Mutation analysis of BBS2 and BBS6 genes in family, affected by Bardet-Biedl Syndrome in Northern Greece

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Introduction

Bardet-Biedl syndrome (BBS; MIM 209900) is a multigene autosomal recessive disorder, characterized by multiple, primary and secondary clinical features. The primary features include rod-cone dystrophy, central obesity, hypogonadism, mental retardation and renal dysplasia. Other features (secondary) of varying frequency include diabetes mellitus, hepatic fibrosis, reproductive abnormalities, endocrinologic deficiencies, short stature, developmental retardation and speech and behavior abnormalities (1-4). Prevalence rates in North America and Europe range from 1:140000 to 1:160000 live births (5-7). However, in Kuwait and Newfoundland the rate is much higher, with an estimated incidence of 1:13500 and 1:17500, respectively. This observation revealed the founder effect hypothesis, in that places (8, 4). As the syndrome is rare, then the frequency with which the BBS gene is being silently carried in the population is uncommon and calculated to be approximately 1:179 (according to Hardy-Weinberg equation). The molecular and genetic bases of oligogenic behavior were observed on BBS, but are also present in several other genetic disorders, such as muscle dystrophy (LGMD). BBS has been shown to display a high degree of genetic heterogeneity. Linkage studies and haplotype analysis in large affected families, revealed at least six independent loci in the human genome: BBS1 on 11q13 (9), BBS2 on 16q21 (10), BBS3 on 3p12 (11), BBS4 on 15q22.2-q23 (12), BBS5 on 2q31 (13) and BBS6 on 20p12 (14). Finally the presence of a seventh (BBS7) (15) as yet unmapped locus, was documented by genetically excluding several pedigrees from all known loci. To date three BBS genes have been cloned: BBS2 which encodes a protein with unknown function, BBS4 which encodes a protein which belongs to yet another functional class of proteins, as it shows significant similarity to O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT) from several species (16), and BBS6 which encodes a putative chaperonin.

It is widely recognized that the substantial genetic heterogeneity in BBS might contribute to the overall phenotypic variation. Genetic analysis of these three genes provides many different types of mutations like missense, nonsense, frameshift and splice mutations in coding sequences, but there is no known hotspot region. The correlation between the nature of the BBS mutations and the severity of the disease is unclear. A stronger phenotype-genotype association can be observed for some BBS6 mutations.

In order to screen a whole BBS family, identified in Northern Greece for the first time, we analyzed the BBS2 and BBS6 genes for known or unknown mutations. We choose these two genes because of their high rate of mutations.

Materials and Methods

All the members of the affected family (10 chromosomes) and fifty healthy individuals (100 chromosomes) used as control samples, have been analyzed for BBS2 and BBS6 genes for known or unknown mutations, in order to determine the spectrum of BBS mutations. It is the first case of BBS affected family in Northern Greece. Genomic DNA was isolated from whole blood according to Phenol-Chlorophorm protocol. Seventeen exons from BBS2 and six exons from BBS6 were amplified via polymerase chain reaction (PCR). The primer sequences for BBS6 were received from the Lupski Lab website while those for BBS2 were received from a previous report (18). At a second step, we screened the BBS2 and BBS6 genes, exon by exon, using the Single Strand Conformation Analysis (SSCP), for all samples. Restriction enzymes (RFLPs) were used to digest the PCR products, as required. For SSCP analysis, PCR products were electrophorised on SSCP gels, using 3 different conditions (table 1). Different SSCP patterns will be sequenced and compared with a control sample to detect any changes from that of the normal sequence.

Results and Discussion

Clinical data

The cardinal features of BBS are1) pigmentary retinopathy in 93% of patients, 2) hypogenitalism 74%, 3) obesity 91%, 4) polysyndactyly 73% and 5) mental retardation 87%. The other are development retardation, defective renal function, sensorineural auditive impairment. Our patient had degenerating retino-choroiditis on posteriol side of both eyes. As reported by Klein and Ammann, only 18% of cases have had typical retinitis pigmentosa. The retinal degeneration is more than a cone-rod dystrophy, than retinitis pigmentosa. Poor night vision is, usually, the first ocular complaint. Also there was hypogenitalism and truncal obesity especially in

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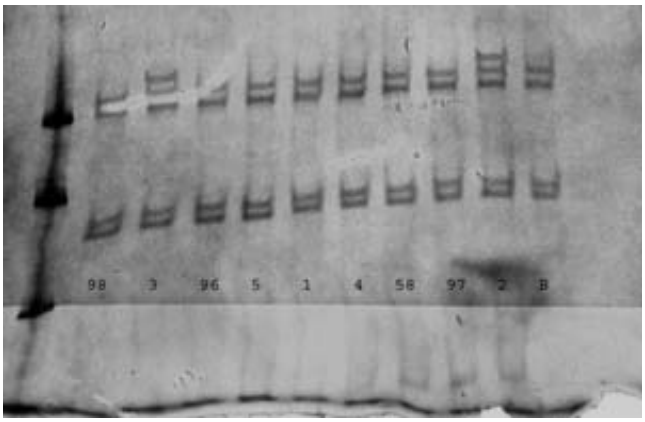
the neck. He has had feet hexadactyly and postaxial polysyndactyly on the right hand. We found a mild mental retardation. We didn't find sensorineural auditive impairment or cardiovascular disorders. A defect in urine-concentrating ability with polyuria there was before becames uraemic. Now is in chronic haemodialysis. Defective renal function occur in many as 90% of BBS cases. The ultrastructural (U/S) change in the glomerular basement membrane is the characteristic renal abnormality in the BBS. The U/S of our patient showed small kidneys without an exact layout and cortex sclerosis (17, 3).

