RESEARCH ARTICLE

Genetic variation and population structure of interleukin genes among seven ethnic populations from Karnataka, India

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Abstract

The extent of genetic variation and the degree of genetic differentiation among seven ethnic populations from Karnataka, India (Bunt, Havyak, Iyengar, Lingayath, Smartha, Vaishya, Vokkaliga), was investigated using four single nucleotide polymorphisms (SNPs: *IL-1A 4845, IL-1B 3954, IL-1B 511* and *IL-1RA 2018*) of the interleukin gene cluster. Allele frequencies varied by threefold among these populations, which also differed for gene diversity and heterozygosity levels. The average degree of population subdivision among these castes was low (F_{ST} = 0.02). However, pair-wise interpopulation differentiation ranged from 0–7%, indicating no detectable differentiation to moderate differentiation between specific populations. The results of phylogenetic analysis based on genetic distances between populations agreed with known social and cultural data on these ethnic groups. Variation in the allele frequencies, as well as differentiation, may be attributed to differential selection and demographic factors including consanguinity among the ethnic groups. Information on the distribution of functionally relevant polymorphisms among ethnic populations may be important towards developing community medicine and public health policies.

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Introduction

Both the cultural and genetic diversity of human populations in India have long been of interest for understanding the origin and evolution of the people of the Indian subcontinent (Singh 1992; Mountain *et al*. 1995; Majumder 1998; Bamshad *et al*. 2001; Basu *et al*. 2003; Cordaux *et al*. 2004). India's population is composed of approximately 50,000 to 60,000 endogamous subpopulations or caste groups (Gadgil *et al*. 1998). Several authors have analysed the genetic diversity among these highly localized populations using both mitochondrial and autosomal markers (Majumder 1998, 2001; Basu *et al*. 2003; Vishwanathan *et al*. 2004). These authors have reported substantial variation and differentiation among populations at all levels: among regional populations and among broad caste groups as well as tribal populations. Based on these studies, Majumder (1998, 2001) concluded that India has more human genetic diversity than any other comparable global region in the world, with the exception of Africa.

Variation and differentiation among populations, including variation influencing infection and disease, could arise due to both demographic history — such as assortative mating, consanguinity and inbreeding — and evolutionary genetic forces, such as selection, mutation and recombination (Jorde *et al*. 2001; Tishkoff and Verelli 2003). However, while demographic processes affect the entire genome, region-specific effects of selection affect specific loci (Tishkoff and Verelli 2003). Among Indians, consanguinity and marriage within caste groups is well known, and these factors may have important clinical consequences and thus, impact on community genetics (Bittles 2001, 2002). A number of complex genetic disorders such as, coronary heart disease, cancer, psychiatric disorders and asthma, have been

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found to be associated with consanguinity and inbreeding in several human populations. For instance, nearly 45% of cardiovascular disease and complex disorders among the Croatian populations have been attributed to inbreeding due to consanguineous marriages (Rudan *et al*. 2003).

Large panels of seemingly neutral markers are preferred for determining genetic diversity and population structure among a broad spectrum of populations. However, neutral markers may not necessarily provide insights into the distribution and maintenance of disease causing variants among populations with divergent evolutionary and demographic histories. Polymorphisms of certain genes implicated in human genetic disorders could show different patterns of distribution among populations, as they are subjected to natural selection. Therefore, Bamshad *et al*. (2004) have recently questioned the generalities resulting from broad population surveys that have been conducted using neutral markers, and listed several examples of specific genetic variants that differed in their distribution among various ethnic groups. They proposed that because variants that influence diseases could have different outcomes in different ethnic groups, variation of specific functional polymorphisms may be relevant not only for defining populations, but also for monitoring community health. For instance, people of the Indian subcontinent are approximately four times more susceptible to cardiovascular diseases than their European counterparts. The incidence of cardiovascular diseases is reaching epidemic proportions both among Indians in the urban parts of India, and among immigrants from India in developed countries, largely due to changing life style (Chandalia and Deedwania 2001). Thus, if the variation in complex diseases is attributable to genetic causes, then the allelic variants underlying these diseases may also vary across populations; which in turn may reflect an interaction between evolutionary and demographic factors. Accordingly, the allelic variants of disease causing genes may also vary among various ethnic populations within a geographical region. Clearly, an understanding of the evolutionary processes involved in determining the pattern of genetic diversity, including variation influencing disease, across different ethnic populations may be important towards identifying genes/alleles that cause disease (Tishkoff and Verelli 2003).

