



Association of Versican (*VCAN*) gene polymorphisms rs251124 and rs2287926 (G428D), with intracranial aneurysm



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ABSTRACT

Intracranial aneurysm (IA) accounts for 85% of Subarachnoid Hemorrhage (SAH) and is mainly caused due to the weakening of arterial wall. The structural integrity of the intracranial arteries is mainly influenced by the extracellular matrix (ECM) remodeling. The Proteoglycan Versican plays an important role in extracellular matrix assembly and plays a major role in the pathogenesis of IA. The linkage studies also indicated *VCAN* as a putative candidate gene for IA in the 5q22–31 region. Using a case-control study design, we tested the hypothesis whether the variants in *VCAN* gene, nonsynonymous variants in the coding region of Glycosaminoglycan α (GAG- α) and GAG- β and two reported SNPs involved in splicing rs251124 and rs173686 can increase the risk of aSAH among South Indian patients, either independently, or by interacting with other risk factors of the disease. We selected 200 radiologically confirmed aneurysmal cases and 250 ethnically, age and sex matched controls from the Dravidian Malayalam speaking population of South India. The present study reiterated the earlier association of rs251124 with intracranial aneurysm ($P = 0.0002$) and also found a novel association with rs2287926 (G428D) in exon 7 coding for GAG- α with intracranial aneurysm ($P = 0.0015$).

Abbreviations: IA, Intracranial aneurysm; *VCAN*, Versican; ECM, Extracellular matrix; GAG- α , Glycosaminoglycan α ; GAG- β , Glycosaminoglycan β ; SNP, Single nucleotide polymorphism; aSAH, aneurysmal Subarachnoid Hemorrhage; LOX, Lysyl oxidase; LD, Linkage disequilibrium; AVM, Arteriovenous malformation; WFNS, World Federation of Neurosurgical Societies; CI, Confidence intervals

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Interestingly, both these SNPs contributed to higher risk for aneurysm in males. In-silico analysis predicted this SNP to have the highest functional relevance in the gene which might have a potentially altered regulatory role in transcription and splicing. Using meta-analysis with available literature rs251124 was found to be the strongest intracranial aneurysm marker for global ethnicities. This study with a novel functional SNP rs2287926 (G428D) further substantiates the potential role of *VCAN* in the pathogenesis of IA.

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Introduction

Intracranial aneurysm (IA) accounts for 85% of Subarachnoid Hemorrhage (SAH), which contributes for 5–15% of strokes, but occurs at a fairly young age (Bederson et al., 2000). Reports have shown a worldwide incidence of aSAH (aneurysmal Subarachnoid Hemorrhage) in 10.5 cases per 100,000 person-years (Ingall et al., 2000). In general population 2–3% individual harbor intracranial aneurysm (Rinkel et al., 1998). Prevalence of intracranial aneurysm in India based on autopsies ranges from 0.2% to 10.3% from various parts of India, with a mean prevalence of 5.3% (Banerjee et al., 1989; Kapoor and Kak, 2003; Ramamurthi, 1969). Familial clustering of intracranial aneurysm is reported with a four-fold risk of aneurysmal rupture in a first degree relative and identical aneurysm location in twins supports the genetic cause (Krischek and Inoue, 2006; Woo and Broderick, 2002). Hypertension and smoking may be causal risk factors which might also modify the effect of genetic factors that could increase susceptibility to aSAH in the Indian population (Koshy et al., 2010).

Various theories have been proposed for the development of aneurysm and most of these theories reflect on the imbalances in the extracellular matrix (ECM) remodeling. ECM remodeling plays an important role in maintaining the structure and integrity of the intracranial arteries. Reduced extracellular matrix is a prominent feature of cerebral aneurysms. In an earlier study we reported that lysyl oxidase (LOX) is an enzyme which is involved in covalent cross linking of these fibrous proteins by the formation of aldehydes between lysine residues that insolubilize these extracellular proteins namely collagen and elastin (Kagan and Li, 2003), thus giving strength to intracranial arteries was not found to be associated with IA in our study population (Sathyan et al., 2013). Proteoglycan Versican (*VCAN*) is a putative candidate gene for IAs as it plays an important role in extracellular matrix assembly and is localized near a previously implicated locus for IAs on chromosome 5q22–31 (Onda et al., 2001).

