Genetic polymorphism of Cytochrome-P450-2C9 (CYP2C9) in Indian populations

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Abstract

Cytochrome-P450-2C9 (*CYP2C9*) metabolizes wide range of drugs and highly express in human liver. Various mutations of *CYP2C9* (R144C, I359L etc.), associated with drug-response, are highly diverse. We aimed to investigate the genetic diversity of *CYP2C9* in Indian-subcontinent, using 1278 subjects from 36 populations. High frequency of *CYP2C9*3* (0-0.179) was observed, comparative to other populations, including Europeans. Subjects having *CYP2C9*3/*3* requires lower dose of warfarin, comparative to *CYP2C9*1/*3* or *CYP2C9*1/*1*. Since, Indians are practicing marriage among their caste system, we predicted and observed high frequency (0-0.05) of *CYP2C9*3/*3*. Out of 21 populations, living outside of Indian subcontinent, only Toscani and Southern Han-Chinese have 0.009 and 0.01 *CYP2C9*3/*3*, respectively, lower than Indians,. We found a non-synonymous mutation (*L362V*), observed only in Indian-subcontinent, and have 0-0.056 allelic, 0-0.037 *L/V* and 0-0.037 *V/V* genotype frequency. We observed unfavorable interatomic interactions between hydroxylation sites of warfarin and reactive oxyferryl heme in mutant, comparative to wild-type *CYP2C9*, in molecular dynamic simulations; and predict lower kinetic activity.

Introduction

Heterogeneous drug response is the major hurdle in the successful treatment of diseases and depends on the genetic variations of drug metabolizing enzyme genes. Cytochrome P450 (CYP) family is an important enzyme of ADME (related to absorption, distribution, metabolism and excretion of drug) genes, of which *CYP2C9* is the major constituent of CYP2C subfamily in human liver. It metabolizes wide range of drugs including anti-coagulant (warfarin), non-steroidal anti-inflammatory (celecoxib, diclofenac), anti-diabetic (netaglinide, tolbutamide), anti-hypertensive (irbesartan, losartan) and anti-epileptic (phenytoin)[1]. Several variations in *CYP2C9* have been reported which affects metabolism of the drug. Most notable variations are *CYP2C9*2* (R144C) and *CYP2C9*3* (I359L) which decreases 12% and 5% enzyme activity, respectively[2]. Interestingly, these variations are highly heterogeneous among world population; (1) 8-19% and 3.3-16.3% in Caucasian; (2) 0-0.1% and 1.1-3.6% in Asian; (3) 2.9% and 2.0% in African-American; and (4) 0-4.3% and 0-2.3% in Black/African, respectively[3]. Moreover, other rare and functionally relevant variations were also reported in various populations, which includes; (1) *CYP2C9*6*, 0.6% frequency in African-Americans[4]; (2) *CYP2C9*4*, 0.5% in African-Americans and 6% in Caucasians [2, 5]; and (3) *CYP2C9*13*, 0.19-0.45% in Asian[6]. Recently, Dai et al. (2013) reported several rare variants in the Han Chinese population[7].

Several studies have been performed on *CYP2C9* in Indian populations. However, most of studies focused only on *CYP2C9*3* and *CYP2C9*2*. Recently, Anil et al (2014) found that *CYP2C9*3* present only in Indo-European population with 0.38–1.85%, while absent in Dravidian, Austro-Asiatic and Tibeto-Burman populations[8]. Indian populations are well known for their endogamy practices and must have very high frequency of homozygous

allele[9], however, Anil et al (2014) did not observe any homozygous *CYP2C9*3/*3* genotype. Many studies have shown that the variations in *CYP2C9* are associated with therapeutic heterogeneity in Indian populations. *CYP2C9*2* and **3* has been reported with less hydroxylation (or metabolism) of phenytoin *in vivo* in South-Indian populations[10], comparative to wild type *CYP2C9*1*. Ramasamy et al. (2007) reported phenytoin toxicity in a patient with normal dose of 300mg/day, who had *CYP2C9*3/*3* genotype[11]. The same symptoms were reported by Thakkar, A. N. et al. (2010) in South-Indian populations[12]. Many studies have also demonstrated that the Indian populations need high dose of warfarin and phenytoin compared to Caucasians[12]. Both of these drugs are metabolized by *CYP2C9*. Some of the drugs, metabolized by *CYP2C9* have narrow therapeutic index *e.g.* warfarin, phenytoin and tolbutamide. This is the reason that small change in the activity of *CYP2C9* may cause major changes in individual's response.

