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Association of fatty acid-binding protein 2 and fat mass and obesity-associated gene polymorphism with primary open-angle glaucoma

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Abstract:

PURPOSE: The present study was carried out to investigate the association of fatty acid-binding protein 2 (*FABP2*) and fat mass and obesity-associated (*FTO*) gene polymorphism with primary open-angle glaucoma (POAG) cases and controls.

MATERIALS AND METHODS: This study includes 122 POAG cases and 112 controls. *FABP2* and *FTO* gene polymorphisms in cases and controls were evaluated by polymerase chain reaction-restriction fragment length polymorphism method.

RESULTS: The mean ages were 49.88 ± 12.34 and 53.74 ± 11.87 years in POAG cases and control groups, respectively. The *FABP2* gene AA, AT, TT genotype frequencies were 12.90%, 62.40%, 24.80% in POAG cases and 20.60%, 64.70%, 14.70% in healthy controls, respectively. The frequencies of A and T allele in POAG cases were 44.06% and 55.94% as compared to 52.94% and 47.06% in the controls. The *FTO* gene AA, AT, TT genotype frequencies were 2.00%, 79.20%, 18.80% in cases and 0%, 75.50%, 24.50% in healthy controls, respectively. The frequencies of A and T allele in POAG cases were 41.58% and 58.42% as compared to 37.75% and 62.25% in the controls. No significant difference in the frequencies of *FABP2* and *FTO* genotype was found between POAG cases and controls.

CONCLUSION: We could not identify the possible association of *FABP2* and *FTO* gene polymorphism with POAG; however, further studies with larger sample size in different population are required to clarify the role of *FABP2* and *FTO* genes in susceptibility to POAG.

Keywords:

Fat mass and obesity associated, fatty acid-binding protein 2, genetic polymorphism, primary open-angle glaucoma

Introduction

Primary open-angle glaucoma (POAG) is a progressive, chronic optic neuropathy in adults, and it is characterized by open anterior chamber angles, visual field abnormalities, and high intraocular pressure (IOP). Glaucoma, affecting at least 90 million people worldwide, is a leading cause of blindness, second only to cataract. It has been estimated that by 2010, almost 60.5 million people will have POAG

worldwide, and by 2020, this number is expected to increase to 79.6 million, which will result in blindness in 11.2 million people by 2020.^[1] Previous studies suggest that the genetic factors may play important roles in the pathogenesis of this disease.^[2,3] Linkage analyses have established three POAG susceptibility genes: myocilin, optineurin, and WD repeat domain 36.^[4-6] Association studies using single nucleotide polymorphisms (SNPs) have substantially contributed to the study of glaucoma over the past few decades; however, the link

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between the genes and the development of the disease is not always clearly demonstrated.

Fatty acid-binding proteins (*FABPs*) are small intracellular polypeptides expressed in the absorptive simple columnar epithelial cells of the intestine (enterocytes).^[7] The *FABP2* gene encodes intestinal *FABP* protein, involved in fatty acid transfer and metabolism.^[8] The gene consists of 3382 nucleotides located in the chromosomal region 4q28–4q31, arranged in four exons containing ~700 bp and three introns containing ~2650 bp.^[7,9] Earlier studies have shown that the Ala54Thr polymorphism of the *FABP2* gene can be commonly found in around 30% of the most populations and is significantly associated with insulin resistance,^[10] dyslipidemia, and obesity.^[11] Genome-wide association (GWA) studies have indicated that the fat mass and obesity-associated (*FTO*) gene has an important genetic effect on body mass index (BMI) and risk of obesity. Obesity has been postulated to exert an effect on IOP by causing excessive intraorbital adipose tissue, increased blood viscosity, increased episcleral venous pressure, and impairment of aqueous outflow facility. Previous studies have shown that overweight and obesity are independent risk factors for increase in IOP.^[12-15] Raised IOP is an important risk factor for the progression to POAG. In cases of POAG as a matter of observation, it can be inferred that the individual ones to intermittently affect with obesity might be susceptible to such conditions. Hence, the present study was carried out to investigate the association of *FABP2* and *FTO* gene polymorphism with POAG cases and controls.

