“A bioinformatic approach to identify pathogenic variants for Stevens-Johnson syndrome”

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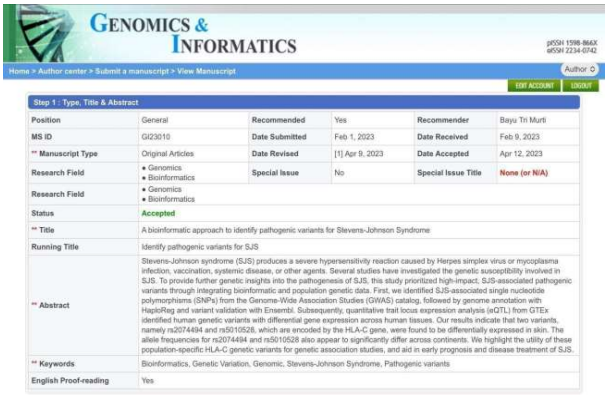
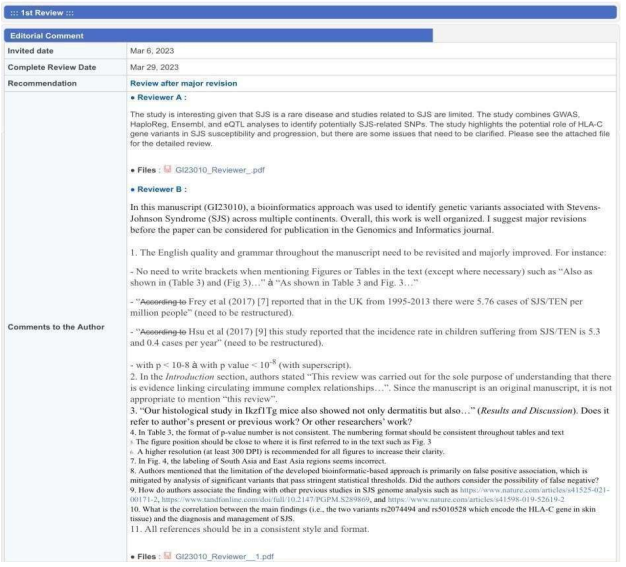
Apt. Lalu Muhammad Irham, M.Farm., Ph.D

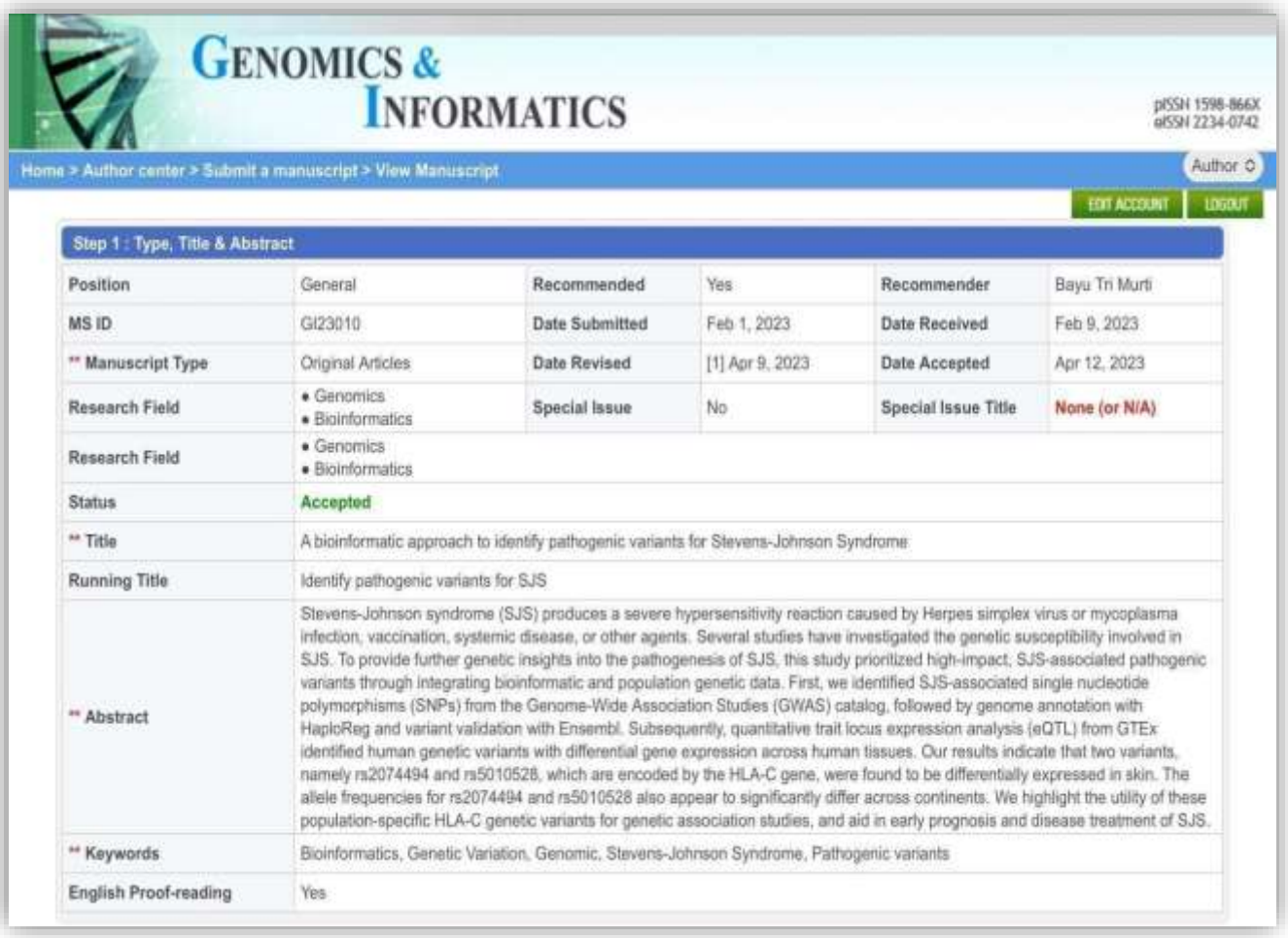
No	Keterangan	Tanggal	Bukti Proses
1	Submit Artikel	1 Februari 2023	 GENOMICS & INFORMATICS Home > Author center > Submit a manuscript > View Manuscript Step 1 : Type, Title & Abstract Position: General; Recommended: Yes; Recommender: Bayu Tri Murti MS ID: GI23010; Date Submitted: Feb 1, 2023; Date Received: Feb 9, 2023 ** Manuscript Type: Original Articles; Date Revised: [1] Apr 9, 2023; Date Accepted: Apr 12, 2023 Research Field: • Genomics • Bioinformatics; Special Issue: No; Special Issue Title: None (or N/A) Research Field: • Genomics • Bioinformatics Status: Accepted ** Title: A bioinformatic approach to identify pathogenic variants for Stevens-Johnson Syndrome Running Title: Identify pathogenic variants for SJS ** Abstract: Stevens-Johnson syndrome (SJS) produces a severe hypersensitivity reaction caused by Herpes simplex virus or mycoplasma infection, vaccination, systemic disease, or other agents. Several studies have investigated the genetic susceptibility involved in SJS. To provide further genetic insights into the pathogenesis of SJS, this study prioritized high-impact, SJS-associated pathogenic variants through integrating bioinformatic and population genetic data. First, we identified SJS-associated single nucleotide polymorphisms (SNPs) from the Genome-Wide Association Studies (GWAS) catalog, followed by genome annotation with HaploReg and variant validation with Ensembl. Subsequently, quantitative trait locus expression analysis (eQTL) from GTEx identified human genetic variants with differential gene expression across human tissues. Our results indicate that two variants, namely rs2074494 and rs5010528, which are encoded by the HLA-C gene, were found to be differentially expressed in skin. The allele frequencies for rs2074494 and rs5010528 also appear to significantly differ across continents. We highlight the utility of these population-specific HLA-C genetic variants for genetic association studies, and aid in early prognosis and disease treatment of SJS. ** Keywords: Bioinformatics, Genetic Variation, Genomic, Stevens-Johnson Syndrome, Pathogenic variants English Proof-reading: Yes
2	Artikel mendapatkan hasil review dari 2 reviewer	6 Maret 2023	 ::: 1st Review ::: Editorial Comment Invited date: Mar 6, 2023 Complete Review Date: Mar 29, 2023 Recommendation: Review after major revision Comments to the Author: • Reviewer A : The study is interesting given that SJS is a rare disease and studies related to SJS are limited. The study combines GWAS, HaploReg, Ensembl, and eQTL analyses to identify potentially SJS-related SNPs. The study highlights the potential role of HLA-C gene variants in SJS susceptibility and progression, but there are some issues that need to be clarified. Please see the attached file for the detailed review. • Files : GI23010_Reviewer_.pdf • Reviewer B : In this manuscript (GI23010), a bioinformatics approach was used to identify genetic variants associated with Stevens-Johnson Syndrome (SJS) across multiple continents. Overall, this work is well organized. I suggest major revisions before the paper can be considered for publication in the Genomics and Informatics journal. 1. The English quality and grammar throughout the manuscript need to be revisited and majorly improved. For instance: - No need to write brackets when mentioning Figures or Tables in the text (except where necessary) such as “Also as shown in (Table 3) and (Fig 3)…” à “As shown in Table 3 and Fig. 3…” - “~~According to~~ Frey et al (2017) [7] reported that in the UK from 1995-2013 there were 5.76 cases of SJS/TEN per million people” (need to be restructured). - “~~According to~~ Hsu et al (2017) [9] this study reported that the incidence rate in children suffering from SJS/TEN is 5.3 and 0.4 cases per year” (need to be restructured). - with $p < 10\text{-}8$ à with p value $< 10^{-8}$ (with superscript). 2. In the *Introduction* section, authors stated “This review was carried out for the sole purpose of understanding that there is evidence linking circulating immune complex relationships…”. Since the manuscript is an original manuscript, it is not appropriate to mention “this review”. 3. “Our histological study in Ikzf1Tg mice also showed not only dermatitis but also…” (*Results and Discussion*). Does it refer to author’s present or previous work? Or other researchers’ work? 4. In Table 3, the format of p-value number is not consistent. The numbering format should be consistent throughout tables and text 5. The figure position should be close to where it is first referred to in the text such as Fig. 3 6. A higher resolution (at least 300 DPI) is recommended for all figures to increase their clarity. 7. In Fig. 4, the labeling of South Asia and East Asia regions seems incorrect. 8. Authors mentioned that the limitation of the developed bioinformatic-based approach is primarily on false positive association, which is mitigated by analysis of significant variants that pass stringent statistical thresholds. Did the authors consider the possibility of false negative? 9. How do authors associate the finding with other previous studies in SJS genome analysis such as https://www.nature.com/articles/s41525-021-00171-2, https://www.tandfonline.com/doi/full/10.2147/PGPM.S289809, and https://www.nature.com/articles/s41598-019-52619-2 10. What is the correlation between the main findings (i.e., the two variants rs2074494 and rs5010528 which encode the HLA-C gene in skin tissue) and the diagnosis and management of SJS. 11. All references should be in a consistent style and format. • Files : GI23010_Reviewer__1.pdf

History Artikel

"A bioinformatic approach to identify pathogenic variants for Stevens-Johnson syndrome"

Dalam proses penerbitan artikel ini dapat diakses melalui https://genominfo.org dengan informasi metadata artikel pada jurnal sebagai berikut.

Artikel disubmit tanggal 1 Februari 2023. Selanjutnya, pada tanggal 9 Februari 2023 artikel yang telah di submit diterima oleh pihak Genomics & Informatics Journal.



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Step 1 : Type, Title & Abstract

Position	General	Recommended	Yes	Recommender	Bayu Tri Murti
MS ID	GI23010	Date Submitted	Feb 1, 2023	Date Received	Feb 9, 2023
** Manuscript Type	Original Articles	Date Revised	[1] Apr 9, 2023	Date Accepted	Apr 12, 2023
Research Field	• Genomics • Bioinformatics	Special Issue	No	Special Issue Title	None (or N/A)
Research Field	• Genomics • Bioinformatics				
Status	Accepted				
** Title	A bioinformatic approach to identify pathogenic variants for Stevens-Johnson Syndrome				
Running Title	Identify pathogenic variants for SJS				
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** Keywords	Bioinformatics, Genetic Variation, Genomic, Stevens-Johnson Syndrome, Pathogenic variants				
English Proof-reading	Yes				

Pada tanggal 6 Maret 2023 artikel mendapatkan hasil review dari 2 orang reviewers. Kemudian, hasil saran dan masukan dari reviewers berupa revisian di submit kembali pada tanggal 29 Maret 2023.