Considering the high genetic diversity in Indian sub-continent, we explore functionally relevant variations in *CYP2C9*, in the present study. The outcome can be utilized to understand heterogeneous therapeutic response and in development of personalized therapy in Indian sub-continent.

Material and methods

Details of samples

In total, 1278 samples were selected for the study. To find the distribution of *CYP2C9* allele/genotype frequency within Indian subcontinent, 36 populations of different linguistic groups and geographical locations were selected (**Table S1)**[9, 13]; and further compared with

populations of 1000 genome project. Present work has been approved by the Institutional Ethical Committee of CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad, India. Informed written consent has been obtained from every participant, prior to collection of blood samples.

Re-sequencing of CYP2C9, genotyping and analysis

All the 9 exons, their respective intron-exon boundary, 3' and 5' UTR of *CYP2C9* have been resequenced. For designing of primer, DNA sequence of ENST00000260682 from Ensembl (v75) has been used. Out of 3 mRNA of *CYP2C9*, only ENST00000260682 translate to protein. Primer3.0 web-based tool (http://simgene.com/Primer3) was used for designing the primers and further primers specificity were checked with NCBI-primer blast. The details of primer sequences are given in **Table S2**. Polymerase chain reaction (PCR) was performed in 10.0 µl solution, which contains 5.0 µl of 2x EmeraldAmp GT PCR master mix, 10-20 ng of genomic DNA and 0.1 pmole (final concentration) of each primer. Thermal cycling conditions used are as follows: initial denaturation step of 5 min at 94ºc, followed by 35 cycles of denaturation step of 30 sec at 94ºc, annealing step of 30 sec at their respective melting temperature, extension step of 2 min at 72ºc, followed by single step of final extension of 7 min at 72ºc. PCR products were cleaned with Exo-SAP-IT (USB, Affymetrix, USA) with recommended protocol of the manufacturer. Cleaned PCR product (1.0 µl) has been subjected to sequencing PCR using BigDye terminator (v3.1) cycle sequencing kit (Applied Biosystem, USA) and analyzed using ABI 3730xl DNA sequencer. AutoAssembler (v1.0) was used for assembling and manual editing of sequence data.

Distal effect of L362V on kinetics of CYP2C9: molecular dynamics simulation

Preparation of 3D (3 dimensional) structure

We performed the molecular dynamics simulation with Gromacs (version 5.0.2)[14]. Starting structure of *CYP2C9* was obtained from PDB (code: 1OG5). We removed warfarin drug from 1OG5 with Chimera (version 1.11)[15] and utilized in the further structure modifications. In total, 7 amino acids (K206E, I215V, C216Y, S220P, P221A, I223L and I224L) were substituted in wild type *CYP2C9*, to enhance the crystallization[16]. Hence, we modified back 1OG5 to wild type sequence with FoldX (version 2.6) and further utilized to generate mutant proteins. We generated 3D structure of L362V, with FoldX.

Docking of warfarin drug

Since, warfarin was located farther from heme center and in catalytically non-reactive stage; we performed docking with AutoDock Vina (version 1.1.2) to obtain putative functional confirmation of drug in the active site of *CYP2C9*[17]. Autodock tools were used for generating partial charges of warfarin and *CYP2C9* using Gasteiger method[18]. Confirmation of warfarin, having lowest binding energy, was selected in further analysis.