Materials and Methods

Patient's selection

A total of 122 blood samples of POAG cases and 112 healthy controls were collected from the Department of ophthalmology of Era's Lucknow Medical College and Hospital, Lucknow. Data collection was done for each patient on clinical variables including age, alcohol consumption, BMI, height, weight, cigarette smoking, and family history. Each individual underwent a complete ophthalmological examination. Patients with POAG were defined by the presence of an open angle, pathological cupping of the optic disc, a glaucoma hemifield test (GHT) outside normal limits with reproducible visual field defects at the same location on two consecutive visits, and an IOP >21 mmHg without antiglaucoma drugs. Cup-to-disc ratios were between 0.4 and 0.9. Patients with a history of eye surgery before the diagnosis of glaucoma, evidence of secondary glaucoma such as exfoliation, pigment dispersion or uveitis, and other causes were excluded from the study. Control group was nonsmokers and had neither diabetes nor any systemic illness. They had no family or personal

history of glaucoma. They had clinical healthy appearing optic discs as demonstrated by indirect ophthalmoscope with a cup-to-disc ratio of 0.3 or lower and GHT within normal limits. Ethical Committee's clearances were obtained from the respective departments, earlier to the recruitment of individuals in this study.

DNA extraction

Five milliliters of peripheral blood was collected from all the individuals in 0.5M EDTA tubes. Genomic DNA was isolated from whole blood using the standard phenol-chloroform extraction method.^[16] The DNA concentration was determined by spectrophotometer and stored at -20°C.

Analysis of polymorphisms

Fatty acid-binding protein 2 polymorphism

Polymerase chain reaction (PCR) was employed for genotyping of the *FABP2* gene polymorphism. Reactions were performed with 10 pmol of each primer:^[17] forward primers 5'-ACAGGTGTTAATATAGTGAAAAG-3' and reverse primer 5'-TACCCTGAGTTCAGTTCGTC-3'. In the final volume of 20 µl containing 0.3 U of Taq DNA polymerase, 10 mmol/l Tris-HCl pH 8.3, 50 mmol/l of KCl, 1.5 mmol/l of MgCl₂, and 100 mmol/l of dNTPs, PCR amplification was carried out under the conditions: 35 cycles for 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C; the PCR products were analyzed on 2% agarose gel stained with ethidium bromide to certify the proper amplification. The amplified PCR products of 180 bp were digested with the addition of 2 U HhaI (New England Biolabs, Hitchin, UK), 10 mmol/l Tris-HCl pH 7.9, 50 mmol/l NaCl, 10 mmol/l MgCl₂, and 1 mmol/l dithiothreitol. After incubation at 37°C for 2 h, digested samples were separated on 10% ethidium bromide-stained polyacrylamide gel electrophoresis and visualized by UVP BIOLMAGING gel doc system. PCR products having an intact HhaI site were cleaved into 99- and 81-bp fragments; the Ala54Thr substitution abolished the restriction site [Figure 1].

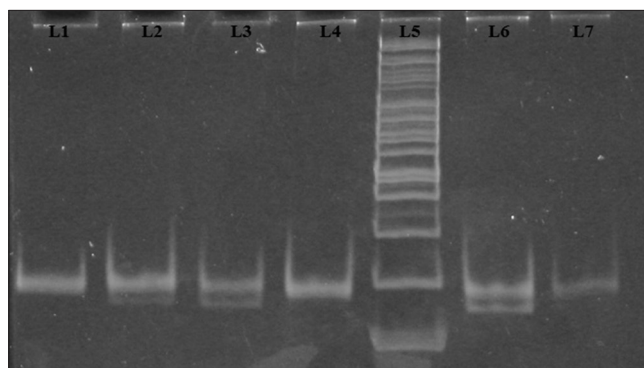


Figure 1: Polyacrylamide gel picture showing digested polymerase chain reaction products for fatty acid-binding protein 2 gene polymorphism. Lane 1: Undigested polymerase chain reaction product of fatty acid-binding protein 2 (180 bp), lane 5-7: TT genotype, lane 2 and 4: AT genotype, lane 3: 100 bp ladder