::: 1st Review :::

Editorial Comment

Invited date	Mar 6, 2023
Complete Review Date	Mar 29, 2023
Recommendation	Review after major revision
Comments to the Author	• Reviewer A : The study is interesting given that SJS is a rare disease and studies related to SJS are limited. The study combines GWAS, HaploReg, Ensembl, and eQTL analyses to identify potentially SJS-related SNPs. The study highlights the potential role of HLA-C gene variants in SJS susceptibility and progression, but there are some issues that need to be clarified. Please see the attached file for the detailed review. • Files : GI23010_Reviewer_.pdf • Reviewer B : In this manuscript (GI23010), a bioinformatics approach was used to identify genetic variants associated with Stevens-Johnson Syndrome (SJS) across multiple continents. Overall, this work is well organized. I suggest major revisions before the paper can be considered for publication in the Genomics and Informatics journal. 1. The English quality and grammar throughout the manuscript need to be revisited and majorly improved. For instance: - No need to write brackets when mentioning Figures or Tables in the text (except where necessary) such as "Also as shown in (Table 3) and (Fig 3)…" à "As shown in Table 3 and Fig. 3…" - "~~According to~~ Frey et al (2017) [7] reported that in the UK from 1995-2013 there were 5.76 cases of SJS/TEN per million people" (need to be restructured). - "~~According to~~ Hsu et al (2017) [9] this study reported that the incidence rate in children suffering from SJS/TEN is 5.3 and 0.4 cases per year" (need to be restructured). - with p < 10-8 à with p value < 10^{-8} (with superscript). 2. In the *Introduction* section, authors stated "This review was carried out for the sole purpose of understanding that there is evidence linking circulating immune complex relationships…". Since the manuscript is an original manuscript, it is not appropriate to mention "this review". 3. "Our histological study in Ikzf1Tg mice also showed not only dermatitis but also…" (*Results and Discussion*). Does it refer to author's present or previous work? Or other researchers' work? 4. In Table 3, the format of p-value number is not consistent. The numbering format should be consistent throughout tables and text 5. The figure position should be close to where it is first referred to in the text such as Fig. 3 6. A higher resolution (at least 300 DPI) is recommended for all figures to increase their clarity. 7. In Fig. 4, the labeling of South Asia and East Asia regions seems incorrect. 8. Authors mentioned that the limitation of the developed bioinformatic-based approach is primarily on false positive association, which is mitigated by analysis of significant variants that pass stringent statistical thresholds. Did the authors consider the possibility of false negative? 9. How do authors associate the finding with other previous studies in SJS genome analysis such as https://www.nature.com/articles/s41525-021-00171-2, https://www.tandfonline.com/doi/full/10.2147/PGPM.S289869, and https://www.nature.com/articles/s41598-019-52619-2 10. What is the correlation between the main findings (i.e., the two variants rs2074494 and rs5010528 which encode the HLA-C gene in skin tissue) and the diagnosis and management of SJS. 11. All references should be in a consistent style and format. • Files : GI23010_Reviewer__1.pdf

Pada tanggal 9 April 2023, revisian yang disubmit kembali telah diterima oleh Editor. Kemudian pada tanggal 12 April 2023 artikel dinyatakan diterima / accepted oleh Editor.

Editorial Comment

Invited date	Apr 9, 2023
Complete Review Date	Apr 12, 2023
Recommendation	Accept as it is
Comments to the Author	None (or N/A)
Files	None (or N/A)

Close

Berikut akan dilampirkan beberapa dokumen perubahan dari artikel yang diterbitkan sejak proses submit, hasil review dari reviewer, artikel revisi, dan artikel versi terbit.

Artikel pertama kali saat disubmit
pada tanggal 1 Februari 2023

Original article

G&I Genomics & Informatics

A bioinformatic approach to identify pathogenic variants for Stevens-Johnson Syndrome

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Abstract

Stevens-Johnson syndrome (SJS) is a syndrome resulting in severe hypersensitivity reactions caused by Herpes simplex virus or Mycoplasma infection, vaccinations, systemic diseases, or certain other agents. SJS has an incidence rate of at about 1.6-9.2 cases per million people per year in the United States as well as in Europe. This study aims to identify genetic variants associated with SJS across continents through a bioinformatics approach. This study identified SJS-associated single nucleotide polymorphisms (SNPs) from the Genome Wide Association Studies (GWAS) catalog, followed by genome annotation with HaploReg and variant validation with Ensembl. Furthermore, expression quantitative trait locus (eQTL) analyses from the GTEx Portal identified human genetic variants with differential gene expression in the skin and sun-exposed (lower leg) tissue. Future studies should validate the effects of these identified variants for the *HLA-C* gene on SJS disease susceptibility and progression.

Keywords: Gene Variation, Genomic, Stevens-Johnson syndrome

Introduction

Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are potentially life-threatening diseases [1]. In particular, SJS is a syndrome resulting from severe hypersensitivity reactions caused by infection with the Herpes simplex or Mycoplasma viruses, vaccinations, systemic diseases, certain agents, food, and drugs [2]. SJS occurs in the skin and mucous membranes in the orifices and eyes in mild to severe conditions, with abnormalities in the skin in the form of erythema, vesicles, or bullae accompanied by purpura [3].

SJS/TEN is widespread in the mucocutaneous immune region, causing exfoliation of the skin on the mucosal surface [4]. The incidence of SJS, SJS/TEN, and TEN in the US reported 9.2, 1.6, and 1.9 cases from 2009-2012 [5]. The incidence of SJS cases that occur in Indonesia is around 12 cases per year, with different causes [6]. According to Frey et al (2017) [7]. reported that in the UK from 1995-2013 there were 5.76 cases of SJS/TEN per million people per year. In Korea, it is reported that the incidence rate of SJS/TEN from 2009–2013 was 3.96–5.03 and 0.94–1.45 per million people per year [8]. According to Hsu et al (2017) [9] this study reported that the incidence rate in children suffering from SJS/TEN is 5.3 and 0.4 cases. The mortality rate of 4.8-9% in SJS, an incidence of 19.4-29% in the SJS/TEN case, and an incidence of 14.8-48% in TEN [10]. SJS can appear with non-specific fever symptoms that cause malaise, headache, cough, and rhinorrhea. On the skin, patients suffering from SJS can have polymorphic lesions and mucous membranes with marked skin blisters and erosion [11].

Therefore, primary prevention is the best mitigation for SJS. SJS is categorized as a severe cutaneous adverse reaction (SCAR), and several drugs have been implicated in its pathogenesis. Non-steroidal anti-inflammatory drugs (NSAIDs) and other multi-ingredient formulations are widely used to relieve the symptoms. There are several studies reporting adverse skin drug reactions that are often SJS with severe ocular complications [12]. Prevention is possible if patients who are susceptible to this SCAR when prescribed certain drugs are identified. Besides, the genomic variants are known to have an important role in SJS progression. However, little information revealed the specific variant as a biological risk gene in SJS. A previous study revealed associated variants in SJS progression (rs2844665, rs3815087, rs3130931, rs3130501, rs3094188, rs9469003). According to Genin et al. (2011) [13], in allopurinol-induced SJS and TEN, rs9469003 can detect an effect similar to that seen with an allele frequency risk of 15% and an associated risk of 99%. Even though several studies have been exploited previously, a limited number of variants summarized the variants associated with expression in tissues-related SJS. This review was carried out for the sole purpose of understanding that there is evidence linking circulating immune complex relationships that can precipitate in areas of the skin, mucosa and cause tissue damage due to complement activation and inflammatory reactions that occur, and how the expression of the *HLA-C* variant gene alleles can influence expression in the various tissues. Here, we use various databases to assess tissue *HLA-C* expression profiles and population allele frequencies of genetic variants. The results will allow future studies to assess whether this agent may be associated with various infectious risks for toxic epidermal necrolysis and susceptibility to SJS progression.

Methods

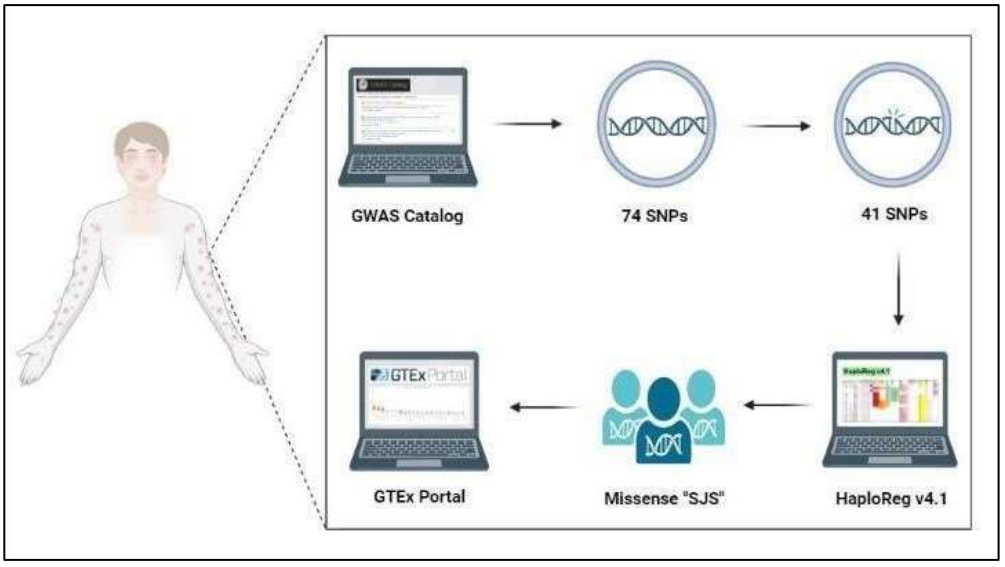

Fig 1. Bioinformatics workflow for the identification of genetic variants associated with Stevens-Johnson syndrome (SJS).

In this research method, we adopted the methodology of Irham et al (2020) [14] in the identification of Genetic variants that influence SARS-CoV-2 receptor TMPRSS2 expression among population cohorts from multiple continents. In this study design, detailed information can be seen in (Fig 1). SJS associated with SNPs was obtained from the GWAS Catalog database of the National Human Genome Research Institute (NHGRI) http://www.ebi.ac.uk/gwas (accessed 19/12/2022), and further analysis was performed using HaploReg (version 4.1). The *p-value* of 10^{-8} is used to account for several tests in the GWAS catalog. These values are widely used to identify the relationship between common genetic variants and traits with adjacent gene expression [15]. Furthermore, an evaluation was carried out between the relationships of various genetic variants and gene expression profiles using expression quantitative trait loci (eQTL) using the GTEx Portal database http://www.gtexportal.org/home/ (accessed 19/12/2022), which was complicated by gene expression from various tissues. A genetic variant of *HLA-C* is present in human skin and sun-exposed (lower leg) tissue obtained from the GTEx Portal database. Then confirm the variant using the Ensembl Genome Browser https://www.ensembl.org/index.html (accessed 19/12/2022). This study uses allele frequencies from populations in Europe, Africa, America, East Asia, and Southeast Asia. Then, to find out the function of the various gene variants, an evaluation was carried out using the SNP Nexus database https://www.snp-nexus.org (accessed 19/12/2022).

Results and Discussion

Identification of genomic variants for Stevens-Johnson syndrome (SJS)

We first identified SNPs associated with SJS from the GWAS database, resulting in 74 SNPs associated with SJS. In (Table 1), we identified 41 SNPs associated with SJS by performing SNPs duplication. Finally, based on the number of SNPs obtained, requirements were made using HaploReg version 4.1, with p < 10-8. Based on the findings presented in (Table 2), we found a risk of two genes of SJS. In this study, we have performed an analysis of tissue expression affecting to SJS with a missense variant *HLA-C*. SJS is an acute mucocutaneous reaction characterized by extensive epidermal necrosis with involvement of at least two mucous membranes caused by drugs [16].

Table 1. SNPs from the GWAS catalog with significance of *p*-value < 10^{-8}

Variant and risk allele	*p*-value
rs6457109	3 x 10^{-16}
rs7760545	4 x 10^{-16}
rs35835721	8 x 10^{-15}
rs137899365	3 x 10^{-14}
rs60581484	2 x 10^{-13}
rs1131151	4 x 10^{-12}
rs2074494	5 x 10^{-12}
rs4917014	8 x 10^{-11}
rs1562468327	6 x 10^{-10}
rs199755581	7 x 10^{-10}
rs11509487	1 x 10^{-9}
rs9469003	2 x 10^{-9}
rs4471527	2 x 10^{-9}
rs28381346	5 x 10^{-9}
rs6500265	6 x 10^{-9}
rs199755581	6 x 10^{-9}
rs114908185	7 x 10^{-9}
rs1297852527	9 x 10^{-9}
rs1371146120	1 x 10^{-8}
rs77491650	1 x 10^{-8}
rs4471527	1 x 10^{-8}
rs77542827	1 x 10^{-8}
rs3130501	2 x 10^{-8}
rs536142737	2 x 10^{-8}
rs16957893	2 x 10^{-8}
rs2734583	2 x 10^{-8}
rs150289893	2 x 10^{-8}
rs1286845082	2 x 10^{-8}
rs548089948	2 x 10^{-8}
rs3094188	3 x 10^{-8}
rs55765602	3 x 10^{-8}
rs77542827	3 x 10^{-8}
rs778096762	3 x 10^{-8}
rs1597607761	3 x 10^{-8}
rs1391213386	3 x 10^{-8}
rs374138762	4 x 10^{-8}
rs879656274	4 x 10^{-8}
rs1211926109	4 x 10^{-8}
rs116953913	5 x 10^{-8}
rs1263106470	6 x 10^{-8}
rs5010528	8 x 10^{-8}

Table 2. Variant Stevens-Johnson syndrome risk allele that codes for prioritized SNPs

Variant and risk allele	Variants near risk allele (r^2 > 0.8)	*p*-value	Gencode	Allele type
rs2074494	rs1050276	5 x 10^{-12}	*HLA-C*	missense
rs5010528	rs1050409	8 x 10^{-8}	*HLA-C*	missense

HLA-C gene expression across 16 human tissues

To evaluate *HLA-C* gene expression in human tissues, we used the GTEx portal database

(http://www.gtexportal.org/), which contains gene expression levels in various tissues. eQTL annotation comprises the most obvious functional consequences of genetic variation. Whole blood, spleen, lung, and lymphocyte cells demonstrate highest *HLA-C* gene expression across the 16 human tissues analyzed from GTEx (Fig 2). Furthermore, we have found that the ID SNPs rs2074494 and rs5010528 have similar gene expression variation in the Sun-Exposed Skin (Lower Leg).