Simulation

To perform molecular dynamics simulation, Gromacs (version 5.0.2) was used[14]. We assigned the partial charges of warfarin and oxyferryl state of heme group, as described by Seifert, A *et. al* (2006)[19], while for generating partial charges in wild-type/mutant protein, we used Amber force field "ff99SB". Before assigning partial charges and structure

modifications, we removed H atom from ligand bound protein, with Babel software[20]. Further, structure was loaded in Tleap (AmberTools)[21] to modify; (1) creating single bond S-Fe, (S of CYM406 and Fe of heme group), (2) creating single bond Fe-O (Fe of heme group and O of water molecule/H_2O464) and (3) removal of H atom from H_2O464.

For all ligand bound models (wild-type/mutant), initially we placed the molecule in 1nm cubic box and then solvated it with TIP3P water molecules; and neutralize the system with sodium (NA) molecules. Energy minimization of the system was performed in 10,000 steps with steepest descent minimization algorithm. The potential energy of system has been demonstrated in **Figure S1**. Further, we equilibrated the system at ~310K (normal human body temperature) and at pressure ~1 bar in 20 and 100 pico seconds (ps) respectively in 2 different steps (**Figure S2**). After this, system was simulated for 15 nano seconds (ns), in 4 replicates. To calculate the rolling mean in 100 ps, we utilized the zoo package of R[22].

Results and discussions

*Diversity of CYP2C9*3 in Indian populations*

The A→C (rs1057910/ *CYP2C9*3*) is a non-synonymous mutation, which replace Isoleucine with Leucine (ATT>CTT; Ile359Leu; low enzyme activity). Considering the higher level of evidence between *CYP2C9*3* and drug response, CPIC (Clinical Pharmacogenomics Implementation Consortium) has been categorized *CYP2C9*3* under level-1A[23]. *CYP2C9*3* has been reported with hypersensitive reaction against phenytoin in epilepsy patients[24], decreased metabolism of celecoxib[25]. It is also reported with high incident of response rate against sulfonamides, urea derivatives[26].

To explore the "C" allele frequency in Indian populations, initially we confirmed Hardy-Weinberg equilibrium (HWE). It was observed that 10 populations were not in HWE (*p*-value < 0.05), which include 1 Indo-European population, Haryana Pandit (p-value = 1.3×10^{-4}) and 9 Dravidians populations; Mudaliar and Madar from Tamil Nadu (p-value = 1.92×10^{-6} and 4.75×10^{-7} respectively), Gawali from Karnataka (p-value = 4.12×10^{-4}), Kurumba from Kerala (p-value = 6.94×10^{-6}) and Telagas, Thoti, Chenchu, Patkar and Vaddera from Andhra Pradesh (p-value = 1.6×10^{-3}, 2.23×10^{-8}, 0, 5.21×10^{-6} and 6.99×10^{-3} respectively) (**Table 1**).

After excluding these 10 populations, we estimated 11.67% (183 out of 1568) "C" allele in Indian populations, similar (p-value = 0.617) to South-Asian populations of 1000 genome project. Further, we categorized these samples on the basis of their linguists and observed that Dravidians have higher percentage of "C" allele (15.32%; 72 out of 470) while Tibeto-Burman have lowest (6.12%; 6 out of 92). Moreover, in Austro-Asiatics and Indo-European, we observed 9.96% (46 out of 416) and 10.96% (59 out of 538), respectively (**Table 1**). Interestingly, Tibeto-Burman are insignificantly different (p-value = 0.21) from East-Asians. Adi-Dravidians (schedule tribe) of TamilNadu, Ho (schedule tribe) of Jharkhand and Baiswar (caste) of Uttar-Pradesh have 17.857%, 15.385% and 16.176% of *CYP2C9*3*, respectively, which is higher in their respective linguistic group; while Bhil of Gujarat, Raj-Gond of Madhya-Pradesh and Gond of Chhattisgarh have 0%, 0%, 2%, respectively (**Table 1)**. In Indian sub-continent, high local heterogeneity was observed and any correlation with geographical location does not exist (**Figure 1A** and **Table 1**). It is evident in isofrequency map that Indian populations have high frequency of *CYP2C9*3*, comparative to other world populations (**Figure 1A**). We observed, decreasing gradient of "C" allele frequency from Indian subcontinent to Europeans (**Figure 1A**).