VCAN gene localized on 5q12–q14 has 15 exons (Iozzo et al., 1992) codes for large 372.82 kDa chondroitin sulfate proteoglycan found abundantly in the extracellular matrix and plays many pivotal roles in maintaining the functions of extracellular matrix. Versican plays an important role in cell adhesion by connecting the cell to the extracellular matrix with interacting partners hyaluronan, type I collagen, tenascin-R, fibulin-1 and -2, fibrillin-1, fibronectin, P- and L-selectins, and chemokines (Yao Jiong et al., 2005) and also in proliferation, migration and angiogenesis (Wight, 2002). Two largest exon 7 and exon 8 encodes for glycosaminoglycan (GAG) attachment sites, GAG alpha and GAG beta respectively. Alternate splicing at this region results in four transcripts; V0 possesses both exon 7 and exon 8, V1 possesses exon 8 but lacks exon 7, V2 possesses exon 7 but lacks exon 8; and V3 possesses neither exons (Wight, 2002). Polymorphism in these regions of the gene can have differential effects which can also have its effects in ethnic specific manner as polymorphisms are known to be ethnicity specific. To date no study has been carried out in these functionally coding regions of the gene and its role in intracranial aneurysms. Linkage studies have indicated 5q12–q14 which harbors the *VCAN* gene and two SNPs rs251124 and rs173686 which are upstream to exon 7 have been reported to be associated with aneurysm in a Dutch population. However, replication studies in different ethnicities on the linkage regions provided conflicting results in regard to intracranial aneurysm (Ruigrok et al., 2006, 2009; Sun et al., 2007; Zhu et al., 2013). In the present study we intend to explore the role of *VCAN* gene variants rs251124 and rs173686 along with four other nonsynonymous SNP variants rs2287926, rs188703, rs309559 and rs160277 in GAG- α and GAG- β coding exon 7 and exon 8 with intracranial aneurysm in the south Indian population.

Materials and methods

Study population

The study populations consist of radiologically confirmed 220 aneurismal cases and 250 ethnically and age matched controls from the Dravidian Malayalam speaking population of South India. This was specifically done to avoid stratification issues (Thomas et al., 2004). Inclusion criteria include saccular intracranial aneurysm detected using CT scan, and location and type of aneurysm were confirmed by digital subtraction angiography (DSA). Exclusion criteria include patient with non-saccular aneurysm, arteriovenous malformation (AVM) and other hereditary connective tissue disorders like autosomal dominant polycystic kidney disease, Marfan syndrome, and Ehler Danlos syndrome. Cases were recruited from two main tertiary care neurosurgical centers in Kerala. All patients were rated based on WFNS scale. The control population consists of age, sex and ethnicity matched individuals who were symptomatically normal and did not possess any symptoms or family history of intracranial aneurysm. The study was as per the ICMR guidelines and approved by Institute Ethical Committee (IEC).

