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Original article

Are ABL and MTP markers at chromosome 4Q28 of patients with abetalipoproteinemia really the same loci?

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SUMMARY

Background: **αbetalipoproteinemia is an extremely rare genetic disorder of lipoprotein metabolism, associated with various biochemical abnormalities, combined with clinical malabsorption. The cause of the disease is considered to be mutations in the Microsomal Triglyceride Protein gene.** ***Purpose:*** **The aim of this study was to see if the locus Microsomal Triglyceride Protein and ABL are identical in patients with αbetalipoproteinemia. Another aim was to provide information concerning the patient's karyotype.** ***Subjects-Methods:*** **The study included seven persons: 3 patients (a girl and two boys aged 32, 30 and 22 years respectively) and their 4 healthy, first-degree relatives (two parents, a brother and a sister). Three chromosome markers (fibrinogen alpha at 4 q 28 (FGA), Gc and MNSs blood groups) were tested. Cytogenetic studies were performed on PHA stimulated peripheral blood cell cultures.** ***Results:*** **Fibrinogen-alpha chromosome marker at 4 q 28 showed absolute correlation in all three patients by inheritance of the same maternal and paternal FGA alleles, whereas the healthy siblings had other allelic constellations. The Gc and MNSs genes were not informative. The karyotype of patients and healthy members of the family was normal.** ***Conclusion:*** **It is concluded that ABL and MTP (at 4 q 22-24) are probably identical loci and should eliminate further on one designation. The karyotype of patients with abetalipoproteinemia is normal.**

Key Words: MTP gene, Abetalipoproteinemia, Greece, Mutations, Chromosomes, Microsomal Transfer Protein

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INTRODUCTION

Abetalipoproteinemia is a rare genetic disorder of lipoprotein metabolism, associated with various serum biochemical abnormalities combined with clinical manifestations of malabsorption.[1-2] The cardinal manifestations include signs of malnutrition, neurologic disturbances, retinitis pigmentosa, and abnormalities in the morphology of erythrocytes. Apolipoprotein B is characteristically absent from both plasma and intestinal mucosa. As a result, chylomicrons, low density and very low-density lipoproteins are absent from plasma. Histology of the small bowel shows that the mucosa is engorged with lipid droplets, which characteristically distend the apical portion of the epithelial cell. Diagnosis is based on the clinical picture of malabsorption and malnutrition, the characteristic neurological signs, unique serum lipidemic profile, as well as on the typical small bowel histological picture. The phenotype of the disease is clinically heterogenous. Differences in presentation may reflect locus heterogeneity. The prognosis varies from patient to patient depending on the time of diagnosis and the proper pharmaceutical treatment applied. So far, less than 100 isolated cases from many parts of the world have been reported.[3-11]

The cause of the disease is considered to be mutations in the Microsomal triglyceride Transfer Protein (MTP) that result in an absence of MTP function.[12] Defects in the MTP genes have been shown to cause αbetalipo-proteinemia and are the predominant cause of hereditary αbetalipo-proteinemia.[13] The MTP gene could be localized on chromosome 4q22-24. However, it remains to be determined if mutations of the MTP gene are the sole, or even main, cause of abetalipoproteinemia.

The aim of this study was to investigate the hypothe-

sis that the locus MTP and ABL are closely linked. To this end we decided to investigate if the locus of MTP is closely linked with a,betalipoproteinemia in a 7-individual family with three affected members. Another aim of the study was to provide information concerning the karyotype of the patients and the healthy members of the family.

SUBJECTS AND METHODS

The study included all seven members of the affected family. There were three patients, a female aged 34 years and two males (brothers), aged 31 and 29 years, as well as the other two healthy brothers and the two healthy parents. Details of the clinical course, outcome and therapeutic strategies applied to these patients have been previously published[1,14] and are summarized in table 1.

On the long arm of chromosome 4, there are at least three marker loci namely Gc (4q12), the FGA microsatellites (4q26-28) and the MNSs blood system. We performed linkage analyses in order to link the still existing gap in the homology of the ABL and MTP loci in the healthy and diseased members of the family. The autosomal microsatellite FGA was analyzed using the same primer sequences and PCR conditions described by Mills et al.[15,16] The alleles were visualized on acrylamide-bisacrylamide denaturating gels in a solution of 5% ethidium bromide.

The chromosome preparations were treated according to GTG-banding protocol. Briefly, the slides were aged for 1 hour at 90 degrees celcius and treated in trypsin solution (0.05%) for 1-2 minutes. They were then stained in 4% Giemsa solution for 10 minutes. The G-banded chromosome preparations were analyzed under microscope. Forty metaphase cells were examined for each subject.

Table 1. Clinical details of the patients studied.

Parameter	Patient 1	Patient 2	Patient 3
Age	33	31	19
Sex	female	male	male
Age of onset of symptoms	infancy	infancy	infancy
Age at diagnosis	25	23	13
Follow-up (years)	17	09	06
Symptoms			
Fatigue	+++	+	-
Gait & balance disturbance	+++	++	+
Muscle atrophy - areflexia	+++	+	+
Dysarthrosis - nystagmus	+++	+	+
Laboratory findings			
Apolipoprotein B	20	20	20
Total lipids	135	204	147
Cholesterol	46	65	43
Triglycerides	0	0	0
Peripheral acanthocytes	+	+	+
Pigmental retinopathy	+	+	+
Outcome			
Alive	+	+	+

(Normal values: Apolipoprotein B: 70-160 mg%, Total lipids:500-700 mg%, Cholesterol: 220 mg%, Triglycerides: 60-165 mg%)

RESULTS

The fibrinogen alpha (at 4 q 28) marker showed absolute correlation in all three patients by inheritance of the same maternal and paternal FGA alleles, whereas the healthy siblings had other allelic constellations (Figure 1). The Gc and MNSs genes were not informative.

