Disease Markers 33 (2012) 145–154
DOI 10.3233/DMA-2012-0915
IOS Press

Genetic association and gene-gene interaction of *HAS2*, *HABP1* and *HYAL3* implicate hyaluronan metabolic genes in glaucomatous neurodegeneration

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Abstract. Hyaluronan (HA) plays a significant role in maintaining aqueous humor outflow in trabecular meshwork, the primary ocular tissue involved in glaucoma. We examined potential association of the single nucleotide polymorphisms (SNPs) of the HA synthesizing gene – hyaluronan synthase 2 (*HAS2*), hyaluronan binding protein 1 (*HABP1*) and HA catabolic gene hyaluronidase 3 (*HYAL3*) in the primary open angle glaucoma (POAG) patients in the Indian population. Thirteen tagged SNPs (6 for *HAS2*, 3 for *HABP1* and 4 for *HYAL3*) were genotyped in 116 high tension (HTG), 321 non-high tension glaucoma (NHTG) samples and 96 unrelated, age-matched, glaucoma-negative, control samples. Allelic and genotypic association were analyzed by PLINK v1.04; haplotypes were identified using PHASE v2.1 and gene-gene interaction was analyzed using multifactor dimensionality reduction (MDR) v2.0. An allelic association (rs6651224; $p = 0.03$; OR: 0.49; 95% CI: 0.25–0.94) was observed at the second intron (C>G) of *HAS2* both for NHTG and HTG. rs1057308 revealed a genotypic association ($p = 0.03$) at the 5' UTR of *HAS2* with only HTG. TCT haplotype (rs1805429 – rs2472614 – rs8072363) in *HABP1* and TTAG and TTGA (rs2285044 – rs3774753 – rs1310073 – rs1076872) in *HYAL3* were found to be significantly high ($p < 0.05$) both for HTG and NHTG compared to controls. Gene-gene interaction revealed *HABP1* predominantly interacts with *HAS2* in HTG while it associates with both *HYAL3* and *HAS2* in NHTG. This is the first genetic evidence, albeit from a smaller study, that the natural polymorphisms in the genes involved in hyaluronan metabolism are potentially involved in glaucomatous neurodegeneration.

Keywords: Hyaluronan, *HAS2*, *HABP1*, *HYAL3*, SNP, POAG

1. Introduction

Glaucoma is a neurodegenerative disorder, caused by retinal ganglion cell (RGC) death, atrophy and axon degeneration, with or without elevated intra-ocular pressure [1]. It is the second largest cause of blindness after cataract affecting more than 80 million people worldwide [2]. Primary open angle glaucoma (POAG) is the major sub-type accounting for more than 50% of the total disease burden [3]. POAG can be sub-divided into two sub-types, high tension glaucoma (HTG) and nor-

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ISSN 0278-0240/12/$27.50

mal tension glaucoma (NTG). Under normal situation, during aqueous humor outflow in the anterior chamber, the fluid exits through the trabecular meshwork (TM) and reaches into Schlemm's canal and aqueous veins. If the TM passage gets blocked, the outflow mechanism is disturbed resulting into elevated intra ocular pressure (IOP) leading to HTG [4]. NTG is usually classified when the RGC death occurs in absence of an elevated IOP [5].

In POAG, the extracellular matrix (ECM) alters in the retina interrupting cell-cell and cell-ECM interactions and eventually causes RGC death by apoptosis. Extensive remodeling of ECM components including collagen I and IV, transforming growth factor-β2 (TGF-β2), matrix metalloproteinase (MMP-1), hyaluronan (HA) and chondroitin sulphate are reported in glaucomatous eye [6]. Being a high molecular weight mucopolysachharide, HA is reported to act as a filter in the TM controlling aqueous humor flow and protecting the anterior chamber from shrinkage. Along with other glycosaminoglycans (GAGs), it is deposited on the trabecular wall reducing the flow channel diameter. Relatively small amounts of HA (0.06 mg/ml) and larger amounts of chondroitin sulphate (0.78 mg/ml) are speculated to be instrumental in aqueous flow resistance in the POAG TM, thus eventually increasing pressure on the retinal ganglion cells and optic nerve and finally lead to glaucoma [7]. A change in its normal distribution pattern has also been reported in several other parts of the eye in POAG [8]. Decrease in aqueous humor outflow resistance by hyaluronidase perfusion illustrates the importance of HA in glaucoma [9]. These observations along with high viscosity and ROS scavenging potentials have made HA a very important component in glaucoma research [10,11].