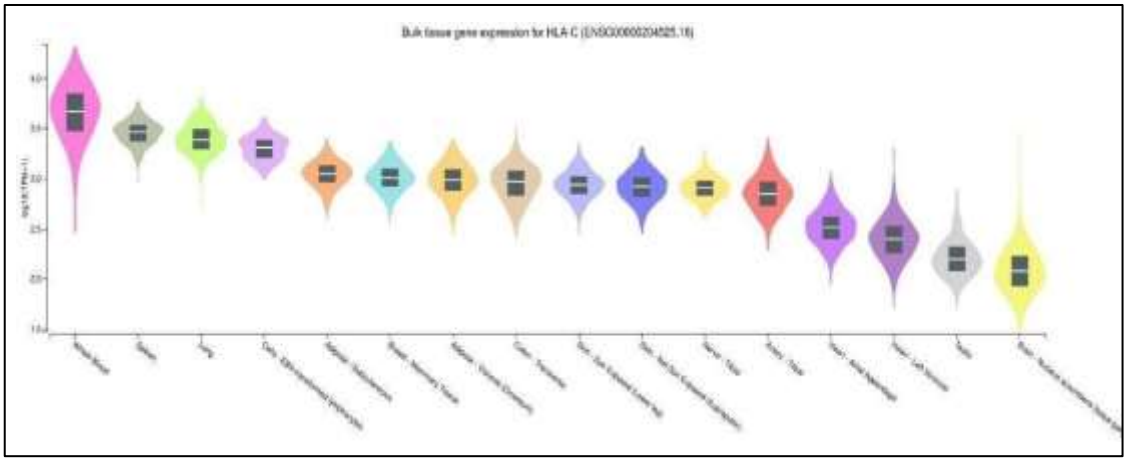

Fig 2. *HLA-C* gene expression associated with Stevens-Johnson syndrome (SJS) in several human tissues from the GTEx Portal.

HLA-C gene expression in the sun-exposed skin

HLA class I genes, including *HLA-A*, *HLA-B*, and *HLA-C*, have been reported as the loci most strongly associated with susceptibility to all types of SJS and TEN, including cold medicine related (CM-SJS) and TEN with severe ocular complications (SOC). Although non-synonymous substitutions affecting peptide binding or *HLA* molecular conformation have been considered major factors in the pathogenesis of immunological diseases, Indeed, different *HLA-C* expression levels have been reported for the different alleles, with higher *HLA-C* expression leading to increased Tc (Trypanosoma cruzi) responses and negative effects in Crohn's disease [17].

When individuals with a genetic background containing SJS/TEN with SOC susceptibility factors are infected by some viral or microbial infection, they develop abnormal immune responses [18]. It is reasonable to presume that there is an interaction of *HLA* multiplication and susceptibility genes such as *HLA-A* and *TLR3* [19], *HLA-A* and *REC14-32*, and *HLA-A* and *PTGER3* [20] and it is possible that some of the susceptibility genes for SJS/TEN CM associated with SOC are involved in the formation of functional networks. An imbalance in this gene can trigger the mucocutaneous inflammation seen in patients with SJS/TEN associated with CM with SOC. SJS/TEN with SOC in the acute stage shows not only inflammation of the skin and ocular surface but also oral mucosal erosions and paronychia. Our histological study in Ikzf1Tg mice also showed not only dermatitis but also inflammation of the tongue, blepharo conjunctivitis and paronychia tissue, similar to patients with SJS/TEN and SOC in the acute stage [12].

In the case of Thailand, we investigated the type of *HLA* in Thai patients with SJS/TEN associated with acetaminophen and SOC. We found a significant association with *HLA-A*33:03* (20 patients, 60 controls; OR = 5.4, p = 0.0030), *HLA-B**44:03 (20 patients, 60 controls; OR = 9 .0, p = 0.0004), *HLA-C**07:01 (20 patients, 60 controls; OR = 9.3, p = 0.0002), and *HLA-B**44:03–*HLA-C* haplotypes *07:01 (20 patients, 60 controls; OR = 9.0, $p < 0.001$) [21]. This suggests that this type of *HLA* plays a role in the pathogenesis of SOC in acetaminophen-associated SJS/TEN.

Relationship between *HLA-C* gene and expression QTLs (eQTLs) from the GTEx database

SJS was evaluated for genetic variation in human tissue using eQTLs via the GTEx Portal database. This aims to determine the gene expression level in various tissues for analysis performed on eQTL. It is necessary to have genetic markers that can be genotyped for all individuals in the population under investigation. With that, eQTL analysis can be pursued to identify position of the variant locus that controls the expression of the target gene [22].

We identified genomic variations from *HLA-C* gene expression using the GWAS catalog database and found 74 SNPs. From these analyses, as many as the top 10 SNPs with the highest *p-values* were further processed with the variant annotation tool SNPnexus to determine the annotation of prioritized SNP variations. After these analyses, two statistically significant SNPs were obtained and prioritized. In this case, we prioritized 2 SNPs at risk for SJS based on an analysis of the number of SNPs expanded using HaploReg ver. 4.1 and a *p*-value of $< 10^{-8}$ to determine the functional annotation of the SNPs. The results of genetic variation are shown in (Table 3).

Table 3. HLA-C expression-Quantitative Trait Loci (eQTL) analysis from the GTEx Portal database

ID SNP	Gencode ID (ENSG00000-)	Gene Symbol	*p*-value	Effect Size	Tissue	Expression Level
rs2074494	204525.16	*HLA-C*	7.2e-9	-0.48	Artery-Tibial	CC>CT>TT
	204525.16	*HLA-C*	0.000013	-0.50	Muscle-Skeletal	CC>CT>TT
	204525.16	*HLA-C*	0.000015	-0.78	Brain-Nucleus accumbens (basal ganglia)	CC>CT>TT
	204525.16	*HLA-C*	0.000017	-0.51	Heart-Left Ventricle	CC>CT>TT
	204525.16	*HLA-C*	0.000046	-0.58	Testis	CC>CT>TT
	204525.16	*HLA-C*	0.00036	-0.39	**Skin-Sun Exposed (Lower leg)**	CC>CT>TT
rs5010528	204525.16	*HLA-C*	5.4e-26	0.56	Adipose-Subcutaneous	AA>AG>GG
	204525.16	*HLA-C*	1.0e-21	0.41	Whole Blood	AA>AG>GG
	204525.16	*HLA-C*	1.4e-14	0.51	Adipose-Visceral	AA>AG>GG
	204525.16	*HLA-C*	4.1e-14	0.49	Lung	AA>AG>GG
	204525.16	*HLA-C*	1.1e-10	0.68	Spleen	AA>AG>GG
	204525.16	*HLA-C*	2.1e-9	0.51	Heart-Atrial Appendage	AA>AG>GG
	204525.16	*HLA-C*	5.7e-8	0.28	Colon-Transverse	AA>AG>GG
	204525.16	*HLA-C*	9.9e-8	0.38	Skin-Not Sun Exposed (Suprapublic)	AA>AG>GG
	204525.16	*HLA-C*	5.1e-7	0.72	Cells-EBV-transformed lymphocytes	AA>AG>GG
	204525.16	*HLA-C*	7.0e-7	-0.25	Nerve-Tibial	AA>AG>GG
	204525.16	*HLA-C*	7.7e-7	0.31	Nerve-Tibial	AA>AG>GG
	204525.16	*HLA-C*	0.0000016	0.34	Breast-Mammary Tissue	AA>AG>GG
	204525.16	*HLA-C*	0.0000018	0.32	**Skin-Sun Exposed (Lower leg)**	AA>AG>GG

Source: Expression Quantitative Trait Loci (eQTL) obtained from the GTEx Portal.

In (Table 3) shows the two identified variants (rs2074494 and rs5010528) for *HLA-C* gene in differential tissue expression in the human skin. Use the GTEx portal (http://www.gtexportal.org/home/). Also as shown in (Table 3) and (Fig 3), the genotype of rs2074494 is associated with a higher expression of rs5010528 for the genotype in *HLA-C* skin tissue.

Allele frequencies of SJS candidate variants across continents

Once we identified both candidate *HLA-C* expression-associated variants, we determined the allele frequencies in the different populations, as shown in (Table 4). The allele frequencies for both variants were evaluated in different African, American, East Asian, European, and Southeast Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia). We extracted the allele frequencies in Africa, America, East Asia, Europe, and Southeast Asia from the Ensemble Genome Browser (http://www.ensembl.org). Allele frequencies across populations differ for each *HLA-C* variant. In (Table 4) and (Fig 4) show gene expression levels at higher frequencies of the rs5010528 associated (G) allele and rs2074494 associated (T) allele. At population frequencies of the rs5010528 (G) allele,

the Asian population (East Asia and Southeast Asia) is expressed at a much lower level than that from the populations of Africa, America, and Europe.

Table 4. Analysis of allele frequencies for the *HLA-C* gene from SNPnexus variant annotation. [AFR: Africa, AMR: America, EAS: East Asia, EUR: Europe; SAS: Southeast Asia; Ref: Reference; Alt: Alternate.]

SNP	Position (hg38)	Gene	Location	Alel		Frekuensi Alel (N)				
				Ref	Eff*	AFR	AMR	EAS	EUR	SAS
rs2074494	Chr6:31271956	*HLA-C*	Missense	C	T	T: 0.008 (10)	T: 0.099 (69)	T: 0.189 (191)	T: 0.037 (37)	T: 0.040 (39)
rs5010528	Chr6:31273255	*HLA-C*	Missense	A	G	G: 0.243 (321)	G: 0.187 (130)	G: 0.056 (56)	G: 0.138 (139)	G: 0.099 (97)

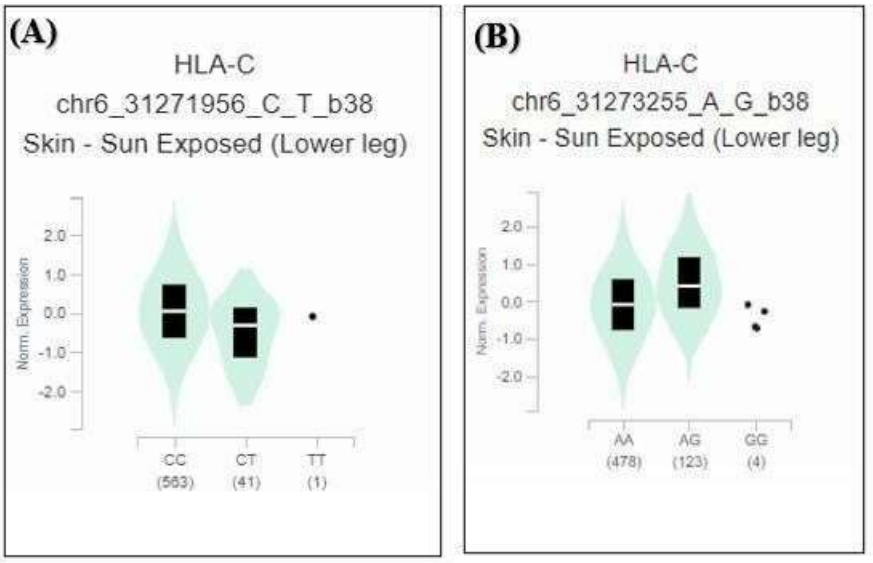

Fig 3. *HLA-C* gene expression for each genotype of the SNPs: (A) rs2074494 and (B) rs5010528.

The population frequency of the "rs2074494" T allele in the African population is expressed at a much lower level than that of the populations of America, Europe, and Southeast Asia. Taken together, the allele frequencies of the variants "rs2074494" and "rs5010528" indicated contribution of differential variant prevalence for *HLA-C* gene expression.