On the basis of founder events and endogamy marriage practices, we have already predicted high frequency of homozygous alleles in Indian populations[9]. Since, patients with *CYP2C9*3/*3* requires lower dose (0.5-2 mg) of warfarin in comparison to those who have *CYP2C9*1/*3* (3-4 mg), it would be interesting to explore in Indian populations. As expected, we observed higher percentage (<5%) of *CYP2C9*3/*3* in Indians, comparative to other world populations, who have 0-1% (**Figure 1B and Table 1**). Out of 21 populations who are living out of Indian subcontinent, only TSI (Italian populations) and CHS (South Chinese populations) have homozygous genotype (0.9 and 1%), while out of 5 populations who are living in Indian sub-continent, 3 (PJL, ITU and GIH) have 1% *CYP2C9*3/*3*. In present Indian populations samples, we observed 0-5% *CYP2C9*3/*3*, of which Bhilala of Madhya-Pradesh and Ho of Jharkhand have 5% and 3%, respectively; higher in Indo-Europeans and Austro-Asiatic linguistic group (**Table 1**). We observed 0% *CYP2C9*3/*3 in Tibeto-Burman. Since, *CYP2C9*3/*3* was not in HWE in Dravidian populations, we did not concluded the frequency.

Novel non-synonymous variant L362V

We observed a non-synonymous mutation L362V in Indian population at higher frequency. Since, this mutation was observed in higher frequency and present only in Indian populations; we further explored it in 1000 genome project samples. We found that this variant (rs578144976; L362V) was reported at very low frequency (0.4%) in South-Asians, while absent in other world populations. Only ITU (Telugu population) and BEB (Bengali population) are reported to have 0.6% and 1.5% (http://browser.1000genomes.org/Homo_sapiens/Variation/Population?db=core;g=ENSG000

00138109;r=10:96698415-96749147;v=ss1338631398;vdb=variation;vf=75731685). In Indian sub-continent, it was observed in all four linguistic groups (13 populations). After excluding the populations in which L362V is not in HWE, we observed 0-5.6% allele, 0-3.7% heterozygous and 0-3.7% homozygous mutant genotype frequency (**Table 2, Figure 2A and 2B**). It can be hypothesize that L362V is originated recently in Indian sub-continent and due to long-term isolation of Indian populations with rest of the world, it was observed only in this region. This might be the reason also, why L362V, deviated from Hardy-Weinberg equilibrium in many populations.

Effect of L362V: In-Silico prediction

Lertkiatmongkol, P. *et. al* (2013)[27] predicted distal effect of amino acid substitution in *CYP2C9* in molecular dynamics simulation. Authors observed that distance between hydroxylation sites of warfarin and reactive oxyferryl heme in mutant (*CYP2C9*3*: FeO-C7=4.85 Å; *CYP2C9*13*: FeO-C7=3.56 Å; and *CYP2C9*2*: FeO-C7=4.46 Å) is higher, comparative to wild type protein (FeO-C7=3.30 Å). This unfavorable interatomic interaction is the reason of lower kinetic activity in mutant proteins. We utilized same molecular dynamics simulation to explore the distal effect of L362V on the kinetic activity of *CYP2C9*, comparative to wild type protein.

We performed 4 different simulations for wild type and mutant (L362V) *CYP2C9*, after re-docking warfarin in active site (**Figure 3**) and equilibrating system (**Figure S2**). The system contains water molecular and wild-type/mutant *CYP2C9* in 1×1×1 nm^3 cubic box, neutralized by sodium ion (Na^+). We observed that in all 4 simulations, wild-type and mutant *CYP2C9* protein were in stable state [radius of gyration (R_g); for wild type, 1st: 2.249±0.0062, 2nd:

