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Genotype Frequency of the Common TLR4 SNPs in a Kurdish Population: Global Reviews and Out-of-African Migrations

Sherko Subhan Niranji
Department of Biology, College of Education, Coronavirus Research and Identification Laboratory, University of Garmian, sherko.subhan@garmian.edu.krd

Sirwan M. Amin Al jaf
Department of Biology, College of Education, Coronavirus Research and Identification Laboratory, University of Garmian, sirwan@garmian.edu.krd

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Sherko Subhan Niranji[1,2]* and Sirwan M. A. Al-Jaf[1,2]

[1]Department of Biology, College of Education, University of Garmian, Bardesur Campus, Kalar, Iraq.

[2]Coronavirus Research and Identification Laboratory, University of Garmian, Bardesur Campus, Kalar, Iraq.

*Correspondence to: Sherko Subhan Niranji, Department of Biology, College of Education, University of Garmian, Bardesur Campus, Kalar, 46021 Kurdistan Region, Iraq, 00964 (0)7707798899. E-mail: Sherko.subhan@garmian.edu.krd.

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Abstract

Toll-like receptors (TLRs) are cellular innate immune receptors that explore microbial molecules. For instance, TLR4 can sense bacterial lipopolysaccharides (LPS) inducing cytokines and anti-microbial peptides against the bacteria. It has been shown that single nucleotide polymorphisms (SNPs) in TLR4 are associated with diseases such as septic shock. Therefore, investigations of common SNPs may help to explain the pathogenesis of diseases and various innate immune responses against infections. This study investigates genotypic frequencies of two common TLR4 SNPs (Asp299Gly and Thr399Ile) in a Kurdish population using Restriction Length Fragment Polymorphisms (RFLP). Furthermore, the global frequencies of both TLR4 SNPs are reviewed in different populations of Sub-Saharan Africa, North Africa, Western Asia, Eurasia and East Asia and used to infer human migrations and past settlements. The RFLP data demonstrate that, in the Kurdish population, the genotypic frequencies of both SNPs are similar to Iranian or other Western Asian populations, which in turn are comparable to Eurasian populations suggesting past admixture due to migrations, population intermixing and common ancestry. The reviewed data reveal that the frequencies of the homozygous wild-types of TLR4 variants are prevalent, but homozygous mutants are rare or lacking in almost all global populations. Frequencies of the heterozygotes varied from population to others. For instance, in Sub-Saharan Africa the frequency of the Asp299Gly SNP is higher than Thr399Ile, whereas in the Arabic peninsula both SNPs are present at higher frequencies. In contrast, East Asian populations lack or have very low frequencies of both TLR4 SNPs of interest. Moreover, co-segregations of the TLR4 SNPs were common in some populations that may indicate their important roles in association with certain diseases. Future studies are required to link the TLR4 SNPs with either resistances against or susceptibility to diseases.

Following the first draft of the human genome and Genome-Wide Association Studies (GWAS), single nucleotide polymorphisms (SNPs), for instance SNPs in Toll-like receptor (TLR) genes, have been an area of scientific interest for understanding diseases and studying genetic variation among populations and ethnic groups (Medvedev 2013). TLRs are innate immune genes that encode evolutionarily conserved cellular membrane proteins. They play vital roles in combating infectious diseases by sensing pathogen molecular patterns (PAMPs) that stimulate innate immune responses to microbes influenced by coding region SNPs in the receptor (Mukherjee and Babu 2019). Humans have 10 TLRs labeled TLR1 to TLR10. Each receptor has the ability to recognize a particular microbial PAMP including nucleic acids, flagella, lipopolysaccharides (LPS), and other molecules constituting microbial parts (Vijay 2018). It is well-known that TLR4 plays a principal role in combating Gram negative bacteria by exploring the bacterial LPS and triggering the nuclear factor kappa B (NF-κB) pathway to induce pro-inflammatory cytokines and antimicrobial peptides against the bacteria (Faure et al. 2001). This TLR4 pathway has been recently suggested to be a target for both diagnosis and treatment of various diseases (Garcia et al. 2020). TLR4 is enhanced by microbial exposure leading to an exaggerated, strong immune response against the pathogen and consequently may lead to sepsis and excessive cytokine secretions (Schröder and Schumann 2005). Research demonstrated that SNPs in TLR4 genes impact the ability of the receptor to respond differently against bacterial LPS endotoxins (Arbour et al. 2000). Polymorphisms in promoter and coding regions of TLRs are generally associated with infections, inflammations, allergies and cancers but these associations are frequently inconsistent or confusing (Medvedev 2013).

Two non-synonym SNPs were recognized in the TLR4 protein target: aspartate 299 (Asp299) and threonine 399 (Thr399) which are substituted with glycine and isoleucine, respectively (Schröder and Schumann 2005). These mutations form two variants termed

TLR4 A>896G (Asp299Gly) and TLR4 C>1196T (Thr399Ile), respectively. A crystal structure of the TLR4 SNP resulting in Asp299Gly, demonstrates particular influence on the helical conformation, surface properties, folding and stability of the receptor protein as well as the ligand binding abilities to it (Ohto et al. 2012).

Investigating TLR4 genetic polymorphisms in various populations is important, in particular when these SNPs are found to be associated with susceptibilities to infections. Since infectious agents often help TLR SNPs to undergo 'adaptive evolutions' (Netea and O'Neill 2012), this may result in resistance to infections due to microbial senses causing better immune responses (Netea et al. 2012).

Both TLR4 Asp299Gly and Thr399Ile are located on chromosome 9q33.1 (Vijay 2018) and both mutations are located within 300 nucleotides of one another. This may explain co-segregation of the TLR4 variants for disease susceptibility or resistance against different microbes in various populations. Previous research has revealed that the roles of co-segregation of TLR4 heterozygous variants may provide a selective advantage in malaria endemic areas of Africa, but might be a disadvantage against bacterial sepsis among Eurasian populations (Ferwerda et al. 2007). Furthermore, it has been shown that studies of TLR4 SNPs in different ethnic groups who have long resided in different locales, help to explain the evolutionary selective pressure of the TLR4 variants against the infectious agents in those areas (Ioana et al. 2012a). Thus, exploring TLR4 SNP co-segregation can be important for better understanding population responses against infectious diseases.

Mostly residing in Iraq, Iran, Turkey and Syria, Kurdish nomadic pastoralists are organized in tribes, clans and families and they practice a conservative marital culture (Izady 1992). Particularly, intra-tribal, -village, -town, -city and relative marriages are still common in most of their provinces. This has resulted in Kurds preserving their genetic information

(e.g., genotypes) among clans and families throughout history since the Kurds have a traditional family system (Ölcer 2020).

Archaeological records have found approximately 70,000 years old Neanderthal skeletons in Shanidar caves located in Bradost mountain, Erbil Province, Iraq (Stewart 1977). Also the oldest agricultural village, nearly 10,000 years old, was discovered in Bestansur on Sharazur plain, Sulaymaniyah Province, Iraq (Matthews et al. 2019). These ancient locations are geographically located among the Zagros mountains, where Kurds have been settled for thousand years. Zagros mountains are a chain of mountains located between Iraq and Iran extending from Persian Gulf to Southern Turkey.

The exact origin(s) of Kurds still remains unresolved. However, they are believed to be descendants of several ancient civilizations, for instance the Halaf culture people who settled in Anatolia, Southern Turkey around 6000 BCE and the Hurrians in Anatolia, Northern Mesopotamia and Levant about 2000 BCE (Arnaiz-Villena et al. 2001). Ancient texts by Sumerians and Greek invaders have been used to trace Kurdish ancestries to the Gutians, Hurrians, and Medians who ruled around the Zagros mountains 1000 years BCE. The discovery of ancient Mesopotamian cuneiform tablets mentioning ‘ the lands of Karda’ is believed to trace the predecessor of Kurds to the Gutians of the Zagros mountains (Hennerbichler 2014) and to Medians or Hurrians from Iran or Anatolia (Arnaiz-Villena et al. 2017).

Genetic studies trace the relationships between Kurds and populations from Iran, Western Asia and Europe. Based on human leukocytes antigen (HLA) polymorphisms analysis, Kurds were found to be closely related to particular Iranian ethnic groups, specifically Persians, Azeris and Gurgans (Amirzargar et al. 2015; Arnaiz-Villena, Palacio-Gruber, et al. 2017). Both mitochondrial and Y- chromosomal DNA indicate that Kurds are closely related to Indo-Iranians, Western Asian and European groups (Nasidze and Stoneking

2005). Kurds are a people who survived around Zagros mountains through which some humans migrated from Africa to the Arabic peninsula, Levant and Anatolia and Mesopotamia then to Eurasia (Izady 1992). Studies have focused on reconstructing migration routes with both mitochondrial and Y-chromosomal lineages in Middle East ethnic populations, including Kurds (Aimé and Austerlitz 2015; Quintana-Murci et al. 2004).