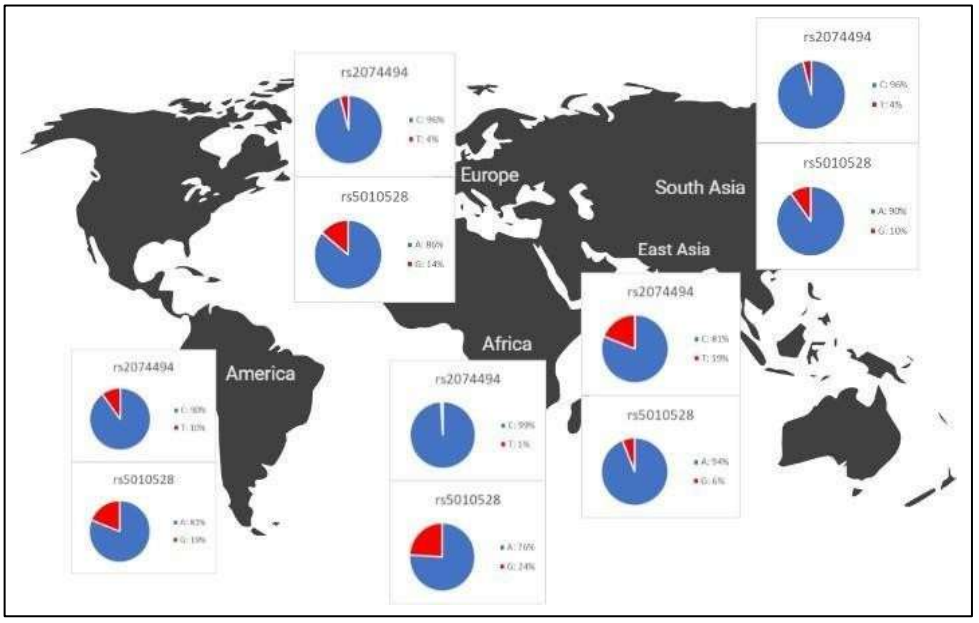

Fig 4. Summary of allele frequency analysis on *HLA-C* gene expression in Africa, America, East Asia, Europe and Southeast Asia.

Irham et al (2023) [23] demonstrated differential tissue expression for the *HLA-S* gene for the chickenpox disease, and this gene has also been implicated as a risk factor for herpes zoster disease. In this study, we investigated the skin tissue expression of the *HLA-C* gene, which has been linked to SJS and can lead to SCAR infection. Importantly, the variants associated with *HLA-C* expression have not been reported for SJS. Considering the global impact of SJS, examining the distribution of *HLA-C* variants may be an important quest that allows further understanding of the global disease susceptibility.

We used publicly available databases, such as the GTEx portal, SNPnexus, and Ensembl, and identified genetic variants associated with *HLA-C* expression in skin tissue, the main site of SCAR infection in SJS disease. The G and A allele frequencies for rs2074494 and rs5010528 were also seen to have a lower prevalence in Southeast Asia (rs2074494, 4%, and rs5010528, 14%), Europe (rs2074494, 4%, and rs5010528, 16%), and East Asia (rs2074494, 19%, and rs5010528, 6%), compared with American (rs2074494, 10%, and rs5010528, 24%) and African (rs5010528) populations. In conclusion, leveraging a bioinformatic-based approach reveals significant variant-associated SJS before clinical validation. However, the limitation of our bioinformatic-based approach is primarily false positive association, which is mitigated by analysis of significant variants that pass stringent statistical thresholds. Nonetheless, clinical validation is recommended as a next step to confirm our findings and to further understand the underlying etiology of the SJS disease.

Conclusion

In this study, we conducted a comprehensive bioinformatic analysis of Stevens-Johnson Syndrome (SJS) from genomic databases, revealing differenetial tissue expression of the *HLA-C* gene across 16 human tissues. We high expression occurs in whole blood, spleen, lung, and lymphocyte cells. In the results of SNPnexus, interestingly, we highlighted that two variants (rs2074494 and rs5010528) encoded the *HLA-C* gene were identified in skin tissue expression. Furthermore, allele frequencies for both variants were highlighted across the Africa, America, East Asia, Europe, and Southeast Asia populations.

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Authors' Contributions

Conceptualization: MM, JCF, MRM, LMI. Data curation: MM, JCF, MRM, LMI, WA, RC. Formal analysis: LMI, WA, RC. Methodology: MM, JCF, MRM. Writing - original draft: MM, JCF, MRM, LMI. Writing - review & editing: MM, JCF, MRM, LMI, NS, WA, RC, AWS.REK, RDS. Supervising: LMI

Conflicts of Interests

The authors declare no conflict of interest.

Acknowledgment

None

References

1. Wang, L., Varghese, S., Bassir, F., Lo, Y. C., Ortega, C. A., Shah, S., Blumenthal, K. G., Phillips, E. J., & Zhou, L. Stevens-Johnson syndrome and toxic epidermal necrolysis: A systematic review of PubMed/MEDLINE case reports from 1980 to 2020. In *Frontiers in Medicine* (Vol. 9). Frontiers Media 2022;9;1-18.
2. Deore, S. S., Dandekar, R. C., Mahajan, A. M., Shiledar, V, Gandhi Vidyamandir's Karmaveer, M., Hirey, B., Gandhi Vidyamandir', M., Bhausaheb, K., & Dental, H. Drug Induced-Stevens Johnson Syndrome: A Case Report. International Journal of Scientific Study 2014;4:84-87.
3. Menaldi, S. S. L., Bramono, K., & Indriatmi, W. *Ilmu Penyakit Kulit dan Kelamin (Edisi Ketujuh)*. Balai Penerbit Fakultas Kedokteran Universitas Indonesia, 2016.
4. Shanbhag, S. S., Chodosh, J., Fathy, C., Goverman, J., Mitchell, C., & Saeed, H. N. Multidisciplinary care in Stevens-Johnson syndrome. Therapeutic Advances in Chronic Disease 2020;11: 1–17
5. Hsu, D. Y., Brieva, J., Silverberg, N. B., & Silverberg, J. I. Morbidity and Mortality of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in United States Adults. Journal of Investigative Dermatology 2016;136:1387–1397.
6. Putri ND, Mutiara H, Hasudungan, Sibero HT, & Sukohar A. Steven-Johnson Syndrom et causa Paracetamol. J Medula Unila 2016;6:101–107.
7. Frey, N., Jossi, J., Bodmer, M., Bircher, A., Jick, S. S., Meier, C. R., & Spoendlin, J. (2017). The Epidemiology of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in the UK. Journal of Investigative Dermatology 2017;137:1240–1247.
8. Yang, M. S., Lee, J. Y., Kim, J., Kim, G. W., Kim, B. K., Kim, J. Y., Park, H. W., Cho, S. H., Min, K. U., & Kang, H. R. Incidence of stevens-johnson syndrome and toxic epidermal necrolysis: A nationwide population-based study using national health insurance database in Korea. PLoS ONE 2016;11:1-12.

9. Hsu, D. Y., Brieva, J., Silverberg, N. B., Paller, A. S., & Silverberg, J. I. (2017). Pediatric Stevens-Johnson syndrome and toxic epidermal necrolysis in the United States. Journal of the American Academy of Dermatology 2017;76:811-817.
10. Paulmann, M., & Mockenhaupt, M. (2019). Severe skin reactions: clinical picture, epidemiology, etiology, pathogenesis, and treatment. Allergo Journal International 2019;28:311-326.
11. Rajput, R., Sagari, S., Durgavanshi, A., & Kanwar, A. Paracetamol induced Steven-Johnson syndrome: A rare case report. Contemporary Clinical Dentistry 2015;6:278–281.
12. Ueta, M. Results of Detailed Investigations into Stevens-Johnson Syndrome With Severe Ocular Complications. Investigative Ophthalmology Visual Science 2018;59:183-1891.
13. Génin, E., Schumacher, M., Roujeau, J. C., Naldi, L., Liss, Y., Kazma, R., Sekula, P., Hovnanian, A., & Mockenhaupt, M. Genome-wide association study of Stevens-Johnson syndrome and Toxic Epidermal Necrolysis in Europe. Orphanet Journal of Rare Diseases 2011;6:1-10.
14. Irham, L. M., Chou, W. H., Calkins, M. J., Adikusuma, W., Hsieh, S. L., & Chang, W. C. Genetic variants that influence SARS-CoV-2 receptor TMPRSS2 expression among population cohorts from multiple continents. Biochemical and Biophysical Research Communications 2020;529:263–269.
15. Chen, Z., Boehnke, M., Wen, X., & Mukherjee, B. Revisiting the genome-wide significance threshold for common variant GWAS. G3: Genes, Genomes, Genetics 2021;11:1-12.
16. Purnamawati, S., Febriana, S. A., Danarti, R., & Saefuddin, T. Topical Treatment for Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis: AReview. Bali Medical Journal 2016;5:82-90.
17. Kawai, Y., Hitomi, Y., Ueta, M., Khor, S, S., Nakatani, K., Sotozono, C., Kinoshita, S., Tokunaga, K. Mapping of susceptible variants for cold medicine-related Stevens–Johnson syndrome by whole-genome resequencing. Genomic Medicine 2021;9:1-8.
18. Tamehiro, N., Mujawar, Z., Zhou, S., Zhuang, D. Z., Hornemann, T., Eckardstein, A. V., Fitzgerald, M. L. Cell polarity factor Par3 Binds SPTLC1 and modulates monocyte serine palmitoyltransferase activity and chemotaxis. Journal of Biological Chemistry 2009;284:81–90.
19. Ueta, M., and Kinoshita, S. Ocular Surface Inflammation Is Regulated by Innate Immunity. Progress in Retinal and Eye Research 2012;31:51-75
20. Ueta, M., Sawai, H., Sotozono, C., Hitomi, Y., Kaniwa, N., Kim, M. K. IKZF1, a New Susceptibility Gene for Cold Medicine-Related Stevens-Johnson Syndrome/toxic Epidermal Necrolysis with Severe Mucosal Involvement. The Journal of Allergy and Clinical Immunology 2015;135:38–45.
21. Jongkhajornpong, P., Ueta, M., Lekhanont, K., Puangsricharern, V., Prabhasawat, P., Chantaren, P. Association of HLA Polymorphisms and Acetaminophen-Related Steven-Johnson Syndrome with Severe Ocular Complications in Thai Population. British Journal of Ophthalmology 2022;106:884-888.
22. Puspitaningrum, A. N., Perwitasari, D. A., Adikusuma, W., Djalilah, G. N., Dania, H., Maliza, R., Faridah, I. N., Sarasmita, M. A., Rezadhini, M., Cheung, R., & Irham, L. M. (2022). Integration of genomic databases and bioinformatic approach to identify genomic variants for sjogren's syndrome on multiple continents. Media Farmasi: Jurnal Ilmu Farmasi 2022;19:71-81.
23. Irham, L. M., Adikusuma, W., Lolita, L., Puspitaningrum, A. N., Afief, A. R., Sarasmita, M. A., Dania, H., Khairi, S., Djalilah, G. N., Purwanto, B. D., & Cheung, R. Investigation of susceptibility genes for chickenpox disease across multiple continents. Biochemistry and Biophysics Reports 2023;33:1-7.