In this study, we report genetic variation and population differentiation of four polymorphisms of the interleukin-gene cluster among seven-ethnic groups of Karnataka, India. Interleukins (cytokines) play a major role in the inflammatory processes, innate immunity and immune responses (Dinarello 1997). Several single nucleotide polymorphisms (SNP) in the three genes ($IL-1\alpha$, $IL-1\beta$ and $IL-1RN$) of the interleukin-1 gene cluster, located on chromosome 2q14, have been implicated in a number of acute and chronic inflammatory, autoimmune, cardiovascular and other inflammatory disorders, such as asthma and rheumatoid arthritis, as well as in infectious diseases (Bidwell *et al*. 2001; Haukim *et al*. 2002). Two genes of the interleukin cluster, $IL-1\alpha$ and $IL-1\beta$ are potent-inflammatory agents (Cantagrel *et al*. 1999), while the third gene (*IL-1RN*) acts as a receptor antagonist and inhibitor of the activity of *IL-1*α and *IL-1*β (Kornman *et al*. 1997). Polymorphisms of the interleukin genes show individual variation in the production of cytokines, and frequencies of these SNPs also vary across major global populations (Ober *et al*. 2000; Meenagh *et al*. 2002). Thus, an understanding of the role of these immune response genes and their variation may help in treating infectious diseases and other complex disorders (Dean *et al*. 2002). The purpose of this study was to estimate (i) the levels of genetic variation at four functional SNPs of the interleukin-1 gene cluster and (ii) the degree of genetic differentiation, based on these SNPs, among seven ethnic populations from Karnataka, India. Patterns of variation among the four variants indicated substantial variation and differentiation among populations, suggesting that both natural selection and demographic forces may have influenced the extent of differentiation among these seven ethnic groups.

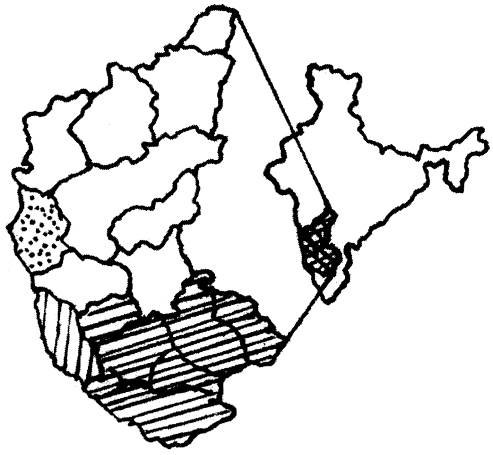

Figure 1. Distribution of the seven ethnic populations in Karnataka, Havyak (stippled); Bunt (straight lines); Iyengar, Lingayat, Smartha, Vaisya and Vokkaliga (diagonal lines).

Material and methods

Population samples

This study included healthy individuals from seven ethnic populations originating from both rural and urban areas of Karnataka, a state in southwestern India (figure 1), living in the Boston area. Members of these caste groups speak mainly Kannada, a Dravidian language, and belong to a relatively homogeneous culture, but to different caste clusters, priest (Brahmins: Havyak, Iyengar and Smartha), merchant (Vaisya), and agricultural class (Bunt, Lingayat and