The city of Sulaymaniyah was established in 1784, as the capital of Baban principality, where its previous capital Qara Cholan and the surrounding villages were transferred (Ágoston and Masters 2008). Sulaymaniyah province is located in Central Zagros, and has been historically and politically important in Iraqi Kurdistan. The rich historical and archaeological records of the people in the Kurdistan region, particularly Sulaymaniyah, suggest that those residing in the region today may contain important genetic clues for reconstructing the past.

Regarding TLR4 SNPs, investigations of genetic polymorphisms are scarce in the Kurdish population that numbers approximately 30 million people. This far, only two studies have been conducted. The first was from Shiraz University in Iran that investigated TLR4 SNPs in various Iranian ethnic groups including Kurds and concluded that TLR4 SNPs are heterogeneous in Iranian populations rejecting associations between the TLR4 SNPs and disease susceptibilities (Ioana et al. 2012a). The other study was conducted by our group in 2020 and looked at only TLR4 Thre399Ile in Kurds attending University of Garmian, concluding that the heterozygous TLR4 Thr399Ile is at 7.1% (Niranji 2020).

In the present study, we investigate the genotypic frequencies of both TLR4 Asp299Gly and Thr399Ile SNPs, from a Kurdish population in Sulaymaniyah province. In particular we aim to explore whether both SNPs Asp299Gly and Thr399Ile are co-segregated in the population.

Genotype frequencies of both Asp299Gly and Thr399Ile SNPs vary in different ethnic groups and even differ within population of the same ethnic background. Therefore, an update of global data on TLR4 SNP variation may help to resolve the discrepancies in findings the genotypic frequencies in global ethnic populations (Mukherjee and Majumder 2013). In fact, migration and gene flow can be of the most important mechanisms that shaped allelic variation. Accordingly, migrations of ancient humans might have influenced the frequency of the TLR4 SNPs. Research has revealed that humans migrated out of Sub-Saharan Africa to Western Asia, Eurasia, and East Asia via North African or Arabian routes (López and Hellenthal 2015; Xiao et al. 2004). Thus, TLR4 SNP frequencies among Kurds will be placed in the context of the genotypic frequencies of the two common SNPs around observed in five principle groups: Sub-Saharan Africans, North Africans, Western Asians, Eurasians, and East Asians.

Materials and Methods

Study Area

The population of interest is located in Sulaymaniyah province, which is divided into several districts. The study was conducted at the University of Garmian, which hosts diverse students and staff from those districts and subdistricts of the province. The province is located in the northeast of the Kurdistan Regional Government and northern Iraq.

Sample Collections

Written consent forms were taken prior to sampling and the study was approved by an ethics committee and adhered to ethical principles of the Declaration of Helsinki in the Department of Biology at the University of Garmian. Blood samples (3 ml) were randomly collected from 114 unrelated healthy individuals in the University of Garmian. The inclusion criterion for

volunteering in this study was that the great grandparents of the participant have Kurdish ethnic origins. The collected blood samples were preserved in tubes containing EDTA and stored at 4°C until DNA was extracted and purified.

Genomic DNA Extraction

The blood samples (200 µl) were treated with 20 mg/mL proteinase K (10 µl) at 56°C for 10 minutes in 1.5 mL microcentrifuge tubes. DNA was extracted according to the kit protocol provided by Genetbio (Korea). The DNA samples were kept at - 20°C until polymerase chain reactions (PCR) were performed.

Polymerase Chain Reaction

Genomic DNA (5 µl), 5 pmole of the forward primer (1 µl), 5 pmole of the reverse primer (1µl), nuclease free water (3 µl) and Addbio master mix 2x (10 µl) were combined in 0.2 ml PCR tubes. The PCR protocol was performed according to the manufacturer's instructions (Addbio, Korea). The primers for TLR4 mutants were used as designed previously (Ajdary et al. 2011; Lorenz and Schwartz 2001). Details of the locations of both forward and reverse primers, and restriction sites with PCR product sizes for the TLR4 variants are shown in Figure 1. The PCR Lightcycler were set up by 95°C for 5 minutes, followed by 40 cycles of (95°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds) and 72°C for 5 minutes.

Restriction Fragments Length Polymorphism (RLFP)

Nco1 and *Hinf1* were used to digest the TLR4 Asp299Gly and Thre399Ile PCR products, respectively. PCR products (10 µl) were digested with 1 µl of restriction enzyme (NEB Biolabs Inc.) and its provided buffer (4 µl) and DEPC water (10 µl) in PCR tubes at 37°C for 2 hours followed by inactivation of the enzyme at 80°C for 20 minutes using a thermal cycler

(Mastercycler nexus, Eppendorf AG, Hamburg, Germany). The digested samples were stored at 4°C until they were identified by gel electrophoresis. Each mutant variant was screened at least twice using independent experiments.

DNA Gel Electrophoresis

Agarose gels (3%) were prepared using agarose powder, TBE buffer and prime safe dye or Ethidium bromide. The digested PCR products were viewed with a UV illuminator. Sizes of the mutant (cleaved) amplicons were confirmed using 10 % polyacrylamide gel.

Global Reviews of the Common TLR4 SNPs

Only data from previous studies having screened healthy controls, with associated ethnic background information and raw genotype frequency data of TLR4 Asp299Gly (rs4986790) and/or Thr399Ile (rs4986791) were combined with data collected in this study. First, the data were subdivided by countries of origin of the participants. As previously mentioned, humans migrated out of Sub-Saharan Africa to Western Asia, Eurasia, and East Asia via North African or Arabian routes (Hellenthal et al. 2014; López et al. 2015; Xiao et al. 2004). Then depending on the human migration routes and mitochondrial lineages (Aimé et al. 2015; Quintana-Murci et al. 2004), the SNP data was categorized into ethnic groups originating in the following regions: (a) Sub-Saharan Africa as the origin of modern humans; (b) North Africa including Egypt, Tunisia, and Morocco; (c) West Asia including Arabia, Mesopotamia, Levant, Anatolia and Indo-Iranian people; (d) Eurasia including European countries, Caucasians and Russia; and (e) East Asian populations including China, Korea and Oceania.

Results and Discussions

This study sought to investigate the genotype frequency of the TLR4 Asp299Gly and TLR4 Thr399Ile SNPs in a Kurdish population at the University of Gramian, one that is reflective of Kurds residing in the Sulaymaniyah province. The RFLP results revealed 98 of 114 (86%) have homozygous wild-type TLR4 genotypes. Expectedly, no homozygous mutants of neither TLR4 Asp299Gly nor TLR4 Thr399Ile were found in this study. This was similar to our global review data from previous studies that the homozygous mutants are either almost absent or seldom in most global populations (Table 1). It is possible that homozygous mutants are so rare in this population that we missed them in our sample.

Sixteen (16) of 114 individuals (14%) carried either one or both heterozygous SNPs. The data showed that nine of the 114 (7.9%) participants have the heterozygous TLR4 Asp299Gly SNP and 11 carry the heterozygous Thr399Ile SNP (9.64%). Four participants shared both SNPs, i.e., four of these participants (16 in total) carried both heterozygous SNPs. Thus, four of 114 (3.5 %) of the population or four of the 16 individuals (25%) possessed both heterozygous variants of TLR4 Asp299Gly and TLR4 Thr399Ile. The results suggest that both non-synonymous SNPs are potentially co-segregated in the Kurdish population. Further research needs to be conducted using a larger number of samples.

Co-segregation of TLR4 Asp299Gly and Thr399Ile variants occurred in Iranian ethnic groups including Kurds (Ioana et al. 2012a). In our global review, few previous studies focused on the co-segregation of both SNPs in the same persons. As shown in the supplementary materials, there is 1.4% co-segregation in the same individuals in Sub-Saharan Africa populations, 1.9% in North African populations, 4.5% in Arabian populations, 1.5% in West Asian populations, and 2.3% in Eurasian populations. These results illustrate co-segregation diversity in populations from different geographical locations. Therefore, further investigations may be required to explain associations of TLR4 SNPs co-segregations and geographical distributions.

Previous research demonstrates that the TLR4 Asp299Gly allele alone is associated with innate immune functions and has protective roles against protozoan infections, such as malaria, that can make the SNP- containing receptor a positively selected variant (Ferwerda et al. 2007). While co-segregation of both Asp299Gly and Thr399Ile do not change immune response, therefore; the receptor, which carries the co-segregated SNPs, responds to diseases as the wild-type does since this makes the haplotype Asp299Gly and Thr399Ile "selectively neutral" (Ferwerda et al. 2007). Ferwerda et al. (2007) explained the importance of TLR4 SNPs in disease resistance against infectious diseases in some places (e.g., in Africa against Malaria), whereas the SNPs are disadvantageous to humans residing other locations (e.g., in Europe against sepsis) around the world. In other words, where malaria is endemic in part of Africa, individuals having TLR4 Asp299Gly SNP are resistant to malarial infection. Yet, this TLR4 SNP is disadvantageous in the absence of malaria, where bacterial diseases, may be the leading cause of sepsis (Ferwerda et al. 2007). This indicates that TLR4 SNPs may have played important roles in early human survival during out-of-African migrations and in any place where malaria poses a selective pressure.