SNP selection and genotyping

SNPs were selected based on the functional relevance and minor allele frequency using genotype data obtained from Caucasian individuals in the HapMap project (HapMap Data Rel 24/Phase II Nov08, on NCBI B36 assembly, dbSNP b126). Four nonsynonymous SNPs were selected which includes SNPs in functionally significant motif GAG- α and GAG- β present in VCAN propeptide, rs2287926 (G428D), rs160277 (D1950Y), rs188703 (R839H) and rs309559 (K529R), along with two other SNPs, rs251124 and rs173686 which were selected based on their presumed role in RNA splicing of this functionally significant motif and previous association in Dutch population (Ruigrok et al., 2006). Genotyping for rs2287926 (G428D), rs160277 (D1950Y), rs188703 (R839H) and rs309559 (K529R) was performed by fluorescence-based competitive allele-specific PCR (KASPar) chemistry (Kbiosciences, UK) while rs251124 and rs173686 were based on sequencing (ABI PRISM Big Dye Terminator v3.1 cycle sequencing kit) according to the manufacturer's instructions, and was analyzed using the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Supplementary Table 1). The KASPar reaction comprised of 8 μ l with 5 ng of DNA, 0.11 μ l of assay mix and 4 μ l of reaction mix and the PCR was performed in ABI 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA). The cycling conditions were as follows: 94 °C for 15 min (Hot-start enzyme activation, 94 °C for 20 s, a touchdown step for 10 cycles over 65–57 °C for 60 s (dropping 0.8 °C per cycle), and a final 26 step cycle with 94 °C for 20 s and 57 °C for 60 s). Further, the genotype calling based on the respective allele specific fluorescence was done by allelic discrimination utility of the SDS 7500 v2.0.5 software at an ambient temperature of 25 °C and genotype clusters were plotted.

Statistical analysis

Genotype and allele frequencies were computed and were checked for deviation from Hardy–Weinberg equilibrium (ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). All statistical analyses were performed using the Graph Pad Prism 5.01, San Diego, CA, USA. We considered P -value of <0.05 as significant. Chi-square test and allelic odds ratios (OR) and 95% confidence intervals (CI) were calculated by Fisher's exact test (two-tailed). To estimate LD between pairs of loci in the patient and control populations, standardized disequilibrium coefficient (D') and squared correlation coefficient (r^2) were calculated using Haploview 4.1 (www.broad.mit.edu/mpg/haploview/) (Barrett et al., 2005). LD blocks were defined in accordance with Gabriel's criteria (Gabriel et al., 2002). Further stratification of the patients based on sex and hypertension status was done to understand the role of VCAN variant within the sexes and between the hypertensive and non-hypertensive groups. However due to the exploratory nature of this study, no adjustment for multiple testing was made, as not to penalize ourselves by missing possibly important findings. rs251124 has been studied in populations including Dutch, Japanese and Chinese. Further, a meta-analysis of the studies with the random-effects and fixed-effects models was performed using Review Manager 5.2 (reviewmanager.software.informer.com/5.2/). The inconsistency index I^2 was used to assess between-study heterogeneity. Functional prediction of the deleterious effect if any, of the associated SNP with respect to the functional categories such as protein coding, splicing regulation, transcriptional regulation,

and post-translation was assessed in-silico using F-SNP program (compbio.cs.queensu.ca/F-SNP/), FastSNP (fastsnp.ibms.sinica.edu.tw), SNP Function Prediction (FuncPred) (snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm), SNP Nexus (www.snp-nexus.org/), and HaploReg (www.broadinstitute.org/mammals/haploreg).

F-SNP extracts information from large number of resources such as PolyPhen, SIFT, SNPeffect, SNPs3D, LS-SNP, Ensembl, ESEfinder, RescueESE, ESRSearch, PESX, TFSearch, Consite, GoldenPath, KinasePhos, OGPET, and Sulfinator to generate a Functional Significance (FS) score.

Results

The demographic and clinical characteristics of the patients and controls are summarized in Table 1. All the six SNPs screened were found to be polymorphic and were in Hardy–Weinberg equilibrium ($P \geq 0.05$) in healthy controls. We observed a novel and significant association of the non-synonymous variant rs2287926 (Gly428Asp) present in the GAG- α domain of VCAN. This association was observed at allelic ($P = 0.0015$, $P_{\text{corrected}} = 0.009$, OR = 1.635, CI = 1.207 to 2.215) and genotypic levels ($P = 0.0031$, $P_{\text{corrected}} = 0.0186$), where A allele poses a higher risk at both allelic and genotypic combinations (Table 2). None of the other non-synonymous variants in VCAN were found to differ in genotype and allele frequency distribution in our study samples (Table 2).

