11S4940*) was accomplished by LD mapping (**Chapter 5**). Support for the location of *BBS1* within the *D11S1883-D11S4940* interval was recently established by a study of recombinations in multiple BBS families that suggests that *BBS1* is located between markers *D11S4205* and *D11S913* (Katsanis et al.1999*)*, a region that totally encompasses the refined disease interval determined in this thesis (**Chapter 5**, fig 5.3). *BBS1* is tightly linked to *PYGM*, the locus for muscle glycogen phosphorylase. This region on chromosome 11q13 is gene rich and well characterized with polymorphic markers (Graff et al. 1997; Manickam et al. 1997), physical maps (Cooper et al. 1997; Guru et al. 1997b; Smith et al. 1997), and transcript maps (Guru et al. 1997a; Sawicki et al. 1997; Zhu et al. 1998) developed in a flurry of activity to clone the genes for mutilple endocrine neoplasia (*MEN1)* (Chandrasekharappa et al. 1997) and Best macular dystrophy (*BMD*) (Petrukhin . et al. 1998).

Although no recombination has been observed between the *BBS1* phenotype and *PYGM,* recombinants were identified with more proximal and distal markers. The

minimal linkage interval for the *BBS1* gene is a 1-Mb region (*D11S1883-D11S4940*). Genes excluded from the disease interval are too numerous to list but include genes previously suggested as candidates for *BBS1*, such as the human homolog (*OVOL1*) of the Drosophila *ovo* gene that results in hypogenitalism, reduced ability to reproduce, and kidney abnormalities in knockout mice (Dai et al. 1998).

The positional candidate genes for *BBS1* include twelve known genes identified between markers *D11S1883* and *D11S4940*: *VRF, FKBP2, PNG, PLCβ3, Neurexin, PYGM, Nu, ZFM1, GC-Kinase, Mu, Kappa* and *H-PAST* (Guru et al. 1997a). A search through the Unigene database at the National Center for Biological Information (NCBI; www.ncbi.nlm.nih.gov) shows that many of these genes are expressed in a wide variety of tissues. Four other genes reportedly map to this interval: a novel human CDC25-like (*HCDC25L*) gene and a dystrophia myotonica protein kinase-like (*DMPKL*) gene map to the 5' end (*D11S1883-PYGM*) of the disease interval; a novel and ubiquitously expressed gene, *GCKNG* (germinal center kinase-neighboring gene), maps telomeric to *PYGM* (Kedra et al. 1997); a homolog of the human p70S6 kinase gene (*p70s6 k*) (relative map position unknown), is universally expressed, including within adipose cells and retina (Zhu et al. 1998).

To distinguish the *BBS1* gene from all other positional candidates in this gene-dense region of the human genome is a formidable task. The only report of mutation testing with any of these candidate genes (*H-PAST*) has failed to find mutations in BBS patients (Haider et al. 1999). The timely identification of *BBS1* will require both informative

families and substantial sequencing efforts. **To meet these requirements, and as a result of my thesis work, our group at Memorial University has embarked on an international collaboration with the sequencing center at Baylor College of Medicine in Houston, Texas, to identify the *BBS1* gene.**