As some early humans passed through and settled in the Middle East on their paths out of Africa, novel environments likely played a role in the evolution of genetic polymorphisms in different ethnic populations (Ioana et al. 2012a). The Zagros mountains, spanning across Iran, Iraq, and Turkey, are home to Kurdish populations which are closely related. However, due to conserved endogamous cultures, Iranian and Iraqi Kurds have developed gene frequencies distinct from each other. Therefore, the lack of TLR SNPs studies in the Iraqi Kurdish population leaves a research gap for completing the human migration history and emergence of genetic polymorphisms associated with human survival against infectious diseases.

Both TLR4 SNPs, Asp299Gly and Thr399Ile, are present in 5-10 % Caucasian populations and they are often co-segregated in patients associated with LPS hyporesponsiveness and sepsis, while they are rare in Asian populations. However, only the TLR4 Asp299Gly SNP is prevalent in African populations as a positively selected variant against malaria (Medvedev 2013). Furthermore, there are inconsistencies in finding associations between the TLR4 SNPs and diseases in different populations including Gambian, South-eastern Chinese, and Korean populations (Medvedev 2013). These encourage further research focusing on ethnic backgrounds who have not been extensively investigated. Thus, populations whose TLR4 SNPs were not investigated, for instance, Kurds are required to be studied. In this study, we have found 9 and 11 individuals bearing heterozygous mutants of both common TLR4 Asp299Gly and Thr399Ile SNPs out of 114 samples, respectively, and both SNPs are co-segregated in 4 individuals. However, in some populations including Korean and Han Chinese, no mutant SNPs or very few were found in thousands of samples (Table 1). Absence or infrequency of these SNPs in East Asian populations while their occurrence in European populations support the importance of Middle Eastern populations e.g., Kurdistan region as an area of future research for tracking ancient mutations that occurred in the past after migrations of humans out of Africa heading toward Eurasia through the Middle East migratory footpaths as shown in Figure 2. There are two migration paths, both are originated from Sub-Saharan Africa (López et al. 2015). One path took humans towards North Africa, then to Levant, Mesopotamia, Anatolia and Iran, and later on to Arabia, India and Europe. The other path was taken by humans through Bab Al Mandab strait (over the Red Sea) to Arabia. Then goes to North reaching the first path or migrated over the Persian Gulf to Iran and India. Basically, Middle East (particularly, Mesopotamia- currently Iraq and Kurdistan) is the convergent of both paths.

Genotype frequency of TLR4 SNP global populations have been shown in Table (1), showed that the homozygous mutants of both SNPs are non-existent or rare in almost all populations in the world. Whereas the heterozygous variants, Asp299Gly and Thr399Ile, varied among populations. For instance, their genotype frequency percentages were 14.3% and 1.6% in Sub-Saharan populations, respectively. However, they were 11.3% and 6.8% in North African people, respectively. These data suggest that both populations are mostly different in the TLR4 Thre399Ile heterozygous variant. Differences in allele frequencies between Sub Saharan and Northern African populations is attributed to a difference between two main groups of African populations to recent migrations and occupations, in addition to emergence of empires and religions. In fact, there were several migrations and invasions among Arabia, Europe, Middle East and North Africa in the past. These have led to cultural intermingling among those populations leading to exchanging genes among each other. A study on autosomal DNA polymorphisms in the Tunisian population revealed that North Africa had been occupied by several invaders from the Middle East and Europe in the past history, in addition to migrations occurred from Sub-Sahara, constituting a genetically diverse population (Cherni et al. 2016).

Table 1 also showed that the Arabic peninsula, Iraq, and Iran have 15.6% and 17%; 10.4% and 9.1%; and 9.1% and 10.1% TLR4 Asp299Gly and Thr399Ile heterozygous genotype frequency, respectively. It can be speculated that the Arabic peninsula, Mesopotamia (modern Iraq) and Persia (modern Iran) had been intermediate locations for both ancient migrations and recent empire invasions among Africa, Europe and Asia. Thus, the heterozygous TLR4 SNPs have been conserved either due to various ethnic background admixtures or as a microbial selective pressure. As we previously stated that there are associations between infectious diseases and TLR4 SNPs. Thus, it could be envisaged that humans who had heterozygous TLR4 SNPs might have similarly evaded microbial infections

as those who carried the wild-type TLR4 haplotypes. In contrast, the homozygous mutants may have faced higher rates of mortality by infectious agents endemic in those locations to which humans migrated or re-migrated. It can be stated that re-migrations back to Africa (López and Hellenthal 2015) have led to admixtures of genetic polymorphisms caused changes in allelic frequencies and genetic diversities. It could be suggested that both microbial pressure and admixture due to re-migrations might have played simultaneous roles for conservations of human TLR4 genetic polymorphisms. However, microbial selective pressure tends to be more probable than re-migrations for shaping TLR4 SNPs as microbes become endemic or find animal reservoirs and then they evolve over time that may escape the SNP variants of the receptor. In contrast, re-migrations occasionally happen to lead to "non-adaptive evolution".

The population of Turkey has 4.6% and 4.8% TLR4 Asp299Gly and Thr399Ile heterozygous genotype frequency, respectively. Turkey is a multi-ethnic country including predominantly Turkish people. The migration of Turkic-speaking people started during the 3rd century A.D. from the Altai region between Mongolia and Kazakhstan to Anatolia, replacing Indo-European speakers (Quintana-Murci et al. 2004). Thus, lower genotype frequencies of the TLR4 SNPs than other West Asian populations might be attributed to their Central Asian genetic background.

The Indian population has 18.5% and 14.3% TLR4 Asp299Gly and Thr399Ile heterozygous genotype frequency, respectively. These higher frequencies of the TLR4 SNPs in India may be associated with 'survival advantage' against neglected tropical diseases such as Malaria, Leishmania, and tuberculosis. It has been shown that both TLR4 Asp299Gly and Thr399Ile SNPs reduce the inflammatory responses against Malaria (Mukherjee et al. 2019).

European populations have 11% and 11.5% TLR4 Asp299Gly and Thr399Ile heterozygous genotype frequency, respectively. This is very similar to West Asian

populations. This supports the evidence that the alleles in modern-day Europe originated from migrations from the Middle East and other Western Asian territories (Xiao et al. 2004). Likewise, the similarity between the genotypic frequency between North African and Levantine populations is probably as a result of re-migration back to Africa as mentioned previously (López et al. 2015).

Han Chinese has almost 0% for both TLR4 SNPs. This might be due to low gene flow among the large Chinese populations. It has been revealed that allele frequencies are reduced because of genetic drift which resulted from migration to a new location, and environmental adaptations might have been another cause (Blair & Feldman 2015). The absence of the mutant SNPs among East Asian populations is possibly due to genetic drift caused by serial founder effects as described by Lopez *et al* 2015 in a manner that a single population migrated into an unoccupied region and then further migrated into another unoccupied place (López et al. 2015).

The absence of mutant TLR SNPs occurred in some populations, however, they are present in other populations and this is may be linked to migrations and invasions. For instance, TLR2 2029C>T is absent in both European and Non-European populations except the Vlax-Roma who possibly have Indo-Aryan roots and migrated into South Asia while both TLR2 1892C>A and 2258G>A polymorphisms are only present in European people that might be arisen from proto-Indo-Europeans (Ioana et al. 2012b). These suggest significant roles of human migrations and globalisations in shaping the gene pools among populations. Although, some alleles are conserved or lost in some populations as a result of genetic drift or founder effects. Extensive population research is essential to find this and link it with disease susceptibility in various people.

Overall, populations in West Asia, which includes Levant, Iraq, Iran, Turkey, India, and Pakistan, have 12.6% and 11.4% TLR4 Asp299Gly and Thr399Ile heterozygous

genotype frequency, respectively. These data support the fact that humans migrated through and settled in these regions after leaving Africa in addition to an admixture of human civilizations during the Neolithic and modern eras in the so-called Fertile Crescent of the Middle East. Using human genomic structures, a study has recognized the influence of genetic admixtures that lead to shaping human populations resulted from main events, including actions (e.g., migrations, invasions, displacements, slavery, and trades) by Mongols, Arab traders, and European colonization (Hellenthal et al. 2014). Figure (2) shows the map of the out-of-African migrations linked with global heterozygous TLR4 SNPs. Up to our best knowledge, no specific studies are available that investigated Native American and Australian TLR4 SNP populations. It would be interesting to investigate whether the genotype frequencies of the TLR4 SNPs of these two aboriginal populations are different from Asian populations. This may shed a light on any genetic drift that occurred in the global evolutionary paths of human out-of-African migrations.

