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A Study on the Diversity of Pharmacogenomic Variants Affecting Dapsone Hypersensitivity: A Comparative Study Based on South Asian and Other World Populations

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Abstract

Recent data from Sri Lanka indicates an increase in leprosy cases, emphasizing the necessity of dapsone as a drug vital for managing it. Ironically, dapsone effectiveness is accompanied by dapsone hypersensitivity syndrome (DHS) which varies among populations. We postulate that this is due to significant differences between SNP frequencies in *HLA-B*13:01, CYP2C9*3, rs701829, rs17211071, and rs201929247.* As per our reading, no comparative study has been done so far on DHS and related genes between South Asian (SAS) and other world populations. Therefore, this study compares the allele frequencies of SNPs from PharmGKB and dbSNP of world populations against SAS using chi-square (χ^2) tests. For *HLA-B*13:01*; it is reported that Europeans, Africans, African others, and African Americans have demonstrated significant differences, and Asians, EAS, Other Asians, and Latin Americans have shown no significant differences. For *CYP2C9*3* and *rs701829*; Americans, Africans, Amish, Ashkenazi Jews, East Asians (EAS), Finns, and Non-Finnish Europeans (NFE) all have demonstrated a significant difference from SAS. For *rs17211071*, Africans, Amish, Americans, East Asians, Finns, and NFE demonstrated no significant difference, and ASJ showed a significant difference. For *rs201929247*, Africans and Finns had no significant differences, whereas Americans, Amish, ASJ, EAS, and NFE had. Hence, compared with other populations, allele frequencies of some studied SNPs were significantly different in SAS, and these may likely account for the variability of DHS occurrence among these populations. Significant allele frequency differences between SAS and the rest of the world populations' impact personalized medicine in leprosy treatment. Clinical research needs to determine the optimal dapsone dose alterations, considering environmental and other factors behind DHS.

Keywords: Pharmacogenomics; Personalized Medicine; Leprosy; DHS; Dapsone

Introduction

Dapsone, known as diamino diphenyl sulfone (DDS), is a popular drug with a complex history mostly used for its many medicinal efficacies, largely to treat leprosy, dermatological problems, and other infectious diseases (Barr, 2011). Leprosy, sometimes called Hansen's sickness, is a chronic contagious illness that has been afflicting people for ages. *Mycobacterium leprae* is a kind of slow-growing bacteria that causes leprosy (Draper, 1983). Every year, a stable amount of more than 200,000 leprosy cases are being registered worldwide (Naaz et al., 2017). In 1981, the WHO (World Health Organization) developed MDT (Multi Drug Therapy), recommending a mix of three specific

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antibiotics to treat leprosy: dapsone, rifampicin, and clofazimine (Fahad, 2019). Even though Sri Lanka achieved the status of elimination (less than 1 leprosy patient per 10,000 of the population) of leprosy as a public health problem at the national level in 1995 (WHO, 2012), an increasing trend of leprosy cases has been detected recently (Dabrera et al., 2016), emphasizing the necessity of dapsone in managing the situation. It is noticed that adverse drug reactions associated with MDT are common among the patients diagnosed with leprosy in Sri Lanka. (Hewawasam et al., 2023) Officials from the Health Ministry of Sri Lanka state that this rising trend has continued since 2023 with a record number of 15,864 leprosy cases identified just in the first seven months of that year. It was reported that 161 of them were from Colombo.

Despite the effectiveness of dapsone as a treatment, DHS is found to be a serious clinical problem. According to Satapornpong et al. (2021), a small proportion of the patients who take dapsone (0.5%-3.6%) may develop DHS, a rare but possibly fatal side response (reported with a mortality rate of 9.9% among who are treated with dapsone). Skin rash, high-grade fever, lymphadenopathy, and multi-organ involvement are a few of the clinical signs of DHS, which are serious and perhaps fatally adverse drug reactions (Wang et al., 2023). The occurrence of DHS varies among SAS and other world populations at clinical levels, with higher rates in some populations and lower rates in others (Gomes et al., 2019). This is a serious clinical problem for medical practitioners in SAS and worldwide in prescribing medications for leprosy. Zhang F.R. et al., 2013 have scientifically proven that the *HLA-B*13:01* SNP (Single Nucleotide Polymorphism) has been linked to a higher risk of developing DHS in the Chinese population. Krismawati et al. (2020), confirmed the genetic predisposition *HLA-B*13:01* to DHS found by Zhang F.R. et al., 2013. A high risk of developing DHS has been linked to the CYP2C9∗3 gene variation (rs1057910) in addition to HLA correlations (Yampayon et al., 2017). Yue Zhenhua et al., 2018 have stated that *rs701827* (A missense variant of the HLADRB1 gene), *rs17211071*(A 5 Prime UTR variant of the *HLA-DRB1* gene), and *rs201929247* (A missense variant of the *HLA-DRB1* gene) are also risk factors behind DHS in Chinese (EAS) populations. So, a study on DHS and relevant genes comparing SAS and world populations is needed. No proper research has been conducted in Sri Lanka regarding the DHS and relevant genes. On the other hand, as per our knowledge, no research has been conducted worldwide, to find out the diversity of pharmacogenomics (PGX) variants affecting DHS, comparing SAS with other world populations. We postulate that SAS populations exhibit a higher significant difference of PGX polymorphism/SNP frequencies linked to DHS when compared to populations worldwide.