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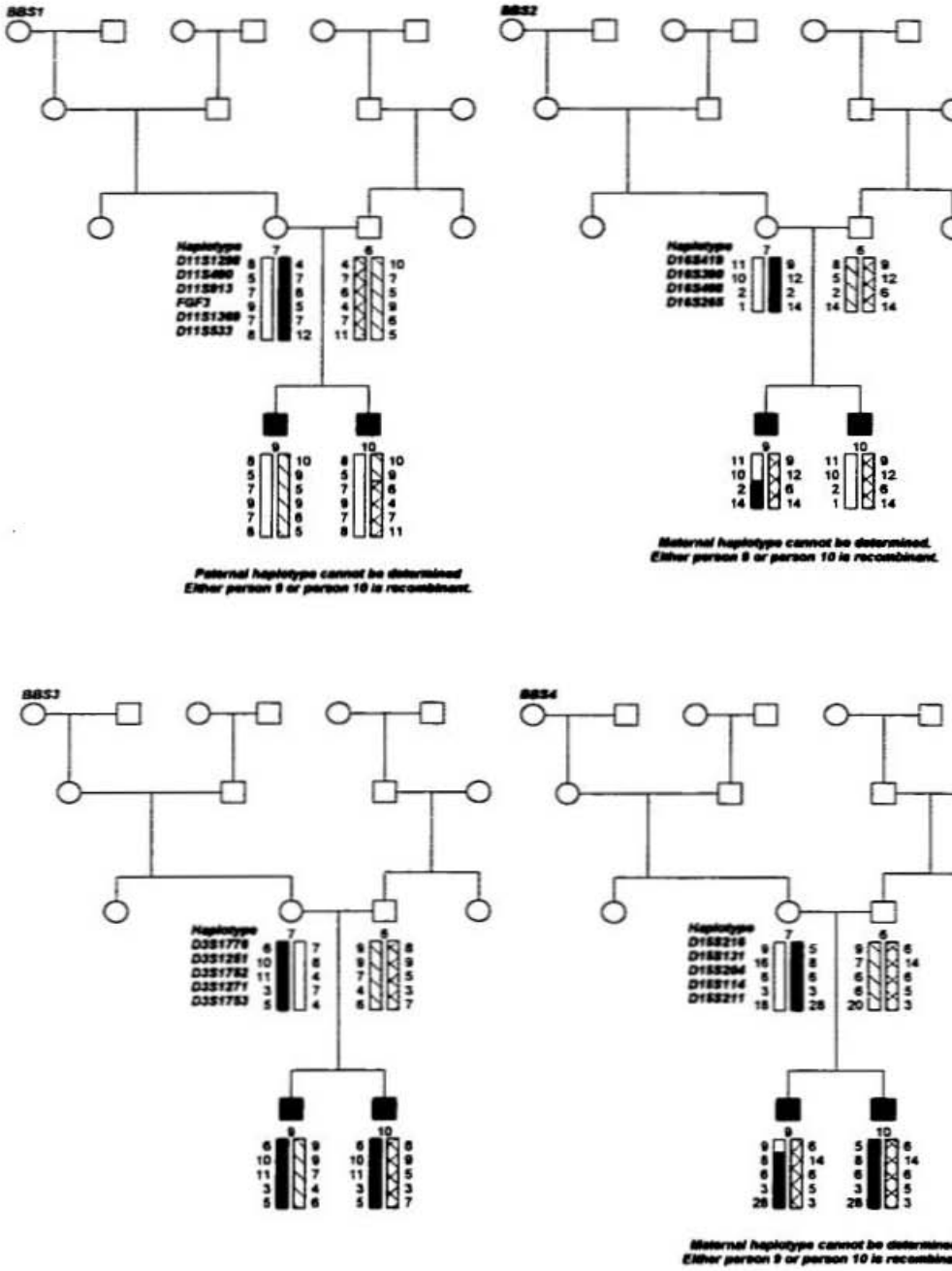
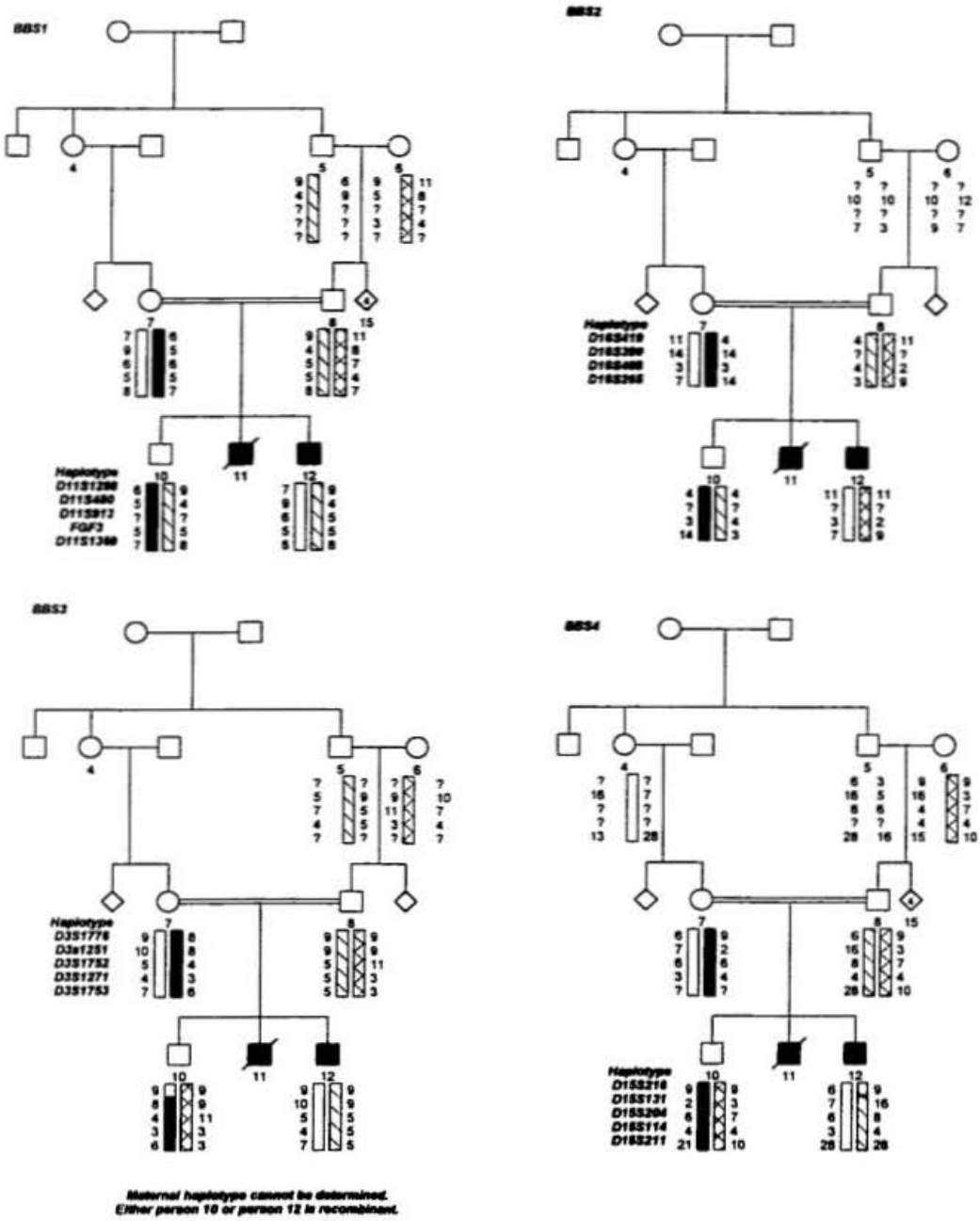
Appendix 1 (Family B1)

