

Original Article

Association of PON2 and PON3 polymorphism with risk of developing cataract



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Abstract

Purpose: Paraoxonases (PON) are calcium bound enzymes offering protection against oxidative stress by working as endogenous free-radical scavenging molecules. Oxidative stress has been implicated in pathophysiology of many diseases including cataract. Lens opacity is an age related disorder which is a principal cause of blindness in Pakistani population. Relationship of PON2 and PON3 polymorphism with genetic predisposition for incidence of cataract has not been investigated till date. Objective of the current study was to explore possible association between PON2 and PON3 polymorphism with incidence of cataract in local population.

Methods: Our study design comprised of fifty-one cataractous and fifty-nine healthy individuals. Identification of single nucleotide polymorphism (SNP) at positions (C311S and G148A) for PON2 and C133A for PON3 was conducted using restriction fragment length polymorphism (RFLP).

Results: Statistical analysis revealed significant association of PON2 G148 allele with incidence of cataract. GG allele was found to be higher in cataract patients as compared to control ($p < 0.001$) suggesting distribution of PON2 G148A genotype and allele frequency is linked with cataractogenesis. There was no noticeable association between PON2 C311S and PON3 C133A. Significant difference was observed in distribution of 311CS/148A combined genotype with highest frequency in control individuals (88.89%), while 311S/148G combined genotypes showed the highest frequencies among the cataract patients (71.42%).

Conclusion: Our data suggests mutation at G148A might be related with incidence of cataract in studied population.

Keywords: PON2, PON3, Polymorphism, Cataract

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Introduction

Lens opacity or cataract is a leading cause of visual impairment throughout the world. WHO estimate indicated 95 million people to be effected from cataract in 2014.¹ Cataract is an age-related disorder that is likely to be initiated at the age of 50 and above.² In low and middle income countries, however, lens opacity has a higher incidence rate with an earlier

onset.³ Although epidemiological studies have implicated various risk factors for age-related cataracts including smoking, exposure to UV radiation, malnutrition, use of steroids etc; involvement of genetic factors in onset of disease cannot be ruled out.⁴ Genetic mutations are likely to increase disposition to environmental factors thus increasing susceptibility to develop cataract. Therefore, genetic variations in relation to risk of developing age related cataract has been studied.⁵

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Studies on association between senile cataract and genetic mutation in OGG1, EPHA2,⁶ FABP2, PPARG²⁷ and MTHFR⁸ have been reported. Among genes regulating oxidative stress, most of the work has been conducted on glutathione S Transferases (GSTs) superfamily members, GSTM1 and GSTT1.⁹

Paraoxonases are calcium dependent antioxidant enzymes constituting three isoforms PON1, PON2 and PON3.¹⁰ PON1 polymorphism has been studied in different disorders including cataract,^{11,12} diabetes,¹³ age related macular degeneration,¹⁴ Alzheimer's disease,¹⁵ Behcet's disease,¹⁶ and atherosclerosis.¹⁷ Data for PON2 and PON3, however, is limited. In PON2, two polymorphic sites C311S and G148A have been studied in Alzheimer's disease,¹⁸ ischemic stroke in type 2 diabetics,¹⁹ coronary artery disease^{20,21} and reduced bone mass in postmenopausal women.²² Most of the polymorphic sites in PON3 belong to non-coding and promoter region. There are some studies on PON3 polymorphisms in CHD,¹⁹ systemic lupus erythematosus (SLE)²³ and Alzheimer's disease.²⁴ A handful of promoter polymorphisms have also been identified suggesting a moderate influence on circulating PON3 levels.^{25,26}

We have earlier reported PON1 polymorphism in patients suffering from cataract.¹¹ In this communication, we studied two polymorphic sites of PON2 and one polymorphic site of PON3 gene in cataract and healthy subjects to identify whether missense mutations can be a risk factor for the development of cataract or not.

Material and methods

Sample collection

The study was conducted after approval from Institutional Review Board, National Center for Proteomics, University of Karachi. Informed written consents were obtained from all participants. We recruited fifty one age related cataract patients and fifty nine healthy individuals for this study. Blood samples were collected in K₂-EDTA coated vials from cataract patients prior to cataract surgery at Department of Ophthalmology, Liaquat National Hospital, Karachi. Cataract status was ascertained through slit lamp examination. Control group comprised of subjects without any ocular disorder. Subjects were excluded from the study if they suffered from any metabolic disorder or secondary cataract due to diabetes or trauma.