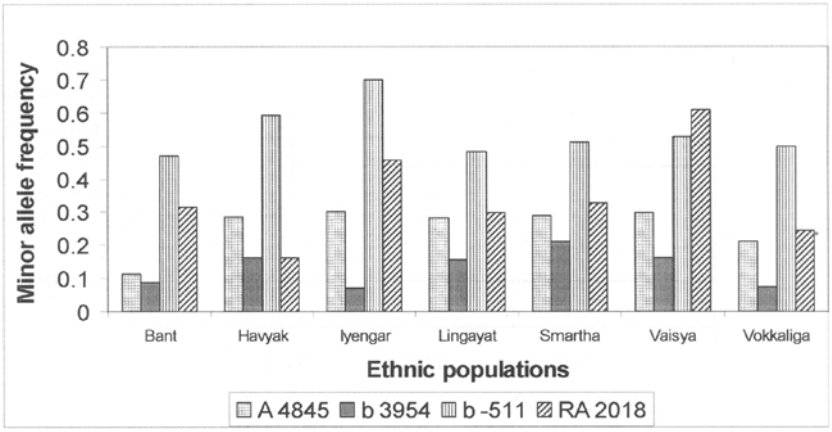

Figure 2. Allele frequencies of the four SNPs among the seven populations.

Vokkaliga). Historical accounts indicate that some of these groups might have switched their caste status in their long cultural history, due to intercaste marriages, migrations and to fulfill religious, political, and administrative needs (Stein 1980; Govindaraju 1995). A majority of the members of these castes in contemporary Indian society practice endogamy, and the level of consanguinity vary both among regions and castes (Bittles 2002). Individuals from known mixed marriages were not included in this study.

DNA isolation and genotyping

The four functional SNPs of the interleukin-1 gene cluster used in this study were *IL-1A 4845, IL-1B 3954, IL-1B 511* and *IL-1RA 2018*. Two buccal (cheek) swab samples were obtained anonymously from each individual. These swabs were air dried for about 3h at room temperature and stored at −40°C until use. Details of DNA extraction and genotyping using the TaqMan (Applied Biosystems, Foster City, CA, USA) assay are provided elsewhere (Raj *et al*. 2006). Briefly, the assay consisted of PCR primers and two allele-specific probes. The PCR reaction was performed using the universal mix in 25 ml reaction tubes containing 20 ng of DNA, 500 nM primer and 50 nM probe. The PCR amplification was carried out in 96-well plates of known allele 1 and allele 2 homozygotes, only reagents and no DNA, and 72 samples of unknown genotypes. The genotypes were inferred using the software provided with the instrument.

Statistical analysis

Estimates of descriptive statistics such as, allele frequencies, observed and expected levels of heterozygosity and fixation indices, and tests for Hardy–Weinberg equilibrium (HWE) followed Weir (1996); genetic distances were calculated following Nei (1987), as implemented in the GDA software (Lewis and Zaykin 2002). The degree of population differentiation, F_{ST}, among all the seven populations, and pair-wise population differentiation between any two populations, was determined according to Weir (1996), using the FSTAT software (Goudet 2001).

Results and discussion

The minor allele frequency (MAF) of *IL-1b 511* ranged from 0.471 in Bunts to 0.700 in Iyengars, thus showing approximately 1.5 fold variation. Similarly, the MAF of *IL-1a 4845* varied from 0.114 in Bunts to 0.300 in Iyengars; *IL-1b 3954* varied from 0.071 to 0.210 in Iyengars and 0.210 in Smarthas. A three-fold variation (0.162 – 0.608) in MAF was found for *IL-1RA 2018* (figure 2). Descriptive statistics for the four SNPs in the seven populations are depicted in table 1. All the four sites were in HWE across populations. The highest level of average gene diversity was found in the Iyengar (0.466), followed by the Vaisya (0.454), and the least average gene diversity was found in the Bunt population (0.321). The highest level of heterozygote deficiency was recorded for the Iyengar and the Smartha populations, respectively.

