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Research Article

Contribution of Nat*2*5* and Nat*2*6* Polymorphisms in Causing Susceptibility to Different Types of Cataract

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Abstract

Aim: Age related cataract (ARC) is caused by a number of risk factors including oxidative stress, ultraviolet radiation, toxic chemicals etc. Arylamine N-acetyltransferases (*NATs*) are xenobiotic metabolising enzymes involved in variety of detoxification processes and thus protect the lens. Alternatively, oxidative dependant inactivation and polymorphisms in *NAT* gene have been implicated in ARC. Hence the present study was conducted to examine the association of *NAT2* polymorphisms with different types of ARC.

Materials and Methods: *NAT2*5* and *NAT2*6* polymorphisms were genotyped in 455 ARC patients and 205 controls by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method.

Results: There was significant difference in the distribution of *NAT2*5* genotypes between the patients and controls (χ^2 = 42.47; P = 0) in general and in different types of cataracts. Risk estimates revealed significant association of CT genotype (intermediate acetylator) with ARC (OR = 2.85; 95%CI = 2.03 - 4.01; P = < 0.0001). This risk was more pronounced in individuals with cortical cataract (OR = 5.27; 95%CI = 3.11-8.92; P < 0.0001) followed by those with posterior subcapsular cataract (OR = 3.81; 95%CI = 2.28 - 6.39; P < 0.0001), nuclear cataract (OR = 2. 34; 95%CI = 1.45 - 3.76; P = 0.0004) and mixed type (OR = 1.88; 95%CI = 1.22 - 2.90; P = 0.003).

Conclusion: Our results suggest high risk for intermediate acetylators for developing ARCs. As *NAT2* is an important xenobiotic metabolising enzyme and theoretically xenobiotics such as smoking, ultraviolet radiation are all involved in cataract formation, it is possible that polymorphisms in *NAT2* may be associated with susceptibility of cataract formation. This is the first study showing association of intermediate acetylator genotype with ARC especially when the subjects were smokers.

Keywords: *Nat2*5; Nat2*6; Cataract*

Introduction

Age-related cataract is the leading cause of blindness in the world today [1]. The aetiology of cataracts is multifactorial with the involvement of both genetic and environmental factors and the mechanisms underlying cataract formation are complex. A number of risk factors have been attributed to the formation of age related cataract which include exposure of human lens epithelial cells (HLE) to endogenous or environmental stress factors such as oxidative stress, ultraviolet radiation (UV) and toxic chemical agents leading to pathologic eye conditions [2- 4]. Peroxynitrite and H_2O_2 are thought to be the major oxidants to which HLE cells are acutely or chronically exposed [5-7].

Tobacco smoke involved in cataract formation, contains aromatic amines, such as 4-aminobiphenyl, that are detoxified by human arylamine *N*-acetyltransferases (*NAT*s) [8]. *NAT*s are xenobiotic metabolizing enzymes that are involved in the detoxification and metabolic activation of numerous drugs and chemicals [9]. In addition, therapeutic drugs such as sulfamides and isoniazid detoxified by *NAT*s have also been implicated in cataract formation [10] and ocular toxicity [11].

Studies on the regulation of human N-acetyl transferases (*NAT*1 and *NAT*2) have shown that hydroxylamine or nitroso intermediates of *NAT* substrates inhibit the enzyme through direct irreversible interaction with its catalytic cysteine residue. Similarly, oxidative molecules such as hydrogen peroxide, s-nitrosothiols and peroxynitrite are known to inactivate the enzyme in a similar manner [9,12]. Thus, the activity of *NAT*1 and *NAT*2 enzymes present in the human lens epithelial cells may be inhibited by the oxidants produced during oxidative and photo-oxidative stresses exposing the lens to harmful toxic chemicals thereby contributing to the development of cataract over time.

Human arylamine *NAT*1 and *NAT*2 exist as isoenzymes with different, though overlapping substrate specificities [13]. The *NAT*2 gene harbours a number of polymorphic sites whose allelic variants have been reported to be co-dominant [8]. The individuals with the alleles encoding the fast (F) or slow (S) enzyme variants may be phenotypically fast (FF) or intermediate (FS) or slow (SS) depending on the presence of these two alleles.

NAT-dependent detoxification pathway has been suggested to play a protective role in lens cells. Further, *NAT*2-slow acetylator individuals have been shown to be at greater risk of developing age-dependent cataract than fast acetylators which suggest that exogenous chemical factors detoxified by *NAT*s may be etiological agents for cataract formation [14]. Therefore, in addition to genetic polymorphisms, oxidative-dependent inactivation of *NAT*s may have important consequences for xenobiotic detoxification in human lens epithelial cells.