While replicating the previously reported associated variants, rs251124 and rs173686 which were presumed to have a role in RNA splicing of GAG domains, we could observe a significant association with rs251124 at allelic ($P = 0.0042$, $P_{\text{corrected}} = 0.0252$, OR = 1.511, CI = 1.141 to 2.002) and genotypic ($P = 0.0002$, $P_{\text{corrected}} = 0.0012$) levels (Table 2). The T allele was found to be significantly higher in aneurismal cases when compared to similar ethnic controls. At genotype level, CT genotype was found to be associated with intracranial aneurysm. Our study could not replicate the association with rs173686 at genotypic and allelic levels with intracranial aneurysm in the south Indian population.

Interestingly, we also observed that rs2287926 (Gly428Asp) A allele contributes to significantly higher risk for males at both allelic ($P = 0.02$, OR = 1.87, CI = 1.19–2.93) and genotypic levels ($P = 0.008$) when stratified based on sex (Table 3). While further stratifying the patient group based on hypertensive and non-hypertensive status we observed that the genetic risk contributed by rs251124 and rs2287926 (Gly428Asp) for intracranial aneurysm was independent of hypertension status (Table 4).

In silico analysis for the functional prediction of the variants in the present study indicated that rs2287926 had the highest functional significance with a functional score (FS) of 0.640. Polyphen predicted that this variant has a potential damaging effect on phenotype. Functional prediction of rs2287926 also suggested that it might have a potentially altered regulatory role in transcription and

Table 1
Clinical characteristics of patients.

Characteristics	Cases	Controls
Mean age \pm SD, years	51.17 \pm 11.37	51.0 \pm 14.1
Men, %	55.7	48.7
Women, %	44.2	51.3
History of hypertension,%	35	16
History of diabetes, %	5.4	14.7
Family history of aSAH, %	3.7	0
Cigarette smoking, %	42.92	18
Alcohol use, %	21.8	12
Intracranial aneurysm location	Percentage	
Anterior communicating artery	39.21	–
Anterior cerebral artery	8.37	–
Middle cerebral arteries	22.91	–
Internal carotid artery	17.18	–
Posterior communicating artery	8.81	–
Basilar artery	2.64	–
Posterior cerebral artery	0.44	–
Vertebral arteries	0.44	–

Table 2Comparison of the genotype and allele frequencies of *VCAN* gene variants between patients and control.

		CC	CT	TT	<i>P</i> -value	C	T	OR (95% CI)	<i>P</i> -value	
rs251124	Cases	81	113	18	0.0002	275	149	0.6616(0.4995 to 0.8764)	0.0042	
		0.382	0.533	0.085		0.649	0.351			
	Controls	142	87	23	371	133	0.563	0.345	0.091	0.736
		GG	AG	AA		G	A			
rs173686	Cases	56	107	46	0.6038	219	199	1.058(0.8156 to 1.371)	0.6913	
		0.268	0.512	0.220		0.524	0.476			
	Controls	69	118	64	256	246	0.275	0.470	0.255	0.510
		AA	AG	GG		A	G			
rs2287926	Cases	13	94	98	0.0031	120	290	1.635(1.207 to 2.215)	0.0015	
		0.063	0.459	0.478		0.293	0.707			
	Controls	10	83	162	103	407	0.039	0.325	0.635	0.202
		CC	CT	TT		C	T			
rs309559	Cases	30	95	74	0.3033	155	243	1.212(0.9220 to 1.594)	0.1841	
		0.151	0.477	0.372		0.389	0.611			
	Controls	26	119	103	171	325	0.105	0.480	0.415	0.345
		CC	CT	TT		C	T			
rs 188703	Cases	117	72	14	0.9368	306	100	1.009(0.7453 to 1.367)	1	
		0.576	0.355	0.069		0.754	0.246			
	Controls	143	93	16	379	125	0.567	0.369	0.063	0.752
		CC	AC	AA		C	A			
rs160277	Cases	118	75	17	0.676	311	109	0.9761(0.7264 to 1.312)	0.8804	
		0.562	0.357	0.081		0.740	0.260			
	Controls	141	98	16	380	130	0.553	0.384	0.063	0.745