Conclusions and Recommendations

This study found that the Kurdish population at the University of Garmian has 7.9% and 9.7% TLR4 Asp299Gly and Thr399Ile heterozygous genotype frequency, respectively, with 3.5% SNP co-segregations but without finding any homozygous mutants. This corresponds with the uncommonness of the homozygous mutants of TLR4 SNPs in the world. Global review data showed that homozygous wild-type genotypes of TLR4 SNPs were common while homozygous mutants were seldom in all populations. The heterozygous TLR4 genotypes of both SNPs varied from a population to another. The geographically distributed TLR4 SNPs were different in genotype frequencies in different groups categorized according to out-of-Africa migrations. Western Asia (Asp299Gly= 12.6%, Thre399Ile= 11.4%) and Eurasia (Asp299Gly= 11 %, Thre399Ile= 11.5%) had similar genotype frequencies of the

TLR4 SNPs. The TLR4 SNPs in North Africa (Asp299Gly= 11.3%, Thre399Ile= 6.8%) were slightly different, only in the Thre399Ile SNP, compared with those of Western Asia and Eurasia. Whereas the frequency of TLR4 Thre399Ile (1.6%) was lower in Sub-Saharan African compared with Western Asia, Eurasia and North Africa. However, TLR4 Asp299Gly (14.3%) of Sub-Saharan Africa was higher than any other global population group studied. Both SNPs were lacking or rare in East Asian population groups. Despite less attention by researchers, global co-segregations of both TLR4 SNPs in the same individuals were different in various populations. Future works will focus on associations of the TLR4 SNPs with infectious diseases in the Kurdish population.

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Table 1. Reviewed Genotype Frequency of the Common TLR4 SNPs of Global Populations

Genotypes	TLR4 Asp299Gly or 896 A/G (rs4986790)			TLR4 Thr399Ile or 1196 C/T (rs4986791)			References
Regions	Asp/Asp **AA**	Asp/Gly **AG**	Gly/Gly **GG**	Thr/Thr **CC**	Thr/Ile **CT**	Ile/Ile **TT**	
Sub-Saharan Africa	2,122 (84.9%)	358 (14.3%)	19 (0.8%)	1,000 (98.4%)	16 (1.6%)	0 (0%)	(Abbas et al. 2016; Allen et al. 2003; Baker et al. 2012; Esposito et al. 2012; Greene et al. 2009; Hassan et al. 2020; Kresfelder, Janssen, Bont, Pretorius, & Venter 2011; Mockenhaupt, Cramer, et al. 2006; Mockenhaupt, Hamann, et al. 2006; Newport et al. 2004)
	n= 2,499			n= 1016			
North Africa	1,520 (86.6%)	198 (11.3%)	37 (2.1%)	1,022 (92.9%)	75 (6.8%)	3 (0.3%)	(Ejghal et al. 2016; Feki et al. 2017; A. Hussein et al. 2018; Y. M. Hussein, Awad, Shalaby, Ali, & Alzahrani 2012; Lachheb, Dhifallah, Chelbi, Hamzaoui, & Hamzaoui 2008; Moaaz, Youssry, Moaz, & Abdelrahman 2020; Omrane et al. 2014; Senhaji et al. 2014; Sghaier et al. 2019; Zouiten-Mekki et al. 2009)
	n= 1,755			n= 1,100			
West Asia	7,085 (86.7%)	1,032 (12.6%)	51 (0.6%)	6,365 (88%)	822 (11.4%)	48 (0.6%)	(Abdolvahabi et al. 2018; Abdul-Mohsen & Chaloob 2014; Abu-Amero, Jaeger, Plantinga, Netea, & Hassan 2013; Ajdary et al. 2011; Al-Hilaly, Salman, & Dakheel 2015; Al-Mayah, Al-Dabagh, & Ali 2014; Al-Qahtani et al. 2014; Awasthi & Pandey 2019; Azzam, Nounou, Alharbi, Aljebreen, & Shalaby 2012; Bagheri et al. 2016; Chua et al. 2016; Degirmenci et al. 2019; Düzgün, Duman, Haydardedeoğlu, & Tutkak 2007; Eed et al. 2020; Gond, Singh, & Agrawal 2018; Güven et al. 2016; Ioana et al. 2012a; Jafari et al. 2016; Jahan et al. 2019; Jahantigh et al. 2013;
	n= 8,168			n= 7,235			

							Janardhanan et al. 2013; Kalkanci et al. 2020; Karaca, Ozturk, Gerceker, Turkmen, & Berdeli 2013; Karakas-Celik et al. 2014; Karasneh et al. 2015; Katrinli, Nigdelioglu, Ozdil, Dinler-Doganay, & Doganay 2018; Kesici, Kargın Kaytez, Özdaş, & Özdaş 2019; Kurt et al. 2016; Meena, Verma, Verma, Ahuja, & Paul 2013; Mohammadpour-Gharehbagh et al. 2019; Mousa et al. 2016; Mutlubas, Mir, Berdeli, Ozkayin, & Sozeri 2009; Myles & Aggarwal 2013; Najmi, Kaur, Sharma, & Mehra 2010; Noori et al. 2018; Nyati et al. 2010; Osman et al. 2016; Pandey et al. 2019; Pirahmadi, Zakeri, & Abouie Mehrizi 2013; Rafiei et al. 2012; Rajendiran, Rajappa, Chandrashekar, & Thappa 2019; Rani, Nawaz, Arshad, & Irfan 2018; Rasouli et al. 2012; Rupasree, Naushad, Rajasekhar, Uma, & Kutala 2015; Sawian, Lourembam, Banerjee, & Baruah 2013; Selvaraj, Harishankar, Singh, Jawahar, & Banurekha 2010; Semlali et al. 2016 2017 2019; Shamran, Al-Obaidi, Jamal, & Latif 2015; A. Singh et al. 2015; K. Singh et al. 2014; V. Singh, Srivastava, Kapoor, & Mittal 2013; Sinha, Singh, Jindal, Birbian, & Singla 2014; SOYDAŞ et al. 2017; Turkey, Abbas, Hathal, & Abdulrasul 2019; Zakeri, Pirahmadi, Mehrizi, & Djadid 2011)
Eurasia	7,493 (88.4%)	938 (11%)	48 (0.6%)	4,504 (88.1%)	585 (11.5%)	22 (0.4%)	(Arabski et al. 2012; Balistreri et al. 2008; Buraczynska, Zukowski, Ksiazek, Wacinski, & Dragan 2016; Despriet et al. 2008; Etokebe et al. 2009; Gębura et al. 2017; Golovkin et al. 2015; Gowin et al. 2017; Greene et al. 2009; Hamann et al. 2005; Heesen, Wessiepe, Kunz, Vasickova, & Blomeke 2003; Iliadi et al. 2009; Jabłońska et al. 2020; James et al. 2007; Jedlińska-Pijanowska et al. 2020; Karananou
	n= 8,479			n= 5,111			

							et al. 2019; Koch, Hoppmann, Pfeufer, Schömig, & Kastrati 2006; Kutikhin, Yuzhalin, Volkov, Zhivotovskiy, & Brusina 2014; Lammers et al. 2005; Marchionni et al. 2020; Molvarec, Jermendy, Kovács, Prohászka, & Rigó 2008; Mrazek, Gallo, Stahelova, & Petrek 2013; Nedoszytko et al. 2018; Noack et al. 2008; Perica et al. 2015; Piñero et al. 2017; Rigoli et al. 2008 2010; Rybka et al. 2016; Salpietro et al. 2011; Sanders et al. 2013; Sheedy, Marinou, O'Neill, & Wilson 2008; Soriano-Sarabia et al. 2008; Varzari et al. 2019; Verweij et al. 2016; Wujcicka, Gaj, Wilczyński, & Nowakowska 2015; Wujcicka, Paradowska, et al. 2015; Wujcicka, Paradowska, Studzińska, Wilczyński, & Nowakowska 2017; Wujcicka, Wilczyński, & Nowakowska 2017)
East Asia	8,382 (99.14%)	70 (0.83%)	3 (0.04%)	7,740 (98.5%)	113 (1.4%)	4 (0.05%)	(Aki et al. 2015; Chen, Lin, Zhan, & Lv 2012; Cheng, Eng, Chou, You, & Lin 2007; Chua et al. 2016; Fan et al. 2014; Fukusaki, Ohara, Hara, Yoshimura, & Yoshiura 2007; Greene et al. 2009; Gu et al. 2014; Guo, Zhu, & Xia 2006; Hang et al. 2004; Jiang, Wang, Jia, Wang, & Liu 2013; E. J. Kim et al. 2012; Y. S. Kim et al. 2008; Lin et al. 2005; Liu et al. 2012; Nguyen et al. 2009; Qing et al. 2013; Shen, Liu, Liu, & Zhang 2013; A.-C. Wang, Wu, Gao, & Sheng 2014; J. Wang et al. 2014; Y. Wang et al. 2017; Xue et al. 2010; Yoon et al. 2006; Zheng et al. 2010)
	n= 8,455			n= 7,857			

Supplementary Table S1.