2.246±0.0058, 3rd: 2.243±0.0058 and 4th: 2.247±0.0064; for mutant, 1st: 2.266±0.0104, 2nd: 2.263± 0.0062, 3rd: 2.59±0.0062 and 4th: 2.253±0.0058] (**Figure S3**). Hydroxylation at C6, C7 and C4 of warfarin needs the hydrogen bond between H6, H7 and H4 of warfarin and FeO (oxygen) of oxyferryl heme. Therefore, we consider only those confirmations in active state, which are having rolling mean distance of H···O < 3Å (distance of C-H···O). We observed unfavorable interatomic interactions for hydroxylation between (1) H6 and FeO and (2) H7 and FeO in simulation of mutant, comparative to wild-type *CYP2C9* (**Figure 4, 6** and **S4**). The simulations predict the less kinetic activity of the mutant (L362V) *CYP2C9*, comparative to wild-type.

Other rare variants

Besides these, a few rare variants have also been observed in this study. The non-synonymous C>T mutation (rs28371685), which replace arginine with tryptophan (R335W) and determine *CYP2C9*11* haplogroup was found only in 3 individuals (0.2347%, 1 each in Chenchu, Telagas of Andhra Pradesh and Mudliar of Tamil Nadu). Besides this, 2 novel non-synonymous mutations F482L and T283S were found in 2 individual (0.0782%), each in Mizo (Mizoram) and Warli (Maharashtra), respectively.

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Figure legend

Figure 1. Isofrequency map of (A) *CYP2C9*3* and (B) *CYP2C9*3/*3* to demonstrate the geospatial distribution. We excluded those populations who were not in HWE. In map, dots represent the sampling location.

Figure 2. Isofrequency map of novel mutant L362V. Figure (A) and (B) represents the allelic and genotype geospatial distribution. Similar to figure 1, we excluded those populations who were not in HWE.

Figure 3. Confirmation of warfarin in (A) original 3D structure (1OG5), dowloaded from PDB and (B) after re-docking.

Figure 4. Distance between FeO of oxyferryl heme group and hydrogen atom (H6) bound to C6 of warfarin. The middle orange line represents rolling mean of distance with window size of 100 ps while blue line represents the cut-off value (< 3 Å) for favorable hydrogen bond. Hydrogen atom H6 attains favorable interactions for hydroxylation (H-bond < 3 Å) only in wild-type *CYP2C9* (during 1^{st}, 2^{nd} and 3^{rd} molecular dynamics simulation).

Figure 5. Distance between FeO of oxyferryl heme group and H7 of warfarin. Orange and blue lines represents similar values, given in figure 4. Hydrogen atom H7 attains favorable interactions for hydroxylation (H-bond < 3 Å) only in wild-type *CYP2C9* (during 2^{nd} molecular dynamics simulation).

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Table 1. Distribution of *CYP2C9*3* (I359L) in Indian populations