Hyaluronan, the unbranched complex polysaccharide is synthesized by hyaluronan synthase in the plasma membrane and degraded by the action of hyaluronidase [12,13]. HAS2, located on chromosome 8q24.12 is principally responsible for polymerization of high molecular weight of HA (up to 2×10^6 Da) and plays a vital role in tissue expansion and developmental growth influenced by growth factors, cytokines and hormones and also reported to synthesize HA in perineuronal net in the central nervous system [14,15]. During glaucoma TGF-β concentration increases in aqueous humor and *in vitro* study shows the HAS2 isomer is maximally upregulated in response to TGF-β [16]. Hyaluronidase is used exogenously to reduce HA and chondroitin sulfate concentration on glaucomatous trabecular wall to maintain normal aqueous outflow [17,18]. HYAL3, located on chromosome 3p21.3 is expressed in brain [19] and retina (our unpublished data) having role in HA catabolism, although the gene has not been explored much [13]. Hyaluronan mediates its multifunctional activity by interacting with a family of proteins named hyaladherin [20]. One of the members of hyaladherin is hyaluronan binding protein 1 (HABP1) isolated and characterized from our laboratory [21]. The gene encoding HABP1 was identified from human fibroblast cDNA expression library and reported to be localized on human chromosome 17p13.3 [22]. Sequence analysis confirms its multifunctional nature due to its identity with globular head of C1q and p32, the protein copurified with splicing factor SF2, but its function was unknown [23]. *HABP1* is represented as a synonym of *C1QBP/p32* in the human genome database.

With this background, we wanted to evaluate the possible association of single nucleotide polymorphisms (SNPs) of genes involved in crucial steps of HA metabolism in glaucomatous neurodegeneration. We selected *HAS2* to represent the synthesis of HA, *HABP1* to represent the binding partner (hyaladherin) of HA and *HYAL3* to represent hyaluronidase. Our study is the first to report association of these genes in POAG and implicate the role of HA in the disease process.

2. Materials and methods

2.1. Selection of the study subjects

For the case-control study, the genomic DNA samples were recruited from a large ethnic group speaking Indo-European language from the eastern part of India. The inclusion and exclusion criteria were used as previously reported [24]. Briefly, patients having IOP above or below 21 mmHg coupled with damaged visual field and/or optic disc cupping were included in the study. Patients with ocular hypertension but no visual field defect were excluded. Further, we divided the POAG cohort into HTG and non-HTG (NHTG) subgroups. The NHTG group is not formally classified as NTG because for practical reasons we could not evaluate IOP at multiple time-points which are critical for clinical confirmation. These patients did not have any record of an IOP $>$ 21 mm of Hg (without medication or surgery) and hence classified as non-HTG patients. The 'unrelated' control samples we used for the association study were tested negative for both IOP and the visual field changes and were matched for gender, ethnicity and age.