Variants	**TLR4 Asp299Gly or 896 A/G (rs4986790)**			**TLR4 Thr399Ile or 1196 C/T (rs4986791)**			
Genotypes	Asp/Asp **AA**	Asp/Gly **AG**	Gly/Gly **GG**	Thre/Thre **CC**	Thre/Ile **CT**	Ile/Ile **TT**	References
Countries							
Kenya	78 (86%)	13 (14%)	-	64 (97%)	2 (3%)	-	Greene 2009
	73 (81%)	17 (19%	0 (0%)	-	-	-	Baker 2012
	120 (83.9%)	22 (15.4%)	1 (0.7%)	-	-	-	
Ghana	234 (78.8%)	59 (19.9%)	4 (1.3%)	291 (98%)	6 (2%) 6 (2%) co-segregated	0 (0%)	Mockenhaupt 2006
	239 (82.4%)	47 (16.2%)	4 (1.4%)	283 (97.6%)	7 (2.4%) 4.3% co-segregated	0 (0%)	Mockenhaupt 2006 PNAS
Uganda	44 (92%)	4 (8%)	0 (0%)	48 (100%)	0 (0%)	0 (0%)	Baker 2012
Tanzania	312 (92%)	25 (7.4%)	2 (0.6%)	-	-		Abbas 2016
Burundi	300 (89.0%)	36 (10.7%)	1 (0.3%)	-	-	-	Esposito 2012

Nigeria	105 (92%)	9 (8%)	0 (0%)	114 (100%)	0 (0%)	0 (0%)	Baker 2012
Gambia	235 (78.9%)	58 (19.4%)	5 (1.7%)	-	-	-	Newport 2004
	198 (78.9%)	51 (20.3)	2 (0.8%)	-	-	-	Allen 2003
South Africa	99 (88%)	14 (12%)	0 (0%)	113 (100%)	0 (0%)	0 (0%)	Kresfelder 2011
	48 (100%)	0 (0%)	0 (0%)	48 (100%)	0 (0%)	0 (0%)	Baker 2012
	37 (92.5%)	3 (7.5%)	0 (0%)	39 (97.5%)	1 (2.5%) 1 (2.5%) co-segregated	0 (0%)	Hassan 2020
Sub-Saharan Africa Average	**2,122 (84.9%)** **n= 2,499**	**358 (14.3%)**	**19 (0.8%)**	**1,000 (98.4%)** **n= 1016**	**16 (1.6%)** **14 (1.4%) co-segregated**	**0 (0%)**	
	223 (99.7%)	0 (0%)	1 (0.04%)	221 (98.6%)	2 (0.09%)	1 (0.04%)	Lachheb 2008
	71 (89%)	9 (11%)	0 (0%)	72 (90%)	8 (10%)	0 (0%)	Zouiten-Mekki 2009
	102 (89.4%)	12 (10.5%)	0 (0%)	-	-	-	
	140 (87.5%)	18 (11.25%)	2 (1.25%)	152 (95%)	8 (5%)	0 (0%)	Feki 2017

Tunisia	120 (85.7 %)	18 (12.9 %)	2 (1.4 %)	123 (87.9 %)	17 (12.1 %) CTAG= 15 (10.7%)	0(0%)	Omrane 2014
	253 (70.3%)	77 (21.4%)	30 (8.3%)	-	-	-	Sghaier 2018
Morocco	42 (97.7%)	1 (2.3 %)	0 (0%)	34 (79.1%)	9 (20.9%)	0 (0%)	Ejghal 2016
	103 (92%)	8 (7.1%)	1 (0.9%)	109(97.3%)	3 (2.7%)	0 (0%)	Senhaji 2014
Egypt	125(88.7%)	15(10.6%)	1(0.7%)	131(92.9%)	9 (6.4%) 4 (1.4%) co-segregated	1(0.7%)	Moaaz 2020
	223 (89 %)	28 (11%)	0 (0%)	-	-	-	Hussein 2012
	188 (94%)	12 (6%)	0 (0%)	180 (90%)	19 (9.5%)	1 (0.5%)	Hussein 2018
North Africa Average	**1,520 (86.6%)** **n= 1,755**	**198 (11.3%)**	**37 (2.1%)**	**1,022 (92.9%)** **n= 1,100**	**75 (6.8%)** **21 (1.9%) co-segregated**	**3 (0.3%)**	
North American Africans	65(74%)	23(26%)	-	84(94%)	5(6%)	-	Greene 2009
Southwest American Africans	48 (90.6%)	4 (7.5%)	1 (1.9%)	-	-	-	Baker 2012

American Africa Average							
Saudi	166 (87%)	20 (11%)	1 (1%)	-	-	-	Semlali 2019
	101 (88%)	14 (12%)	0 (0%)	-	-	-	Semlali 2017
	92 (92%)	7 (7%)	1 (1%)	-	-	-	Semlali 2016
	178 (88.6%)	23 (11.4%)	0 (0%)	184 (91.5%)	17 (8.6%) 15 (7.5% co-segregated)	0 (0%)	Abu-amero 2013
	475 (79.2%)	123 (20.5%)	2 (0.3%)	469 (78.1%)	130 (21.7%) 36 (6.1%) co-segregated Protective role against HCV	1 (0.2%)	Al-Qahtani 2014
	-	-	-	32 (64.0%)	14 (28.0%)	4 (8.0%)	Azzam 2012
	-	-	-	87 (91.6%)	8 (8.4%)	0 (0%)	Mousa 2016
	95 (92.2%)	8 (7.8%)	0 (0%)	94 (91.3%)	9 (8.7%)	0 (0%)	Osman 2016
	56 (70%)	21 (26.3%)	3 (3.8)	63 (78.8%)	14 (17.5%)	3 (3.8)	Eed 2020
Arabia Average	**1,163 (83.9%)** **n= 1,386**	**216 (15.6%)**	**7 (0.5%)**	**929 (82.3%)** **n= 1,129**	**192 (17%)**	**8 (0.7%)**	

					51 (4.5%) co-segregated		
Israel	No TLR4 SNPs (n=189)= 89.6%, Any TLR4 SNP= 10.4%. Co-segregation= 4.67%						LESHINSKY 2005
	n= 90, TLR4 Asp299G= 4 (4.4%)			n= 90, TLR4 Thr399Ile= 4 (4.4%)			Tal 2004
Turkey	137 (97%)	4 (3%)	0 (0%)	138 (98%)	3 (2%)	0 (0%)	Düzgün 2007
	196 (98%)	3 (1%)	1 (1%)	196 (98%)	2 (1%)	2 (1%)	Güven 2015
	62 (87.3%)	7 (9.9%)	2 (2.8%)	59 (83.1%)	8 (11.3%)	4 (5.6%)	Kesici 2019
	74 (94.9%)	4 (5.1%)	0 (0%)	74 (94.9%)	4 (5.1%)	0 (0%)	Degirmenci 2019
	27 (81.8%)	6 (18.2%)	0 (0%)	25 (75.8%)	8 (24.2%) 4 (12.1%) co-segregated	0 (0%)	Kalkanci 2020
	97 (97%)	2 (2%)	1 (1%)	95 (95%)	4 (4%)	1 (1%)	Karaca 2012
	78 (96.3%)	3 (3.7%)	0 (0%)	80 (98.8%)	1 (1.2%)	0 (0%)	Karakas-celik 2014
	165 (98.2%)	0 (0%)	3 (1.8%)	-	-	-	Katrinli 2017
	99 (99%)	1 (1%)	0 (0%)	91 (91%)	9 (9%)	0 (0%)	Kurt 2016
	109 (94.7%)	6 (5.3%)	0 (0%)	110 (95.7%)	5 (4.3%)	0 (0%)	Mutlubas 2009
	181 (89%)	23 (11%)		191 (93.5%)	10 (5%)	3 (1.5%)	SOYDAŞ 2017
Turkey (Average)	**1,225 (94.9%)** **n= 1,291**	**59 (4.6%)**	**7 (0.5%)**	**1,059 (94.3%)** **n= 1,123**	**54 (4.8%)**	**10 (0.9%)**	
Iraq	41(91.11%)	4 (4.89%) 0	0 (0%)	36 (80%)	9 (20%)	0 (0%)	Shamran 2015
	-	-	-	25 (100%)	0 (0%)	0 (0%)	Al-Hilaly 2015
	49 (94.23%)	3 (5.77%)	0 (0%)	48 (92.3%)	4 (7.69%)	0 (0%)	Chaloob 2014
	34 (94.45%)	2 (5.55%)	0 (0%)	34 (94.45%)	2 (5.55%)	0 (0%)	Al-Mayah 2014
	40 (80%)	10 (20%)	0 (0%)	46 (92%)	4 (8%)	0 (0%)	Turkey 2019