Hasil review dari 2 reviewers pada tanggal 6 Maret 2023

::: 1st Review :::

Editorial Comment

Invited date	Mar 6, 2023
Complete Review Date	Mar 29, 2023
Recommendation	**Review after major revision**
Comments to the Author	• **Reviewer A :** The study is interesting given that SJS is a rare disease and studies related to SJS are limited. The study combines GWAS, HaploReg, Ensembl, and eQTL analyses to identify potentially SJS-related SNPs. The study highlights the potential role of HLA-C gene variants in SJS susceptibility and progression, but there are some issues that need to be clarified. Please see the attached file for the detailed review. • **Files :** GI23010_Reviewer_.pdf • **Reviewer B :** In this manuscript (GI23010), a bioinformatics approach was used to identify genetic variants associated with Stevens-Johnson Syndrome (SJS) across multiple continents. Overall, this work is well organized. I suggest major revisions before the paper can be considered for publication in the Genomics and Informatics journal. 1. The English quality and grammar throughout the manuscript need to be revisited and majorly improved. For instance: - No need to write brackets when mentioning Figures or Tables in the text (except where necessary) such as "Also as shown in (Table 3) and (Fig 3)…" à "As shown in Table 3 and Fig. 3…" - "~~According to~~ Frey et al (2017) [7] reported that in the UK from 1995-2013 there were 5.76 cases of SJS/TEN per million people" (need to be restructured). - "~~According to~~ Hsu et al (2017) [9] this study reported that the incidence rate in children suffering from SJS/TEN is 5.3 and 0.4 cases per year" (need to be restructured). - with $p < 10\text{-}8$ à with p value $< 10^{-8}$ (with superscript). 2. In the *Introduction* section, authors stated "This review was carried out for the sole purpose of understanding that there is evidence linking circulating immune complex relationships…". Since the manuscript is an original manuscript, it is not appropriate to mention "this review". 3. "Our histological study in Ikzf1Tg mice also showed not only dermatitis but also…" (*Results and Discussion*). Does it refer to author's present or previous work? Or other researchers' work? 4. In Table 3, the format of p-value number is not consistent. The numbering format should be consistent throughout tables and text 5. The figure position should be close to where it is first referred to in the text such as Fig. 3 6. A higher resolution (at least 300 DPI) is recommended for all figures to increase their clarity. 7. In Fig. 4, the labeling of South Asia and East Asia regions seems incorrect. 8. Authors mentioned that the limitation of the developed bioinformatic-based approach is primarily on false positive association, which is mitigated by analysis of significant variants that pass stringent statistical thresholds. Did the authors consider the possibility of false negative? 9. How do authors associate the finding with other previous studies in SJS genome analysis such as https://www.nature.com/articles/s41525-021-00171-2, https://www.tandfonline.com/doi/full/10.2147/PGPM.S289869, and https://www.nature.com/articles/s41598-019-52619-2 10. What is the correlation between the main findings (i.e., the two variants rs2074494 and rs5010528 which encode the HLA-C gene in skin tissue) and the diagnosis and management of SJS. 11. All references should be in a consistent style and format. • **Files :** GI23010_Reviewer__1.pdf

Artikel hasil dari revisi setelah mendapatkan masukkan dan saran oleh 2 reviewer pada 29 Maret 2023

Maret 29, 2023

Dear Editors,

Please find our attached revised manuscript, entitled "A bioinformatic approach to identify pathogenic variants for Stevens-Johnson Syndrome," which we are submitting for consideration for publication as an original research article in *Genomics & Informatics* (GI23010). We are thankful for your kind encouragement regarding our manuscript. Herewith, we are sending this revised manuscript in accordance with the comments given by the reviewers. The revised parts of the manuscript are highlighted in yellow. Finally, we would like to thank you once again for giving us the opportunity to improve our manuscript. We very much hope that these revisions are adequate. We appreciate your review and assistance, and look forward to hearing from you.

Sincerely yours,

Apt Lalu Muhammad Irham M.Farm Ph.D.

Faculty of Pharmacy,

Universitas Ahmad Dahlan, Yogyakarta, Indonesia

Jl. Prof. DR. Soepomo SH, Warungboto,

Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta

Recommendation Reviewer 1:

We sincerely thank the reviewer for taking the time to review our work, and have addressed the critical comments point-by-point.

Q1: The result in abstract section is unclear. Please add more detail about the findings.

A1: We appreciate the reviewer's comments. We now revised the results in the abstract, as well as included research findings [Page 1, lines 22-28]., in order to provide more details, and substantiate the results in abstract section.

Q2: The figure is lack of information. To be more informative and enhanced the readability, i suggest to add more detail related to what and the study addressed.

A2: We revised the Figure in the Methods section and modified Figure 1 as shown below. [Page 2, lines 68-69].

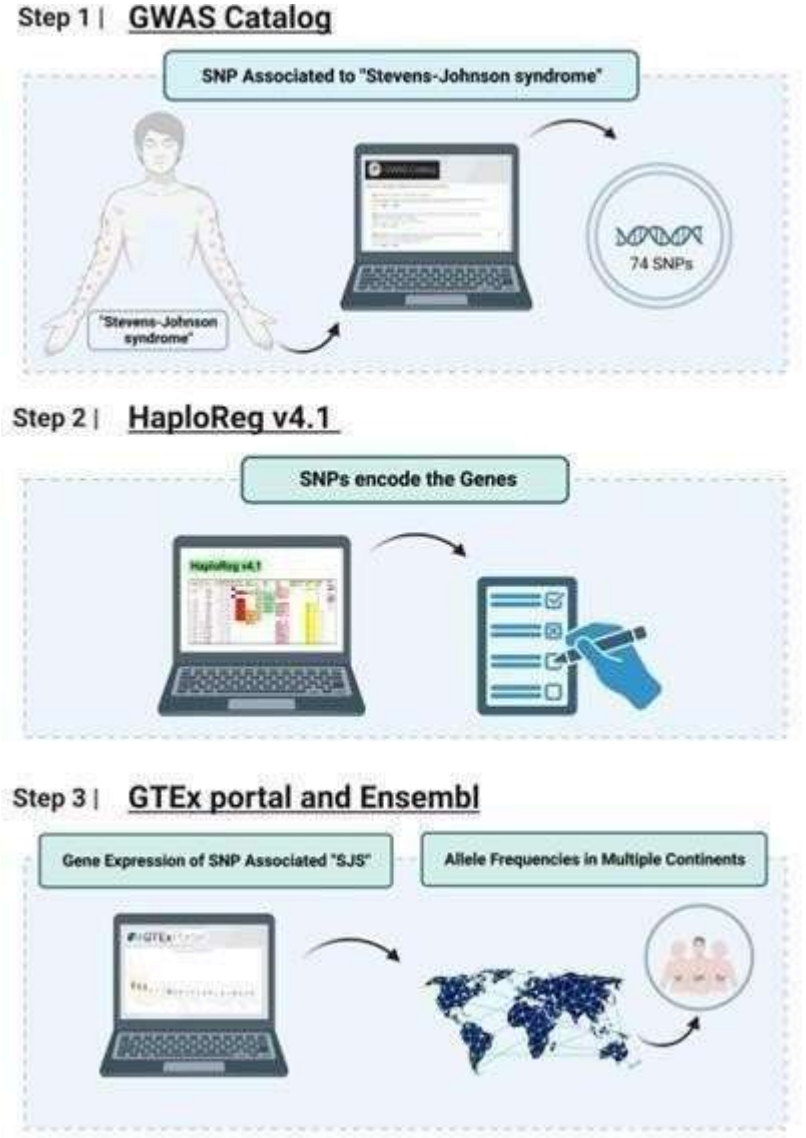


Figure 1. Bioinformatics workflow for the identification of genetic variants associated with Stevens-Johnson syndrome (SJS).

Q3: In methods. Please mentioned how the obtaining data addressed instead of using several test. It is necessary to mention the detail of the process.

A3: Thank you for the suggestion, and we revised the Methods section accordingly [Page 3, lines 76-80]. The sentences are revised as below:

"In this study design, detailed information can be seen in Figure 1. We used the keyword "Stevens-Johnson syndrome (SJS)" to derive SJS associated from the GWAS National Human Genome Research Institute (NHGRI) Catalog database http://www.ebi.ac.uk/gwas (accessed 19/12 /2022). SJS associated with 74 SNPs were obtained and further analysis was carried out using HaploReg (version 4.1) and found a total of 41 SNPs with a significance value of p-value $< 10^{-8}$*."*

Q4: In methods. If possible, add the detail participant such numbers, percentage and etc.

A4: Thank you for the suggestion. We revised the manuscript according to your suggestions. We revised the method description to provide detailed explanation [Page 3, lines 88-91] as below:

"This study used allele frequencies from populations in Europe, Africa, America, and Asia. The allele frequencies for both variants were evaluated in different African, American, East Asian, European, and Southeast Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia)."

Q5: In results and discussion about identification of genomic variants for Stevens-Johnson syndrome (SJS), How you performed this issue?

A5: Thank you for the comments. We added the rationale for this study to identifying genomic variants for Stevens-Johnson syndrome (SJS) [Page 3, lines 102-103]. The sentences are revised as below:

"In this study, we investigated two variants with missense mutation (rs2074494, rs5010528) encoded the HLA-C genes as the biological risk SNPs for SJS."

Q6: In results and discussion about *HLA-C* gene expression across 16 human tissues, Please add the citation and why it is important.

A6: We appreciate the reviewer's comments. We now added citations regarding the section about HLA-C gene expression across 16 human tissues [Page 4, lines 123-127] as below:

"Stevens-Johnson syndrome (SJS) is acute and can cause death occurs, therefore this disease is an emergency disease on the skin [16]. One Sun-Exposed Skin that is often exposed is exposure to ultraviolet radiation (UVR). In general, exposure to ultraviolet radiation (UVR) has been reported as a risk factor for Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [17]."

Q7: In results and discussion about *HLA-C* gene expression in the sun-exposed skin. In paragraph 2, I think this sentence is not proper. Did the study addresses by your lab ?

A7: Thank you for the reviewer's suggestion. We deleted the sentence because it does not have a relationship that is in accordance with the discussion section that has been written. [Page 5, lines 145-149]. Several susceptibility genes for SJS/TEN CM associated with SOC may be involved in the formation of functional networks. An imbalance in this gene can trigger the mucocutaneous inflammation seen in patients with SJS/TEN associated with CM with SOC. SJS/TEN with SOC in the acute stage shows inflammation of the skin and ocular surface and oral mucosal erosions and paronychia [12].

Q8: To make the findings of this study more meaningful, it would be useful to compare the results of this study with other studies on HLA-C and its role in autoimmune diseases, as well as with studies on genetic factors that contribute to SJS. This may help provide a broader context for the

potential role of HLA-C gene variants in SJS susceptibility and development, and further elucidate the genetic mechanisms involved in this disease.

A8: Thank you for the suggestion. We now added 3 citations from references related to the results and discussion.

Citation 1: [Page 5, lines 139-141]. The detailed sentence has now been added as below:

"*It was also reported that genetic variation in HLA-A and other autosomal genes have been identified as a risk factor for SJS/TEN associated with flu drugs with severe ocular complications such as CM-SJS or TEN with SOC [18]."*

Citation 2: [Page 7, lines 214-219]. The detailed sentence has now been added as below:

"*Notably, the variants associated with HLA-C expression have not been reported for SJS. Basic research on the genetics of SJS and TEN to date has focused on human leukocyte antigen (HLA), the system associated with the presence of specific receptors, cytotoxic proteins, and the part of immunocytes during disease pathogenesis [23]. Considering the global impact of SJS, examining the distribution of HLA-C variants may be an essential quest that allows further understanding of global disease susceptibility."*

Citation 3: [Page 8, lines 227-230]. The detailed sentence has now been added as below:

"We identified genetic variants associated with HLA-C expression in skin tissue, the leading site of SCAR infection in SJS disease. It has previously been reported that specific human leukocyte antigen (HLA) genotypes have been associated with the occurrence of severe skin disease due to drug-induced side effects (SCARs), which cases are included in SJS/TEN [26]."

Q9: On the third line in the conclusion, incomplete sentence. Please modified it.

A9: We are grateful for the reviewer's comments. **We revised the sentence in the conclusion section [Page 8, lines 245]. The detailed citation has been added as below:**

"The high expression occurs in whole blood, spleen, lung, and lymphocyte cells."

Recommendation Reviewer 2:

In this manuscript (GI23010), a bioinformatics approach was used to identify genetic variants associated with Stevens-Johnson Syndrome (SJS) across multiple continents. Overall, this work is well organized. I suggest major revisions before the paper can be considered for publication in the Genomics and Informatics journal.

Answer: We sincerely thank the reviewer for taking the time to review our work.

Q1: The English quality and grammar throughout the manuscript need to be revisited and majorly improved. For instance:

- No need to write brackets when mentioning Figures or Tables in the text (except where necessary) such as "Also as shown in (Table 3) and (Fig 3)…" ➔ "As shown in Table 3 and Fig 3…"
- "~~According to~~ Frey et al (2017) [7] reported that in the UK from 1995-2013 there were 5.76 cases of SJS/TEN per million people" (need to be restructured).
- "According to Hsu et al (2017) [9] this study reported that the incidence rate in children suffering from SJS/TEN is 5.3 and 0.4 cases per year" (need to be restructured).
- with $p < 10\text{-}8$ ➔ with p value $< 10^{-8}$ (with superscript).

A1: We sincerely thank the reviewer for taking the time to review our work. We revised the main text according to the suggestions from the reviewers:

- **For parts of Tables and Figures, we removed the brackets as suggested.**
- **For the citation, we removed the word "according", page 1, in the last line.**
- **For p-value (numeric value), which has not been properly marked with superscript, we now corrected and revised to $p < 10^{-8}$**

Q2: In the *Introduction* section, authors stated "This review was carried out for the sole purpose of understanding that there is evidence linking circulating immune complex relationships…". Since the manuscript is an original manuscript, it is not appropriate to mention "this review".

A2: We are grateful for the reviewer's comments. We revised part of the sentence based on reviewer's input [Page 2, lines 61-64]. The detailed sentence has been added as below: This study aims to invesitgate the variants associated SJS through bioinformatic based approach and further prioritize the biological risk variants. Besides, the pattern of gene expression profiles and population allele frequencies of genetic variants were assessed using various databases.

Q3: "Our histological study in Ikzf1Tg mice also showed not only dermatitis but also…" (*Results and Discussion*). Does it refer to author's present or previous work? Or other researchers' work?

A3: Thank you for the feedback from reviewers that have been given to us. We have removed the sentence as suggested by reviewer.

Q4: In Table 3, the format of p-value number is not consistent. The numbering format should be consistent throughout tables and text.