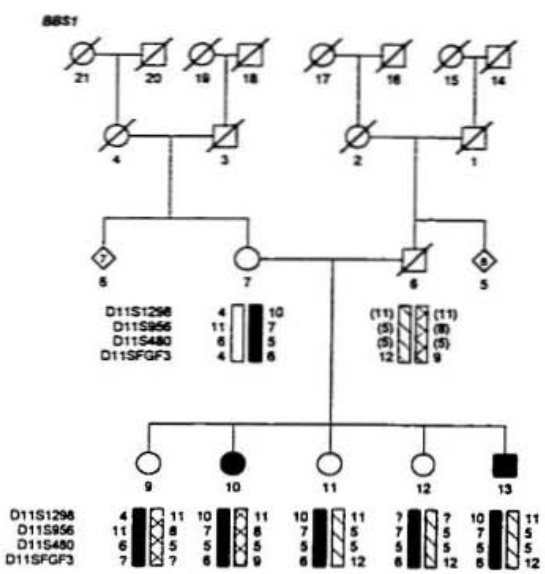


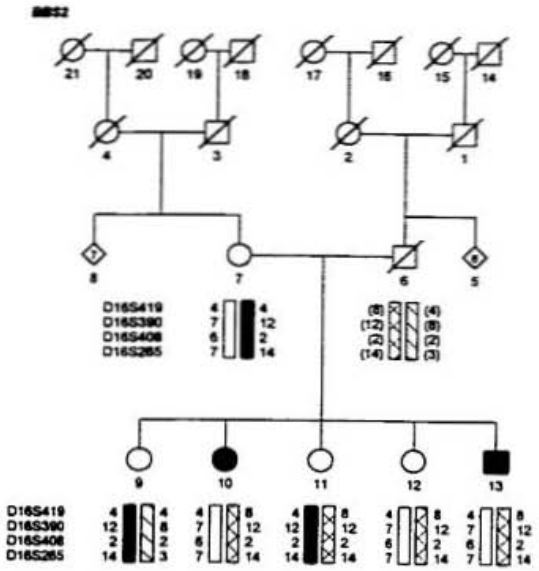
Appendix 2 (Family B3)

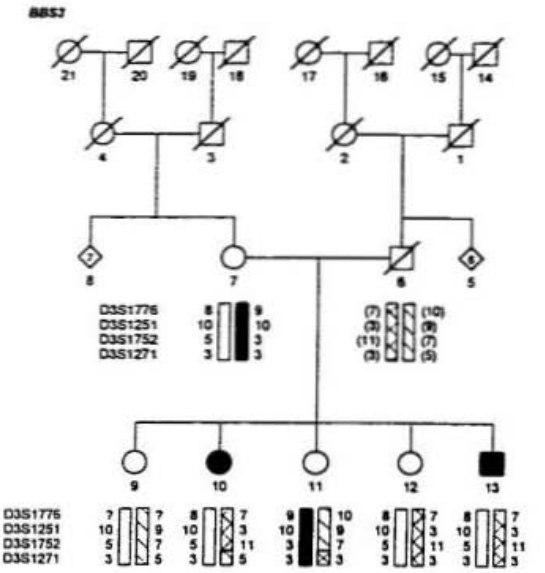


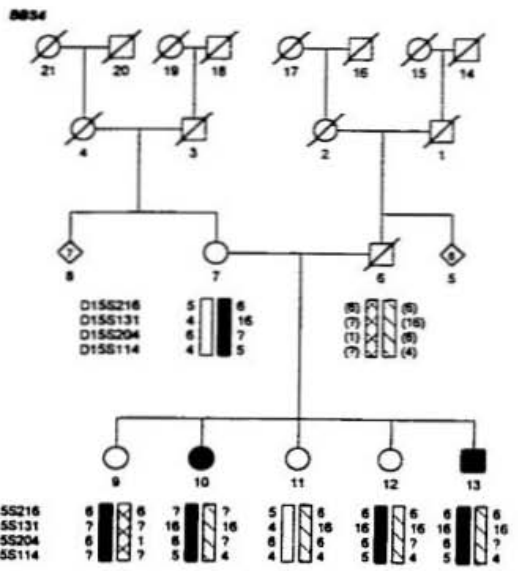
Maternal haplotype cannot be determined. Either person 10 or person 12 is recombinant.

Appendix 3 (Family B4)