Iraq Average	**164 (89.6%)** **n= 183**	**19 (10.4%)**	**0 (0%)**	**189 (90.9%)** **n= 208**	**19 (9.1%)**	**0 (0%)**	
Jordon	146 (95.4%)	7 (4.6%)	0 (0%)	-	-	-	Karasneh 2015
Iran	146 (98%)	3 (2%)	0 (0%)	141 (94.6%)	7 (4.7%)	1 (0.7%)	Jahantigh 2013
	660 (90.41%)	66 (9.04%)	4 (0.55%)	657 (90%)	68 (9.31%)	4 (0.55%)	Ioana 2012
	73 (97.3%)	2 (2.7%)	0 (0%)	74 (86.7%)	1 (1.3%)	0 (0%)	Ajdary 2011
	39 (86.7%)	6 (13.3%)	0 (0%)	38 (84.4%)	7 (15.6%)	0 (0%)	
	287 (89.7%)	33 (10.3%)	0 (0%)	270 (84.4%)	50 (15.6%)	0 (0%)	Zakeri 2011
	29 (65.9%)	15 (34.1)	0 (0%)	-	-	-	Bagheri 2016
	315 (90%)	35 (10%)	0 (0%)	294 (84%)	56 (16%) Co-segregated 23 (6.6%)	0 (0%)	Pirahmadi 2013
	115 (94.3%)	7 (5.7%)	0 (0%)	120 (98.4%)	2 (1.6%)	0 (0%)	Jafari 2016
	98(85%)	16(14%)	1(1%)	105(91%)	9(8%)	1(1%)	Mohammad pourgharehbagh 2019
	79 (98.7%)	1 (1.3%)	0 (0%)	80 (100%)	0 (0%)	0 (0%)	Noori 2018
	89 (91.7%)	7 (7.2%)	1(1%)	89 (91.7%)	8(8.2)		Abdolvahabi 2018
	137 (88.4%)	18 (11.6%)	0 (0%)	137 (88.4)	18 (11.6) Co-segregated 7 (4.5 %)	0 (0%)	Rasouli 2012

	155 (91.2%)	14 (8.2)	1 (0.6)	-	-	-	Rafiei 2012
Iran (Average)	**2,222(90.62%)** **N= 2,452**	**223 (9.1%)**	**7 (0.28 %)**	**2,005(89.63%)** **N= 2,237**	**226(10.1%)**	**6(0.27%)**	**ALL**
Pakistan	196 (87%)	26 (11%)	4 (2%)	-	-	-	Rani 2018
India	65 (82%)	10 (13%)	4 (5%)	-	-	-	Sawian 2013
	206 (82.4%)	44 (17.6%)	0 (0%)	206 (82.4%)	43 (17.2%)	1 (0.4%)	Najmi 2010
	-	-	-	173 (86.5%)	26 (13%)	1 (0.5%)	Singh 2013
	497 (89%)	61 (11%)	-	531 (95%)	28 (9%)	-	AWASTHI 2019
	211 (81.08%)	47 (18.15%)	2 (0.77%)	217 (83.46%)	41 (15.77%)	2 (0.77%)	Gond 2017
	116 (86.5%)	16 (11.9%)	2 (1.4%)	101 (77%)	28 (21.3%)	2 (1.5%)	Janardhanan 2013
	107 (75.9%)	32 (22.7%)	2 (1.4%)	-	-	-	Pandey 2019
	154 (76.61%)	46 (22.88%)	1 (0.49%)	183 (91.10%)	18 (8.95%)	0 (0%)	Meena 2013
	247 (77.2%)	73 (22.8%)	0 (0%)	262 (81.9%)	54 (16.9%)	4 (1.2%)	Singh 2014
	181 (90.5%)	18 (9%)	1 (0.5%)	189 (94.5%)	10 (5%)	1 (0.5%)	Myles 2013
	127 (84.7%)	22 (14.7%)	1 (0.7%)	140 (93.3%)	9 (6.0%) 9 (6%) co-segregated	1 (0.7%)	Nyati 2010
	185 (70.1%)	70 (26.5%)	9 (3.4%)	-	-	-	Rajendiran 2019
	158 (70.9%)	63 (28.3%)	2 (0.9%)	161 (72.2%)	61 (27.4%) 41.55 (9%)	1 (0.4%)	Rupasree 2015
	151 (73 %)	53 (25.6%)	3 (1.4%)	152 (74.9%)	46 (22.6%) Co-segregated 22 (10.8%)	5 (2.5)	Selvaraj 2010

	117 (87.3%)	14 (10.4%)	3 (2.2%)	120 (89.6%)	11 (8.2%)	3 (2.2%)	Singh 2015
	381 (78.9%)	95 (19.7%)	7 (1.4%)	384 (79.5%)	92 (19 %)	7 (1.5%)	Sinha 2014
	-	-	-	67 (74.4%)	19 (21.1%)	4 (4.4%)	Chua 2016
India Average	**2,903 (80.6%)** **n= 3,600**	**664 (18.5%)**	**33 (0.9%)**	**2,886 (84.8%)** **n= 3,404**	**486 (14.3%)**	**32 (0.9%)**	
Bangladesh	124 (83%)	25 (17%)	0 (0%)	123 (82.5%)	26 (17.5%)	0 (0%)	Jahan 2019
Kurds	-	-	-	79 (92.9 %)	6 (7.1%)	0 (0%)	Niranji 2020
	105 (92.1%)	9 (7.9%)	0 (0%)	103 (90.3%)	11 (9.7%) 4 (3.5%) co-segregated	0 (0%)	This study (blood draws from Kurdish students and staff)
West Asia average	7,085 (86.7%) n= 8,168	1,032 (12.6%)	51 (0.6%)	6,365 (88%) n= 7,235	822 (11.4%) 110 (1.5%) co-segregated	48 (0.6%)	
Croatia	32 (80%)	2 (5%)	6 (15%)	31 (77.5%)	3 (7.5%)	6 (15%)	Perica 2015
	84 (84.8%)	15 (15.2%)	0 (0%)	-	-	-	Etokebe 2009

Greece	98 (89.9%)	9 (8.26%)	2 (1.84%)	96 (88.07%)	12 (11.01)	1 (0.92)	Karananou 2019
	187 (89.5%)	22 (10.5%)	1 (0.48%)	186 (89%)	22 (10.5%)	1 (0.48%)	Iliadi 2009
Italy	208 (92.9%)	16 (7.1%)	0 (0%)	-	-	-	Lammers 2005
	80 (91.9%)	7 (8.1%)	0 (0%)	81 (93.1%)	6 (6.9%)	0 (0%)	Rigoli 2010
	140 (93.4%)	10 (6.6%)	0 (0%)	139 (92.7%)	11 (7.3%)	0 (0%)	SALPIETRO 2011
	161 (84.7%)	28 (14.7%)	1 (0.53%)	-	-	-	Balistreri 2008
	-	-	-	141 (94%)	9 (6%)	0 (0%)	Marchionni 2020
	95 (92.2%)	8 (7.8%)	0 (0%)	97 (94.1%)	6 (5.9%)	0 (0%)	Rigoli 2008
Italy Average							
	171 (85.9%)	26 (13.1%)	2 (1.0%)	173 (86.9%)	24 (12.1%)	2 (1.0%)	Soriano-sarabia 2008

Spain	448 (89.1%)	52 (10.3%)	3 (0.6%)	-	-	-	Piñero 2017
Poland (Caucasian)	47 (94 %)	1 (2%)	2 (4%)	36 (87.8 %)	3 (7.3 %)	2 (4.9 %)	Wujcicka 2015
	53 (86.9%)	8 (13.1%)	0 (0%)	55 (87.3%)	8 (12.7%)	0 (0%)	Wujcicka 2017
	15 (83.3%)	1 (5.6%)	2 (11.1%)	14 (77.8%)	3 (16.7%)	1 (5.6%)	Wujcicka 2015 Plos
	130 (92.9%)	9 (6.4%)	1 (0.7%)	-	-	-	Jabłońska 2020
	41 (83.67%)	8 (16.33%)	0 (0%)	-	-	-	Gowin 2017
	-	-	-	44 (88%)	6 (12%	0 (0%)	Arabski 2012
	149 (87.1%)	22 (12.9%)	0 (0%)	-	-	-	Nedoszytko 2018
	107 (87%)	16 (13%)	0 (0%)	104 (85.2%)	18 (14.8%)	0 (0%)	Rybka 2016
	-	-	-	126 (89.4%)	13 (9.2%)	2 (1.4%)	Jedlińska-Pijanowska 2020

	658 (91.9%)	57 (8%)	1 (0.1%)	657 (91.8%)	59 (8.2%)	0 (0%)	Buraczynska 2016
	107 (87 %)	16 (13 %)	0 (0%)	104 (85.2%)	18 (14.8%)	0 (0%)	Gebura 2017
Poland Average							
Czeck (Caucasian)	166 (87.8%)	22 (11.6%)	1 (0.5%)	166 (87.8%)	23 (12.2%)	0 (0%)	Mrazek 2013
Netherlands (Dutch Caucasian)	374 (89 %)	33 (7.9%)	13 (3.1%)	-	-	-	Sanders 2013
	352 (86.1%)	57 (13.9%)	0 (0%)	-	-	-	Verweij 2016
	329 (89.3%)	39 (10.7%)	0 (0%)	323 (87.7%)	45 (12.3%)	0 (0%)	Despriet 2008
United Kingdom (Caucasian)	163 (86%)	25 (13%)	1 (1%)	-	-	-	Hamann 2005
	78 (83 %)	16 (17 %)	0 (0%)	74 (80.4%)	18 (19.6%) Complete co-segregation	0 (0%)	James 2007
	841 (87.2%)	121 (12.5%)	3 (0.3%)	-	-	-	Sheedy 2008
	83 (89%)	10 (11%)	0 (0%)	-	-	-	Heesen 2003