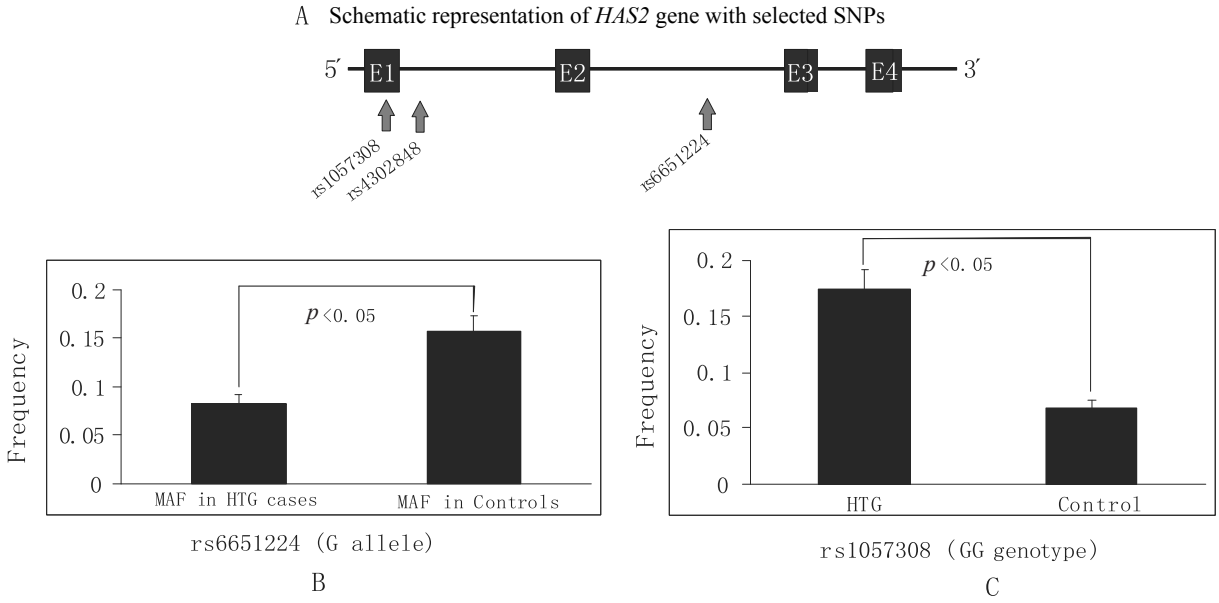

Fig. 1. The hyaluronan synthesizing gene *HAS2* is associated with HTG: Panel (A) shows a schematic representation of the genomic region of *HAS2* with selected SNPs marked with arrow. Black boxes represent the exonic regions (E) of the gene. Panel (B) depicts the frequency variation of the minor allele (G) of rs6651224 in the HTG patients compared to the controls. The histogram shows protective allelic association in HTG patients. Panel (C) shows difference of genotypic frequency of GG in the HTG patients with respect to controls for rs1057308 (*HAS2*). The data were presented as the mean $+/-$ SD.

2.2. Selection of SNPs

In the present study, 13 tagged SNPs (rs3910552, rs1057308, rs4302848, rs11992999, rs6651224 and rs2385924 of *HAS2* (Fig. 1A); rs8072363, rs2472614 and rs1805429 of *HABP1* (Fig. 2A); rs1076872, rs13100173, rs3774753 and rs2285044 of *HYAL3* (Fig. 3A)) were selected using CEU as a reference population, with minor allele frequency (MAF) cut-off at 0.1 and the $r^2 \geqslant 0.8$. Tagged SNPs (tSNP) reported in Caucasian (CEU) populations in HapMap database got priority as the previous study [25] suggests SNPs in CEU population are portable to Indian populations.

2.3. Genotyping

Genotyping was carried out in The Centre for Genomic Application (TCGA, New Delhi, India), using MALDI-TOF based chemistry on the Sequenom platform. The multiplexed assays for the mentioned SNP IDs were designed using the assay design software provided by the Massarray platform. The primers were synthesized at the oligo facility in TCGA. For accuracy of the genotyping data, 10% of the samples were duplicated.

2.4. Data analysis

Initially as a quality check step Hardy-Weinberg Equilibrium was tested ($p < 0.05$) for the genotypes by Fisher's Exact Test using PLINK v1.04 version [26] (http://pngu.mgh.harvard.edu/∼purcell/plink). Minimum minor allele frequency was kept at 0.01. On the basis of these quality control criteria, three SNPs of *HAS2* (rs2385924, rs11992999 and rs3910552) were filtered out and case-control association study was carried forward using the remaining 10 SNPs. For association analysis, allelic and genotypic frequencies were compared by chi-square test having one and two degrees of freedom, respectively. For test of association a p-value of less than 0.05 was considered significant. Haplotypes and their frequencies were analyzed from phase-unknown genotype data by using the PHASE version 2.1 [27]. In order to check for gene-gene interaction, multifactor dimensionality reduction (MDR) ver 2.0 was used based on the principle of non-parametric and genetic model-free alternative to logistic regression [28]. The data were presented as the mean $+/-$ SD. An unpaired Student's t-test was used to compare the data obtained.