Fat mass and obesity-associated polymorphism

The *FTO* SNP (rs9939609) was genotyped by PCR (MJ Mini Thermo Cycler BioRad, USA) and restriction fragment length polymorphism analysis. Genomic DNA (20 ng) was incubated in a 10- μ l solution containing 1X NH4 buffer, 2.5 mmol/l magnesium, 200 μ mol/l each dNTP, 20 pmol forward (5'-AACTGGCTCTTGAATGAAATAGGAT TCAGA-3') and reverse (5'-AGAGTAACAGAGA CTATCCAAGTG CAGTAC-3') oligonucleotide primers,^[18] and 0.5 U Taq DNA polymerase (Bioline Ltd., London, UK). The PCR mix was incubated at 94°C for 5 min followed by 20 cycles of 94°C for 45 s, 61°C for 45 s (dropping 0.5 C per cycle), and 72°C for 45 s. After this, the PCR mix was incubated for 15 cycles of 94°C for 45 s, 51°C for 45 s, and 72°C for 45 s, followed by the final incubation at 72°C for 10 min. The PCR products thus obtained were incubated at 37°C for 16 h with 2 U *ScaI* (New England Biolabs, Hitchin, UK). Upon running the final products on a 3% agarose gel, the T allele produced a 182-bp band and the A allele produced 154- and 28-bp bands [Figure 2].

Statistical analysis

All the figures are presented as means \pm standard deviation. The genotyping data were compared between cases and controls using Chi-square test. Other variables were compared using Student's *t*-test for normally distributed variables. All statistical tests were performed using Statistical Package for the Social Sciences version 12 software (IBM, USA).

Results

Our study included 122 POAG cases (65 were males and 57 were females) and 112 controls (59 were males and 53 were females). The mean ages were 49.88 ± 12.34 and 53.74 ± 11.87 years in POAG cases and control groups, respectively. Clinical and biochemical parameters of cases and controls are shown in Table 1. In our population, the

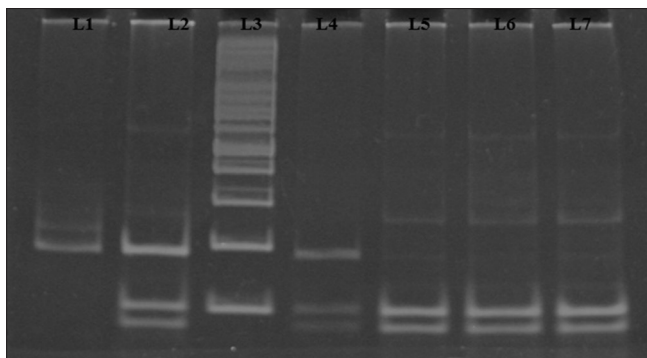


Figure 2: Polyacrylamide gel picture showing digested polymerase chain reaction products for fat mass and obesity-associated gene polymorphism. Lane 2, 3, and 6 show AT genotype (182 and 154), lane 4 and 7 show TT genotype (182 bp), lane 5 shows 100 bp ladder, and lane 1 shows undigested polymerase chain reaction product of *FTO* (182 bp)