splicing. The SNP rs251124 has also been predicted to have altered transcriptional regulation (Table 5) but was found to be of lesser functional significance when compared to rs2287926.

Subsequently we also carried out a meta-analysis of our observation with the previously reported studies to compare the prevalence of the associated variants of *VCAN* rs251124 and rs173686 among IA and controls, using both fixed and random-effects models. A significant association was observed in both random-effects and fixed-effects models for rs251124 (T v/s C; $P = 0.0001$, OR = 1.26, CI = 1.11–1.46), and heterogeneity among the studies was observed to be 24% from the I^2 values (Fig. 1). The south Indian population displayed a similar trend of association for aneurismal risk to the European population. However, the meta-analysis of rs173686 could not predict any significant association with IA susceptibility by either models (A v/s G; $P = 0.06$, OR = 1.15, CI = 1.00–1.33) and the heterogeneity was high ($I^2 = 68\%$) among the studies.

Discussion

The characteristic feature of IA is the ballooning of intracranial arteries which is indicative of weakening of arterial walls and thus one can speculate the role of defective ECM maintenance in IA pathogenesis (Wight et al, 2014). Versican plays a prominent role in the maintenance of extracellular matrix structure. It has also been implicated in cell proliferation (Ricciardelli et al., 2009; Wight, 2002), cell

Table 3

Comparison of the genotype and allele frequencies of rs251124 and rs2287926 *VCAN* gene variants within males and females in cases and control.

	Male	CC	CT	TT	P-value	C	T	OR (95% CI)	P-value																																																																										
rs251124	Case	0.400	0.530	0.070	0.0017	0.665	0.335	0.5326(0.3283 to 0.8639)	0.0124																																																																										
	Control	0.654	0.269	0.077		0.788	0.212				Female	CC	CT	TT		C	T			rs251124	Case	0.549	0.378	0.073	0.0773	0.738	0.262	1.635(1.022 to 2.615)	0.0443	Control	0.373	0.518	0.108	0.633	0.367		Male	AA	AG	GG		A	G			rs2287926	Case	0.070	0.440	0.490	0.0391	0.290	0.710	1.805(1.100 to 2.962)	0.0204	Control	0.012	0.345	0.643	0.185	0.815		Female	AA	AG	GG		A	G			rs2287926	Case	0.073	0.427	0.500	0.3209	0.287	0.713	1.465(0.8810 to 2.436)	0.1583	Control	0.038
	Female	CC	CT	TT		C	T																																																																												
rs251124	Case	0.549	0.378	0.073	0.0773	0.738	0.262	1.635(1.022 to 2.615)	0.0443																																																																										
	Control	0.373	0.518	0.108		0.633	0.367				Male	AA	AG	GG		A	G			rs2287926	Case	0.070	0.440	0.490	0.0391	0.290	0.710	1.805(1.100 to 2.962)	0.0204	Control	0.012	0.345	0.643	0.185	0.815		Female	AA	AG	GG		A	G			rs2287926	Case	0.073	0.427	0.500	0.3209	0.287	0.713	1.465(0.8810 to 2.436)	0.1583	Control	0.038	0.354	0.608	0.215	0.785																						
	Male	AA	AG	GG		A	G																																																																												
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	Control	0.012	0.345	0.643		0.185	0.815				Female	AA	AG	GG		A	G			rs2287926	Case	0.073	0.427	0.500	0.3209	0.287	0.713	1.465(0.8810 to 2.436)	0.1583	Control	0.038	0.354	0.608	0.215	0.785																																																
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	Control	0.038	0.354	0.608		0.215	0.785																																																																												