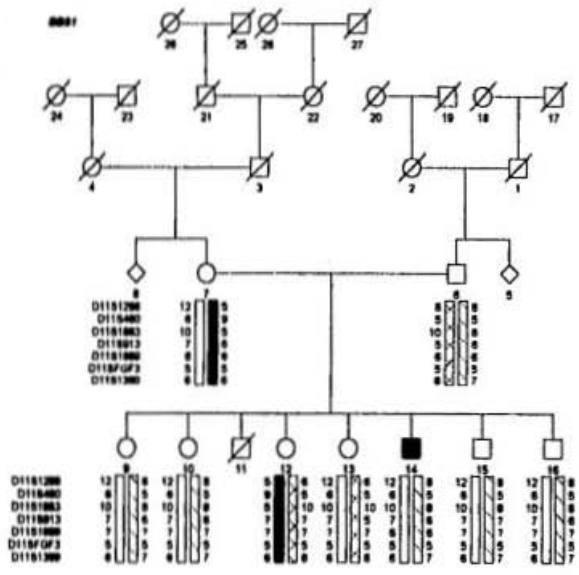


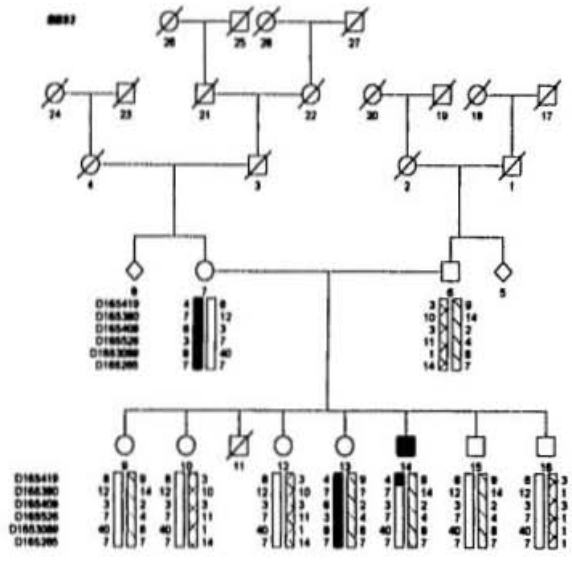


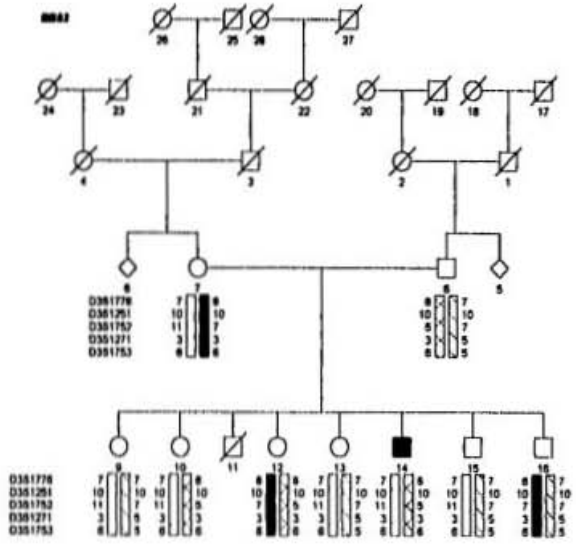


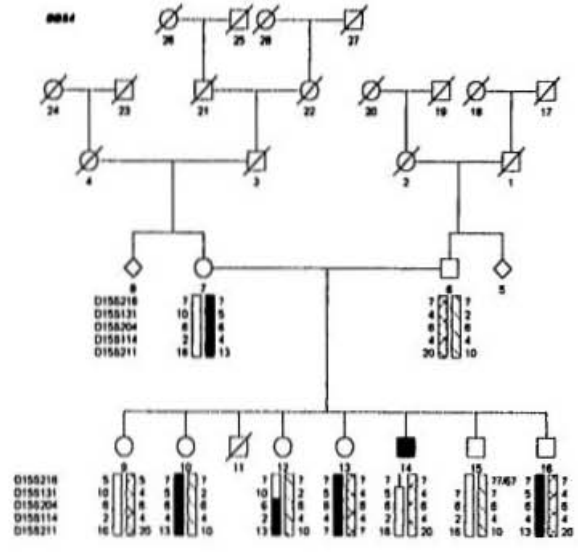


Appendix 4
(Family B5)