mean red blood cell (RBC) lysate glutathione (GSH) levels were significantly lower in cases as compare to the controls ($P < 0.05$). *FABP2* gene AA, AT, TT genotype frequencies were 12.90%, 62.40%, 24.80% in POAG cases and 20.60%, 64.70%, 14.70% in healthy controls, respectively. Odds ratio (OR) for AA was 0.569 (95% confidence interval [CI] 0.27–1.21, $P = 0.141$, $\chi^2 = 2.17$, power = 0.875), for AT 0.904 (95% CI 0.51–1.60, $P = 0.73$, $\chi^2 = 0.12$, power = 0.644), and for TT 1.908 (95% CI 0.94–3.88, $P = 0.072$, $\chi^2 = 3.24$, power = 0.925). The frequencies of A and T allele in POAG cases were 44.06% and 54.94% as compared to 52.94% and 47.06% in the controls. OR for A was 0.700 (95% CI 0.47–1.03, $P = 0.074$, $\chi^2 = 3.21$, power = 0.845) and for T 1.428 (95% CI 0.97–2.11, $P = 0.074$, $\chi^2 = 3.21$, power = 0.845). The *FTO* gene AA, AT, TT genotype frequencies were 2%, 79.20%, 18.80% in cases and 0%, 75.50%, 24.50% in healthy controls, respectively. OR for AA was not available (NA) (95% CI NA, $P = 0.153$, $\chi^2 = 2.05$, power = 0.958), for AT 1.237 (95% CI 0.64–2.39, $P = 0.527$, $\chi^2 = 0.40$, power = 0.843), and for TT 0.714 (95% CI 0.36–1.40, $P = 0.324$, $\chi^2 = 0.97$, power = 0.892). The frequencies of A and T allele in POAG cases were 41.58% and 58.42% as compared to 37.75% and 62.25% in the controls. OR for A was 1.174 (95% CI 0.79–1.75, $P = 0.429$, $\chi^2 = 0.63$, power = 0.844) and for T 0.852 (95% CI 0.57–1.27, $P = 0.429$, $\chi^2 = 0.63$, power = 0.844). The genotype, allele's frequencies of *FABP2*, *FTO* and statistical analysis among the cases and controls are also shown in Table 2. Genotype distribution for all investigated SNPs was in Hardy–Weinberg equilibrium in both cases and controls.

Discussion

Glaucoma is accurately defined as an optic neuropathy involving a characteristic atrophy of the optic nerve head. It is usually results from decreased outflow of aqueous fluid due to an acceleration and exaggeration of normal

Table 1: Clinical and biochemical profile of primary open-angle glaucoma cases and controls

Parameters	POAG cases (n=122)	Control (n=112)
Gender (male/female)	65/57	59/53
Age (years)	49.88 ± 12.34	53.74 ± 11.87
BMI	23.61 ± 4.71	21.24 ± 3.93
SBP (mm Hg)	128.32 ± 22.15	122.30 ± 20.19
DBP (mm Hg)	79.06 ± 22.14	82.15 ± 19.30
RBS (mg/dl)	113.97 ± 24.22	124 ± 61.47
Hb (g%)	14.10 ± 4.11	14.45 ± 1.65
TLC (cell/cumm)	7183.83 ± 1841.84	7571.70 ± 1690.06
Neutrophils (%)	66.43 ± 8.49	65.35 ± 6.73
Lymphocytes (%)	27.78 ± 8.24	28.70 ± 6.73
Eosinophils (%)	4.07 ± 2.64	4.58 ± 2.89
Monocytes (%)	2.02 ± 4.76	1.19 ± 3.20
Platelet count (lakh/cumm)	1.98 ± 0.65	1.99 ± 0.68

n = Number of participants. POAG = Primary open-angle glaucoma, BMI = Body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, RBS = Random blood sugar, Hb = Hemoglobin, TLC = Total lymphocyte count

Table 2: Genotypes and alleles frequency of *FABP2* and *FTO* genes in primary open-angle glaucoma cases and controls