adhesion (Yamagata and Kimata, 1994), cell migration and invasion (Huang et al., 2006). *VCAN* gene is known to produce four isoforms V0, V1, V2 and V3 by alternate splicing of exon 7 and exon 8 (Dours-Zimmermann and Zimmermann, 1994). V1 isoform contains GAG- β and lacks GAG- α domain while V2 isoform contains GAG- α and lacks GAG- β domain (Lemire et al., 1999). The GAG- α and GAG- β are the key glycosaminoglycan binding G2 domain of Versican proteoglycan which are encoded by exons 7 and 8. In the present study we analyzed the genetic variants in these domains represented by rs2287926 in exon 7 (coding GAG- α) and rs160277, rs188703 and rs309559 in exon 8 (coding GAG- β). Our study revealed a novel association for nonsynonymous variant rs2287926 (G428D) in exon 7 coding for GAG- α domain where A allele was found to be a risk allele for intracranial aneurysm in the south Indian population. We also carried out a replication study of the two previously reported associated variants rs251124 and rs173686 for intracranial aneurysm in the south Indian population. rs251124 and rs173686 are present in the intronic regions upstream to exon 7. Interestingly one of the SNP rs251124 was found to be strongly associated with aneurysm in our study population. This observation is interesting as rs251124 was found to be associated across all ethnicities as evidenced from our meta-analysis. However, rs173686 was not found to be associated in the study population as well as in the meta-analysis. This could be due to the influence of Asian ethnicities as in Chinese population rs173686 was also not found to be associated with aneurysm (Ruigrok et al., 2009; Sun et al., 2007). The odds ratio for rs251124 in the south Indian population was similar to the European population. Interestingly the allele and genotype frequencies for rs251124 when compared to the 1000 genome population were also similar to the European population while rs2287926 was similar to Asian ethnic populations (Supplementary Figs. 1 and 2). From the allele and genotype frequencies of the 1000 genome population it is evident that the intronic SNPs in the South Indian population were similar to the European population while the exonic associated SNPs were similar to the Asian ethnicity. This suggests for a different level of selection pressures in intronic and the functional exonic regions of the *VCAN* gene.

Table 4

Comparison of the genotype and allele frequencies of rs251124 and rs2287926 *VCAN* gene variants within patient with hypertension and non-Hypertensive status.

SNP	Hypertension status	CC	CT	TT	P-value	C	T	OR (95% CI)	P-value																						
rs251124	Hyp+	0.391	0.563	0.047	0.2119	0.672	0.328	1.103(0.6950 to 1.749)	0.7258																						
	Hyp-	0.418	0.464	0.118		0.650	0.350					AA	AG	GG		A	G			rs2287926	Hyp+	0.091	0.364	0.545	0.339	0.273	0.727	0.8967(0.5576 to 1.442)	0.7185	Hyp-	0.060
		AA	AG	GG		A	G																								
rs2287926	Hyp+	0.091	0.364	0.545	0.339	0.273	0.727	0.8967(0.5576 to 1.442)	0.7185																						
	Hyp-	0.060	0.470	0.470		0.295	0.705																								

Table 5
Prediction of effect of studied SNPs in this study using F-SNP database.