DNA extraction

DNA was isolated from whole blood within 24 h of collection as described earlier.²⁷ Briefly, 300 µl of blood was added

to 900 µl of cell lysis buffer (0.31 M sucrose, 0.01 M Tris, 0.002 M MgCl₂, 0.0003 M sodium azide, pH 7.6) and centrifuged at 13,000g for one minute. The procedure was repeated twice. The pellet was broken by addition of 300 µl red cell lysis buffer (0.064 M Tris, 0.017 M Na₂EDTA, 0.069 M SDS pH 8.0) followed by incubation for 30 min at 37 °C. Afterwards, 120 µl protein precipitation solution was added and centrifuged for 5 min. The supernatant was then transferred to a tube containing 300 µl iso-propanol and mixed gently for 15–20 times to visualize the DNA, followed by centrifugation. DNA thread was washed using 500 µl ethanol and air dried after centrifugation. Later, DNA was rehydrated with 50 µl of nuclease free water and stored –20 °C till further use.

Restriction fragment length polymorphism

Analysis of PON2 polymorphic site 311 was carried out as described earlier.²¹ Mismatch PCR assay for genotyping the polymorphic site at codon 148 in PON2 gene was performed using method of Mochizuki et al.²⁸ In case of PON3, polymorphism at codon 133 was evaluated.¹⁹ Primer sequences, restriction enzymes and product size in each case are depicted in Table 1.

Polymerase chain reaction was performed using PCR master mix DreamTaq Green PCR Master Mix 2X (Thermo Scientific Company EU, Lithuania) containing 25 µl PCR master mix, 5 µl DNA template (>150 ng/µl), 1 µl of each forward and reverse primer (10 µM) and 16 µl of nuclease free water giving a total reaction volume of 50 µl. The cycling condition for position C311S PON2 polymorphisms was 4 min at 94 °C for initial denaturation, followed by 30 cycles of 1 min at 94 °C for denaturation, 1 min 30 s at 50 °C for annealing and 2 min at 72 °C for extension. The conditions for PON2 G148A polymorphism were essentially same as of C311S with only once exception of 40 cycles instead of 30 in denaturation step. In case of PON3 C133A, PCR condition was 5 min for 95 °C, followed by 30 cycles of 94 °C for 45 s, 53 °C for 40 s and 72 °C for 45 s with final extension for 7 min at 72 °C. Products obtained from PCR were digested with 10 U restriction enzymes of Ddel for C311S, Fnu4HI for G148A and HinfI for polymorphic site of C133A respectively and visualized on 3% agarose gel electrophoresis using ethidium bromide.

Statistical analysis

Data was analyzed with SPSS version 20.0 and descriptive statistics were used. Demographic characteristics were compared by Pearson χ^2 test. Gene counting method was used to calculate allele frequencies. Odds ratios were calculated with 95% confidence interval. *P* value < 0.05 was considered as significant.

Table 1. Primers used for PON2 and PON3 polymorphic positions C311S, G148A and C133A respectively.

Polymorphic sites	Primer Sequence	PCR Products	Restriction Enzymes	Restricted bands
PON 2	C311S	5'- ACATGCATGTACGGTGGTCT-3' (forward)	Ddel	C allele: 142 bp
		5'-AGCAATTCATAGATTAATTGTTA-3' (reverse)		S allele: 75 bp + 67 bp
	G148A	5'-AGTGAAAATTTTAAATTTGAAGCAG-3' (forward)	Fnu4HI	G allele: 130 bp
		5'-TTGTTTGCAAATGCTGGGGAT-3' (reverse)		A allele: 110 bp + 20 bp
PON 3	C133A	5'-TTACCTATCATGTAGACTGTGAGA*GT-3' (forward)	HinfI	A allele: 168 bp
	5'-ATTCACAACATAAAGATACACAGTA-3' (reverse)	C allele: 143 bp		

* 120 bp band found in all genotype because of common restriction site.

Results

Two genetic variants in PON2 (C311S, G148A) and one in PON3 (C133A) were analyzed in blood samples from cataract patients and healthy controls using RFLP. The distribution of C311S codon for PON2 polymorphism in controls and patients is shown in Table 2. We did not observe any significant association of polymorphic marker C311S with incidence of cataract in studied groups (Table 2). The odds ratio for PON2 C vs S allele was found to be 1.448 ($p = 0.179$).