	Control (102)		POAG (101)		OR	95% CI	χ^2	P	Bonferroni corrected P	Power
	n	Frequency (%)	n	Frequency (%)						
<i>FABP2</i>										
Genotype										
AA	23	20.60	16	12.90	0.569	0.27-1.21	2.17	0.141	0.423	0.875
AT	72	64.70	76	62.40	0.904	0.51-1.60	0.12	0.73	1.000	0.644
TT	17	14.70	30	24.80	1.908	0.94-3.88	3.24	0.072	0.216	0.925
Allele										
A	119	52.94	108	44.06	0.700	0.47-1.03	3.21	0.074	0.147	0.845
T	105	47.06	136	55.94	1.428	0.97-2.11	3.21	0.074	0.147	0.845
<i>FTO</i>										
Genotype										
AA	0	-	2	2.00	NA	NA	2.05	0.153	0.460	0.958
AT	85	75.50	97	79.20	1.237	0.64-2.39	0.40	0.527	1.000	0.843
TT	27	24.50	23	18.80	0.714	0.36-1.40	0.97	0.324	0.974	0.892
Allele										
A	85	37.75	101	41.58	1.174	0.79-1.75	0.63	0.429	0.858	0.844
T	139	62.25	143	58.42	0.852	0.57-1.27	0.63	0.429	0.858	0.844

OR = Odds ratio, CI = Confidence interval, NA = Not available, POAG = Primary open-angle glaucoma, FABP2 = Fatty acid-binding protein 2

aging changes in the anterior chamber angle, iris, and ciliary body tissues of the eye. If it is untreated, the natural course is toward blindness or significant visual loss disability. We have observed that the mean RBC lysate GSH levels were significantly lower in cases as compare to the controls, similar finding were observed in the UK population where patients with glaucoma exhibit low levels of circulating GSH.^[19]

Fatty acid-binding protein 2 polymorphism

FABP2 gene is expressed only in the absorptive simple columnar epithelial cells of the small intestine.^[20] According to Hongmei *et al.*, the evidence of decreased blood flow to the optic nerve is directly influenced by fatty acids, and further investigation result shows abnormal blood fatty acid composition in POAG patients compared to a healthy control. Indeed, the POAG patients studied had reduced total omega-3 long-chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) fatty acids.^[7] These findings may be significant as EPA and DHA could modulate impaired systemic microcirculation, ocular blood flow, and positively impact optic neuropathy, which are the main physiological changes associated with glaucoma.^[21] Numerous studies have assessed *FABP2* gene variants and their association with insulin resistance and type 2 diabetes mellitus.^[7] This is the first time we are reporting this gene polymorphism in POAG cases. We have observed that the frequency of *FABP2* AA, AT, TT genotype were 12.90%, 62.40%, 24.80% in POAG cases and 20.60%, 64.70%, 14.70% in healthy controls, respectively. In our study, we have found no significant association of *FABP2* gene polymorphism in North Indian POAG cases [Table 2].

Fat mass and obesity-associated polymorphism

FTO gene is associated with an increased BMI, risk of myocardial infarction, and cardiovascular death. GWA studies first describe the link between genetic variation in *FTO* and obesity,^[22] and later, it was confirmed in different populations all over the world. Obesity is one of the major risk factors for several diseases such as type 2 diabetes, hypertension, stroke, osteoarthritis, and sleep apnea syndrome; some eye diseases such as glaucoma,^[23] cataract,^[24] diabetic retinopathy,^[25] and age-related macular degeneration were reported to have potential relation to obesity.^[26] In our study, we found that *FTO* gene AA, AT, TT genotype frequencies were 2%, 79.20%, 18.80% in cases and 0%, 75.50%, 24.50% in healthy controls, respectively. Frequencies of A and T allele in POAG cases were 41.58% and 58.42% as compared to 37.75% and 62.25% in the controls. Our results show no significant association between the AA, AT, TT genotype of *FABP2* and POAG ($P = 0.153, 0.527, 0.324$). Adequate data of *FTO* polymorphism and POAG were not available.

Conclusion

We could not identify the possible association of *FABP2* and *FTO* gene polymorphism with POAG; however, further studies with larger sample size in different population are require to clarify the role of *FABP2* and *FTO* genes in susceptibility to POAG.

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Conflicts of interest

There are no conflicts of interest.

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