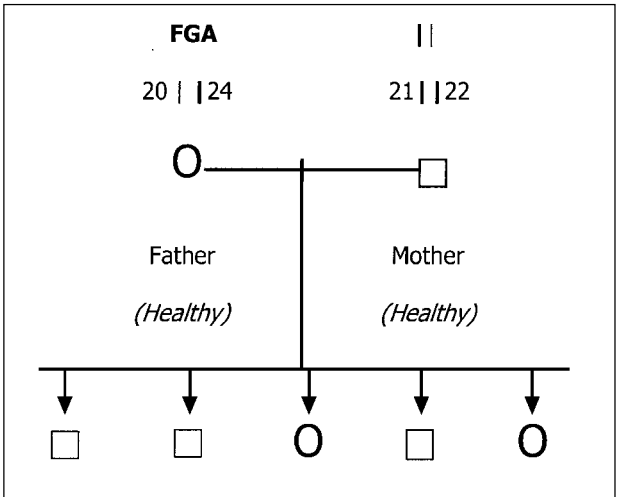
Cytogenetic analysis revealed normal karyotype in all patients. Neither clonal or sporadic chromosome abnormalities were detected, except for a small fragment-like acentric structure observed in only one cell in the case of one male patient (brother).

DISCUSSION

Abetalipoproteinemia is caused by the homozygous state of a recessive mutation in the gene encoding the 97-kDa subunit of the Microsomal triglyceride Transfer Protein (MTP). MTP is a dimeric lipid transfer protein consisting of protein disulfide isomerase and a unique 97-kDa subunit. In vitro MTP accelerates the transport of triglyceride, cholesteryl ester, and phospholipid between membrane.[17] MTP is required to produce triglyceride rich droplets in the smooth endoplasmic reticulum, which may supply the core lipids for conversion of nascent, dense apoB-48 particles to mature VLDL.

The gene symbol for αbetalipoproteinemia is ABL.[13] The rare defect allele is recessively inherited; some additional lipoprotein characteristics led to the suggestion that mutations of the MTP gene are the predominant cause of hereditary ABL. In fact eight apparently unrelated ABL patients showed the same MTP gene defect.[13]

In the present study the FGA marker showed absolute correlation in all three patients, thus confirming the



Subject	FGA
Son (patient)	21 24
Son (healthy)	22 24
Daughter (patient)	21 24
Son (patient)	21 24
Daughter (healthy)	20 22
Mother (healthy)	20 24
Father (healthy)	21 22

Figure 1. Fibrinogen alpha expression in the healthy and diseased members of the family with abetalipoproteinemia.

initial hypothesis that MTP and ABL loci are in fact identical and must be described under one designation. This finding becomes more interesting as all patients in this family showed inheritance of HLA B18. Indeed, the analysis of the HLA pattern showed that the two patients with the most severe disease were homozygous for HLA antigen B18, whereas the third patient with milder symptoms had no alleles for B18. On the other hand, the chromosomal study revealed normal karyotype in all members of the family. Only in the case of one patient (brother) an aberrant metaphase was detected. This cell bore small, fragment-like acentric chromosomal material that seemed to be an extrachromosomal structure rather than a fragment belonging to a delete chromosome. The abnormality cannot be characterized as clonal in the present study, since it was detected in only one cell. However, its presence in the karyotype could be of importance in the clinical/pathological picture, especially if it were detected as a clonal aberration in at least two cells.

Only a limited small number of families have screened for mutations in the MTP gene. Yang et al[18] found that abetalipoproteinemia was inherited as a homozygous intron 9 splice acceptor G(-1)-to-A mutation of the transfer protein gene. The authors analyzed chromosome 4, including MTP gene (4q22-24), using short tandem repeat markers. The proband has only his mother's genes in chromosome 4q spanning a 150-centimogran region ie, segmental maternal isodisomy 4q21-35, due to mitotic recombination. Maternal isodisomy (maternal UPD 4q) was the basis for homozygosity of the MTP gene mutation in the above-mentioned patient.

Wang and Hegele[19] sequenced the MTP gene in six Canadian subjects with abetalipoproteinemia, of whom four were found to be single homozygotes and two were found to be compound heterozygotes for MTP gene mutations. Of the 8 MTP gene mutations identified, 6 had not previously been described, including two new nonsense mutations, two missense mutations, one new frameshift mutation and one new splice donor site mutation.

Ohashi et al[20] screened four unrelated patients with abetalipoproteinemia looking for mutations in MTP gene and were able to identify three novel mutations: a frameshift mutation caused by a single adenine deletion at position 1389 of the cDNA, a missense mutation, Asn780Tyr, (each in homozygous forms), and a splice site mutation, 2218-2A→G, in a compound heterozygous form.

Our observation and the published knowledge that the expression of MTP with ApoB in Hela and Cos-1 cells is sufficient to mediate the secretion of triglyceride-rich lipoproteins[12] suggest that mutations of the MTP gene, on chromosome 4q22-24, are the principal cause of classical recessive inherited ABL. It seems to present a new target for research in order to understand the heterogeneity in clinical appearance among the patients as well as to improve our efforts at treatment through investigation of the pathophysiology of this rare disease.

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