Restriction pattern of PCR amplified 130 bp product digested with Fnu4HI for genotyping at 148 position (Fig. 1). Genotype and allele frequency for PON2 G148A polymorphism is shown in Table 2. PON2 G148A polymorphism was found to be statistically significant ($p < 0.05$ in PON2) suggesting an association of this position with incidence of cataract. The prevalence of homozygous G allele was found to be more frequent in the cataract patients as compared to control (62.50% vs 40.35%) whereas healthy subjects had more homozygous A allele in contrast to cataract patients (59.64% vs. 37.50%), as depicted in Table 2. The odds ratio for the G allele versus A allele of PON2 G148A was (OR = 2.464, $p = 0.001$). Allelic patterns are presented in Fig. 1.

The distribution of genotype and allele frequency for PON3 polymorphic site C133A is shown in Table 2. We did not observe any significant association at this polymorphic site. Odds ratio between C allele versus A allele was found to be 0.957 ($p = 0.872$). Restriction pattern of PCR product is shown in Fig. 2.

Combined genotype analysis of G148A and C311S polymorphic sites of PON2 indicated a significant association.

The highest frequencies were observed in two groups: The first one is 311Cys/Ser vs 148Ala which showed higher frequency in control subjects as compared to diseased. The other genotype combination 311Ser vs 148Gly was more prevalent in cataractous group (Table 3).

Discussion

Identification of genetic predisposition to cataract might be beneficial for early detection and disease management. Cataract is an age-related disorder responsible for 48% of world blindness.²⁹ Among various heterogeneous factors contributing in development of lens opacity in elderly, genetic component is reported to be critically important.³⁰ Genetic factors are likely to increase susceptibility to environmental insult such as oxidative stress in aging population thus making them prone to develop lens opacity.³¹ Oxidative stress has been implicated in pathophysiology of cataract and several other eye related complications.³² PON2 and PON3 are members of Paraoxonase antioxidant family and have an established role in reducing oxidative damage through Q10.³³ Both enzymes are capable to restrain LDL oxidation³⁴ and trigger antioxidant properties along with cholesterol efflux of HDL.^{35,36} Association of PON2 and PON3 polymorphism with various disorders has sparingly been studied.¹⁴⁻²¹ Present study was conducted to explore association between senile cataract and PON2 and PON3 polymorphism.

The relationship between PON2 polymorphism and senile cataract was studied for two positions including C311S and G148A. Our results indicated an insignificant association

Table 2. Statistical analysis of PON2 and PON3 polymorphic sites.

Polymorphic positions			Control (F %)	Cataract F %	Odds ratio	95% CI	χ^2 Test
PON2 C311S (C- →S)	Genotypes	CC	19/57 (33.33%)	15/52 (28.84%)	1.125	0.502–2.519	0.775
		CS	15/57 (26.31%)	15/52 (28.84%)	0.969	0.414–2.269	0.943
		SS	23/57 (40.35%)	22/52 (42.30%)	1.084	0.505–2.326	0.836
	Alleles	CC	53/114 (46.49%)	45/104 (43.26%)	1.448	0.843–2.488	0.179
		SS	61/114 (53.50%)	59/104 (56.73%)			
PON2 G148A (G- →A)	Genotypes	GG	18/57 (31.57%)	29/52 (55.76%)	0.366	0.167–0.800	0.011*
		GA	10/57 (17.54%)	07/52 (13.46%)	1.368	0.479–3.904	0.557
		AA	29/57 (50.54%)	16/52 (30.76%)	2.330	1.063–5.110	0.033*
	Alleles	GG	46/114 (40.35%)	65/104 (62.50%)	2.464	1.428–4.251	0.001*
		AA	68/114 (59.64%)	39/104 (37.50%)			
PON3 C133A (C- →A)	Genotypes	CC	20/57 (35.08%)	14/52 (26.92%)	1.467	0.647–3.329	0.358
		CA	27/57 (47.36%)	32/52 (61.53%)	0.563	0.262–1.207	0.138
		AA	10/57 (17.54%)	06/52 (11.53%)	1.631	0.548–4.855	0.376
	Alleles	CC	67/114 (58.77%)	60/104 (41.22%)	0.957	0.558–1.640	0.872
		AA	47/114 (57.69%)	44/104 (42.31%)			

* Significance = ($p < 0.05$).