The PGX Knowledge Base, shortly popular as PharmGKB is a priceless tool for researchers, scientists, medical professionals, and the general public, seeking to draw connections between our genetic composition with how drugs affect us, and, eventually, expecting better health results. The National Human Genome Research Institute (NHGRI) and the National Centre for Biotechnology Information (NCBI) collaborated to develop dbSNP (Single Nucleotide Polymorphism Database), a publicly available resource that records and annotates SNPs from a variety of species, with an emphasis on humans (Sherry et al., 2001). We have extracted allele frequencies from both PharmGKB and dbSNP databases for the current study. The major objective of this research is to describe the diversity of PGX variants affecting DHS, comparing SAS with other world populations. The overall objective of the study is to reduce hypersensitivity reactions to dapsone during leprosy treatment. Another major objective of the research is to provide an initiation step/foundation for researchers to conduct research in this context. On the other hand, it provides medical practitioners with insights into how to treat individuals based on the principles of personalized medicine. We have utilized χ^2 tests for the current study which is a statistical technique utilized to ascertain whether there is a significant correlation between two categorical variables (Ali & Bhaskar, 2016).

Materials and Methods

SNPs associated with DHS were identified after a thorough literature search. The rs IDs (Reference SNP cluster ID) and wild type/variant allele of every SNP identified above were obtained through literature review and utilizing the NCBI dbSNP and PharmGKB databases. SAS allele frequencies of *rs2844573, rs1057910, rs701829, rs17211071,* and *rs201929247* SNPs were retrieved utilizing PharmGKB and NCBI dbSNP databases (*rs1057910, rs701829, rs17211071* and *rs201929247* from PharmGKB and *rs2844573* from NCBI dbSNP). For *HLA-B*13:01*, European, AFR(African), AFR others, AFR AMR (African American), Asian, EAS (East Asian), Other Asian, Latin AMR 1(Latin American 1), and Latin AMR 2(Latin American 2) world population allele frequencies were obtained from the NCBI dbSNP database. For *CYP2C9*3, rs701829, rs17211071, and rs201929247*; the AFR, AMI(Amish), AMR(American), ASJ(Ashkenazi-Jewish), EAS, FIN(Finnish), and NFE (Non-Finnish European) world population allele frequencies were obtained from the PharmGKB database. Mutation and wild-type numbers were calculated based on the sample sizes available in PharmGKB and NCBI dbSNP for all the above-retrieved allele frequencies. X^2 analyses were conducted. A total of 37 χ^2 analyses were performed, comparing SAS allele frequencies with those of the global populations, SNP-wise for 5 SNPs of 3 genes. The null hypothesis was stated as there is no significant difference between the SNP distribution across world populations compared to SAS and the alternative hypothesis was stated vice-versa. Finally, the p-values were ascertained. Distribution of the analysed variants across world population groups and differences in the allelic frequencies of these variants between SAS were compared using the $\chi 2$ test and $p < 0.05$ was considered significant.