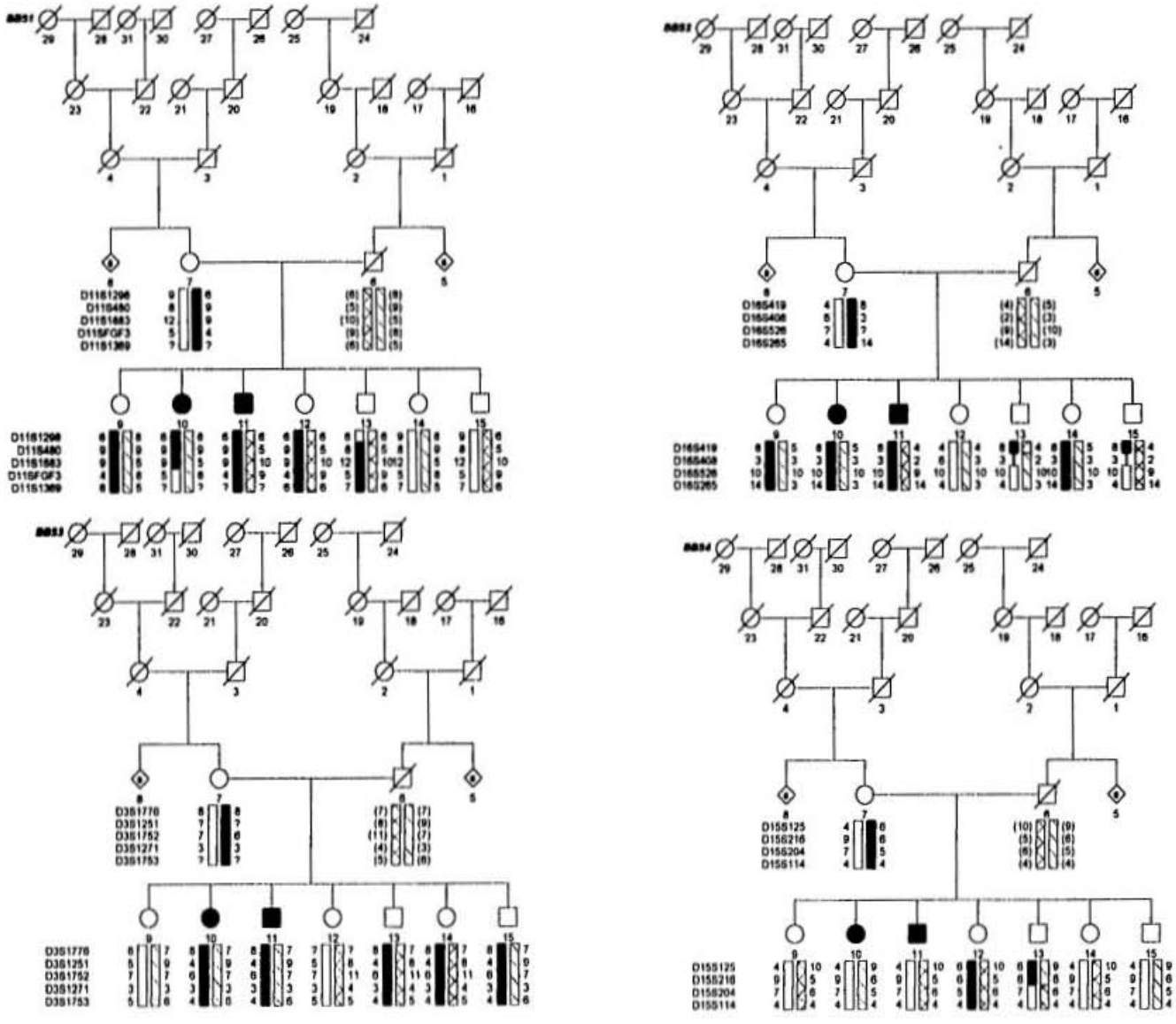




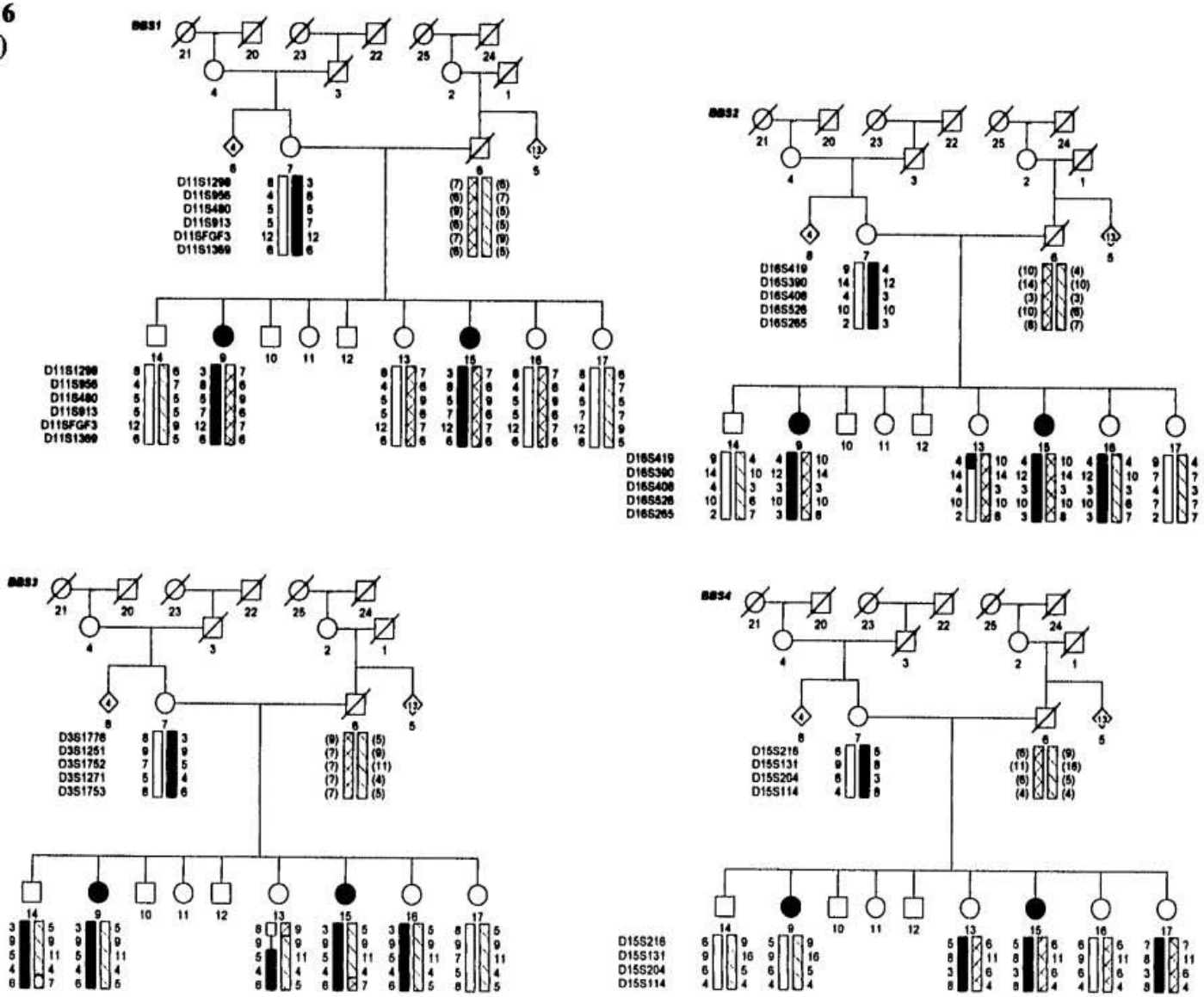




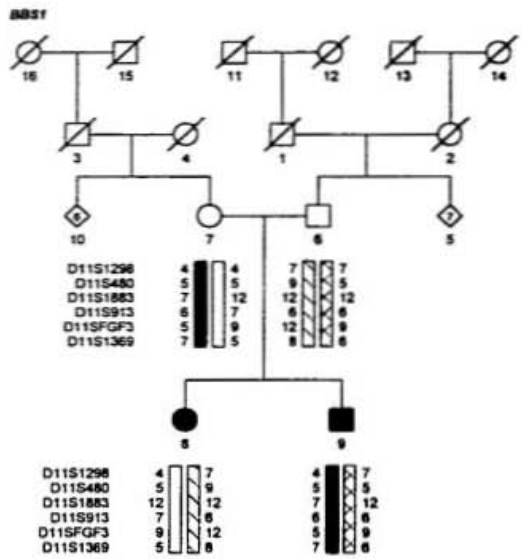
Appendix 5
(Family B6)

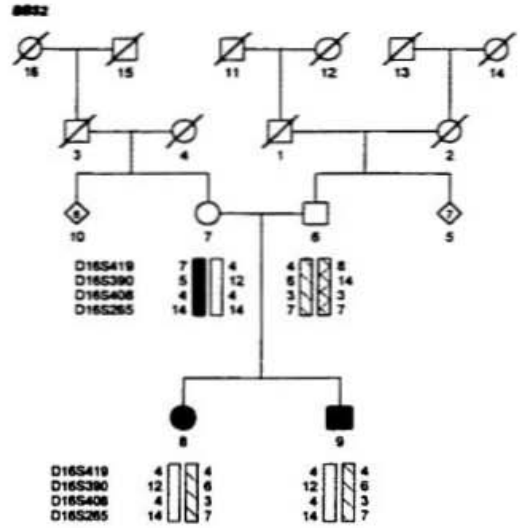


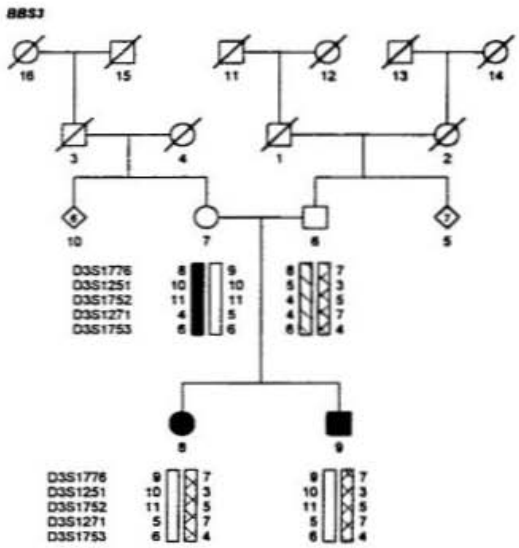
Appendix 6
(Family B7)