Materials and Methods

A total of 455 age related cataract cases [108 - Nuclear Cataract (NC), 105 - Cortical cataract (CC), 96 - Posterior Subcapsular cataract (PSC) and 146 - Mixed type (MT)] and 205 healthy non-cataractous controls were genotyped for *NAT2*5* and *NAT2*6* polymorphisms in *NAT2* gene. All the patients included in the study were recruited from among the inpatients of Sarojini Devi Eye Hospital and Institute of Ophthalmology, Hyderabad, India. The type of cataract was determined by the ophthalmologists at the time of surgery following LOC- III classification. Controls were selected at random by personal contacts, by house visits and from among the employees of government and private organizations with provision for annual health check-up. All the subjects were explained about the purpose and outcome of the study and only those who gave their consent to participate in the investigations by providing the blood samples and demographic history were considered. Data pertaining to sex, age, age at onset, duration of disease, type of cataract, information on habit of smoking and alcohol consumption, diet and detailed medical history along with 3 - 4 generation pedigrees was collected from all the subjects using a specified proforma. The study was approved by the Institutional Ethical Committee following Helsinki guidelines.

DNA was isolated from the whole blood collected in EDTA vaccutainers from patients and controls using rapid non-enzymatic method described by Lahiri and Nurnberger [15]. The genotyping of c.481C > T (*NAT2*5*) polymorphism and c.590G > A (*NAT2*6*) polymorphism in *NAT*2 gene was carried out by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. PCR amplification was performed in a total reaction volume of 10 µl containing 1X PCR buffer, 200 mM each dNTP, 0.25U Taq Polymerase and 25 pmol of forward and reverse primers with initial denaturation of 5 mins at 94^0C followed by 30 cycles of denaturation at 94^0C for 45 seconds, annealing at 60^0C for 1 minute, extension at 72^0C for 1 minute and a final extension at 72^0C for 7 minutess. Restriction digestion was done by incubating 5 µl of the PCR product with Kpn1 (*NAT2*5*) and Taq1 (*NAT2*6*) restriction enzyme overnight followed by electrophoresis on 10% polyacrylamide gels. Based on the number of fragments generated the samples were genotyped as CC (433 and 114bp), CT (547, 433 and 114bp) and TT (547 bp) for c. 481C > T polymorphism and as GG (222, 170 and 155bp), GA (547, 222, 170 and 155bp) and AA (547bp) for c.590G > A polymorphism. The presence of mutation in both the alleles of *NAT*2 was assumed to represent a slow acetylation phenotype while wild types and heterozygotes were classified as fast acetylators.

Statistical Analysis

SPSS (version 17) package was used to analyze the data for descriptive statistics and computation of means and standard deviations. χ^2 statistics was computed for significance of differences in the distribution of genotypes between patients and controls. The frequencies of the marker alleles were estimated by allele counting method and tested for Hardy-Weinberg equilibrium. Odds ratios (OR) and 95%

confidence interval (CI) were computed for different combinations of genotypes under different models to estimate the risk contributing to the onset of cataracts.

Results

Table 1 shows the demographic features of the patients and controls. There was preponderance of female patients (54.3%) as compared to males (45.7%) the frequency being highest in cases of NC (58.3%) indicating high risk for females for developing age related cataracts specially the nuclear type. The mean age recorded for patients in general was 58.7 ± 0.51 (NC- 61.4 ± 0.92, CC- 58.3 ± 1.14, PSC-55.8 ± 1.22, MT-58.7 ± 0.81) and 50.74 ± 0.74 for controls. The mean age at onset of cataract was 57.3 ± 0.51 for the patients in general with delayed onset in patients of NC (60.2 ± 0.89) followed by MT (57.5 ± 0.81), CC (56.4 ± 1.13) and PSC (54.4 ± 1.23) cases. In 20.2% cases the onset was early and 21.7% of the patients were found to have overweight (BMI ≥ 25.0kgms/m^2). BMI showed significant association with the condition wherein individuals with normal weight were found to be at risk for developing age related cataract (χ^2 = 50.64; p≤0.00). In general the incidence of smokers was more in cataract patients (45.6%) as compared to controls (35.5%). As the frequency of females with the habit of smoking was negligible, we considered the frequency of smokers among males only in the present study. Considering different types of cataract, the incidence of smokers was more in NC (48.9%) followed by CC (48.3%), MT (46.0%) and PSC (38.1%). The frequency of alcoholics was lesser in patients (22.2%) as compared to controls (30.2%) (Table 1).