Figure 1. SSCP different pattern in exon 3a of BBS6 gene in samples 2 (mother) and 3 (son) of the affected family.



Genetic analysis and comments

In this study one pedigree (10 chromosomes) of BBS affected family was analyzed for BBS2 and BBS6 variations. Our results were compared with data that was obtained by the analysis of other 100 chromosomes from healthy individuals from Northern Greece. SSCP analysis of all six exons of BBS6 gene revealed a pattern in exon 3a of one parent and one son (figure1) that was different from the analyzed controls. SSCP analysis of BBS2 and Sequencing analysis of the suspiciously samples is being contacted at present. It is significant to note that this is the first reported case of Bardet-Biedl syndrome patients in Greece, who are being analyzed for the BBS2 and BBS6 gene variations. Many other cases of patients and pedigrees affected with McKusic-Kaufman syndrome (MKS) in Greece remain to be screened for BBS mutations. This is because of the clinical and molecular overlap between MKS and BBS. It is well known that many cases of BBS have been misdiagnosed as MKS in infancy or early childhood prior of the development of other manifestations of BBS (19). It must be seriously considered whether MKS is part of the spectrum of BBS. The choice of BBS2 and BBS6 genes was based on previous reports, referring to the possible interactions of these gene products. The aim was to understand the heterogeneity and pleiotropism of the BBS in inter and intra family patients (5,12, 3, 20, 4, 21). Many studies have tried to correlate the phenotype and genotype of this syndrome.

Different hypotheses have been reported so far, each one trying partly to explain this heterogeneity. The most important is that of the triallelic inheritance, in which three mutations are necessary for pathogenesis of this disorder. The third mutation is located in a different BBS locus and has a modifying effect (22).

The epistatic effect hypothesis that complements the previous hypothesis, suggest that the BBS2 and BBS6 proteins may interact with each other in a common metabolic pathway. It is possible that the BBS2 protein plays an unrecognized chaperonin role like BB6 protein or is possible to be a part of a chaperonin complex. Other possibility is that BBS2 protein is a substrate for the best chaperonin function (18). In another report (23) recent observations support the above hypothesis. They suggest an additional layer of complexity in the genetics of BBS. The initial triallelic hypothesis in which three mutations are necessary for pathogenesis, may oversimplify the true contribution of each BBS locus to the phenotype. Their observation, in which individuals with two mutations in one locus are asymptomatic but individuals with three mutations (two in one locus and one in the other) are affected, may reveal a new epistatic model, for the interaction between genes in mendelian disorders (23). Another hypothesis that could help explain the epistatic effect, is the consideration of one ancestral founder mutation. This mutation behaves as a dominant susceptibility locus, and may be paired and interact with other mutations at various known BBS loci to cause the disease (22). The hypothesis mentioned above support the model of the triallelic inheritance (22). However in this report we suggest that the pathogenesis and the severity of this disorder is not simply the result of inter or intragenic interaction of the mutations but there must be an additional mechanism. We suggest the hypothesis of the SNPs map (Single Nucleotide Polymorphisms Map). Our hypothesis was based on a previous report by Nishimura et al., 2001 who identified homozygous BBS patients for V75G (Val75Gln) mutation in exon 2 of BBS2 gene, that was not identified in any of the healthy individuals. In addition the V75G patients were homozygous for the I123V (Ile123Val) polymorphism, while some of the healthy individuals and the parents of V75G patients were found heterozygous for the same polymorphism.

With this hypothesis we introduce the possibility that the heterogeneity of the disorder is the result not only of the interaction of certain mutations on BBS loci but of the interaction between the different every time association of SNPs located across the BBS loci with mutations as well. In this way we believe that we have a SNPs map that interacts with the BBS mutations contributing to the severity of the disease. Still much remains to be learned about both the genetic and the physiological dysfunction in BBS. In addition, the question of the multiallelic inheritance must be thoroughly investigated. Reconciliation of the pleiotropic BBS phenotype with mutations in a single gene or combination of genes will remain difficult until all BBS proteins are elucidated. Also the elucidation of the relative effect of each mutation at each locus still remains unclear. Finally answers must be given about the function of all BBS proteins, the

way in which may interact with each other, the possibility of the existence of other BBS loci that remain undetectable and the relationship between interactions of the known genetic components of this disorder. The answer to these and other related questions will provide new insights for various cellular functions such as the retinal or kidney development.

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