Populations	State	Lat.	Long.	Linguistic	Sample size	Genotype/Allele percentage (number of individuals): rs1057910						HWE p-value
						Missing data	AA	AC	CC	A	C	
Mahli	Jharkhand	23.46	85	Austro-Asiatic	38	4(10.526)	27(79.412)	6(17.647)	1(2.941)	60(88.235)	8(11.765)	0.3712145
Gond	Chattisgarh	19.87	81.6	Austro-Asiatics	37	8(21.622)	28(96.552)	0(0)	1(3.448)	56(96.552)	2(3.448)	0.018
Kharia	Chattisgarh	23.33	85.44	Austro-Asiatics	86	14(16.279)	62(86.111)	10(13.889)	0(0)	134(93.056)	10(6.944)	1
Gond	Madhya-Pradesh	26.12	77.4	Austro-Asiatics	38	7(18.421)	25(80.645)	6(19.355)	0(0)	56(90.323)	6(9.677)	1
Ho	Jharkhand	23.35	85.33	Austro-Asiatics	67	2(2.985)	47(72.308)	16(24.615)	2(3.077)	110(84.615)	20(15.385)	0.633
Kolhas	Andhra-Pradesh	14.46	79.98	Dravidian	14	2(14.286)	10(83.333)	2(16.667)	0(0)	22(91.667)	2(8.333)	1
Adi-Dravidar	Tamilnadu	11.35	77.73	Dravidian	15	1(6.667)	9(64.286)	5(35.714)	0(0)	23(82.143)	5(17.857)	1
Telagas	Andhra-Pradesh	18.17	83.53	Dravidians	12	0(0)	9(75)	0(0)	3(25)	18(75)	6(25)	*0.001634521*
Thoti	Andhra-Pradesh	16.51	80.64	Dravidians	29	0(0)	20(68.966)	0(0)	9(31.034)	40(68.966)	18(31.034)	*2.23E-08*
Naidu	Andhra-Pradesh	13.22	79.6	Dravidians	21	11(52.381)	9(90)	1(10)	0(0)	19(95)	1(5)	1
Reddy	Andhra-Pradesh	17.37	78.48	Dravidians	24	1(4.167)	17(73.913)	6(26.087)	0(0)	40(86.957)	6(13.043)	1
Mudaliar	Tamilnadu	12.92	79.13	Dravidians	48	3(6.25)	41(91.111)	0(0)	4(8.889)	82(91.111)	8(8.889)	*1.92E-06*
Gammavokklu	Karnataka	12.93	74.83	Dravidians	19	4(21.053)	13(86.667)	2(13.333)	0(0)	28(93.333)	2(6.667)	1
Vysya	Andhra-Pradesh	14.68	77.65	Dravidians	60	10(16.667)	40(80)	10(20)	0(0)	90(90)	10(10)	1
Gawli	Karnataka	13.33	74.77	Dravidians	89	10(11.236)	63(79.747)	10(12.658)	6(7.595)	136(86.076)	22(13.924)	*0.000412713*
Medari	Andhra-Pradesh	16.56	80.61	Dravidians	4	0(0)	3(75)	1(25)	0(0)	7(87.5)	1(12.5)	1
Madar	Karnataka	15.33	75.05	Dravidians	70	9(12.857)	55(90.164)	1(1.639)	5(8.197)	111(90.984)	11(9.016)	*4.75E-07*
Patkar	Andhra-Pradesh	15.8	78.1	Dravidians	20	1(5)	12(63.158)	0(0)	7(36.842)	24(63.158)	14(36.842)	*5.21E-06*
Raj-Gond	Madhya-Pradesh	23.87	78.7	Dravidians	28	19(67.857)	9(100)	0(0)	0(0)	18(100)	0(0)	1
Adhiyan	Tamilnadu	13.72	79.41	Dravidians	44	4(9.091)	37(92.5)	3(7.5)	0(0)	77(96.25)	3(3.75)	1
Kurumba	Tamilnadu	12.94	79.09	Dravidians	15	2(13.333)	11(84.615)	2(15.385)	0(0)	24(92.308)	2(7.692)	1
Chenchu	Andhra-Pradesh	17.37	78.47	Dravidians	27	2(7.407)	17(68)	0(0)	8(32)	34(68)	16(32)	*0*
Kurumba	Madhya-Pradesh	22.71	75.83	Dravidians	26	6(23.077)	14(70)	0(0)	6(30)	28(70)	12(30)	*6.94E-06*
Vaddera	Andhra-Pradesh	18.72	79.48	Dravidians	8	0(0)	5(62.5)	0(0)	3(37.5)	10(62.5)	6(37.5)	*0.006993007*
Brahmin-Tiwari	Uttar-Pradesh	25.73	82.68	Indo-European	44	13(29.545)	28(90.323)	3(9.677)	0(0)	59(95.161)	3(4.839)	1
Kashmiri pandit	JammuKashmir	34.37	75.83	Indo-European	21	0(0)	17(80.952)	3(14.286)	1(4.762)	37(88.095)	5(11.905)	0.235
Bhil	Gujarat	23.03	72.67	Indo-European	4	0(0)	4(100)	0(0)	0(0)	8(100)	0(0)	1
Gamit	Gujrat	21.17	72.83	Indo-European	45	7(15.556)	35(92.105)	3(7.895)	0(0)	73(96.053)	3(3.947)	1
Tharu	Uttarakhand	29.38	79.5	Indo-European	30	3(10)	23(85.185)	3(11.111)	1(3.704)	49(90.741)	5(9.259)	0.183
Warli	Maharastra	19.17	72.95	Indo-European	70	7(10)	48(76.19)	15(23.81)	0(0)	111(88.095)	15(11.905)	0.5832885
Baiswar	Uttar-Pradesh	25.15	82.6	Indo-Europeans	40	6(15)	23(67.647)	11(32.353)	0(0)	57(83.824)	11(16.176)	0.5620674