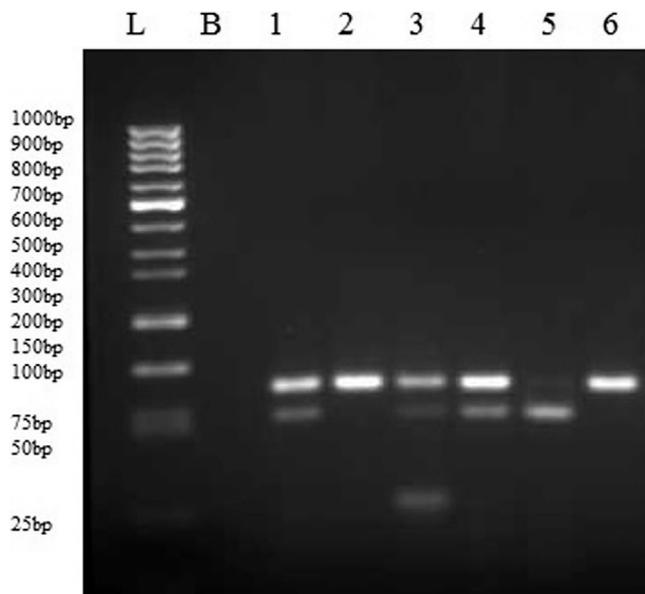


Fig. 1. Agarose gel electrophoresis of Restriction fragment length polymorphism for PON2. G148A PCR products show cleavage of amplified 130 bp DNA fragment. Fragment containing GG allele remained intact (lane 1). Lanes 2, 3, 4, 5 represent A/G (i.e heterozygous Alanine/Glycine). AA allele containing homozygous Alanine fragment (lane 6). L and B stand for ladder and blank, respectively.

between C311S mutation with risk of developing cataract in studied samples. Earlier investigations in coronary arterial disease for polymorphic position C311S remained inconclusive. One study stated significant association of C311 allele with the susceptibility to develop CAD,³⁶ while other suggested 311S to be important in same disorder.²¹ Martinelli²⁰ found no association at all. We also analyzed PON2 polymorphism for G148A which indicated that frequency of G148 allele was significantly higher than 148A allele in subjects suf-

fering from cataract. Presence of G allele at 148 position has been reported in patients suffering from coronary artery disease.³⁷ Another report showed same mutation to be the responsible for high plasma cholesterol and Apo. B.³⁸ New-born babies with PON2 148GA/GG genotype were also found to be at high risk of low birth weights and short birth length, when exposed to Di-n-butyl phthalate (MBP) and di-2-ethylhexyl phthalate (MEHP).³⁹

Combine genotype analysis for PON2 polymorphism revealed the combination of G148 and 311S was more frequent in cataract subjects while heterozygous allele at 311 and 148A are more likely to be present in subjects without lens opacity. It seems likely that presence of subsequent mutations at 148 and 311 might be implicated as a risk factor for development of cataract.

Studies on PON3 mutations are limited. However, available data mainly includes investigations on promoter and intronic region polymorphism.²³⁻²⁵ The most frequently studied position in coding region is C133A probably because it is located in potential binding site for transcriptional factor LFA1T.¹⁹ The same site was also checked in current study. Our analysis did not reveal any considerable association between PON3 C133A polymorphism with senile cataract. An earlier study also reported insignificant association of this mutation with CHD.¹⁹ Still, further studies with larger dataset are needed to validate the association.

Exact physiological substrates for PON2 and PON3 have not been identified yet but initial studies have demonstrated that like PON1, PON2 and PON3 not only act as anti-atherogenic agents but can also be used as a potential target for therapy.⁴⁰ PON1 and PON2 share common structural features thus it is likely that they share functional features also. However, wide expression in different tissues and multiple transcripts also suggest independent function for PON2 product³⁸ that needs to be further explored. Some reports suggest interaction of PON2 and PON3 through coenzyme Q10

