Original article

Cytogenetic studies and H.L.A. pattern in Greek patients with A,B-Lipoproteinemia

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SUMMARY

A,β-lipoproteinemia is an extremely rare genetic disorder of lipoprotein metabolism, associated with various biochemical abnormalities combined with clinical malabsorption. The aim of this study was to provide information concerning the cytogenetic and HLA patterns of three patients with A,β-lipoproteinemia, as well as to see if there is any correlation between specific HLA type and clinical course of the disease. The study included seven persons. There were 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). Cytogenetic studies were performed on PHA stimulated peripheral blood cell cultures of the three patients. Forty metaphase cells were analyzed for each one of them. The HLA typing was carried-out using the standard NIH microlymphotoxicity assay in all seven members of the family. B-cells were separated by the use of immunumagnetic beads CD19 coated. The karyotypic study resulted in normal picture. No sporadic chromosome abnormalities were observed except of a small fragment-like acentric structure seen in only one cell in the case of one male patient (brother). The HLA pattern in the two most severely affected patients was identical (A2, 24, B18 (Bw6) Cw15, w7. DR8, 11, DQ4, 7). However, the only detectable difference between severely ill patients and the other members of the family was homozygosity for the HLA-B18. The HLA pattern of the third patient was A2, A24/B35, B44/Bw4, Bw6/Cw4, Cw7/DR11, DR14, DR52/DQ5, DQ18. This patient had no alleles for HLA B18. It is concluded that the karyotype of patients with A,β-lipoproteinemia is normal. Despite the identical pattern seen in the two most severely affected patients, no association could be found between any particular HLA type and this rare genetic disorder.

Key words: Immunogenetics, A,β-lipoproteinemia, HLA, Karyotype, Genetic disorders

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INTRODUCTION

A,β-lipoproteinemia 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 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 Transfer Protein (MTP) that result in an absence of MTP function.[12] Defects in the MTP genes have been shown to cause A,β-lipoproteinemia and are the predominant cause of hereditary A,β-lipoproteinemia.[13]

The aim of this study was to provide information concerning Cytogenetic and Human Leucocyte Antigen (HLA) patterns in all three patients, members of a Greek family with A,β-lipoproteinemia diagnosed and followed-up by us during the last 7 years. Another aim of the study was to see if there is any correlation between specific HLA abnormality and clinical course of the disease.

PATIENTS 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 respectively, as well as the other two healthy brothers and the two healthy parents (total number of subjects: seven). Details of the clinical course, outcome and therapeutic strategies applied on these patients, have been previously published.[1,14]

Cell cultures

Heparinized peripheral blood was obtained from the three patients. Peripheral blood mononuclear cells (PBMC) were separated according to the ficoll centrifugation technique. The PBMC were suspended in McCoy's 5A medium supplemented with 10% heat inactivated fetal calf serum (FCS), glutamine (2mM) and antibiotics (penicilline/streptomycin solution 100U/100 μg/ml). The mitogen phytohaemagglutinine (PHA) was added at a concentration of 5μg/ml to induce proliferation and the cell cultures were maintained at 37o C in CO2 incubator for 48 hours. The cells were cultured in duplicate and the cell density was 1X106 PBMC per ml of complete medium. Two to three hours prior to cell harvesting, colcemide was added at a concentration of 0.2 μg/ml.[15] After the end of incubation period, the cells were treated with hypotonic solution (KCl 0.075 mM) and fixed in methanol/acetic acid (3:1). Next, they were dropped on wet slides and air-dried.

Cytogenic analysis

The chromosome preparations were treated according to GTG-banding protocol.[16] Briefly, the slides were aged for 1 hour at 90o C and treated in trypsin solution (0.05%) for 1-2 minutes. After, they were stained in 4% Giemsa solution for 10 minutes. The G-banded chromosome preparations were analyzed under microscope. Forty metaphase cells were examined for each patient.