Pandit	Haryana	29.96	76.87	Indo-Europeans	40	12(30)	23(82.143)	1(3.571)	4(14.286)	47(83.929)	9(16.071)	*0.000129726*
Bhilala	Madhya-Pradesh	22.6	75.3	Indo-Europeans	49	9(18.367)	33(82.5)	5(12.5)	2(5)	71(88.75)	9(11.25)	0.05670192
Chakhesang_Naga	Nagaland	26.12	94.48	Tibeto-Burmans	33	19(57.576)	14(100)	0(0)	0(0)	28(100)	0(0)	1
Naga-sema	Nagaland	25.7	93.81	Tibeto-Burmans	40	21(52.5)	16(84.211)	3(15.789)	0(0)	35(92.105)	3(7.895)	1
Mizo	Mizoram	23.2	92.83	Tibeto-Burmans	23	7(30.435)	13(81.25)	3(18.75)	0(0)	29(90.625)	3(9.375)	1

Table 2. Distribution of novel non-synonymous mutation L362V, observed in exon-7 of *CYP2C9*

Populations	State	Lat.	Long.	Linguistic	Sample size	number of individuals (Genotype/Allele percentage): Novel; Leu>Val						HWE P-value
						Missing data	CC	CG	GG	C	G	
Mahli	Jharkhand	23.46	85	Austro-Asiatic	38	4(21.053)	15(100)	0(0)	0(0)	30(100)	0(0)	1
Ho	Jharkhand	23.35	85.33	Austro-Asiatics	67	3(6.25)	41(91.111)	0(0)	4(8.889)	82(91.111)	8(8.889)	1.92E-06
Gond	Madhya-Pradesh	26.12	77.4	Austro-Asiatics	38	11(52.381)	10(100)	0(0)	0(0)	20(100)	0(0)	1
Gond	Chattisgarh	19.87	81.6	Austro-Asiatics	37	7(18.421)	31(100)	0(0)	0(0)	62(100)	0(0)	1
Kharia	Chattisgarh	85.44	23.33	Austro-Asiatics	86	13(29.545)	31(100)	0(0)	0(0)	62(100)	0(0)	1
Adi-Dravidar	Tamilnadu	11.35	77.73	Dravidian	15	7(15.556)	38(100)	0(0)	0(0)	76(100)	0(0)	1
Kolhas	Andhra-Pradesh	14.46	79.98	Dravidian	14	2(14.286)	12(100)	0(0)	0(0)	24(100)	0(0)	1
Reddy	Andhra-Pradesh	17.37	78.48	Dravidians	24	6(23.077)	14(70)	0(0)	6(30)	28(70)	12(30)	6.94E-06
Thoti	Andhra-Pradesh	16.51	80.64	Dravidians	29	12(30)	23(82.143)	1(3.571)	4(14.286)	47(83.929)	9(16.071)	0.000129726
Raj-Gond	Madhya-Pradesh	23.87	78.7	Dravidians	28	2(2.985)	63(96.923)	0(0)	2(3.077)	126(96.923)	4(3.077)	0.000183117
Chenchu	Andhra-Pradesh	17.37	78.47	Dravidians	27	9(18.367)	38(95)	0(0)	2(5)	76(95)	4(5)	0.000493178
Kurumba	Madhya-Pradesh	22.71	75.83	Dravidians	26	9(45)	9(81.818)	0(0)	2(18.182)	18(81.818)	4(18.182)	0.007518797
Kurumba	Tamilnadu	12.94	79.09	Dravidians	15	0(0)	20(95.238)	0(0)	1(4.762)	40(95.238)	2(4.762)	0.02439024