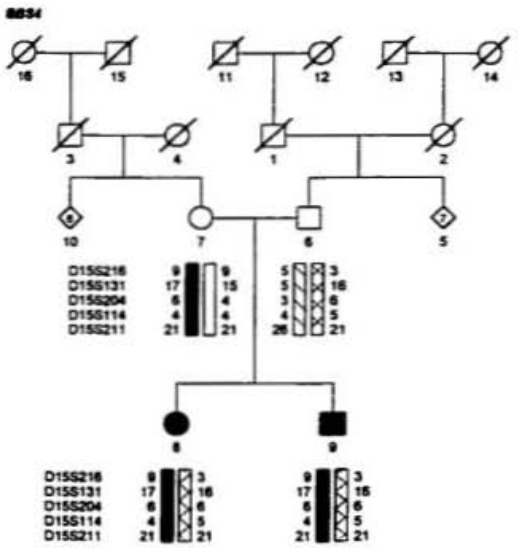


Appendix 7 (Family B11)

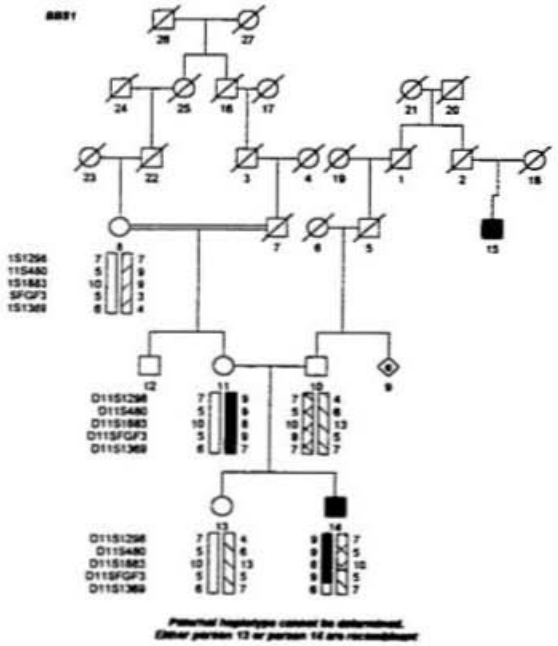


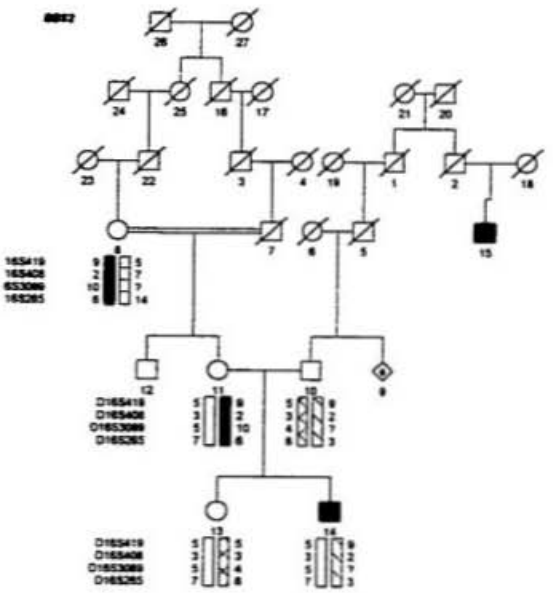


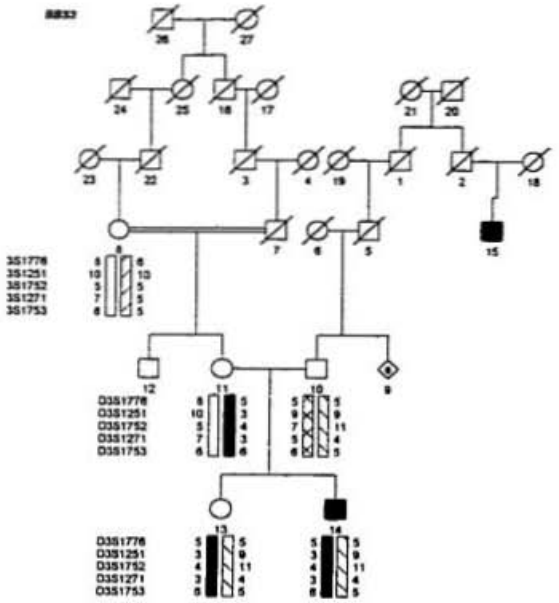


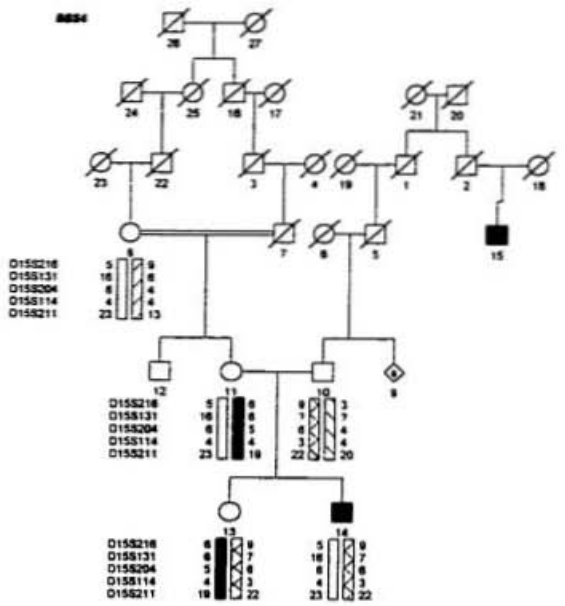


Appendix 8 (Family B12)