SNP	Functional category	Prediction tool	Prediction result	Functional information
rs160277 (D1950Y)	protein_coding	PolyPhen	Possibly damaging	FS score = 0.560
		SIFT	Damaging	
		SNPeffect	Deleterious	
	splicing_regulation	LS-SNP	Benign	
		SNPs3D	No entry	
		Ensembl-NS	Nonsynonymous	
		ESEfinder	Changed	
		ESRSearch	changed	
		PESX	Changed	
	transcriptional_regulation post_translation	RESCUE_ESE	Not changed	
		GoldenPath	Exist	
		OGPET	Not exist	
rs188703 (R839H)	protein_coding	Sulfinator	Not exist	FS score = 0.5
		PolyPhen	Benign	
		SIFT	Tolerated	
	splicing_regulation	SNPeffect	Benign	
		LS-SNP	Benign	
		SNPs3D	No entry	
		Ensembl-NS	Nonsynonymous	
		ESEfinder	Changed	
		ESRSearch	Changed	
	transcriptional_regulation post_translation	PESX	Changed	
		RESCUE_ESE	Not changed	
		GoldenPath	Exist	
rs309559 (K529R)	protein_coding	OGPET	Exist	FS score = 0.347
		Sulfinator	Not processed	
		PolyPhen	Benign	
	splicing_regulation	SIFT	Tolerated	
		SNPeffect	Deleterious	
		LS-SNP	Benign	
		SNPs3D	No entry	
		Ensembl-NS	Nonsynonymous	
		ESEfinder	Changed	
	transcriptional_regulation post_translation	ESRSearch	Changed	
		PESX	Not changed	
		RESCUE_ESE	Not changed	
rs2287926 (G428D)	protein_coding	GoldenPath	Exist	FS score = 0.640
		OGPET	Not exist	
		Sulfinator	Not exist	
	splicing_regulation	PolyPhen	Possibly damaging	
		SIFT	Tolerated	
		SNPeffect	Benign	
		LS-SNP	Benign	
		SNPs3D	No entry	
		Ensembl-NS	Nonsynonymous	
	transcriptional_regulation post_translation	ESEfinder	Changed	
		ESRSearch	Changed	
		PESX	Changed	
transcriptional_regulation post_translation	RESCUE_ESE	Not changed		
	GoldenPath	Exist		
	OGPET	Not exist		
rs251124	transcriptional_regulation	Sulfinator	Not exist	FS score = 0.101
	transcriptional_regulation	GoldenPath	Exist	FS score = 0.101

To date no study has implicated the role of functional variants in *VCAN* to be associated with intracranial aneurysm. However, functional variants in this gene have been implicated in cancer. In an earlier study in a Korean population the genetic variants in the GAG- β domain of *VCAN* A1826H and D2937Y have been reported to influence susceptibility to intestinal-type gastric cancer (Ju et al., 2010).