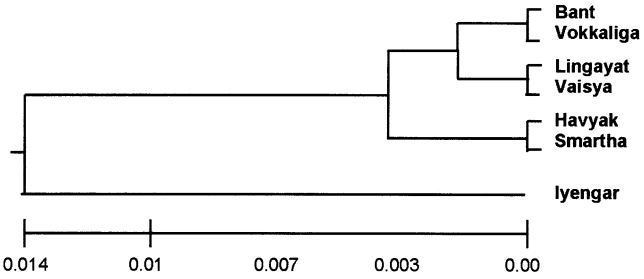

Figure 3. UPGMA dendrogram based on the genetic distance among the seven populations.

Variation in allele frequencies among geographically close and culturally related populations has been reported

Table 1. Genetic diversity statistics for the seven populations.

Ethnic group	N	Observed heterozyote frequency	Expected heterozyote frequency	f	Average gene diversity
Bunt	35	0.307	0.327	0.061	0.321
Havyak	37	0.372	0.363	−0.024	0.344
Iyengar	35	0.336	0.373	0.100	0.466
Lingayat	32	0.437	0.403	−0.088	0.416
Smartha	36	0.461	0.387	−0.194	0.352
Vaisya	37	0.405	0.422	0.040	0.454
Vokkaliga	33	0.348	0.341	−0.024	0.356

Table 2. Pair-wise F_{ST} values and genetic distances.

Population	Bunt	Havyak	Iyengar	Lingayat	Smartha	Vaisya	Vokkaliga
Bunt	–	0.0299*	0.0429**	0.0094	0.0331*	0.0154	0.0000
Havyak	0.0303	–	0.0549**	0.0061	0.0000	0.0225*	0.0058
Iyengar	0.0438	0.0564	–	0.0352*	0.0713**	0.0117	0.0479*
Lingayat	0.0095	0.0061	0.0358	–	0.0002	0.0000	0.0000
Smartha	0.0337	0.0000	0.0739	0.0002	–	0.0191	0.0076
Vaisya	0.0154	0.0227	0.0117	0.0000	0.0193	–	0.0100
Vokkaliga	0.0000	0.0058	0.0409	0.0000	0.0077	0.0100	–

F_{ST} values above the diagonal and genetic distances below the diagonal.
*,**significant at P = 0.05 and 0.01 percent, respectively.

among various ethnic groups in India. For example, Vishwanathan *et al*. (2004) reported high diversity among tribal populations of South India. Likewise, Roychoudhury *et al*. (2001) reported genetic similarities and differences among various Indian populations. Because all the four SNPs included in this study are involved in immunological, inflammatory and complex diseases such as cardiovascular and respiratory diseases (Bidwell *et al*. 2001; Meenagh *et al*. 2002), the similarities and differences in their allele frequencies could possibly be due to both evolutionary and demographic factors. Genetic differences among the eight populations indicate the effects of selection, drift and migration, which influence the inbreeding load among populations (Glemin *et al*. 2003). One of these ethnic groups, the Havyaks, were found to be less than 30 families and have assimilated the local population into its fold over time (Hegde and Hegde 1995). The distribution of heterozygosities and the associated fixation index (f-values) among populations suggests that populations with higher levels of inbreeding show positive f-values, and vice versa. Accordingly, for the studied loci, while the Iyengar population showed greater levels of heterozygote deficiency, the Smartha population showed heterozygote in excess.

Population differentiation as suggested by F_{ST} is a good surrogate of genetic distance (Reynolds *et al*. 1983) and these measures are often used interchangeably. None or very little differentiation was found between the Bunts and Vokkaligas. Similarly, very low differentiation was apparent between the Lingayats and Iyengars. However, the largest differentiation was recorded between Smartha and Havyak populations on one hand, and the Iyengar population on the other. These results agreed with the genetic distances among the populations studied (table 2). Using the F_{ST} measures, Zhou *et al*. (2004) have shown large degree of differentiation for the *IL-13* locus among Chinese, Europeans and Africans. Similarly, Sakagama *et al*. (2004) demonstrated that polymorphisms of the *IL-4* and *IL-13* regions show clear differentiation between the European and Japanese populations, which they attributed for selection.