Germany (Caucasian)	1069 (88.3%)	138 (11.4%)	4 (0.3%)	1066 (88 %)	140 (11.6%) Co-segregated: 135 (11.1%)	5 (0.4%)	Koch 2006
	71 (988.8%)	9 (11.3%)	0 (0%)	71 (988.8%)	9 (11.3%)	0 (0%)	Noack 2008
Hungary (Caucasian)	150 (87.2%)	21 (12.2%)	1 (0.6%)	149 (86.6%)	22 (12.8%)	1 (0.6%)	MOLVAREC 2008
Maldova	228 (93.8%)	15 (6.2%)	0 (0%)	-	-	-	Varzari 2019
Russia (Caucasian)	253 (84.3%)	46 (15.3%)	1 (0.3%)	252 (84%)	47 (15.7%)	1 (0.3%)	Golovkin 2015
	258 (86 %)	39 (13 %)	3 (1%)	255 (85 %)	45 (15 %)	0(0%)	Kutikhin 2014
North America Caucasians	67 (83%)	14 (17%)	-	64 (81%)	15 (19%)	-	Greene 2009
Eurasia Average	**7,493 (88.4%)** **n= 8,479**	**938 (11%)**	**48 (0.6%)**	**4,504 (88.1%)** **n= 5,111**	**585 (11.5%)** **153 (2.3%) co-segregated**	**22 (0.4%)**	
China (Han)	105 (100%)	0 (0%)	0 (0%)	105 (100%)	0 (0%)	0 (0%)	Wang 2014
	519 (99.4%)	1 (0.2%)	2 (0.4%)	517 (99.0%)	3 (0.6%)	2 (0.4%)	Shen 2013
	66 (100%)	0 (0%)	0 (0%)	466 (100%)	0 (0%)	0 (0%)	Liu 2012
	491 (100%)	0 (0%)	0 (0%)	491 (100%)	0 (0%)	0 (0%)	Hang 2004
	250 (100%)	0 (0%)	0 (0%)	250 (100%)	0 (0%)	0 (0%)	Cheng 2007
	126 (100%)	0 (0%)	0 (0%)	126 (100%)	0 (0%)	0 (0%)	Wang, Simayi 2014
	203 (99.5%)	1 (0.5%)	0 (0%)	-	-	-	Gu 2014

	285 (91.3%)	27 (8.7%)	-	304 (97.4%)	8 (2.6%)	-	Fan 2014
	160 (100%)	0 (0%)	0 (0%)	-	-	-	Guo 2006
	835 (100%)	0 (0%)	0 (0%)	835 (100%)	0 (0%)	0 (0%)	Jiang 2013
	226 (100%)	0 (0%)	0 (0%)	226 (100%)	0 (0%)	0 (0%)	Qing 2013
	164 (100%)	0 (0%)	0 (0%)	164 (100%)	0 (0%)	0 (0%)	Chen, Lin 2012
	203 (100%)	0 (0%)	0 (0%)	203 (100%)	0 (0%)	0 (0%)	Xue 2009
	247 (100%)	0 (0%)	0 (0%)	247 (100%)	0 (0%)	0 (0%)	Zheng 2010
	-	-	-	86 (100.0)	0 (0%)	0 (0%)	Chua 2016
China (Uygur)	1671 (98.8%)	21 (1.2%)	0 (0%)	1590 (94%)	100 (5.9%)	2 (0.1%)	Wang, Chen 2017
	210 (91%)	20 (8.6%)	1 (0.4%)	-	-	-	Gu 2014
Taiwan	457 (100%)	0 (0%)	0 (0%)	-	-	-	Lin 2005
	200 (100%)	0 (0%)	0 (0%)	200 (100%)	0 (0%)	0 (0%)	Cheng 2007
Korea	153 (100%)	0 (0%)	0 (0%)	153 (100%)	0 (0%)	0 (0%)	Kim 2008
	178 (100%)	0 (0%)	0 (0%)	178 (100%)	0 (0%)	0 (0%)	Kim 2012
	179 (100%)	0 (0%)	0 (0%)	179 (100%)	0 (0%)	0 (0%)	Yoon 2006
Japan	45 (100%)	0 (0%)	0 (0%)	45 (100%)	0 (0%)	0 (0%)	Aki 2015
	100 (100%)	0 (0%)	0 (0%)	100 (100%)	0 (0%)	0 (0%)	Fukusaki 2007
Vietnam	372 (100%)	0 (0%)	0 (0%)	284 (100%)	0 (0%)	0 (0%)	Hue 2009
Malaysia	-	-	-	72 (97.3%)	2 (2.7%)	0 (0%)	Chua 2016
Papua New Guinea	906 (100%)	0 (0%)	-	906 (100%)	0 (0%)	-	Greene 2009
North America Asians	76(92%)	7(8%)	-	77(93%)	6(7%)	-	Greene 2009
Asians Average	8,382 (99.14%) n= 8,455	70 (0.83%)	3 (0.04%)	7,740 (98.5%) n= 7,857	113 (1.4%)	4 (0.05%)	

Figure Captions

Figure 1. Human TLR4 partial gene sequencing (NCBI gene bank AB445638.1) showing the sites where primers are designed and fitted with the restriction sites: In the Asp299Gly forward primer (GATTAGCATACTTAGACTACTACCTCCATG), the nucleotide G was designed to be substituted with C (underlined) to fit with the restriction site, *Nco1* (ccatgg). Thus, in the wild type *Nco1* restriction site is not formed (ccatgA) for allele A ((Aspartate 299 (gAt)) and the PCR product remained as an uncut band (249 bp) on gel electrophoresis. While the restriction site (ccatgG) is formed in case of the presence of the mutant g allele ((Glycine 299 (gGt)) and the PCR product appears as cleaved bands (223 bp) on the gel electrophoresis. Similarly, for the Thr399Ile forward primer (GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA), the nucleotide C in the gene sequence was designed to be replaced with G in order to fit with the *Hinf1* restriction site (gaNtc) when N is any nucleotide. When the wildtype is present, the enzyme can not cleave the sequence (406 bp) as it contains no restriction site (gaaCc) for the *Hinf1* enzyme. The aCc is the codon for Threonine 399. In contrast, the mutant sequence (gaaTc) fits with the *Hinf1* restriction site and it is cleaved by the enzyme (379 bp). The aTc is the codon for Isoleucine 399. The reverse primers for both SNPs are also determined.

Figure 2. Map of the Old World. The arrows show paths where out-of-African migrations occurred. The percentage of the heterozygous TLR4 SNPs, Asp/Gly 299 and Thre/Ile 399 were displayed. The map was generated by Landsatlook viewer (USGS Products, from the U.S. Geological Survey: https://landsatlook.usgs.gov/).

Figure 1.