HLA typing

HLA typing was carried out by the standard NIH microlymphotoxicity assay in all seven members of the family. B-cells were separated by immunomagnetic beads CD19 coated.

RESULTS

Cytogenic analysis revealed normal karyotypes in the three patients. Neither clonal or sporadic chromosome abnormalities were detected, except of a small fragment-like acentric structure observed in only one cell in the case of one male patient (brother) (Figure 1).

The HLA patterns of the 3 patients and the 4 healthy members of the family are shown in Table 1. As it can be seen the HLA pattern in the two most severely affected patients was identical. However, the only detectable difference between severely ill patients and the other members of the family was homozygosity for HLA -B18. The HLA pattern of the third patient is shown in table 1. As it is obvious this patient had no alleles for HLA B18.

DISCUSSION

Although A,β, lipoproteinemia is caused by the homozygous state of a recessive mutation, we looked for an influence of chromosomal or HLA abnormality on this rare condition bearing in mind that the disease exhibits a rather different clinical behavior from patient to patient. On the other hand it is well known that a quite large number of gastroenterological disorders are considered to be the result of genetic abnormalities, although different from that observed in a,β, lipoproteinemia. So, a review of documented cases demonstrated a significant association of Turner's syndrome with Ulcerative colitis and Crohn's disease due to an abnormal X chromosome.[17] Other cases include Trisomy 918 and myelodysplastic syndromes[19] associated with Crohn's disease. A very recent report links Mucosa-associated lymphoid tissue (MALT) lymphoma of the rectum with chromosomal translocation of the t (11;18) (q21;q22) and an additional aberration of trisomy 3.20 The t (11;18) (q21;q-21) has been reported to be a frequent and specific aberration in MALT lymphomas of the stomach, lung, thyroid and parotic gland and breast. Trisomy 3 and no rearrangement of the bcl-2 gene have also been indicated. Many other congenital abnormalities of gastrointestinal