A4: Thank you for the reviewer's suggestion. We now revised all table numbering formats, *p*-value numbers, tables, and text to be consistent [Page 6, lines 165-166].

Table 3. *HLA-C* expression-Quantitative Trait Loci (eQTL) analysis from the GTEx Portal database

ID SNP	Gencode ID (ENSG00000-)	Gene Symbol	*p*-value	Effect Size	Tissue	Expression Level
rs2074494	204525.16	*HLA-C*	7.2×10^{-10}	-0.48	Artery-Tibial	CC>CT>TT
	204525.16	*HLA-C*	13×10^{-6}	-0.50	Muscle-Skeletal	CC>CT>TT
	204525.16	*HLA-C*	15×10^{-6}	-0.78	Brain-Nucleus accumbens (basal ganglia)	CC>CT>TT
	204525.16	*HLA-C*	17×10^{-6}	-0.51	Heart-Left Ventricle	CC>CT>TT
	204525.16	*HLA-C*	46×10^{-6}	-0.58	Testis	CC>CT>TT
	204525.16	*HLA-C*	36×10^{-5}	-0.39	**Skin-Sun Exposed (Lower leg)**	CC>CT>TT
rs5010528	204525.16	*HLA-C*	5.4×10^{-26}	0.56	Adipose-Subcutaneous	AA>AG>GG
	204525.16	*HLA-C*	1×10^{-21}	0.41	Whole Blood	AA>AG>GG
	204525.16	*HLA-C*	1.4×10^{-14}	0.51	Adipose-Visceral	AA>AG>GG
	204525.16	*HLA-C*	4.1×10^{-14}	0.49	Lung	AA>AG>GG
	204525.16	*HLA-C*	1.1×10^{-10}	0.68	Spleen	AA>AG>GG
	204525.16	*HLA-C*	2.1×10^{-9}	0.51	Heart-Atrial Appendage	AA>AG>GG
	204525.16	*HLA-C*	5.7×10^{-8}	0.28	Colon-Transverse	AA>AG>GG
	204525.16	*HLA-C*	9.9×10^{-8}	0.38	Skin-Not Sun Exposed (Suprapublic)	AA>AG>GG
	204525.16	*HLA-C*	5.1×10^{-7}	0.72	Cells-EBV-transformed lymphocytes	AA>AG>GG
	204525.16	*HLA-C*	7.0×10^{-7}	-0.25	Nerve-Tibial	AA>AG>GG
	204525.16	*HLA-C*	7.7×10^{-7}	0.31	Nerve-Tibial	AA>AG>GG
	204525.16	*HLA-C*	1.6×10^{-6}	0.34	Breast-Mammary Tissue	AA>AG>GG
	204525.16	*HLA-C*	$1,8 \times 10^{-6}$	0.32	**Skin-Sun Exposed (Lower leg)**	AA>AG>GG

Source: Expression Quantitative Trait Loci (eQTL) obtained from the GTEx Portal.

Q5: The figure position should be close to where it is first referred to in the text such as Fig. 3.

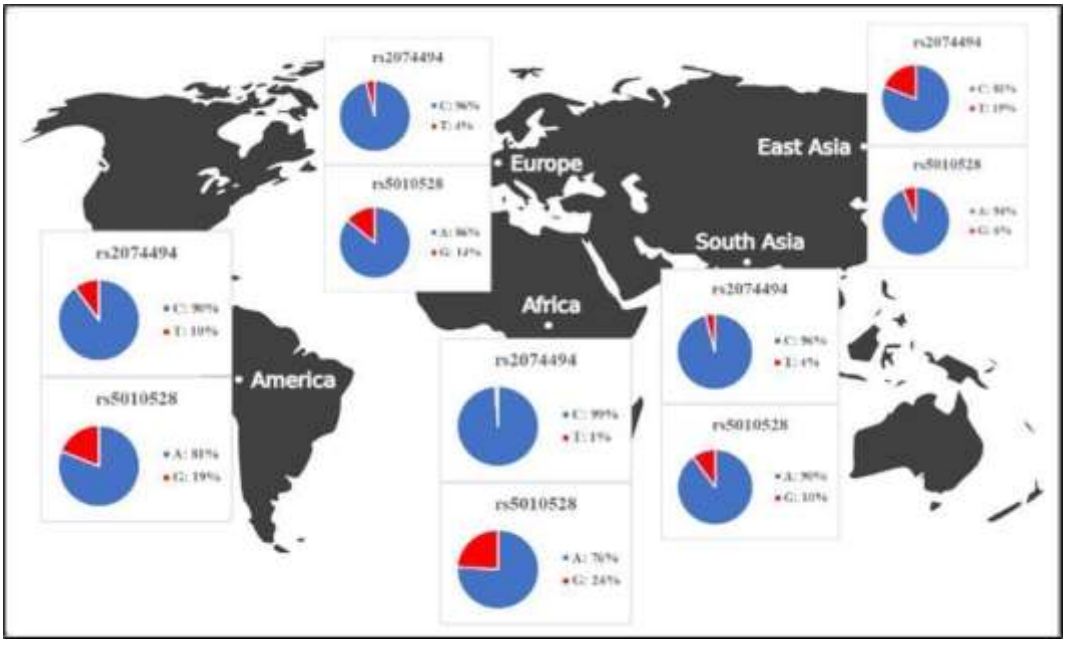
A5: **We are grateful for the reviewer's comments. We revised the figure as suggested by the reviewer [Page 6, Figure 3, line 171].**

Q6: A higher resolution (at least 300 DPI) is recommended for all figures to increase their clarity.

A6: **Thank you for the suggestion to improve figure clarity. We now revised all figures to a higher resolution greater than 300 DPI.**

Q7: In Fig. 4, the labelling of South Asia and East Asia regions seems incorrect.

A7: **Thank you for the reviewer's suggestion with regards to labelling of Figure 4. We revised the figure by swapping the position of the correct labels for the South Asia and East Asiaregions. [Page 7, Figure 4, line 209]. The revised version is depicted by the following figure below:**



Q8: Authors mentioned that the limitation of the developed bioinformatic-based approach is primarily on false positive association, which is mitigated by analysis of significant variants that pass stringent statistical thresholds. Did the authors consider the possibility of false negative?

A8: We appreciate this comment and revised the limitation as below [Page 8, lines 238 241]:

"However, we acknowledged that the limitation of our bioinformatic-based course is no all the variants has encoded the genes, with lack of pharmacological activity. Nonetheless, clinical validation is recommended as a next step to confirm our findings and further understand the underlying etiology and functional effect of the SJS disease"

Q9: How do authors associate the finding with other previous studies in SJS genome analysis such as https://www.nature.com/articles/s41525-021-00171-2, https://www.tandfonline.com/doi/full/10.2147/PGPM.S289869, and https://www.nature.com/articles/s41598-019-52619-2 ?

A9: To address this important point, we have added these references as citations:

Citation 1: [Page 5, lines 139-141] The detailed sentence has now been added as below:

"*It was also reported that genetic variation in HLA-A and other autosomal genes has been identified as a risk factor for SJS/TEN associated with flu drugs with severe ocular complications such as CM-SJS or TEN with SOC [18].****"***

Citation 2: [Page 7, lines 214-219] The detailed sentence has now been added as below:

"*Notably, the variants associated with HLA-C expression have not been reported for SJS. Basic research on the genetics of SJS and TEN to date has focused on human leukocyte antigen (HLA), the system associated with the presence of specific receptors, cytotoxic proteins, and the part of immunocytes during disease pathogenesis [23]. Considering the global impact of SJS, examining the distribution of HLA-C variants may be an essential quest that allows further understanding of global disease susceptibility.*"

Citation 3: [Page 8, lines 227-230]. The detailed sentence has now been added as below:

"We identified genetic variants associated with HLA-C expression in skin tissue, the leading site of SCAR infection in SJS disease. It has previously been reported that specific human leukocyte antigen (HLA) genotypes have been associated with the occurrence of severe skin disease due to drug-induced side effects (SCARs), which cases are included in SJS/TEN [26]."

Q10: What is the correlation between the main findings (i.e., the two variants rs2074494 and rs5010528 which encode the HLA-C gene in skin tissue) and the diagnosis and management of SJS?

A10: Thank you for this critical remark. The findings' relevance to the skin tissue is now described in the section on *HLA-C* gene expression across 16 human tissues [Page 4, lines 122-127] as below:

"Furthermore, we have found that the ID SNPs rs2074494 and rs5010528 have similar gene expression variation in the Sun-Exposed Skin (Lower Leg). Stevens-Johnson syndrome (SJS) is acute and can cause death occurs, therefore this disease is an emergency disease on the skin [16]. One Sun-Exposed Skin that is often exposed is exposure to ultraviolet radiation (UVR). In general, exposure to ultraviolet radiation (UVR) has been reported as a risk factor for Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [17]."

Q11: All references should be in a consistent style and format.

A11: We appreciate this comment, and have revised all the formats contained in the reference section [Pages 9-10, lines 269-336] to maintain style and format consistency.

G&I Genomics & Informatics

A bioinformatic approach to identify pathogenic variants for Stevens-Johnson Syndrome

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Abstract

Stevens-Johnson syndrome (SJS) produces a severe hypersensitivity reaction caused by Herpes simplex virus or mycoplasma infection, vaccination, systemic disease, or other agents. Several studies have investigated the genetic susceptibility involved in SJS. To provide further genetic insights into the pathogenesis of SJS, this study prioritized high-impact, SJS-associated pathogenic variants through integrating bioinformatic and population genetic data. First, we identified SJS-associated single nucleotide polymorphisms (SNPs) from the Genome-Wide Association Studies (GWAS) catalog, followed by genome annotation with HaploReg and variant validation with Ensembl. Subsequently, quantitative trait locus expression analysis (eQTL) from GTEx identified human genetic variants with differential gene expression across human tissues. Our results indicate that two variants, namely rs2074494 and rs5010528, which are encoded by the *HLA-C* gene, were found to be differentially expressed in skin. The allele frequencies for rs2074494 and rs5010528 also appear to significantly differ across continents. We highlight the utility of these population-specific *HLA-C* genetic variants for genetic association studies, and aid in early prognosis and disease treatment of SJS.

Keywords: Bioinformatics, Genetic Variation, Genomic, Stevens-Johnson syndrome, Pathogenic variants

Introduction

Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are potentially life-threatening diseases [1]. In particular, SJS is a syndrome resulting from severe hypersensitivity reactions caused by infection with the Herpes simplex or Mycoplasma viruses, vaccinations, systemic diseases, certain agents, food, and drugs [2]. SJS occurs in the skin and mucous membranes in the orifices and eyes in mild to severe conditions, with abnormalities in the skin in the form of an erythema, vesicles, or bullae accompanied by purpura [3].

SJS/TEN is widespread in the mucocutaneous immune region, causing exfoliation of the skin on the mucosal surface [4]. The incidence of SJS, SJS/TEN, and TEN in the United States (US) reported 9.2, 1.6, and 1.9 cases from 2009-2012 [5]. The incidence of SJS cases that occur in Indonesia is around 12 cases per year, with different causes [6]. The incidence in the United Kingdom (UK) from 1995-2013 there were 5.76 cases of SJS/TEN per million people per year [7]. In Korea, it is reported that the incidence rate of SJS/TEN from 2009–2013 was 3.96–5.03 and 0.94–1.45 per million people per year [8]. Events caused by SJS, SJS-TEN, and TEN have an average of 5.3, 0.8, and 0.4 cases per million

children each year [9]. The mortality rate of 4.8-9% in SJS, 19.4-29% in the SJS/TEN case, and 14.8-48% in TEN [10]. SJS can appear with non-specific fever symptoms that cause malaise, headache, cough, and rhinorrhea. On the skin, patients suffering from SJS can have polymorphic lesions and mucous membranes with marked skin blisters and erosion [11].

Therefore, primary prevention is the best mitigation for SJS. SJS is categorized as a severe cutaneous adverse reaction (SCAR), and several drugs have been implicated in disease pathogenesis. Non-steroidal anti-inflammatory drugs (NSAIDs) and other multi-ingredient formulations are widely used to relieve the symptoms. Several studies are reporting adverse skin drug reactions that are often SJS-associated with severe ocular complications [12]. Prevention is possible if patients who are susceptible to this SCAR when prescribed certain drugs are identified. Besides, the genomic variants are known to have an important role in SJS progression. However, little information revealed the specific variant as a biological risk gene in SJS. A previous study revealed associated variants in SJS progression (rs2844665, rs3815087, rs3130931, rs3130501, rs3094188, rs9469003). In allopurinol-induced SJS and TEN, rs9469003 can detect an effect similar to that seen with an allele frequency risk of 15% and an associated risk of 99% [13]. Even though several studies have been exploited previously, a limited number of variants summarized the variants associated with expression in tissue-related SJS. This study aims to investigate the variants associated with SJS through a bioinformatic-based approach and further prioritize the biological risk variants. Besides, the pattern of gene expression profiles and population allele frequencies of genetic variants were assessed using various databases. Here, The results will enable future studies to assess whether these variants may be associated with various infectious risks for Stevens-Johnson syndrome/toxic epidermal necrolysis, as well as SJS progression and disease susceptibility.