	ARC		NC		CC		PSC		MT		CT	
	n	%	n	%	n	%	n	%	n	%	n	%
Total	455		108		105		96		146		205	
Males	208	45.7	45	41.7	58	55.2	42	43.8	63	43.2	138	67.3
Females	247	54.3	63	58.3	47	44.8	54	56.3	83	56.8	67	32.7
Overweight	99	21.7	49	45.3	15	14.3	12	12.5	23	15.8	101	50.7
Normal Weight	356	78.3	59	54.6	90	85.7	84	87.5	123	84.2	104	30.2
Smokers	95	45.6	22	48.9	28	48.3	16	38.1	29	46.0	49	35.5
Alcohols	101	22.2	25	23.1	19	18.1	20	20.8	37	25.3	62	30.2
Familial	24	5.30	6	5.60	8	7.60	3	3.10	7	4.80	4	2.0
Mean Age	58.65 ± 0.51		61.40 ± 0.92		58.30 ± 1.14		55.80 ± 1.22		58.70 ± 0.81		50.74 ± 0.74	
Age@Onset	57.32 ± 0.51		60.15 ± 0.89		56.35 ± 1.13		54.42 ± 1.23		57.48 ± 0.81		---	

Table 1: *Baseline characteristic features in different types of cataract and control subjects studied.*

ARC: Age Related Cataract; NC: Nuclear Cataract; CC: Cortical Cataract; PSC: Posterior Sub Capsular Cataract; MT: Mixed Type; CT: Controls.

Table 2 depicts the genotypic and allelic distributions of c.481C > T (*NAT2*5*) and c.590G > A (*NAT2*6*) polymorphisms in *NAT2* gene in both cases (i.e. in age related cataract patients in general and in different types of cataracts) and controls.

Marker	c.481C > T			C allele	P-Value	c.590G > A			G allele	P-Value
	CC	CT	TT			GG	GA	AA		
Total (455)	135 (29.7)	283 (62.2)	37 (8.1)	0.61	0	171(37.6)	231 (50.8)	53 (11.7)	0.63	0.71
NC (108)	42 (38.9)	62 (57.4)	4 (3.7)	0.68	0.000	45(41.7)	44 (40.7)	19 (17.6)	0.62	0.34
CC (105)	24 (22.9)	79 (75.2)	2 (1.9)	0.60	0	33 (31.4)	61 (58.1)	11 (10.5)	0.6	0.19
PSC (96)	20 (20.8)	66 (68.8)	10 (10.4)	0.55	0.000	36 (37.5)	51 (53.1)	9 (9.4)	0.64	0.59
MT (146)	49 (33.6)	76 (52.1)	21 (14.4)	0.60	0.015	57 (39.0)	75 (51.4)	14 (9.6)	0.65	0.65
Control (205)	88 (42.9)	75 (36.6)	42 (20.5)	0.61	--	83 (40.5)	97 (47.3)	25 (12.2)	0.64	--

Table 2a: *Distribution of genotypes and allele frequencies of c.481C>T (NAT2*5) and c.590G>A (NAT2*6) polymorphisms studied in different types of cataract.*

NC: Nuclear Cataract; CC: Cortical Cataract; PSC: Posterior Sub Capsular Cataract; MT: Mixed Type; P ≤ 0.05 considered significant.

There was significant difference in the distribution of *NAT2*5* genotypes between the patients and controls (χ^2 = 42.47; P = 0) in general and when different types of cataracts was considered (Table 2). The allele frequencies deviated from Hardy Weinberg equilibrium in controls (P = 0.001) and in patients (P = 0) in general and in different types of cataract (NC = 0.0013; CC = 0; PSC = 0.0001) except for MT (P = 0.33). Risk estimates revealed significant association of the polymorphism with age related cataract in general under heterogenous co-dominant, dominant, recessive and overdominant models wherein individuals with intermediate acetylator genotype i.e. CT were at risk for developing the condition under overdominant model (OR = 2.85; 95%CI = 2.03 - 4.0; P = < 0.0001; Table 3). When different types of cataracts was considered it was observed that the risk for intermediate acetylator genotype was more pronounced in individuals with cortical cataract (OR = 5.27; 95%CI = 3.1 - 8.97; P < 0.0001) followed by those with PSC (OR = 3.81; 95%CI = 2.28 - 6.39; P < 0.0001), NC (OR = 2. 34; 95%CI = 1.45 - 3.76; P = 0.0004) and MT (OR = 1.88; 95%CI = 1.22 - 2.90; P = 0.003; Table 3).