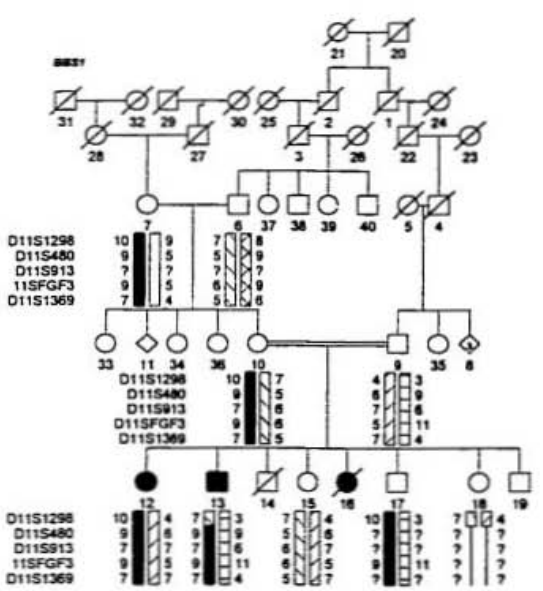


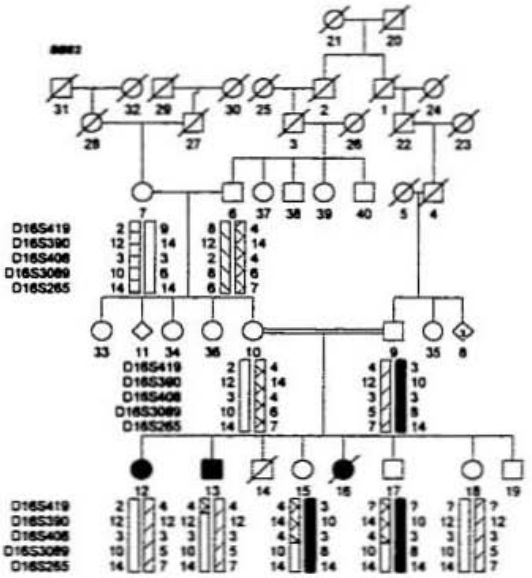


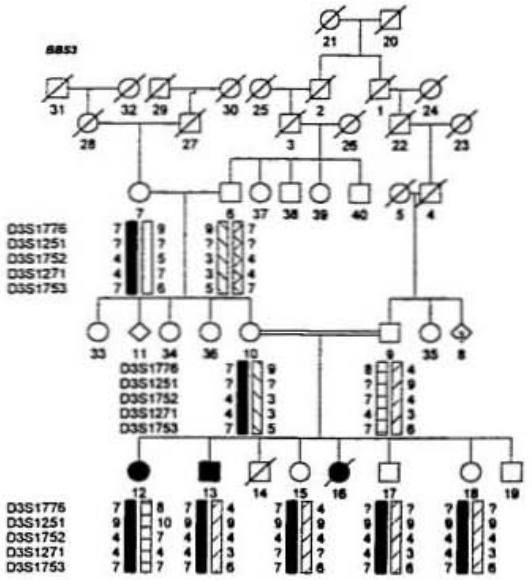


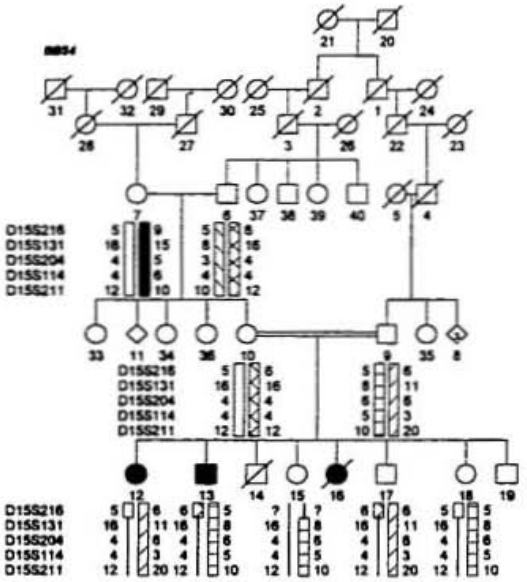


Appendix 9 (Family B13)