Methods

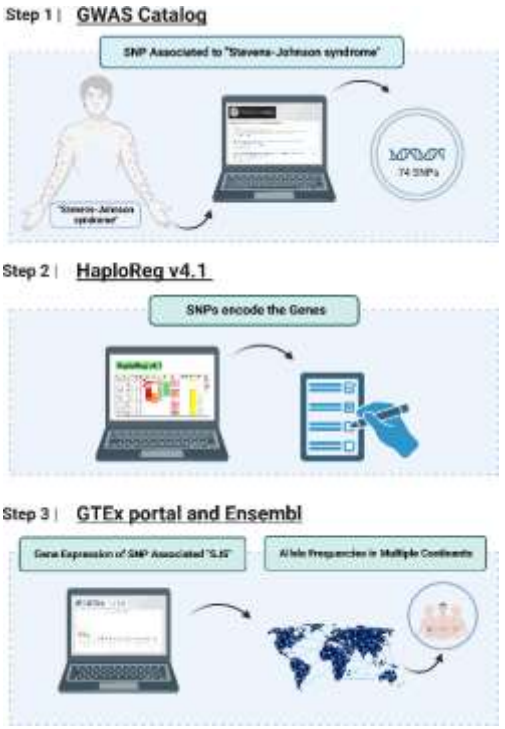

Fig 1. Bioinformatics workflow for the identification of genetic variants associated with Stevens-Johnson syndrome (SJS).

Genomic information not only can be leveraged to identify the variant-associated disease, but it can also be translated into actionable knowledge for the disease. SJS is one of the severe skin reaction due

to genomic risk factors. In this study, we used a bioinformatic-based approach to prioritize the pathogenic variants that potentially trigger the SJS. Detailed information regarding the study design has been depicted in Figure 1. We used the keyword "Stevens-Johnson syndrome (SJS)" to derive SJS associated from the GWAS National Human Genome Research Institute (NHGRI) Catalog database http://www.ebi.ac.uk/gwas (accessed 19/12 /2022). SJS associated with 74 SNPs were obtained and further analysis was carried out using HaploReg (version 4.1). Further analysis yielded a total of 41 SNPs with a significance value of *p-value* < 10^{-8}. This value is used to account for several tests in the GWAS catalog. These values are widely used to identify associations between variants and shared genetic traits with adjacent gene expression [14]. Furthermore, an evaluation was carried out between the relationships of various genetic variants and gene expression profiles using quantitative expression trait loci (eQTL) using the GTEx Portal database http://www.gtexportal.org/home/ (accessed 19/12/2022), which was found by gene expression from various networks. The *HLA-C* genetic variant is present in human skin and tissue (lower extremities) exposed to sunlight obtained from the GTEx Portal database. Then confirm the variant using the Ensembl Genome Browser https://www.ensembl.org/index.html (accessed December 19, 2022). Furthermore, the allele frequencies of variants associated SJS were evaluated in different populations including African, American, East Asian, European, and Southeast Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia). Then, to find out the function of the various gene variants, an evaluation was carried out using the SNP Nexus database https://www.snp-nexus.org (accessed December 19, 2022).

Results and Discussion

Identification of genomic variants for Stevens-Johnson syndrome (SJS)

We first identified SNPs associated with SJS from the GWAS database, resulting in 74 SNPs associated with SJS. We identified 41 unique SNPs associated with SJS after remove all SNPs duplication (Table 1). Based on the number of SNPs obtained, candidate SNPs were further constrained and prioritized using HaploReg version 4.1, with a *p-value* of < 10^{-8}. Based on the findings presented in Table 2, we focused on two genomic variants from the same gene that qualify as the biological risk SNPs for SJS from this study.

Through our integrative bioinformatics approach, two variants with a missense mutation (rs2074494, rs5010528) that encoded the *HLA-C* genes were prioritized as the biological risk SNPs for SJS. SJS is characterized by necrosis and shedding of the epidermis, known as the triad of disorders of the vesiculobullous skin, orifice mucosa, and eyes, accompanied by severe general symptoms [6]. It was also reported that the *HLA-C* gene has an important role in protecting against cancer and viruses. However, the *HLA-C* gene may also be involved in allograft rejection, the state of preeclampsia, and is also present in autoimmune diseases [15]. The diagnosis of SJS/TEN is a blistering autoimmune disease. which is included in linear IgA dermatosis and paraneoplastic pemphigus but is also present in pemphigus vulgaris and bullous pemphigoid, acute generalized exanthematous pustulosis (AGEP), and later disseminated drug persistently erupting bullosa and staphylococcal scalded skin syndrome (SSSS) [16].

Table 1. SNPs from the GWAS catalog with a significance of *p*-value < 10^{-8}

Variant and risk allele	*p*-value
rs6457109	3 x 10^{-16}
rs7760545	4 x 10^{-16}
rs35835721	8 x 10^{-15}
rs137899365	3 x 10^{-14}
rs60581484	2 x 10^{-13}

rs1131151	4×10^{-12}
rs2074494	5×10^{-12}
rs4917014	8×10^{-11}
rs1562468327	6×10^{-10}
rs199755581	7×10^{-10}
rs11509487	1×10^{-9}
rs9469003	2×10^{-9}
rs4471527	2×10^{-9}
rs28381346	5×10^{-9}
rs6500265	6×10^{-9}
rs199755581	6×10^{-9}
rs114908185	7×10^{-9}
rs1297852527	9×10^{-9}
rs1371146120	1×10^{-8}
rs77491650	1×10^{-8}
rs4471527	1×10^{-8}
rs77542827	1×10^{-8}
rs3130501	2×10^{-8}
rs536142737	2×10^{-8}
rs16957893	2×10^{-8}
rs2734583	2×10^{-8}
rs150289893	2×10^{-8}
rs1286845082	2×10^{-8}
rs548089948	2×10^{-8}
rs3094188	3×10^{-8}
rs55765602	3×10^{-8}
rs77542827	3×10^{-8}
rs778096762	3×10^{-8}
rs1597607761	3×10^{-8}
rs1391213386	3×10^{-8}
rs374138762	4×10^{-8}
rs879656274	4×10^{-8}
rs1211926109	4×10^{-8}
rs116953913	5×10^{-8}
rs1263106470	6×10^{-8}
rs5010528	8×10^{-8}

Table 2. Stevens-Johnson syndrome variant and risk allele that codes for prioritized SNPs

Variant and risk allele	Variants near risk allele ($r^2 > 0.8$)	*p*-value	Gencode	Allele type
rs2074494	rs1050276	5×10^{-12}	*HLA-C*	missense
rs5010528	rs1050409	8×10^{-8}	*HLA-C*	missense

HLA-C gene expression across 16 human tissues

To evaluate *HLA-C* gene expression in human tissues, we used the GTEx portal database (http://www.gtexportal.org/), which contains gene expression levels in various tissues. eQTL annotation comprises the most apparent functional consequences of genetic variation. Whole blood, spleen, lung, and lymphocyte cells demonstrate the highest *HLA-C* gene expression across the 16 human tissues analyzed from GTEx in Figure 2. Furthermore, we have found that the ID SNPs rs2074494 and rs5010528 have similar gene expression variation in the Sun-Exposed Skin (Lower Leg). SJS is acute and can cause death occurs, therefore this disease is an emergency disease on the skin [16]. One Sun-Exposed Skin that is often exposed is exposure to ultraviolet radiation (UVR). In general, exposure to ultraviolet radiation (UVR) has been reported as a risk factor for SJS and TEN [17].

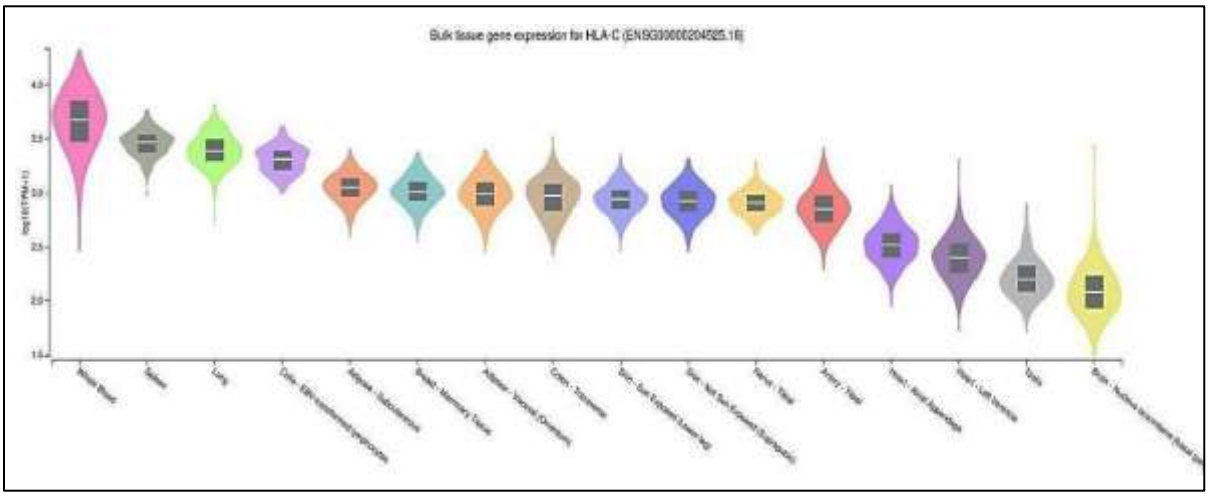

Fig 2. *HLA-C* gene expression associated with Stevens-Johnson syndrome (SJS) in several human tissues from the GTEx Portal.

HLA-C gene expression in the sun-exposed skin

HLA class I genes, including *HLA-A*, *HLA-B*, and *HLA-C*, have been reported as the loci most strongly associated with susceptibility to all types of SJS and TEN, including cold medicine-related (CM-SJS) and TEN with severe ocular complications (SOC). Although non-synonymous substitutions affecting peptide binding or *HLA* molecular conformation have been considered significant factors in the pathogenesis of immunological diseases, Indeed, different *HLA-C* expression levels have been reported for the different alleles, with higher HLA-C expression leading to increased Tc (Trypanosoma cruzi) responses and adverse effects in Crohn's disease. It was also reported that genetic variation in *HLA-A* and other autosomal genes has been identified as a risk factor for SJS/TEN associated with flu drugs with severe ocular complications such as CM-SJS or TEN with SOC [18].

When individuals with a genetic background containing SJS/TEN with SOC susceptibility factors are infected by some viral or microbial infection, they develop abnormal immune responses [19]. It is reasonable to presume that there is an interaction between HLA multiplication and susceptibility genes such as *HLA-A* and *TLR3* [20], *HLA-A* and *REC14-32*, and *HLA-A* and *PTGER3* [21]. Several susceptibility genes for SJS/TEN CM associated with SOC may be involved in the formation of functional networks. An imbalance in this gene can trigger the mucocutaneous inflammation seen in patients with SJS/TEN associated with CM with SOC. SJS/TEN with SOC in the acute stage shows inflammation of the skin and ocular surface and oral mucosal erosions and paronychia [12].

Relationship between *HLA-C* gene and expression QTLs (eQTLs) from the GTEx database

Gene expression of SJS in human tissue was evaluated via the GTEx Portal database. This aims to determine the gene expression level in various tissues including in the skin tissue. We identified genomic variations from *HLA-C* gene expression using the GWAS catalog database and found 74 SNPs. From these analyses, we determined top 10 SNPs with the highest *p-values*. *We* were further processed with the variant annotation tool SNPnexus to determine the annotation of prioritized SNP variations. After these analyses, two statistically significant SNPs were obtained and prioritized. In this case, we prioritized 2 SNPs at risk for SJS based on an analysis of the number of SNPs expanded using HaploReg ver. 4.1 and a *p*-value of $< 10^{-8}$ to determine the functional annotation of the SNPs. The results of genetic variation are shown in Table 3.