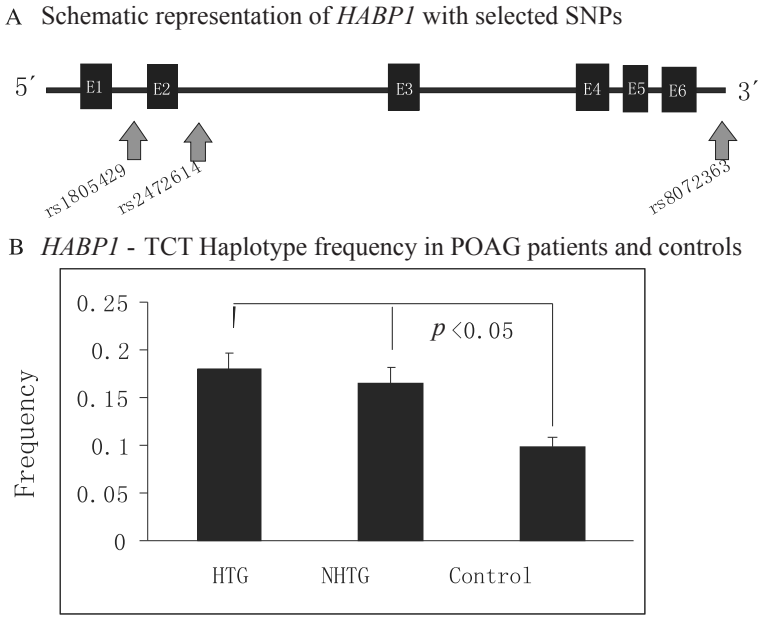

Fig. 2. Risk haplotype of hyaluronan binding gene *HABP1* for POAG: Panel (A) shows a schematic representation of the genomic region of *HABP1* with selected SNPs marked with arrow. Black boxes represent the exonic regions (E) of the gene. Panel (B) depicts the variation in the frequency of TCT haplotype of *HABP1* in HTG and NHTG patients compared to the controls. The histogram represents significant association ($p < 0.05$) of TCT haplotype in both categories of POAG patients.

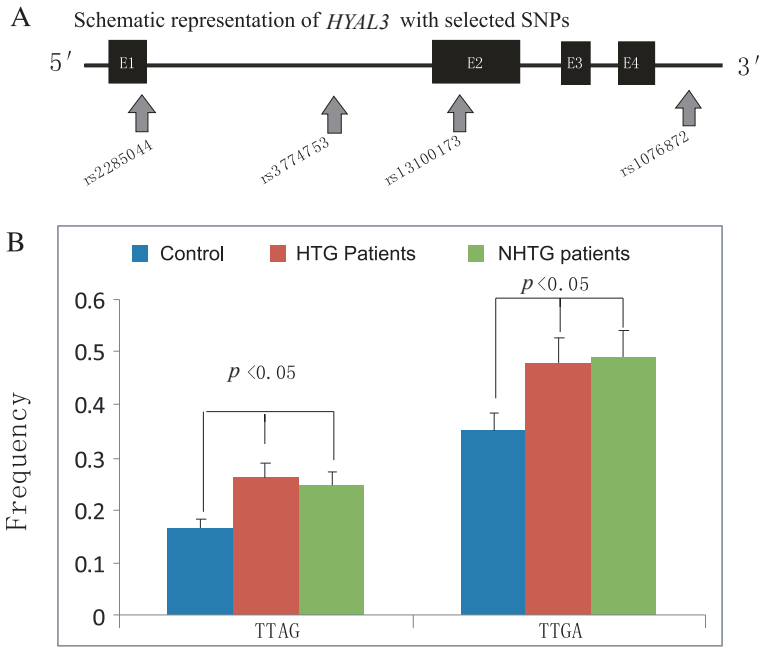

Fig. 3. Haplotypic association of hyaluronan catabolic gene *HYAL3* in POAG: Panel (A) represents a cartoon of HYAL3 gene with the selected tagged SNPs. Black boxes represent the exonic regions (E) of the gene. Panel (B) depicts the frequency variation of TTGA and TTAG haplotypes of *HYAL3* in the HTG (red) and NHTG (green) patients compared to the controls (blue). The data shows the haplotypes are significantly associated with both HTG and NHTG. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2012-0915)

Table 1
Allelic association of the hyaluronan metabolic genes with POAG in Indian population

Gene	SNP	DNA variation	Minor Allele frequency		Chi-square	p-value	OR	95% C.I.	Type of glaucoma	Remark
			Case	Control						
HAS2	rs1057308	A>G	0.35	0.33	0.21	0.64	1.10	0.71–1.71	HTG	
			0.35	0.33	0.23	0.63	1.09	0.75–1.57	NHTG	
	rs4302848	A>T	0.48	0.50	0.15	0.69	0.92	0.61–1.37	HTG	
			0.47	0.49	0.13	0.71	0.93	0.67–1.31	NHTG	
	rs6651224	C>G	*0.08*	*0.16*	*4.63*	*0.03*	*0.48*	*0.25–0.94*	HTG	*Significant*
			0.11	0.15	2.93	0.08	0.64	0.39–1.06	NHTG	
HABP1	rs1805429	T>C	0.21	0.16	1.26	0.26	1.35	0.79–2.28	HTG	
			0.19	0.16	0.41	0.52	1.15	0.73–1.82	NHTG	
	rs2472614	C>G	0.34	0.38	0.64	0.42	0.84	0.55–1.27	HTG	
			0.32	0.38	1.79	0.18	0.78	0.55–1.11	NHTG	
	rs8072363	C>T	0.46	0.5	0.49	0.47	0.86	0.58–1.28	HTG	
			0.47	0.5	0.35	0.55	0.90	0.64–1.26	NHTG	
HYAL3	rs2285044	C>T	0.03	0.02	0.74	0.38	1.72	0.49–5.98	HTG	
			0.02	0.02	0.15	0.69	1.24	0.40–3.84	NHTG	
	rs3774753	C>T	0.07	0.06	0.10	0.74	1.13	0.53–2.37	HTG	
			0.07	0.06	0.005	0.94	1.02	0.53–1.94	NHTG	
	rs13100173	G>A	0.26	0.25	0.05	0.82	1.05	0.67–1.63	HTG	
			0.25	0.25	0.01	0.91	0.98	0.67–1.43	NHTG	
	rs1076872	G>A	0.46	0.52	1.47	0.22	1.27	0.86–1.87	HTG	
			0.48	0.53	1.49	0.22	0.81	0.58–1.13	NHTG	

