Association of serum lipids and apolipoprotein E gene polymorphism with the risk of colorectal adenomas

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ABSTRACT

Objective: To investigate the relationship of serum lipids and apolipoprotein (apoE) gene polymorphism to colorectal adenomas.

Methods: This study took place in the Department of Gastroenterology, Renmin Hospital of Wuhan University, PR China from June 2003 to March 2005. Ninety-eight patients with colorectal adenomas and 40 healthy subjects were enrolled, and their serum levels of triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were determined. The apoE gene polymorphism was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: Serum TC levels of colorectal adenomas group (5.32 ± 0.85 mmol/L), distal colorectal adenomas group (5.58 ± 0.63 mmol/L), and villous adenoma group (5.49 ± 0.69 mmol/L) were higher than the control group (4.28 ± 0.62 mmol/L, p=0.016), proximal colorectal adenomas group (4.82 ± 0.58 mmol/L, p=0.038) and non-villous adenoma group (4.76 ± 0.58 mmol/L, p=0.03). Serum HDL-C levels of colorectal adenomas group (1.39 ± 0.25 mmol/L) were lower than the control group (1.51 ± 0.29 mmol/L) (p=0.035). Serum lipids levels of each genotype in colorectal adenomas group were not statistically significant. Apolipoprotein ε3/ε4 genotypic frequency in colorectal adenomas group (7.1%) was lower than the control group (17.5%) (p=0.012).

Conclusions: The findings suggest that altered lipid metabolism may be differentially associated with colorectal adenomas and the persons with apoE ε3/ε4 genotype have lower risk suffering from colorectal adenomas than those with other genotypes.

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Epidemiological data indicate that there is a close relation of high fat diet and obesity to the raising incidence rate of colorectal tumor.[1,2] And there are some relations of serum lipids level to colorectal adenomas, too.[3] Apolipoprotein E (apoE) gene is polymorphic, consisting of 3 common isoforms (E2, E3, and E4) encoded by 3 alleles (ε2, ε3, and ε4) in exon 4 of apoE.[4] Lipoproteins that have different apoE isomer have a close relation with variation of serum lipids level.[5] At present there are some researchers who study the relationship of serum lipids and apoE gene polymorphism to colorectal adenomas. But, as the studied race is different, the results have some difference.[6] This study is to examine the relationship of serum lipids and apoE gene polymorphism to colorectal adenomas for Chinese people through the detection of serum lipids metabolism and apoE gene, which can provide the new data for the prevention and cure of colorectal adenomas.

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Methods. ***Objects.*** This study was carried out from June 2003 to March 2005 at the Department of Gastroenterology, Renmin Hospital of Wuhan University, PR China. A total of 138 individuals, non-related were studied. Among them, 84 (60.9%) were males and 54 (39.1%) were females. The individuals were separated into colorectal adenomas group, consisting of 98 patients (average age 58.2 ± 7.5 years) who was diagnosed as colorectal adenomas by colonoscopy (all were confirmed with pathology) and control group, consisting of healthy individuals (average age 55.2 ± 8.1 years). The groups were matched by age and gender. The histological categories of colorectal adenomas are tubular adenoma, tubulovillous adenoma that also is named as mixed adenoma and villous adenoma. Patients that had a history of colon operation, colon malignant tumor, hyperlipoidemia and use of drugs affecting serum lipids were rejected. All samples were Chinese people. Distal colon was from anus to flexura lienalis coli. Proximal colon was from flexura lienalis coli to blindgut. Fifty-one patients were distal colorectal adenomas, 42 patients were proximal colorectal adenomas and 5 patients were distal and proximal colorectal adenomas. Patients having both distal and proximal adenomas were excluded when the relation of serum lipids with the location of adenoma was analyzed. The study was approved by the Hospital Ethics Committee.

Detection of apoE gene polymorphism. Patients ate vegetable diet for 3 days before colonoscopy. Fasting plasma samples were collected in 10 ml EDTA-coated vacutainer tubes. Plasma was separated and preserved in -20^{O}C to detect lipids. Genome DNA was abstracted with NaI method to detect apoE gene polymorphism. The apoE genotype was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The DNA was amplified by PCR using oligonucleotide primers; the upstream primer was 23-mer dTCGCGGGCCCCG GCCTGGTACAC, and the downstream primer was 23-mer dACGCGGGCACGGCTGTCCAAGGA. The PCR product was digested with the restriction enzyme HhaI. Each reaction mixture was loaded onto an 8% polyacrylamide gel.[7] At the end, result was observed with viltalight lamp. The apoE PCR production was 299bp segment. After it was digested by restriction enzyme, 6 kinds of segments appeared. They represented 6 genotypes: ε2/ε2 was 91, 83 and 61bp; ε2/ε3 was 91, 83, 61 and 48bp; ε2/ε4 was 91, 83, 72, 61 and 48bp; ε3/ε3 was 91, 61 and 48bp; ε3/ε4 was 91, 72, 61 and 48bp; ε4/ε4 was 72, 61 and 48bp.