Model	Genotype	ARC		NC		CC		PSC		MT	
		Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value
Co-dominant	TT Vs CC	0.57 (0.34 - 1.00)	0.03	0.20 (0.07 - 0.59)	0.003	0.17 (0.05 - 0.86)	0.02	1.05 (0.45 - 2.44)	0.92	0.90 (0.48 -1.69)	0.74
	CT Vs CC	2.46 (1.70 - 3.56)	< 0.0001	1.73 (1.05 - 2.85)	0.03	3.86 (2.33 - 6.70)	< 0.0001	3.87 (2.15 - 6.97)	< 0.0001	1.82 (1.13 -2.92)	0.01
Dominant	CT-TT CC	1.78 (1.27 - 2.51)	0.008	1.18 (0.73 - 1.90)	0.49	2.54 (1.49 - 4.33)	0.0004	2.89 (1.65 - 5.09)	0.000	1.49 (0.96 -2.31)	0.08
Recessive	TT CC-CT	0.34 (0.21 - 0.55)	< 0.0001	0.15 (0.05 - 0.43)	0.0001	0.08 (0.02 - 0.32)	< 0.0001	0.45 (0.22 - 0.94)	0.03	0.65 (0.37 -1.16)	0.14
Overdominant	CT CC-TT	2.85 (2.03 - 4.01)	< 0.0001	2.34 (1.45 - 3.76)	0.0004	5.27 (3.11 - 8.92)	< 0.0001	3.81 (2.28 - 6.39)	< 0.0001	1.88 (1.22 -2.90)	0.003

Table 3: *Risk Estimates of c.481C>T (NAT2*5) genotypes under different models for developing age related cataract in general and different types of cataract. ARC: Age Related Cataract; NC: Nuclear Cataract; CC: Cortical Cataract; PSC: Posterior Sub Capsular Cataract; MT: Mixed Type; P ≤ 0.05 considered significant.*

Considering the *NAT2*6* polymorphism, the allele frequencies were in accordance with Hardy-Weinberg equilibrium among the cases (p = 0.07) and controls (p = 0.76). No statistically significant difference was found for the genotypic and allelic distributions of *NAT2*6* polymorphism between the patients in general and in different types of cataracts when compared to controls (Table 4).

Model	Genotype	ARC		NC		CC		PSC		MT	
		Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value	Odd's Ratio (95%) CI	P-Value
Co-dominant	TT Vs CC	1.03 (0.60 - 1.77)	0.92	1.40 (0.70 - 2.82)	0.34	1.11 (0.49 - 2.50)	0.80	0.83 (0.35 - 1.95)	0.67	0.81 (0.39 - 1.70)	0.58
	CT Vs CC	1.16 (0.81 -1.65)	0.42	0.84 (0.50 - 1.39)	0.49	1.58 (0.95 - 2.65)	0.08	1.21 (0.72 - 2.03)	0.47	1.13 (0.72 - 1.77)	0.61
Dominant	CT-TT CC	1.12 (0.80 - 1.57)	0.51	0.95 (0.59 - 1.52)	0.81	1.47 (0.90 - 2.42)	0.13	1.24 (0.68 - 1.95)	0.65	1.05 (0.68 - 1.63)	0.81
Recessive	TT CC-CT	0.95 (0.57 - 1.58)	0.84	1.54 (0.80 - 2.94)	0.19	0.84 (0.40 -1.79)	0.65	0.75 (0.33 - 1.66)	0.47	0.76 (0.38 - 1.53)	0.44
Overdominant	CT CC-TT	1.15 (0.83 - 1.60)	0.41	0.77 (0.48 - 1.23)	0.27	1.54 (0.96 - 2.48)	0.07	1.26 (0.78 - 2.05)	0.35	1.17 (0.77 - 1.80)	0.45

Table 4: *Risk Estimates of c.590G > A (NAT2*6) genotypes under different models for developing age related cataract in general and different types of cataract. ARC: Age Related Cataract; NC: Nuclear Cataract; CC: Cortical Cataract; PSC: Posterior Sub Capsular Cataract; MT: Mixed Type; P ≤ 0.05 considered significant.*