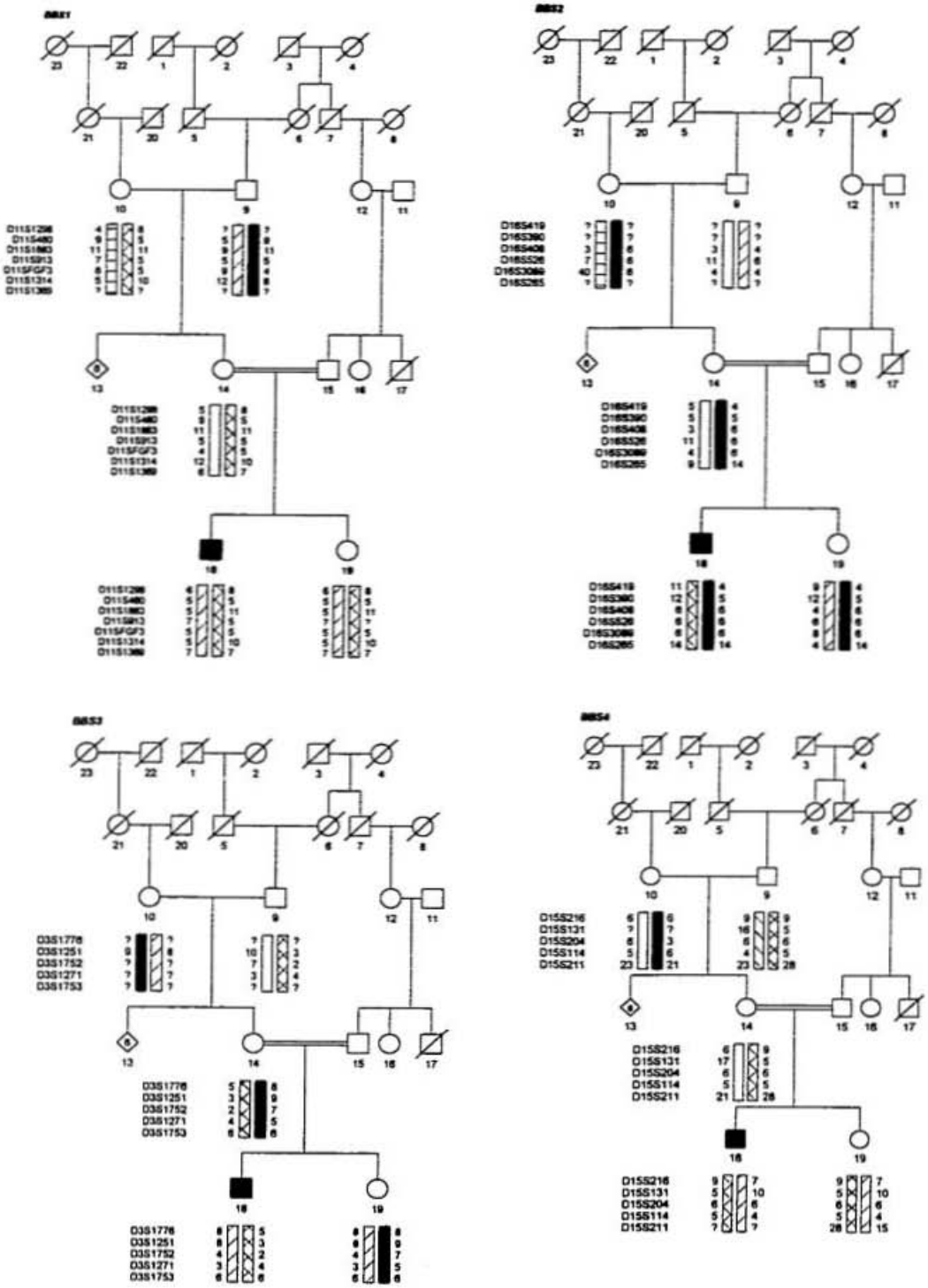




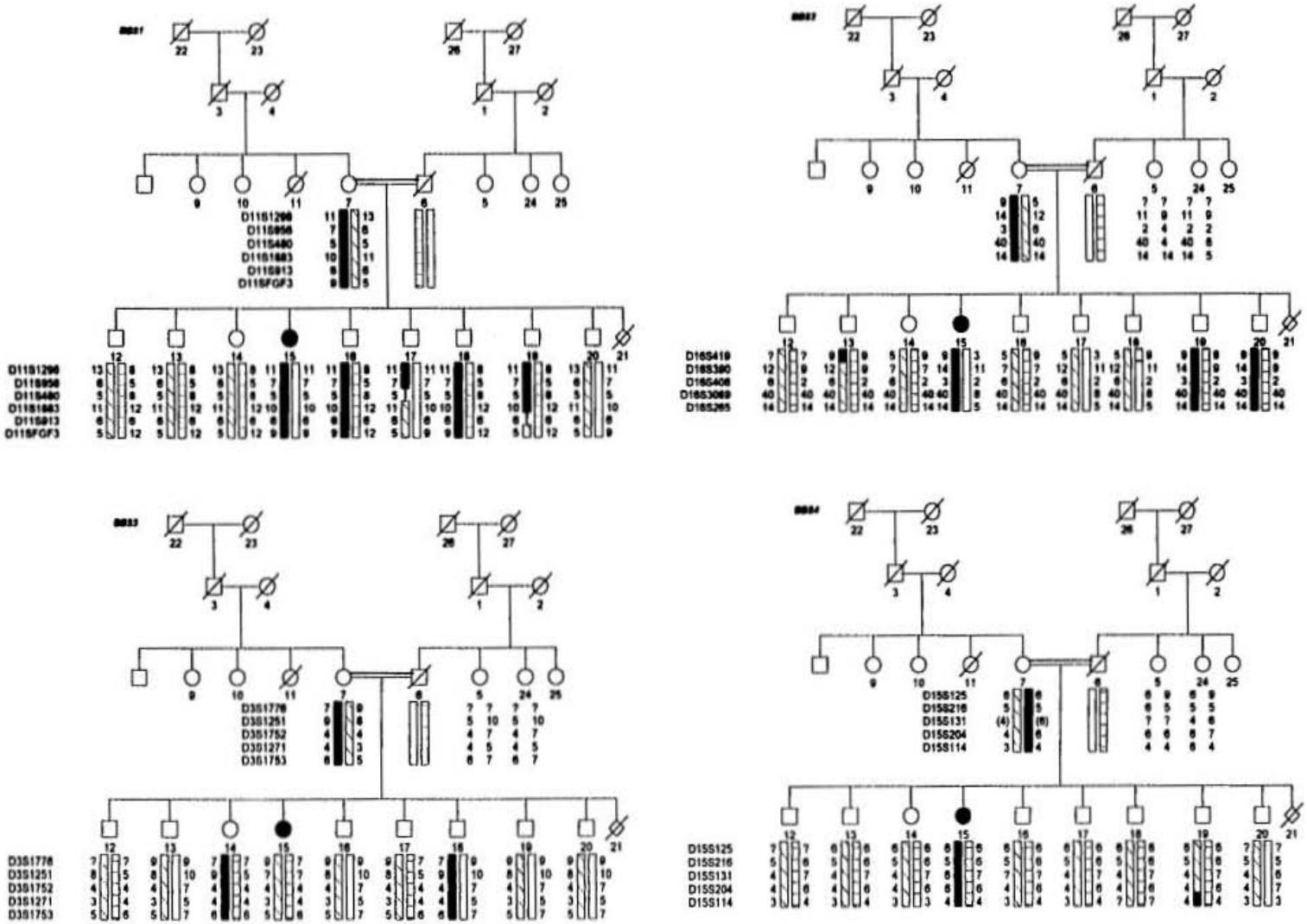




Appendix 10 (Family B14)



Appendix 11 (Family B15)



Appendix 12 (Family B16)