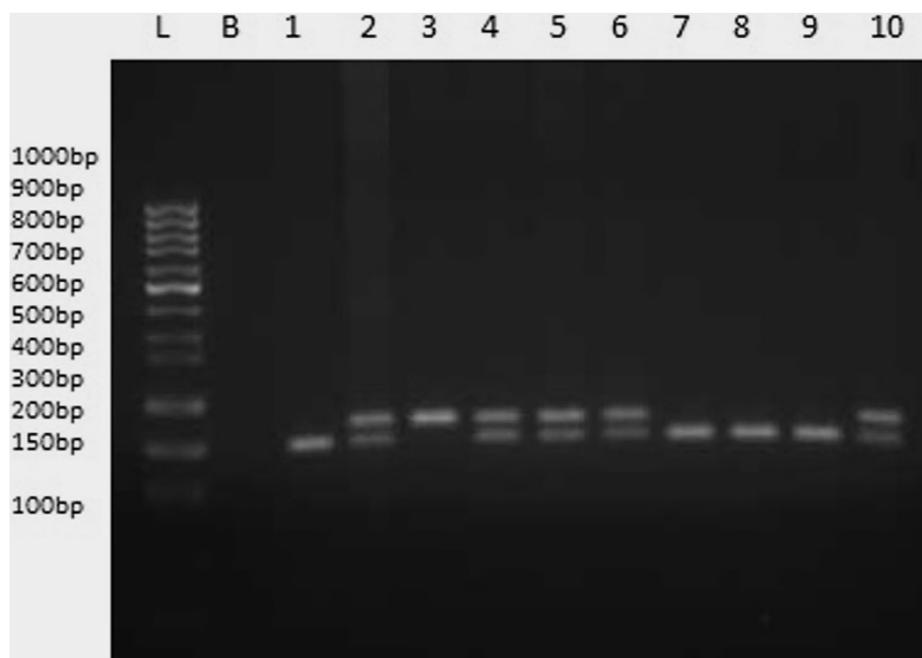


Fig. 2. Agarose gel electrophoresis of PCR-RFLP for PON3 C133A using HinfI. Lanes 1, 7, 8, 9 represent CC allele at 2, 4, 5, 6 represent CA while 10 represent AA. L and B represents ladder and blank.

Table 3. Frequencies of combine genotype of C311S and G148A polymorphism for PON2 in control and cataract subjects.

Combine Genotype	Control N Frequency (%)	Cataract N Frequency (%)	Odds ratio	95% CI	χ^2 Test
311Cys 148Gly	09 (47.36%)	10 (52.63%)	0.788	0.292–2.122	0.636
311Cys 148Ala	07 (63.63%)	04 (36.36%)	1.680	0.462–6.108	0.427
311Cys 148Gly/Ala	03 (75%)	01 (25%)	2.833	0.285–28.128	0.354
311Cys/Ser 148 Ala	08 (88.89%)	01 (11.1%)	8.327	1.004–69.058	0.022*
311Ser 148Gly	04 (28.57%)	10 (71.42%)	0.317	0.093–1.083	0.050*
311Ser 148Ala	14 (51.85%)	13 (48.14%)	0.977	0.409–2.333	0.958
311Ser 148Gly/Ala	04 (80%)	01 (20%)	3.849	0.416–35.609	0.204
311Cys/Ser 148Gly/Ala	02 (928.57%)	05 (71.42%)	0.342	0.063–1.844	0.194
311Cys/Ser 148Gly	05 (941.67%)	07 (58.3%)	0.618	0.183–2.083	0.435

* Significant value ($p < 0.05$).

thus implicating their possible role in maintaining oxidative balance.³³ PON2 can reduce levels of lipid peroxides⁴¹ and its expression is accelerated in cells during oxidative stress.⁴² PON3 can also inhibit LDL oxidation and its anti-atherogenic role is 100 times more effective than PON1.⁴³ Since oxidative imbalance plays crucial role in pathophysiology of most of the diseases including cataract, the role of PON2 and PON3 in keeping oxidative homeostasis might be important. Recently, high PON2 and PON3 mRNA expression was reported in cataract probably reflecting compensatory mechanism against oxidative stress.⁴⁴ Studies on polymorphism, therefore, may suggest functional outcome of genetic variation in PON3 and PON2.²⁶

Identification of polymorphic site is gaining popularity as it may help in screening of population to predict susceptibility for the disease.⁴⁵ Earlier diagnosis of population at risk through genetic testing might not only be beneficial for therapeutic intervention but may also contribute in designing strategies to delay or prevent opacities since delay of ten year in the development of opacity may reduce cataract surgeries by 50%.⁴⁶ Furthermore, counseling of high risk individuals to adopt preventive measure through life style changes, healthy diet and physical activity could be additional benefit having a positive impact on patient care.

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Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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