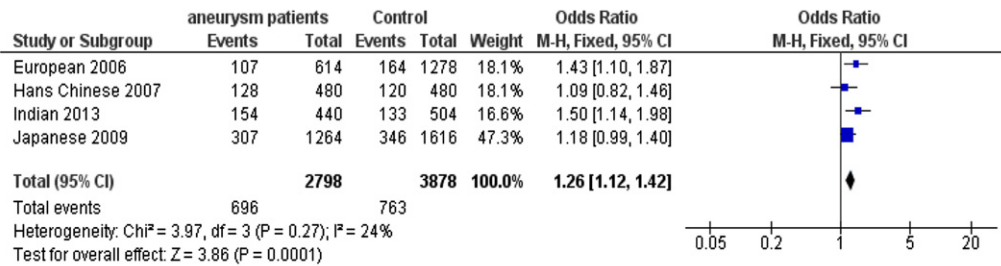
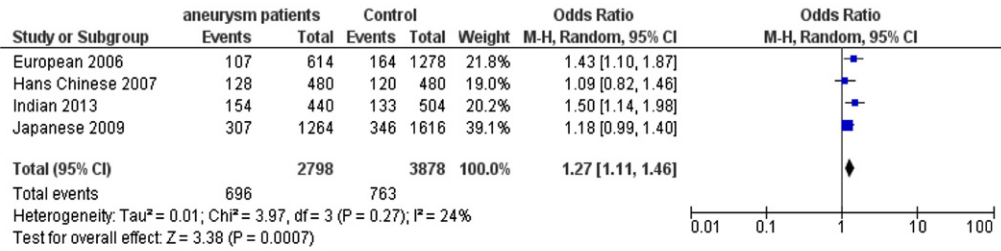
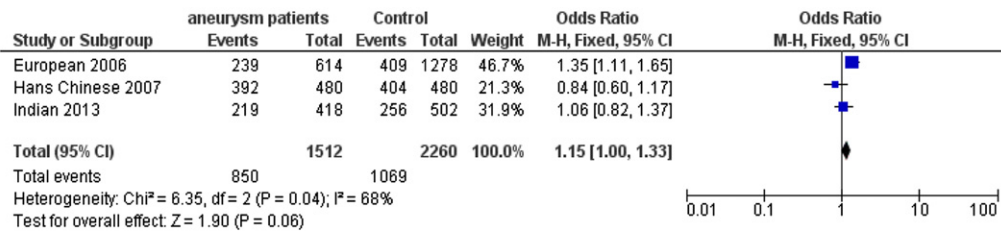
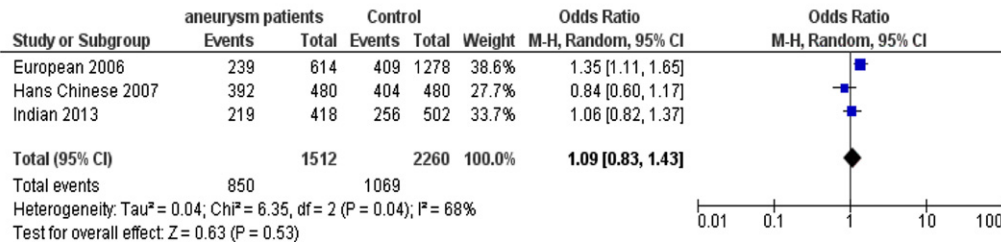
a)rs251124**b)rs173686**

Fig. 1. Metanalysis of VCAN gene variants rs251124 (a) and rs173686 (b) with intracranial aneurysm.

This observation is interesting as we observe that it is not the GAG- β domain but instead the genetic variant in the GAG- α domain rs2287926 (G428D) in exon 7 results in the development of aneurysm. This study clearly demonstrates that different domains in the VCAN gene associate differently with intracranial aneurysm and gastric cancer phenotype. This contrasting observation in the present study could be explained with following reasons at functional level. It has been reported that V1 and V2 isoforms behave differently with opposite biological activity (Sheng et al., 2005). V1 isoform harboring GAG- β domain enhances cell proliferation, modulates cell cycle progression and protects cells from apoptosis, which clearly supports the variants' role in causing a cancerous phenotype while V2 isoform harboring the

GAG- α domain inhibits cell proliferation and expression of EGFR and cyclin A and does not protect cell from apoptosis (Sheng et al., 2005). Further VCAN V1 isoform induces neuronal differentiation and promotes neurite outgrowth (Wu et al., 2004) while V2 inhibit axonal growth (Schmalfeldt et al., 2000). A similar phenotype shown by the V2 isoform may be contributing to the inhibition of the growth of smooth muscle and other vascular entities causing intracranial aneurysm. Interestingly the associated variants rs251124 and rs2287926 (Gly428Asp) for intracranial aneurysm was found to be independent of confounding factors such as hypertension status.

We conclude that this study confirms the association of rs251124 with intracranial aneurysm as a global marker however, its exact functional role is still not very clear. In addition we report a novel function variant rs2287926 (G428D) in the GAG- α domain of VCAN gene which might have a potentially altered regulatory role in transcription and splicing. Interestingly, this variant poses a higher risk for males independent of its confounding factors such as hypertension. Thus VCAN is an important candidate gene involved in the pathogenesis of intracranial aneurysm.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.mgene.2014.07.001>.

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