3. Results and discussion

In this study we explored the role of genetic variations in three genes, involved in HA metabolism, for glaucomatous neurodegeneration by case-control study. We genotyped 437 POAG patients (116 HTG & 321 non-HTG) and 96 glaucoma-negative controls followed by haplotypic and allelic association analyses.

3.1. The hyaluronan synthesizing gene, HAS2 is associated with HTG

rs6651224 (C>G) located in intron-II of *HAS2* showed significant association (chi square: 4.63; p-value: 0.03; OR: 0.49; 95% CI: 0.25–0.94) with the HTG patients. The frequency of the minor 'G' allele in the cases was significantly less than that in controls (0.08 vs 0.16), implicating its protective role in HTG [Fig. 1B(i)]. In NHTG group the variant showed the same trend but was not significant ($p = 0.08$; OR $=$ 0.65; 95% CI: 0.39–1.06) (Table 1). Another variation in *HAS2* rs1057308 (A>G), located at 5' UTR (exon 1) showed significant genotypic (GG) association with the HTG patients (Chi square: 7.18; p-value: 0.02). The higher frequency of GG (0.17) in the HTG with respect to the control groups (0.068) implicates a higher risk conferred by this genotype for HTG patients in the study population (Fig. 1C (ii)). We could not observe any association of this variant ($p = 0.18$) in NHTG group. Interestingly, rs1057308 is also located in the intergenic sequence of the natural antisense of *HAS2* (*HAS2AS*) complimentary to the 5' UTR of *HAS2*. *HAS2AS* is reported to hinder the hyaluronan synthesizing function of HAS2 [29]. rs1057308 can potentially alter the function of *HAS2AS* consequently altering the regulation of hyaluronan synthesis. As mentioned earlier, if produced in larger quantities, the HA polysaccharide deposits on the TM wall and may eventually elevate IOP by blocking the aqueous humor outflow [7].

Our data on genetic association of *HAS2* with POAG is supported by the previous observation on upregulation of HAS2 both in transcriptional as well as in translational level in bovine trabecular meshwork under treatment of the growth factors, TGF-beta and PDGF-BB simulating glaucomatous condition *in vitro* [16], implicating probable role of HAS2 in glaucomatous neurodegeneration.

3.2. A risk haplotype of hyaluronan binding gene HABP1 for both HTG and NHTG

In *HABP1*, the frequency of TCT haplotype (rs180 5429, rs2472614, rs8072363) was significantly higher in both HTG and NHTG patients ($p < 0.05$) compared to matched controls (0.16 in HTG, 0.18 in NHTG vs. 0.09 in controls), indicating that this is a risk haplotype for POAG (Fig. 2B). Our present observation on

Table 2
Haplotype association of the hyaluronan metabolic genes with POAG in Indian Population: Haplotypes with frequency greater than 5% in controls have been represented

Gene	Type of glaucoma	Haplotype	Frequency (%)		Haplotype association		Remark
			Case	Control	Chi-square	P-value	
HAS2	NHTG	GTA	9.64	14.06	1.39	> 0.05	
		CAA	48.55	48.95	0.003	> 0.05	
		CTG	36.17	33.34	0.24	> 0.05	
	HTG	GTA	6.9	14.06	3.64	> 0.05	
		CAA	51.29	48.95	0.11	> 0.05	
		CTG	39.22	33.34	1.03	> 0.05	
HABP1	NHTG	*TCT*	*16.55*	*9.89*	*4.48*	*< 0.05*	*Significant*
		TGT	32.95	38.54	0.81	> 0.05	
		CCT	32.63	34.89	0.14	> 0.05	
		CCC	15.11	14.06	0.07	> 0.05	
	HTG	*TCT*	*18.1*	*9.89*	*6.81*	*< 0.01*	*Significant*
		TGT	34.48	38.54	0.42	> 0.05	
		CCT	28.87	34.89	1.03	> 0.05	
		CCC	17.24	14.06	0.71	> 0.05	
HYAL3	NHTG	TTGG	19.13	15.1	1.07	> 0.05	
		TTAG	*24.75*	*16.43*	*4.21*	*< 0.05*	*Significant*
		TTGA	*49.03*	*34.93*	*5.69*	*< 0.05*	*Significant*
	HTG	TTGG	18.1	15.1	0.59	> 0.05	
		TTAG	*26.29*	*16.43*	*5.91*	*< 0.05*	*Significant*
		TTGA	*47.84*	*34.93*	*4.77*	*< 0.05*	*Significant*