Assay of serum lipids. Total cholesterol (TC) and triacylglycerol (TG) in serum was assayed by enzymatic procedures; high-density lipoprotein cholesterol (HDL-C) was measured by analyzing the supernatant fluid obtained after precipitation of a plasma aliquot with dextran sulphate and Mg^{2+}; low-density lipoprotein cholesterol (LDL-C) was measured with homogeneous methods. All the measurement of biochemical data was carried out on one special machine by one people, and the quality control was strictly obeyed.

Statistical analysis. All data were collected and input into computer and analyzed with SPSS 11.0. Gene frequency was calculated with gene notation. The comparison for allele frequency was carried out with chi-square test. The comparison for mean of serum lipids was carried out with t-test.

Results. ***Assay of serum lipids.*** **Table 1** showed that serum TC of colorectal adenomas group was higher than control group but serum HDL-C of colorectal adenomas group was lower than control group. These differences had statistical significance. Total cholesterol of distal colorectal adenomas group and villous adenoma group was higher than proximal colorectal adenomas group and non-villous adenoma group.

Assay of apoE gene. There were total 5 kinds of apoE genotype in our study: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, and ε3/ε4. There was no ε4/ε4 (**Figure 1**). The apoE gene of colorectal adenomas group and control group was showed in **Table 2**. The apoEε3/ε4 of colorectal adenomas group was lower than the control group. The apoEε2 allele includes 3 kinds of genotype: ε2/ε2, ε2/ε3 and ε2/ε4. The apoEε3 allele includes one kind of genotype: ε3/ε3. The apoEε4 allele includes 2 kinds of genotype: ε3/ε4 and ε4/ε4. In theses genotypes, ε2/ε3, ε3/ε3 and ε3/ε4 can be found most frequently. Adenomas patients were divided into 3 groups: ε2/ε3, ε3/ε3, and ε3/ε4 3 groups. The difference of serum lipids of these groups had no statistical significance (p>0.05, showed in **Table 3**). The average age and gender of colorectal adenomas group and control group had no statistical difference. The apoE genotypic frequency in gender had no statistical difference too.

Discussion. Many studies indicate that there is a certain relation between the raise of serum lipids and colorectal adenomas and difference constituent in serum lipids has different relationship to colorectal adenomas. Park et al[3] find that high triglyceride and hypercholesteremia has positive correlation to occurrence of colorectal adenomas after they analyze serum lipids of 134 colorectal adenomas patients. Shinomiya et al[6] prove that the raise of serum lipids

Table 1 - Comparison of serum TC, TG, HDL-C and LDL-C from healthy individuals and patients with colorectal adenomas.

Levels of serum lipid (mmol/L)	Control group (n=40)	Colorectal adenomas group (n=98)	Proximal colorectal adenomas group (n=51)	Distal colorectal adenomas group (n=42)	Mixed+tubular adenoma group (n=46)	Villous adenoma group (n=52)
TC	4.28 ± 0.62	5.32 ± 0.85*	4.82 ± 0.58‡	5.58 ± 0.63	4.76 ± 0.58§	5.49 ± 0.69
TG	1.08 ± 0.31	1.15 ± 0.32	1.15 ± 0.30	1.16 ± 0.33	1.16 ± 0.35	1.14 ± 0.30
HDL-C	1.51 ± 0.29	1.39 ± 0.25†	1.40 ± 0.23	1.39 ± 0.24	1.38 ± 0.25	1.39 ± 0.26
LDL-C	2.56 ± 0.30	2.55 ± 0.30	2.53 ± 0.25	2.57 ± 0.35	2.54 ± 0.29	2.56 ± 0.31

Data presented as mean ± SD, *p=0.016, †p=0.035 colorectal adenomas group vs control group; ‡p=0.038 proximal colorectal adenomas vs distal colorectal adenomas group; §p=0.03 mixed+tubular adenoma group vs villous adenoma group, TC - total cholesterol, TG - triglyceride, HDL-C - high density lipoprotein-cholesterol, LDL-C - low density lipoprotein -cholesterol

Table 2 - Apoliprotein E gene distribution of colorectal adenomas group and control group. [n (%)]

Groups	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
Control (n=40)	0 (0)	4 (10)	1 (2.5)	28 (70)	7 (17.5)	0 (0)
Colorectal adenomas (n=98)	1 (1)	11 (11.2)	4 (4.1)	75 (76.5)	7 (7.1)*	0 (0)

Table 3 - Serum lipids for different genotypes in colorectal adenomas groups.