Although this study is based on a few functional SNPs of the inflammatory pathway, their discriminating power along the cultural and caste line is surprising. Culturally similar groups formed clusters. For instance, both Vokkaligas and Bunts are agriculturists and claim cultural identity; hence, intermarriage between the members of these two caste groups is fairly common in present day Karnataka. Lingayats and Vaisyas are often classified between Brahmins and the agricultural class. Interestingly, Majumder (1998) also placed both Lingayats and Vysyas between the Brahmins and the Vokkaligas. Among the three Brahmin castes, Smarthas and Havyaks formed one group, but the Iyengars formed a separate branch. Lingayats as a caste group originated approximately 800 years ago, largely from the agricultural class, and over time, this caste has absorbed members from several other castes groups in south–central India, including brahmins. Therefore, their position in the UPGMA dendrogram between the two dominant agricultural classes and the brahmin castes is not surprising (figure 3). Similarly, Iyengars

have inherited a history of over 800 years and have absorbed members from various caste groups in the region over the centuries. The admixture might have influenced their position in the cladogram as defined by separate branch in this study.

Variation in the allele frequencies and differentiation among the seven castes indicates that the alleles may be influenced by differential selection intensities acting upon these populations in different parts of Karnataka. Interleukin genes, as noted earlier, are involved in a number of infectious, autoimmune and inflammatory diseases. For instance, allele-2 of the *IL-1A 4845* locus has shown to be associated with periodontal disease (Kornman *et al*. 1997), and variation at a common SNP of the *IL-4* region among human populations has been attributed to natural selection (Rockman *et al*. 2003). Also, if the F_{ST} measure based on functional variants exhibit different patterns of geographical variation, it may be taken as evidence for different selective pressures across populations (Bowcock *et al*. 1991; Salamon *et al*. 1999; Fullerton *et al*. 2002; Hamblin *et al*. 2002). These are also influenced by other demographic factors such as consanguinity and inbreeding among the seven ethnic groups. Additionally, environmental factors such as rainfall, or endemic diseases such as malaria, might have also played a role in moulding the levels of genetic variation. Interleukin genes are known to be associated with responses to both infectious and other tropical diseases such as malaria (Gyan *et al*. 2004; Verra *et al*. 2004). Additionally, as suggested by Bittles (2002) and Rudan *et al*. (2003), different levels of consanguinity and inbreeding may be differentially influencing the incidence of complex diseases among these populations. Therefore it may be hypothesized, that various ethnic populations represented in this study may have experienced differential mortality due to infectious diseases and epidemics during their long evolutionary history in the region. Indeed, selection and demographic forces impact the patterns and distribution of genetic variation among populations in relation to infectious diseases (Dean *et al*. 2002)

In conclusion, interplay between the genetic factors on the one hand, and environmental and demographic factors on the other, may be contributing to the differential distribution of the four functional polymorphisms of the interleukin genes among the seven ethnic groups included in this study. The large variation among allele frequencies found in the present study clearly suggests that moderate differentiation among disease-causing variants could occur even among evolutionarily closely related and geographically narrowly distributed regional ethnic populations. Clearly, generalities based on regional or entire Indian populations on either linguistic or cultural affinities could be misleading. Therefore, it may be important to know not only the broader aspects of ethnic origins, but also the distribution of specific disease-causing variants among well-defined ethnic groups. Information on the distribution of the causal variants of specific genes among ethnic populations may be important for monitoring the health of migrant populations. South Asians are forming a significant part of minority populations in the western societies and similarly, members of various groups migrate from rural to urban areas within South Asia, which could impact their life style. Modified life style is influencing the incidence of several complex diseases such as diabetes and cardiovascular diseases, which are reaching epidemic proportions among South Asians, both in the rural and urban centers within the India subcontinent, as well as among the members of the South Asian diaspora in western societies. Thus, as pointed out by Weatherall (2003), knowledge of the distribution of specific disease-causing variants among specific ethnic groups may be important for monitoring the health of minority and migrant populations, as well as in developing genomic medicines and public health policies.

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