the association of haplotype of *HABP1* in POAG is strongly supported by a recent report on significant up-regulation and synaptic relocalization of the complement component 1q (C1q) in adult retina during early stage of glaucoma [30] as well as the protective nature of mutant C1qa in glaucomatous mice model [31]. As mentioned earlier, C1q is one of the important ligands of the multifunctional *HABP1* [23,32], implicating its role in glaucoma. In addition, in a recent report, HABP1 has been found interacting with Forkhead Box C1 (FOXC1), which is principally responsible for axenfeld-rieger malformations in human with reports of glaucoma as a secondary complication [33]. HABP1 is predominantly cytoplasmic and its nuclear domain co-localizes with FOXC1 and helps in its transcription activation. Mutation (p.Phe112Ser) in FOXC1 disturbed its interaction with HABP1 that might eventually lead to eye disease [34]. Thus, our observation corroborating with these reports justifies the probable involvement of HABP1 in glaucomatous neurodegeneration. This can be independent through both FOXC1 and C1q or they might be working in synergy.

3.3. Hyaluronan catabolic gene HYAL3 represents two risk haplotypes for both HTG and NHTG

Four SNPs were selected (rs2285044, rs3774753, rs1310073, and rs1076872) from *HYAL3* in the present study. As depicted in Fig. 3C, TTAG and TTGA, the two major haplotypes were found to be significantly over represented ($p < 0.05$) in both HTG and NHTG compared to controls [(TTAG: HTG, 26.29%; NHTG, 24.75%; Controls, 16.43%) and (TTGA: HTG, 47.84%; NHTG, 49.03%; Controls, 34.93%)] (Table 2).

3.4. Gene-gene interaction network of hyaluronan metabolic genes in glaucoma

We explored possible interactions between the genotypes using a regression approach and observed *HABP1* plays an important role in both HTG and NHTG. In HTG, it interacts only with *HAS2*, not with *HYAL3* while in NHTG it associates with both of them. We observed AA of rs1057308 (*HAS2*) significantly co-occur more ($p < 0.01$) with TC of rs1805429 (*HABP1*) and CC of rs2472614 (*HABP1*) in HTG patients (8.62%) compared to controls (3.12%) (Fig. 4A). The same three SNPs as above in *HAS2* and *HABP1* with GG, TT and CG genotypes, respectively, co-occur with significantly higher frequency in HTG samples compared to normal individuals (4.31% vs 1.04%, $p < 0.01$) [data not shown]. This indicate that the two loci in *HABP1*, rs1805429 and rs2472614 are interacting in a dominant fashion (both one type homozygote and heterozygote co-occur more in HTG patients) via rs1057308 (*HAS2*) independent of its genotype (both AA and GG shows interaction). Interestingly, when TT of rs1805429