ApoE genotypes	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
ε2/ε3 (n=11)	5.36 ± 0.78	1.17 ± 0.29	1.40 ± 0.19	2.57 ± 0.26
ε3/ε3 (n=75)	5.41 ± 0.96	1.15 ± 0.36	1.39 ± 0.31	2.55 ± 0.36
ε3/ε4 (n=7)	5.47 ± 0.83	1.15 ± 0.30	1.38 ± 0.25	2.55 ± 0.29

ApoE - Apoliprotein E, TC - total cholesterol, TG - triglyceride, HDL-C - high density lipoprotein-cholesterol, LDL-C - low density lipoprotein -cholesterol

has a certain relation to occurrence of distal colorectal adenomas but, has no relation to occurrence of proximal colorectal adenomas. Houghton et al[8] study shows that rise of serum cholesterol level has nonlinear positive correlation to augment of villiform tissue of colorectal adenomas. Augment of villiform tissue of colorectal adenomas hints that canceration degree of colorectal adenomas increases. This study manifests that serum cholesterol of colorectal adenomas group was higher than control group (p=0.016) and serum HDL-C of colorectal adenomas group was lower than control group (p=0.035); TC of distal colorectal adenomas group and villous adenoma group was higher than proximal colorectal adenomas group (p=0.038) and non-villous adenoma group (p=0.03). This result is partly coincident with the results of foreign researcher. The identical point is the consistency of cholesterol's variance, which suggests that augment of serum cholesterol in our territory is a risk for the incidence of colorectal adenomas and raise of serum HDL-C is a protecting factor for the decreasing incidence of colorectal adenomas. The relation of other serum lipids level and colorectal adenomas is not obvious. The apoE gene is located on chromosome 19. Genetic polymorphism of apoE is mainly controlled by 3 allelomorphic genes (ε2, ε3 and ε4) that are on the same gene locus, which encodes 3 isomers in plasma and constitutes 6 genotypes: 3 homozygotes (ε2/ε2, ε3/ε3 and ε4/ε4) and 3 heterozygotes (ε2/ε3, ε2/ε4 and ε3/ε4).[9] The apoEε2 includes ε2/ε2, ε2/ε3, and ε2/ε4. The apoEε3 includes ε3/ε3. The ApoEε4 includes 2 genotypes: ε3/ε4 and ε4/ε4. The distribution of apoE

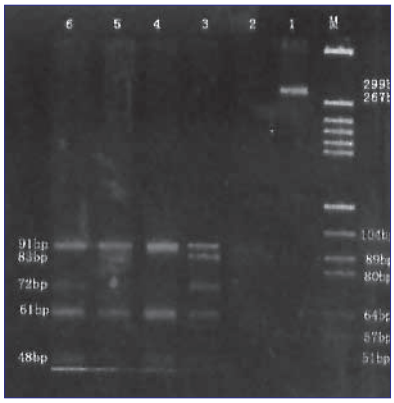

Figure 1 - Apolipoprotein E (ApoE) polymerase chain reaction (PCR) product and restriction fragment length polymorphism pattern of the PCR products digested with HhaI. M: DNA markers; Lane 1: apoE PCR product ; Lane 2: apoEε2/ε2; Lane 3: apoEε2/ε4; Lane 4: apoEε3/ε3; Lane 5: apoEε2/ε3; Lane 6: apoEε3/ε4.

gene frequency in normal population has obviously common character. The apoE gene frequencies of oriental are more consonant: the most phenotype is ε3/ε3 that is more than 50%.[10] Genotype distribution of ε2/ε2, ε4/ε4 and ε2/ε4 is lower. Our data display 5 genotypes ε2/ε4, ε3/ε4, ε3/ε3, ε2/ε2, and ε2/ε3. There is no ε4/ε4. The apoE, one of important ingredients of plasma lipoprotein, directly participates the composition, transformation and metabolism of lipoprotein. The apoE is an important role in the metabolism of cholesterol. The study of individual that has allelomorphic gene ε4 shows that absorption of cholesterol in intestinal tract raises[11] and metabolic of bile acid deoxycholic acid decreases.[12] Secondary bile acid such as deoxycholic acid can induce proliferation of colorectal adenomas cells through the way of activation for α-PKC and P42/44MAP (mitogen-activated protein) kinase, which leads to canceration of colorectal adenomas.[13] Kervinen et al[14] find that disease incidence of proximal colorectal adenomas and colon carcinoma for individual carrying apoEε4 obviously decreases. Our data show that the relation of apoE allelomorphic gene and colorectal adenomas maybe relates to allelomorphic gene ε4. The apoE ε3/ε4 frequency of occurrence for colorectal adenomas group is obviously lower than control group. But, the difference of blood lipids for different genotypes in colorectal adenomas groups has no statistical significance. As the genotype of the main apoE ε4 is apoE ε3/ε4, cholesterol decreases in intestinal tract for apoE ε4 population for increase of absorption. Metabolic of bile acid deoxycholic acid in intestinal tract decreases too for increase of excretion. The apoE gene polymorphism interferes in generation of colorectal adenomas through the affection of cholesterol's absorption and metabolism. The lower frequency of the apoE ε3/ε4 genotype confirmed its role as a risk factor associated with colorectal adenomas.

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