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 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 are:1) pigmentary retinopathy in 93% of patients, 2) hypogenitalism 74%, 3) obesity 91%, 4) polydactyly 73% and 5) mental retardation 87%. The other are development retardation, defective renal function, sensorineural auditive impairment. Our patient had degenerating retino-choroiditis on posterior 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...
the neck. He has had hexadactyly and postaxial poly-
syndactyly on the right hand. We found a mild mental retar-
dation. We didn’t find sensorineural auditive impairment or
cardiovascular disorders. A defect in urine-concentrating
ability with polyuria there was before became 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
cortex sclerosis (17, 3).

Our results were compared with data that was obtained by
the analysis of other 100 chromosomes from healthy individ-
uals from Northern Greece. SSCP analysis of all six ex-
on of BBS6 gene revealed a pattern in exon 3a of one par-
tent and one son (figure1) that was different from the ana-
alyzed 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
McKusie-Kauffman syndrome (MKS) in Greece remain to
be screened for BBS mutations. This is because of the clini-
cal 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 previ-
ous 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 pa-
tients (5,12, 3, 20, 4, 21). Many studies have tried to corre-
late the phenotype and genotype of this syndrome.

Different hypotheses have been reported so far, each one
trying partly to explain this heterogeneity. The most impor-
tant is that of the triallelic inheritance, in which three muta-
tions 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 previ-
ous hypothesis, suggest that the BBS2 and BBS6 proteins
may interact with each other in a common metabolic path-
way. It is possible that the BBS2 protein plays an unrecog-
nized 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 inter-
action between genes in mendelian disorders (23). Another
hypothesis that could help explain the epistatic effect, is the
consideration of one ancestral founder mutation. This muta-
tion behaves as a dominant susceptibility locus, and may be
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(22). However in this report we suggest that the pathogene-
sis 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 hypothe-
sis 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 pa-
tients for V75G (Val75Gln) mutation in exon 2 of BBS2
gene, that was not identified in any of the healthy individu-
als. In addition the V75G patients were homozygous for the
1123V (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 in-
teraction of certain mutations on BBS loci but of the inter-
action 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 dis-
ease. Still much remains to be learned about both the ge-
netic and the physiological dysfunction in BBS. In addition,
the question of the multiallelic inheritance must be thor-
oughly 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 elu-
cidated. Also the elucidation of the relative effect of each
mutation at each locus still remains unclear. Finally answers
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Genetic analysis and comments
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cidated. 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.

References