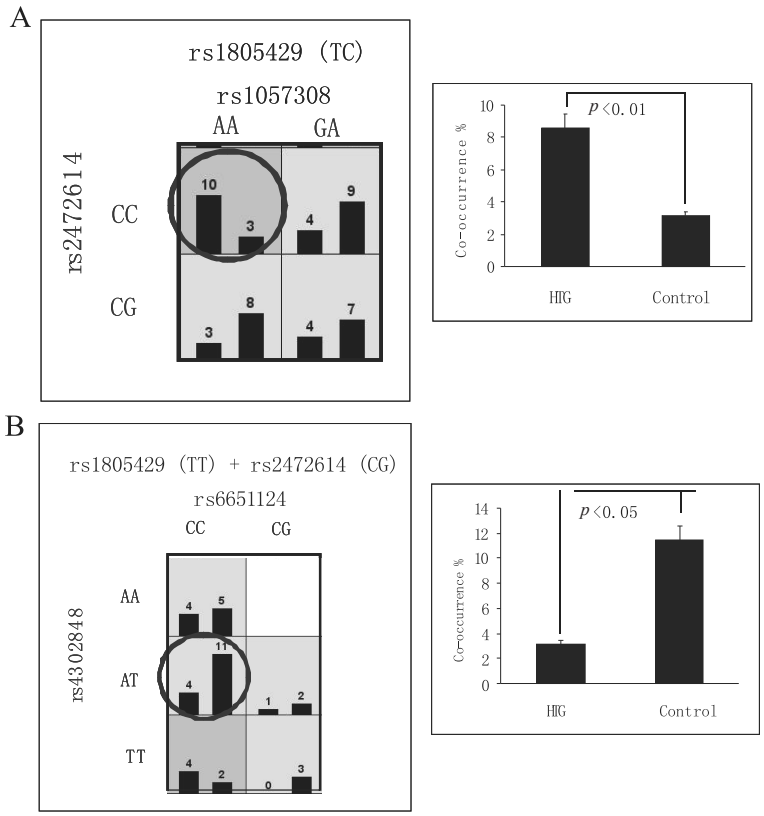

Fig. 4. Gene-gene interaction of hyaluronan synthesizing gene, *HAS2* and hyaluronan binding gene, *HABP1* in high tension glaucoma. In panel (A) MDR output shows AA of rs1057308 (*HAS2*) significantly co-occur ($p < 0.01$) with TC of rs1805429 (*HABP1*) and CC of rs2472614 (*HABP1*) in higher frequency in HTG patients (8.62%) compared to controls (3.12%) implicating risk interaction for HTG. Panel (B) shows significant co-occurrence ($p < 0.001$) of CC of rs6651224 (*HAS2*) and AT of rs4302848 (*HAS2*) with TT of rs1805429 (*HABP1*) and CG of rs2472614 (*HABP1*) in higher frequency in normal individuals (11.45%) in comparison to the HTG group (3.44%) implicating a protective interaction for HTG. The black bars represent distributions of cases (left) and controls (right). For each gene-gene interaction panel, in the left numbers above bars represent the numbers of cases and controls while in the right percentage is represented. In top row, high-risk cells are indicated by dark colour, low-risk cells by light colour, and empty cells by no colour. Case-control pairs marked with circle are magnified in the histogram and represented according to the frequency of co-occurrence.

(*HABP1*) and CG of rs2472614 (*HABP1*) were observed together with CC of rs6651224 (*HAS2*) and AT of rs4302848 (*HAS2*), the co-occurrence revealed putative protective effect for HTG (3.44% vs 11.45% in controls, $p < 0.05$). The results are depicted in Fig. 4B.

The overall interaction map from the gene-gene interaction analysis shows, rs1805429 of *HABP1* is the common member in both the best-fit protective and risk genetic interactions in HTG (Fig. 5). In this model, information gained about case-control status from knowledge about genotypes at one or more SNPs is measured by removal of entropy. The interaction map shows that rs6651224 of *HAS2* has the strongest synergistic binding with rs1805429 (8.98% of the total entropy). This observation corroborates with the protective allelic association data of rs6651224. On the other hand, interaction between rs1057308 of *HAS2* and rs1805429 has the maximum synergistic interaction (3.22% of the total entropy) potentially at-risk for HTG patients. We hypothesize that rs1805429 of *HABP1* may influence the effect of rs1057308 (present in both *HAS2* and *HAS2-AS*), to hinder the normal activity of natural antisense, and thus induces over-production of hyaluronan implying indirect role of the polymorphism (rs1805429) in aetiology of HTG. Interestingly, the locus rs2472614 of *HABP1* reveals a detrimental interaction with *HAS2* in HTG but it indicates beneficial interaction with *HAS2* and *HYAL3* in NHTG. This might lead to discovery of biological cross-talk between these molecules in the disease pathology.

Our present results indicate that the HA metabolic genes are involved in glaucomatous neurodegeneration probably by two different routes. In both, HTG and NHTG, hyaluronan binding protein (hyaladherin)