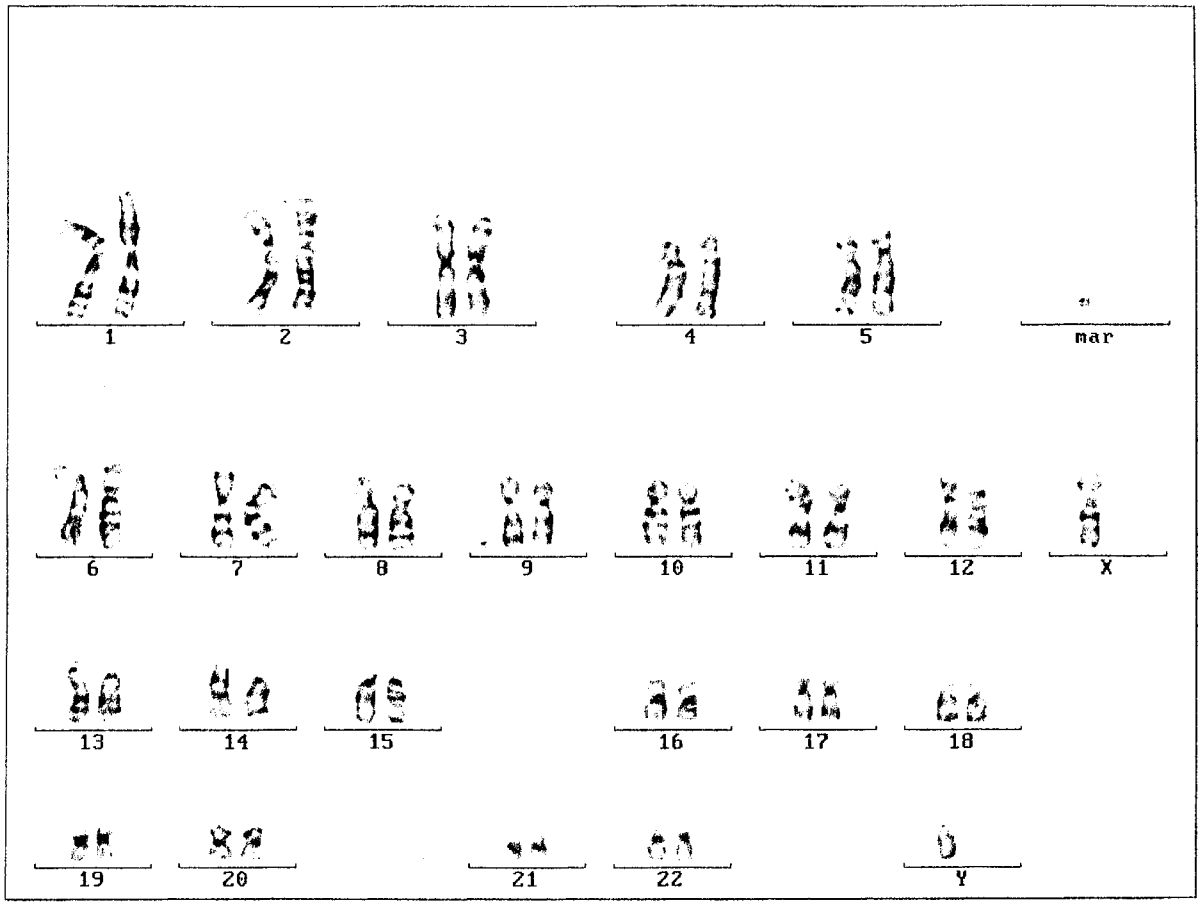

Figure 1. Karyotypic picture of one male patient showing a small fragment-like acentric structure.

Table 1. HLA antigens in the diseased and healthy members of the family with A,β-lipo-proteinemia

Father (healthy)	Mother (healthy)
A24 B18 Cw5 DR8 DQ4	A2 B18 Cw7 DR11 DQ7
Ax(24) B44 Cw7 DR11 DQ7	Ax(2) B35 Cw4 DR14 DQ5
Son (ill)	Son (Healthy)
A24 B44 Cw7 DR11 DQ7	
A2 B35 Cw4 DR14 DQ5	
Daughter (healthy)	Daughter (ill) / Son (ill)
A24 B44 CW7 DR11 DQ7	A24 B18 Cw5 DR8 DQ4
A2 B18 Cw7 DRx(11) DQx(7)	A2 Bx(18) Cw7 DR11 DQ7

tract have been linked with chromosomal abnormalities including congenital bilateral agenesis of diaphragm[21] and anorectal malformations.[22] Moreover, the association of some caryotypic abnormalities with a certain number of gastrointestinal malignancies including hepatoblastomas,[23] gastric and colorectal cancer[24,25] and hereditary non-popyposis colorectal cancer[26] seems to be well established.

So far, there is no information in the international literature concerning the HLA and karyotypic pattern of patients with A,β-lipoproteinemia. The chromosome study in our patients with A,β-lipoproteinemia resulted in normal karyotype in all subjects. Only in the case of one patient (brother), an aberrant metaphase was detected. This cell was bearing a small, fragment-like acentric chromosomal material that seemed to be an extra

chromosomal structure rather, than a fragment belonging to a deleted 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 of this rare genetic disease, especially if it could be detected as a clonal aberration (found in at least two cells). More cases required to be examined in order to investigate the presence of any chromosomal alteration within this disorder.

The analysis of the HLA pattern of the seven members of the family showed that the two patients with the most severe disease were homozygous for HLA antigen B18, whereas the third patient in whom the disease was running with milder symptoms, had no alleles for B18. No association could be demonstrated however, between any particular HLA type and this disorder.

From the results of the present study therefore it seems certain that there is no any abnormality of HLA or karyotypic type in patients with a,β-lipoproteinemia. Despite the identical type of HLA pattern seen in the most severely affected patients, no association could be found between any particular HLA type and clinical course of this very rare genetic disorder.

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