Results

Table 1 below contains the identified SNPs and their genomic information (rs IDs, and wild type/variant allele of each SNP). Results in Table 2 below contain allele frequencies and sample sizes of rs1057910, rs701829, rs17211071, and rs201929247 of SAS and other world populations. Results in Table 3 contain allele frequencies and sample sizes of rs2844573 of SAS and other world populations. Results in Tables 4 and 5 show the results of the χ^2 analyses. They show the p-values of each world population compared to SAS SNP-wise. *CYP2C9*3*: - AMRs, AFRs, AMI, ASJ, EASs, FIN, and NFE have all demonstrated p-values significantly lower than 0.05 compared to SAS. *HLA-B*13:01*: - Europeans, AFR s, AFR others, and AFR AMRs have demonstrated p-values less than 0.05 when compared with SAS. On the other hand, Asians, EASs, Other Asians, and Latin AMRs have shown p-values greater than 0.05 in the current study when compared with SAS. *rs701829*: - AMRs, AFRs, AMI, ASJ, EASs, FIN, and NFE have all demonstrated p-values significantly lower than 0.05 when compared to SAS. *rs17211071*: - AFRs, AMI, AMRs, EASs, FIN, and NFE have p-values greater than 0.05 in the current study when compared with SAS. When compared to SAS, ASJ had a p-value of less than 0.05. *rs201929247*: - In this study, AFRs and FIN have a p-value greater than 0.05 for the rs201929247 allele frequency when compared with SAS. In the current analysis, p-values for the rs201929247 allele frequency compared to SAS were significantly lower than 0.05 for AMRs, AMI, ASJ, EASs, and non-FIN Europeans.

Table 1. Gene variants/SNPs behind DHS and their corresponding gene, rs ID(SNV ID), and wild type/variant allele

Gene	Gene variant name	rs ID	Wild type/ Mutation
HLA-B	*HLA-B*13:01*	*rs2844573*	A/C
CYP2C9	*CYP2C9*3*	*rs1057910*	A/C
HLA-DRB1	*A missense variant of HLA-DRB1 gene*	*rs701829*	T> A/C/G
HLA-DRB1	*A 5 Prime UTR variant of HLA-DRB1 gene*	*rs17211071*	G> A/T
HLA-DRB1	*A missense variant of HLA-DRB1 gene*	*rs201929247*	G/A

Table 2. Allele frequencies and sample sizes of SAS and world populations of gene variants CYP2C9*3, rs701829, rs17211071, and rs17211071 and rs201929247

		SAS	AFR	AMI	AMR	ASJ	EAS	FIN	NFE
CYP2C9*3	Allele frequency	11.38%	1.26%	2.96%	4.91%	8.27%	3.07%	5.58%	6.62%
	Sample Size	4834	41436	912	15262	3472	5184	10616	68024
rs701829	Allele frequency	62.52%	72.03%	90.16%	82.60%	89.85%	75.40%	78.22%	80.28%
	Sample Size	2866	22592	768	9604	2502	3602	6582	50538
rs17211071	Allele frequency	0.18%	0.11%	0.59%	0.19%	0.63%	0.49%	0.07%	0.28%
	Sample Size	2170	21248	512	6290	1594	2056	5884	36370
rs201929247	Allele frequency	10.18%	9.90%	15.21%	17.49%	16.22%	17.27%	9.82%	13.88%
	Sample Size	2938	26526	572	9146	2330	2924	6884	44528

Table 3. Allele frequencies and sample sizes of SAS and world populations of gene variant HLA-B*13:01

		SAS	European	AFR	AFR others	AFR - AMR	Asians	EASs	Other Asians	Latin-AMR 1	Latin-AMR 2
HLA-B*13:01	Allele frequency	7.00%	27.25%	1.34%	0.80%	1.37%	4.50%	5.70%	0.00%	4.60%	7.00%
	Sample Size	92	55108	2682	120	2562	132	106	26	196	1072

Table 4. p-values of X_2 analysis of CYP2C9*3, rs701829, rs1721107, and rs201929247 allele frequencies of world populations, compared with SAS (p<0.05)

SNP	AFR	AMI	AMR	ASJ	EAS	FIN	NFE
*CYP2C9*3*	0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
rs701829	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
rs17211071	0.359	0.1091	0.9524	0.0273	0.0876	0.1413	0.4062
rs201929247	0.629	0.0004	<0.001	<0.001	<0.001	0.5878	<0.001

Table 5. p-values of X_2 analysis of HLA-B*13:01 allele frequencies of world populations, compared with SAS (p<0.05)

SNP	European	AFR	AFR others	AFR – AMR	Asians	EASs	Other Asians	Latin-AMR 1	Latin- AMR 2
*HLA-B*13:01*	<0.001	<0.001	0.0216	<0.001	0.5181	0.799991	0.181354	0.491913	0.863703

Discussion

To summarize the core experimental design and methodology of the current research, firstly a considerable number of reputed journals were studied to identify the SNPs behind DHS. There, we managed to identify 5 major SNPs, while retrieving allele frequencies related to each SNP for world populations utilizing PharmGKB and NCBI dbSNP databases. Then, for each SNP, we performed χ^2 analyses between the allele frequencies of SAS and other world populations to compare and see whether there is a significant difference between them. We have obtained highly significant results, with p-values less than 0.05.