Discussion and Conclusion

The human arylamine *N*-acetyltransferases (*NAT*1 and *NAT*2) are important xenobiotic-metabolizing enzymes involved in the detoxification and metabolic activation of numerous drugs and chemicals [9]. Although functional *NAT*1 and *NAT*2 are present in human lens epithelial (HLE) cells, oxidative damage to the macromolecules of HLE cells due to increases in the levels of cellular oxidants by UV-dependent photo-oxidative processes, exposure to toxic chemicals or failure of antioxidant defense systems is causally related to age-dependent cataract formation [2,5]. It was shown by Dairou., *et al.* [9] that exposure of HLE cells to UVB irradiation and oxidants such as H_2O_2 and peroxynitrite led to dose dependent inactivation of both *NAT* isoforms leading to cataract formation.

Further, humans are exposed to many toxic *NAT* substrates including the food-derived heterocyclics present in the diet as well as arylamines such as 4-aminobiphenyl and naphthylamine present in tobacco smoke which have been implicated in cataract formation [8,16-19]. Further, therapeutic drugs detoxified by *NAT*s, such as sulfamides and isoniazid, have also been implicated in cataract formation [10] and ocular toxicity [11].

In addition, the *NAT*2 gene contains a number of polymorphic sites, whose allelic variants have been reported to be codominant [8]. Polymorphic aromatic amine *NAT*2 catalyzes the N-acetylation of aromatic amines and the metabolic activation of N-hydroxyarylamines (via O-acetyaltion) and N-hydroxy-N-acetylarylamines (via N,O-acetylation) to electrophilic intermediates that mutate DNA. N-acetylation forms the amide derivative which is often non-toxic. However, O-acetylation of N-hydroxyarylamine (following oxidation) yields an acetoxy arylamine derivative that breaks down spontaneously to highly reactive arylnitrenium ion that is responsible for mutagenic and carcinogenic properties.

Individuals with homozygous TT genotype for *NAT*2 polymorphisms are considered as slow acetylators, those with CT are intermediate acetylators, while those with CC genotype are fast acetylators. Slow acetylator status has been reported to be associated with age related cataract and other conditions such as urinary bladder cancer [20], epilepsy [21] etc. Fast acetylator status, on the other hand, has been associated with conditions such as type 1 diabetes mellitus [22], lung cancer [23], benign breast cancer [24], laryngeal cancer [25] and phenylketonuria [26].

Because N-acetylation is involved in a wide variety of detoxification processes, the present study was carried out to examine the association of *NAT*2 polymorphisms with age-related cataracts. In the present study, patients with intermediate acetylator genotype of *NAT2*5* polymorphism were found to be at higher risk for developing age-dependent cataract. An explanation regarding the risk for rapid acetylator genotype was given by King., *et al*. [27] who reported that tobacco activates heterocyclic amines, and if exposed to tobacco smoke, rapid acetylators are more likely to O-acetylate the N-hydroxy heterocyclic amines forming compounds capable of creating DNA adducts. The risk for intermediate acetylator genotype in the present study can be attributed to the fact that the habit of smoking was observed more in the patient group (45.6%) as compared to controls (35.5%; Table 1). Further, 62.1% of the smokers were found to have CT genotype in the patient group as compared to 38.8% in the controls. Considering, different types of cataract, the risk of CT genotype was more pronounced in patients with cortical cataract followed by PSC, NC and MT. This is in accordance with the frequency of smokers with CT genotype which was found to be more in patients with cortical cataract (71.4%) followed by PSC (68.8%), NC (59.1%) and MT (51.7%) as compared to controls.

Considering *NAT2*6* polymorphism no significant association was found with age related cataract in the present study. This polymorphism however showed association with age related cataract in Turkish population [28] wherein slow acetylator genotype was reported to be at risk for developing the condition.

So far only few studies have reported the association of *NAT*2 polymorphism with age related cataract [14,28] wherein individuals with slow acetylator genotype were found to be at risk for developing the condition. The present study is the first one to report the association of intermediate acetylator genotype of *NAT2*5* polymorphism with age related cataract. This inconsistency may be attributed to be due to variation in *NAT*2 allele distribution between different ethnic populations, as well as to exposure to environmental factors. Further it is the first study reporting the association of *NAT*2 polymorphism with different types of cataract showing risk of intermediate acetylator genotype for CC followed by PSC, NC and MT cataract. Replication of these results in other populations may further elucidate the role of *NAT*2 gene in causing susceptibility to age related cataracts.

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Conflict of Interest

The authors declare no conflict of interest.

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