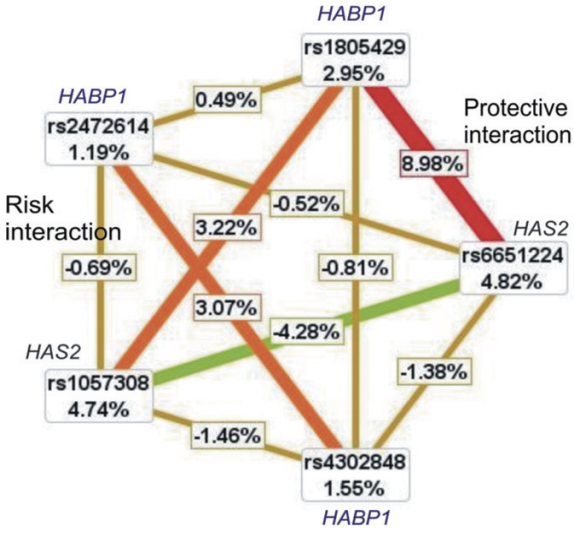

Fig. 5. Gene-gene interaction network of hyaluronan metabolic genes in glaucoma. Entropy-based interaction map generated from MDR analysis shows, *HABP1* and *HAS2* predominantly interact in the high tension glaucoma patients. rs1805429 of *HABP1* is the common member in both the protective and risk genetic interactions. Interaction among rs1805429 (*HABP1*), rs6651224 (*HAS2*), rs2472614 (*HABP1*) and rs4302848 (*HAS2*) is beneficial or protective for HTG while co-occurrence of rs2472614 (*HABP1*), rs1805429 (*HABP1*) and rs1057308 (*HAS2*) is detrimental for HTG. Strength of the genetic interactions has been represented by different shades of colour. Red is the strongest interaction, followed by orange then yellow ochre, while green represents the weakest interaction. The numerical value within box indicates % of entropy removed either by genetic connection or by the SNPs at nodes. The interaction map shows, strongest interaction between rs1805429 (*HABP1*) and rs6651224 (*HAS2*) (removing 8.98% entropy) while interaction between rs6651224 and rs1057308 (both of *HAS2*) is the weakest. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2012-0915)

HABP1 putatively act as an interacting partner. In case of HTG, it indicates interaction with the HA synthesizing *HAS2* while during NHTG pathogenesis, instead of *HAS2* the evidence points to its interaction with hyaluronidase *HYAL3*. Our finding is preliminary conducted in a small number of samples. Further in-depth genetic studies in large number of samples as well as functional assays are required to support our initial observations.

Hyaluronan is a major component of the human eye, predominantly present in vitreous humor [35] and also in the aqueous humor, conjunctiva, corneal stroma, iris, optic nerve etc [8,36]. Due to its viscous nature, the vitreous fluid has the potential to absorb shock and thus prevent trauma to the eye. It also plays an important role in optic nutrient transportation and ocular wound healing [8] and acts as a temporary matrix during the initial phase of wound healing in the corneal stroma. Together with its different binding protein partners (hyaladherin) like CSPG2/Versican, SPACR and SPACRCAN, HA helps in retinal development and retinal physiology [37]. It is reported, after the 5^{th} decade of life, eyes usually stop producing HA leading to several eye problems like poor vision, dry eyes and floaters [38]. In diseases like macular degeneration, retinitis pigmentosa and glaucoma, its level is altered [39,40]. Accumulation of HA due to upregulation of choroidal HAS2 is thought to play important role in stromal swelling during recovery from myopia [41]. Interestingly, in another recent study myopia has been indicated as a risk factor for POAG [42]. Modulation in expression of HA-binding proteins like CD44 and CSPG2/Versican has been reported in glaucoma [43, 44]. Inspite of excess-synthesis of HA on TM [7], its concentration was found decreased in aqueous humor in POAG patients in a different study [39]. In primary angle closure glaucoma (PACG), SPACR was upregulated in iris [45].

Since our observations along with other published works justify the importance of HA metabolism in the biological process of glaucoma and this field has not yet been explored much, in the present study we carefully ignored rigorous statistical correction so that any important signal might not get lost due to marginal association. It is advisable to further check the associations in large cohorts. Our present study on evaluating the association of genetic variants in the genes involved in HA metabolism provides the first genetic support to the earlier reports about the important role of HA and related genes in glaucomatous neurodegeneration. Interestingly, our results suggest that their involvement in the disease process might be via different routes for HTG and NHTG. Further studies on this area would provide more insight into these preliminary but important observations and, if proven, might provide new clues to disease management and therapy.

Acknowledgements

The authors wish to thank Dr. Mitali Mukerji for the initial support to carry out this research. We are thankful to Dr. Sudhir Giddaluru for helping in running PHASE software to generate haplotypes. Financial assistance for this study was obtained from grant numbers SIP-0006, SIP-0007, MLP-0016 and 60(0078)/07/EMR-II from Council for Scientific and Industrial Research (CSIR), Govt. of India. The senior research fellowship to K.B. has been provided by the CSIR, Govt. of India, Department of Science and Technology (DST), Govt. of India and Department of Biotechnology (DBT), Govt of India.

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