When the SNP distribution in SAS and the relevant world population differs significantly (p-value<0.05); the same dosages of dapsone pharmaceuticals might not be advised_for leprosy in these 2 population categories, or alternatives for dapsone might be utilized.

When the SNP distribution across SAS and the relevant world population is not significantly different (p-value>0.05); similar dapsone regimens are likely to be recommended for leprosy in these populations. However, it must be insisted that this dosage determination or recommendation of alternative drugs for dapsone should be made after considering all the SNPs, environmental factors, and disease heterogeneity factors behind DHS at clinical level research. Discussing the clinical practice implications, if we use the same dosage of dapsone for leprosy in two populations where there is a significant difference in allele frequencies, the high allele frequency population would develop more adverse drug reactions. One of the research drawbacks is SAS allele frequencies which might not be the same as Sri Lankan allele frequencies. An improved strategy could use genomic data from Whole Exome Sequencing (WES) databases in Sri Lanka. Therefore, we propose to utilize Sri Lankan allele frequency data (even ethnicity-wise) for DHS SNPs as the next step of this research commences. Dapsone therapy could receive stronger recommendations for personalized medicine in Sri Lanka with this approach. Screening programs (maybe utilizing qPCR rapid detection kits) could be conducted in high-risk areas in Sri Lanka before prescribing dapsone for leprosy. A Genome Wide Association Study (GWAS) would be ideal. However, it would need higher levels of funds. Clinical-level research utilizing primary data/ extracted blood DNA from a statistically significant number of DHS patients should be conducted to claim firm conclusions regarding dapsone dosages/ alternative medications.

If a patient shows hypersensitivity to dapsone, an alternative treatment plan may involve replacing dapsone with a mixture of minocycline, clarithromycin, and ofloxacin (Florian & Deps, 2023). Another suggested alternative regime for WHO-MDT (consists of dapsone) is ROM (Rifampicin, Ofloxacin, and Minocycline). Narang et al., 2019, a group of North Indian researchers have researched, proving ROM a safer and more effective alternative anti-leprosy treatment. They have concluded that in cases when patients do not respond well to a year (12 months) of WHO-MDT, ROM is a safe and effective treatment for leprosy. As per Kar & Gupta, 2015, these regimens might not be as successful as conventional MDT as dapsone is cheaper and a more effective drug. Considering the market prices worldwide, alternatives to dapsone are more expensive, and as most of the SAS countries, including Sri Lanka, consist of a majority of low and middle-income families, alternative regimes seem to be unaffordable for them. At the same time from the current research, pharmaceutical companies might be encouraged to create safer, more effective and cost-friendly medications or formulations for usage in Sri Lankan (SAS) and other regions with comparable genetic profiles as certain genetic markers linked to DHS and their worldwide population significant differences/ non-significant differences have been shown in the current research. Secondary data, such as allele frequencies from PharmGKB and dbSNP databases, can provide valuable information about DHS, but it may not accurately represent the broader population due to inherent biases and the need for functional investigations or primary data analysis. The accuracy and consistency of secondary data may vary based on the quality of primary research and data-gathering procedures. To mitigate these limitations, in the current study we have been open about the drawbacks of using secondary data, combined information from primary sources, studied patterns in various populations, and avoided generalizing based solely on secondary data. Since χ^2 analysis works well with large sample sizes and there are no hard and fast rules for the sample sizes, works well with categorical data, and allows for frequency comparison, it is important for evaluating the diversity of PGX variants affecting DHS among various populations making it useful in the current scenario.

Conclusions

Compared with Western and other world populations, allele frequencies of some studied SNPs were significantly different in SAS, and these are likely to

account for the variability of DHS occurrence among these populations. A significant difference has been seen between the *HLA-B*13:01* SNP distribution in Europe, AFR, AFR others, and AFR AMRs; the *CYP2C9*3* SNP distribution in AMRs, AFRs, AMI, ASJ, EASs, FIN, and NFEs; the *rs701829* SNP distribution among all other world populations considered; the *rs17211071* SNP distribution in ASJ; the *rs201929247* SNP distribution in AMRs, AMI, ASJ, EASs, and NFE, compared to South Asians. These findings could significantly benefit medical professionals for personalized medicine in dapsone therapy. The study underscores the significance of incorporating local pharmacogenomics data in treatment decisions before prescribing dapsone to improve patient care in Sri Lanka and other South Asian regions.

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