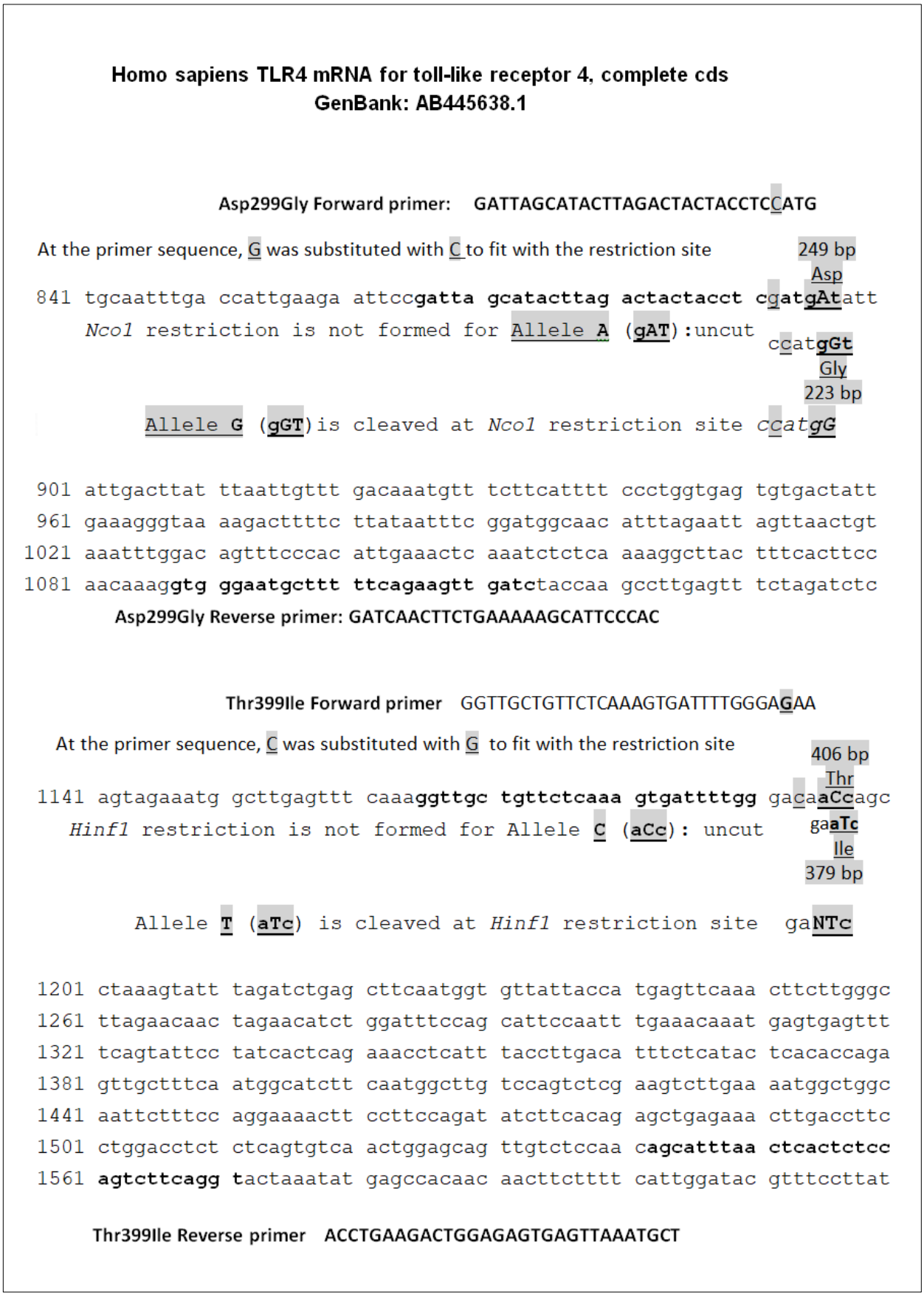
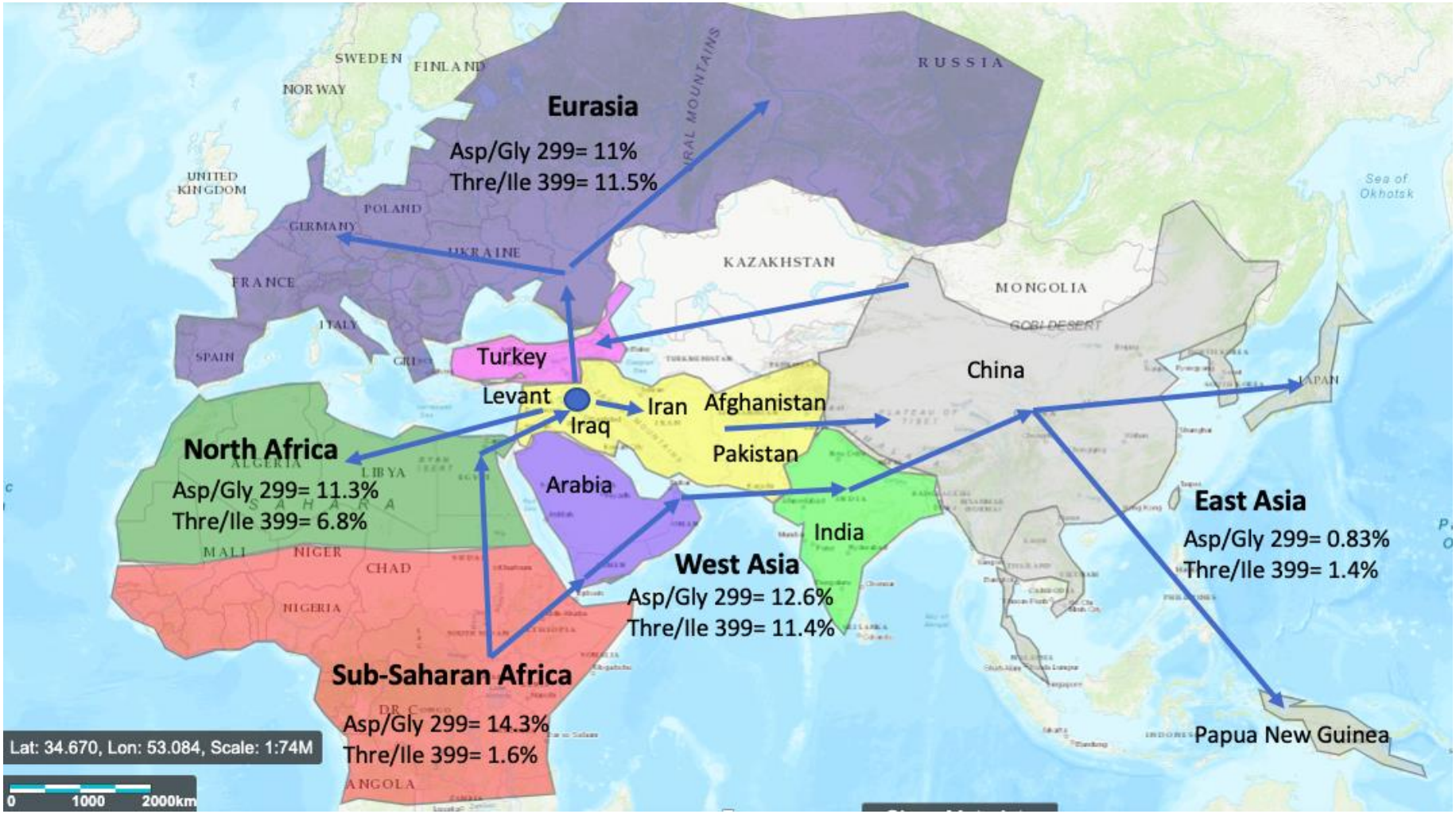

Homo sapiens TLR4 mRNA for toll-like receptor 4, complete cds
GenBank: AB445638.1
Asp299Gly Forward primer: GATTAGCATACTTAGACTACTACCTCCATG
At the primer sequence, G was substituted with C to fit with the restriction site
249 bp
Asp
841 tgcaatttga ccattgaaga attccgatta gcatacttag actactacct cgatgAtatt
Nco1 restriction is not formed for Allele A (gAT):uncut
ccatgGt
Gly
223 bp
Allele G (gGT)is cleaved at Nco1 restriction site ccatgG
901 attgacttat ttaattgttt gacaaatgtt tcttcatttt ccctggtgag tgtgactatt
961 gaaagggtaa aagacttttc ttataatttc ggatggcaac atttagaatt agttaactgt
1021 aaatttggac agtttcccac attgaaactc aaatctctca aaaggcttac tttcacttcc
1081 aacaaaggtg ggaatgcttt ttcagaagtt gatctaccaa gccttgagtt tctagatctc
Asp299Gly Reverse primer: GATCAACTTCTGAAAAAGCATTCCCAC
Thr399Ile Forward primer GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA
At the primer sequence, C was substituted with G to fit with the restriction site
406 bp
Thr
1141 agtagaaatg gcttgagttt caaaggttgc tgttctcaaa gtgattttgg gacaaCcagc
Hinf1 restriction is not formed for Allele C (aCc): uncut
gaaTc
Ile
379 bp
Allele T (aTc) is cleaved at Hinf1 restriction site gaNTc
1201 ctaaagtatt tagatctgag cttcaatggt gttattacca tgagttcaaa cttcttgggc
1261 ttagaacaac tagaacatct ggatttccag cattccaatt tgaaacaaat gagtgagttt
1321 tcagtattcc tatcactcag aaacctcatt taccttgaca tttctcatac tcacaccaga
1381 gttgctttca atggcatctt caatggcttg tccagtctcg aagtcttgaa aatggctggc
1441 aattctttcc aggaaaactt ccttccagat atcttcacag agctgagaaa cttgaccttc
1501 ctggacctct ctcagtgtca actggagcag ttgtctccaa cagcatttaa ctcactctcc
1561 agtcttcagg tactaaatat gagccacaac aacttctttt cattggatac gtttccttat
Thr399Ile Reverse primer ACCTGAAGACTGGAGAGTGAGTTAAATGCT

Figure 2.


Eurasia
Asp/Gly 299= 11%
Thre/Ile 399= 11.5%
Turkey
Levant
Iraq
Iran
Afghanistan
Pakistan
Arabia
India
China
North Africa
Asp/Gly 299= 11.3%
Thre/Ile 399= 6.8%
West Asia
Asp/Gly 299= 12.6%
Thre/Ile 399= 11.4%
East Asia
Asp/Gly 299= 0.83%
Thre/Ile 399= 1.4%
Sub-Saharan Africa
Asp/Gly 299= 14.3%
Thre/Ile 399= 1.6%
Papua New Guinea
RUSSIA
KAZAKHSTAN
MONGOLIA
SWEDEN
FINLAND
NORWAY
UNITED KINGDOM
POLAND
GERMANY
UKRAINE
FRANCE
SPAIN
ITALY
JAPAN
LIBYA
MALI
NIGER
CHAD
NIGERIA
ANGOLA
Lat: 34.670, Lon: 53.084, Scale: 1:74M
0
1000
2000km