Gammavokklu	Karnataka	12.93	74.83	Dravidians	19	3(10)	25(92.593)	1(3.704)	1(3.704)	51(94.444)	3(5.556)	0.05660377
Adhiyan	Tamilnadu	13.72	79.41	Dravidians	44	2(7.407)	25(100)	0(0)	0(0)	50(100)	0(0)	1
Gawli	Karnataka	13.33	74.77	Dravidians	89	4(9.091)	40(100)	0(0)	0(0)	80(100)	0(0)	1
Madar	Karnataka	15.33	75.05	Dravidians	70	0(0)	12(100)	0(0)	0(0)	24(100)	0(0)	1
Medari	Andhra-Pradesh	16.56	80.61	Dravidians	4	19(67.857)	9(100)	0(0)	0(0)	18(100)	0(0)	1
Mudaliar	Tamilnadu	12.92	79.13	Dravidians	48	1(5)	19(100)	0(0)	0(0)	38(100)	0(0)	1
Naidu	Andhra-Pradesh	13.22	79.6	Dravidians	21	10(16.667)	50(100)	0(0)	0(0)	100(100)	0(0)	1
Patkar	Andhra-Pradesh	15.8	78.1	Dravidians	20	19(57.576)	14(100)	0(0)	0(0)	28(100)	0(0)	1
Telagas	Andhra-Pradesh	18.17	83.53	Dravidians	12	7(10)	63(100)	0(0)	0(0)	126(100)	0(0)	1
Vaddera	Andhra-Pradesh	18.72	79.48	Dravidians	8	7(30.435)	16(100)	0(0)	0(0)	32(100)	0(0)	1
Vysya	Andhra-Pradesh	14.68	77.65	Dravidians	60	1(4.167)	23(100)	0(0)	0(0)	46(100)	0(0)	1
Kashmiri pandit	Jammu Kashmir	34.37	75.83	Indo-European	21	10(11.236)	73(92.405)	0(0)	6(7.595)	146(92.405)	12(7.595)	8.44E-10
Warli	Maharastra	19.17	72.95	Indo-European	70	9(12.857)	56(91.803)	0(0)	5(8.197)	112(91.803)	10(8.197)	4.32E-08
Bhil	Gujarat	23.03	72.67	Indo-European	4	14(16.279)	72(100)	0(0)	0(0)	144(100)	0(0)	1
Brahmin-Tiwari	Uttar-Pradesh	25.73	82.68	Indo-European	44	0(0)	4(100)	0(0)	0(0)	8(100)	0(0)	1
Gamit	Gujrat	21.17	72.83	Indo-European	45	1(6.667)	14(100)	0(0)	0(0)	28(100)	0(0)	1
Tharu	Uttarakhand	29.38	79.5	Indo-European	30	0(0)	29(100)	0(0)	0(0)	58(100)	0(0)	1

Pandit	Haryana	29.96	76.87	Indo-Europeans	40	1(2.174)	35(77.778)	0(0)	10(22.222)	70(77.778)	20(22.222)	6.26E-11
Baiswar	Uttar-Pradesh	25.15	82.6	Indo-Europeans	40	2(13.333)	13(100)	0(0)	0(0)	26(100)	0(0)	1
Bhilala	Madhya-Pradesh	22.6	75.3	Indo-Europeans	49	0(0)	4(100)	0(0)	0(0)	8(100)	0(0)	1
Naga-sema	Nagaland	25.7	93.81	Tibeto-Burmans	40	4(10.526)	33(97.059)	0(0)	1(2.941)	66(97.059)	2(2.941)	0.01492537
Chakhesang_Naga	Nagaland	26.12	94.48	Tibeto-Burmans	33	8(21.622)	28(96.552)	0(0)	1(3.448)	56(96.552)	2(3.448)	0.01754386
Mizo	Mizoram	23.2	92.83	Tibeto-Burmans	23	0(0)	8(100)	0(0)	0(0)	16(100)	0(0)	1