Table 3. *HLA-C* expression-Quantitative Trait Loci (eQTL) analysis from the GTEx Portal database

SNP ID	Gencode ID (ENSG00000-)	Gene Symbol	*p*-value	Effect Size	Tissue	Expression Level
rs2074494	204525.16	*HLA-C*	7.2×10^{-10}	-0.48	Artery-Tibial	CC>CT>TT
	204525.16	*HLA-C*	13×10^{-6}	-0.50	Muscle-Skeletal	CC>CT>TT
	204525.16	*HLA-C*	15×10^{-6}	-0.78	Brain-Nucleus accumbens (basal ganglia)	CC>CT>TT
	204525.16	*HLA-C*	17×10^{-6}	-0.51	Heart-Left Ventricle	CC>CT>TT
	204525.16	*HLA-C*	46×10^{-6}	-0.58	Testis	CC>CT>TT
	204525.16	*HLA-C*	36×10^{-5}	-0.39	**Skin-Sun Exposed (Lower leg)**	CC>CT>TT
rs5010528	204525.16	*HLA-C*	5.4×10^{-26}	0.56	Adipose-Subcutaneous	AA>AG>GG
	204525.16	*HLA-C*	1×10^{-21}	0.41	Whole Blood	AA>AG>GG
	204525.16	*HLA-C*	1.4×10^{-14}	0.51	Adipose-Visceral	AA>AG>GG
	204525.16	*HLA-C*	4.1×10^{-14}	0.49	Lung	AA>AG>GG
	204525.16	*HLA-C*	1.1×10^{-10}	0.68	Spleen	AA>AG>GG
	204525.16	*HLA-C*	2.1×10^{-9}	0.51	Heart-Atrial Appendage	AA>AG>GG
	204525.16	*HLA-C*	5.7×10^{-8}	0.28	Colon-Transverse	AA>AG>GG
	204525.16	*HLA-C*	9.9×10^{-8}	0.38	Skin-Not Sun Exposed (Suprapublic)	AA>AG>GG
	204525.16	*HLA-C*	5.1×10^{-7}	0.72	Cells-EBV-transformed lymphocytes	AA>AG>GG
	204525.16	*HLA-C*	7.0×10^{-7}	-0.25	Nerve-Tibial	AA>AG>GG
	204525.16	*HLA-C*	7.7×10^{-7}	0.31	Nerve-Tibial	AA>AG>GG
	204525.16	*HLA-C*	1.6×10^{-6}	0.34	Breast-Mammary Tissue	AA>AG>GG
	204525.16	*HLA-C*	$1{,}8 \times 10^{-6}$	0.32	**Skin-Sun Exposed (Lower leg)**	AA>AG>GG

Source: Expression Quantitative Trait Loci (eQTL) obtained from the GTEx Portal.

Table 3 shows the two identified variants (rs2074494 and rs5010528) encoded the *HLA-C* gene in differential tissue expression in the human skin. By Using the GTEx portal (http://www.gtexportal.org/home/), we further emphasized that the variants of rs2074494 and rs5010528 encoded the *HLA-C* genes were a higher expression in the skin tissue to Table 3 and Fig 3.

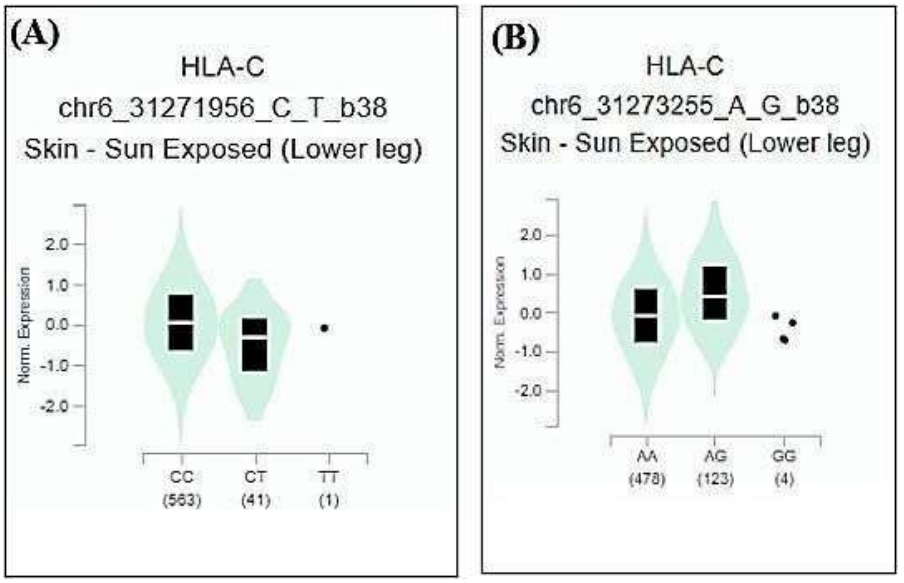

Fig 3. *HLA-C* gene expression for each genotype of the SNPs: (A) rs2074494 and (B) rs5010528.

Allele frequencies of SJS candidate variants across continents

Once we identified the candidate *HLA-C* expression-associated variants, we set out to determine the allele frequencies across transcontinental populations as shown in Table 4. The allele frequencies for both variants were evaluated in different population including African, American, East Asian, European, and Southeast Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia).

We extracted the allele frequencies in Africa, America, East Asia, Europe, and Southeast Asia from the Ensemble Genome Browser (http://www.ensembl.org). Allele frequencies across populations differ for each *HLA-C* variant. Table 4 and Fig 4 show gene expression levels at higher frequencies of the rs5010528 associated (G) allele and rs2074494 associated (T) allele. At population frequencies of the rs5010528 (G) allele, the Asian population (East Asia and Southeast Asia) is expressed at a much lower level than that from the populations of Africa, America, and Europe.

Table 4. Analysis of allele frequencies for the *HLA-C* gene from SNPnexus variant annotation. [AFR: Africa, AMR: America, EAS: East Asia, EUR: Europe; SAS: Southeast Asia; Ref: Reference; Alt: Alternate.]

SNP ID	Position (hg38)	Gene Symbol	Location	Allele		Allele Frequency (N)				
				Ref	Alt	AFR	AMR	EAS	EUR	SAS
rs2074494	Chr6:31271956	*HLA-C*	Missense	C	T	T: 0.008 (10)	T: 0.099 (69)	T: 0.189 (191)	T: 0.037 (37)	T: 0.040 (39)
rs5010528	Chr6:31273255	*HLA-C*	Missense	A	G	G: 0.243 (321)	G: 0.187 (130)	G: 0.056 (56)	G: 0.138 (139)	G: 0.099 (97)

The allele frequency of the "rs2074494" T allele in the African population is expressed at a much lower level than that of the populations of America, Europe, and Southeast Asia. Taken together, the allele frequencies of the variants "rs2074494" and "rs5010528" indicated the contribution of differential variant prevalence for *HLA-C* gene expression.

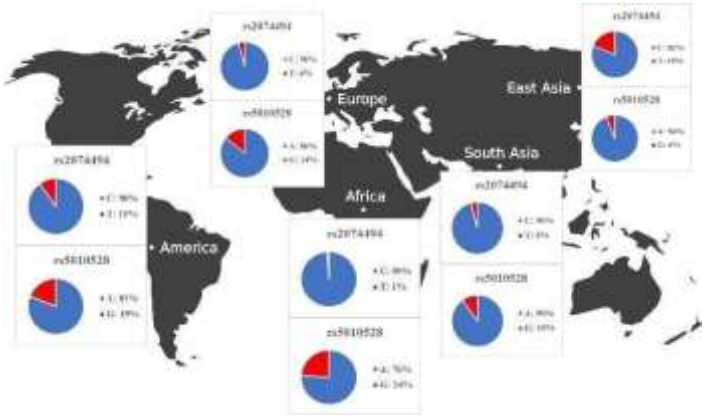

Fig 4. Summary of allele frequency analysis on *HLA-C* gene expression in Africa, America, East Asia, Europe, and Southeast Asia.

Another study revealed that *HLA-S* gene which were reported to be potential associated in chickenpox disease[22]. In this study, we investigated the skin tissue expression of the *HLA-C* gene, which has been linked to SJS and can lead to SCAR infection. Notably, the variants associated with *HLA-C* expression have not been reported for SJS. Basic research on the genetics of SJS and TEN to date has focused on human leukocyte antigen (HLA), the system associated with the presence of specific receptors, cytotoxic proteins, and the part of immunocytes during disease pathogenesis [23]. Considering the global impact of SJS, examining the distribution of *HLA-C* variants may be an essential quest that allows further understanding of global disease susceptibility.

The *HLA* gene encodes several molecules that are crucial to the immune system. With that in mind, a strong relationship between *HLA* genes and autoimmune diseases (AIDs) has been demonstrated for more than half a century [24]. Findings that most patients with carbamazepine-induced Stevens-Johnson syndrome and CBZ-SJS/TEN toxic epidermal necrolyses have an associated *HLA-B*15:02* in an Asian population. In contrast, the association with *HLA-A*31:01* was only reported in Japan and Europe. and

has a novel association between *HLA-A*31:01* and CBZ-SJS/TEN in Indians [25]. The association with various HLA genes can then be analyzed using publicly available databases. We used publicly available databases like the GTEx portal, SNPnexus, and Ensembl. We identified genetic variants associated with *HLA-C* expression in skin tissue, the leading site of SCAR infection in SJS disease. It has previously been reported that specific *HLA* genotypes have been associated with the occurrence of severe skin disease due to drug-induced side effects (SCARs), which cases are included in SJS/TEN [26].

The allele frequencies in all populations differ for each SNP, as shown in Figure 4. In general, it is known that the G and A allele frequencies for rs2074494 and rs5010528 were also seen to have a lower frequency in Southeast Asia (rs2074494, 4%, and rs5010528, 14%), Europe (rs2074494, 4%, and rs5010528, 16%), and East Asia (rs2074494, 19%, and rs5010528, 6%), compared with American (rs2074494, 10%, and rs5010528, 24%) and African (rs5010528, 24%) populations. In conclusion, by leveraging a bioinformatic-based approach it is revealed the pathogenic variants that are potentially associated with SJS. We propose that these variants could be used for further study to identify the SJS diagnostic biomarker as well as for prognosis. However, we acknowledged that there are limitations to the bioinformatic-based approach used to investigate the genetic variants associated with SJS. One of the main limitations is that not all the variants necessarily have genes that encode them (i.e. non-coding variants), and even if they do, these genes or genetic variants may not be suitable drug targets. Nonetheless, clinical validation is recommended as a next step to confirm our findings and gain a better understanding of the underlying etiology and functional effect of the SJS disease.

Conclusion

In this study, we conducted a comprehensive bioinformatic analysis of Stevens-Johnson Syndrome (SJS) from genomic databases, revealing differential tissue expression of the *HLA-C* gene across 16 human tissues. Even though *HLA-C* is highly expressed in whole blood, spleen, lung, and lymphocyte cells, the relevant disease variants (rs2074494 and rs5010528) are differentially expressed in the skin tissue. Overall, alleles for rs2074494 and rs5010528 have lower frequencies in Southeast Asia (rs2074494, 4%, and rs5010528, 14%), Europe (rs2074494, 4%, and rs5010528, 16%), and East Asia (rs2074494, 19%, and rs5010528, 6%), as compared to the American population (rs2074494, 10%, and rs5010528, 24%) and the African (rs5010528, 24%) population.

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Authors' Contributions

Conceptualization: MM, JCF, MRM, LMI. Data curation: MM, JCF, MRM, LMI, WA, RC. Formal analysis: LMI, WA, RC. Methodology: MM, JCF, MRM. Writing - original draft: MM, JCF, MRM, LMI. Writing - review & editing: MM, JCF, MRM, LMI, NS, WA, RC, AWS.

Conflicts of Interests

The authors declare no conflict of interest.

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References

1. Wang L, Varghese S, Bassir F, Lo YC, Ortega CA, Shah S, et al. Stevens-Johnson syndrome and toxic epidermal necrolysis: A systematic review of PubMed/MEDLINE case reports from 1980 to 2020. Vol. 9, Frontiers in Medicine. Frontiers Media S.A.; 2022.
2. Deore SS, Dandekar RC, Mahajan AM, Shiledar V V, Gandhi Vidyamandir's Karmaveer M, Hirey B, et al. Drug Induced-Stevens Johnson Syndrome: ACase Report. International Journal of Scientifi c Study. 2014.
3. Menaldi SL, Bramono K, Indriatmi W. Ilmu Penyakit Kulit dan Kelamin (Edisi Ketujuh). Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia; 2016.
4. Shanbhag SS, Chodosh J, Fathy C, Goverman J, Mitchell C, Saeed HN. Multidisciplinary care in Stevens-Johnson syndrome. Vol. 11, Therapeutic Advances in Chronic Disease. SAGE Publications Ltd; 2020.
5. Hsu DY, Brieva J, Silverberg NB, Silverberg JI. Morbidity and Mortality of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in United States Adults. Journal of Investigative Dermatology. 2016;136(7):1387–97.
6. Putri ND, Mutiara H, Hasudungan, Sibero HT, Sukohar A. Steven-Johnson Syndrom et causa Paracetamol. J Medula Unila. 2016;6(1):101–7.
7. Frey N, Jossi J, Bodmer M, Bircher A, Jick SS, Meier CR, et al. The Epidemiology of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in the UK. Journal of Investigative Dermatology. 2017;137(6):1240–7.
8. Yang MS, Lee JY, Kim J, Kim GW, Kim BK, Kim JY, et al. Incidence of stevens-johnson syndrome and toxic epidermal necrolysis: A nationwide population-based study using national health insurance database in Korea. PLoS One. 2016;11(11).
9. Hsu DY, Brieva J, Silverberg NB, Paller AS, Silverberg JI. Pediatric Stevens-Johnson syndrome and toxic epidermal necrolysis in the United States. J Am Acad Dermatol. 2017;76(5):811-817.e4.
10. Paulmann M, Mockenhaupt M. Severe skin reactions: clinical picture, epidemiology, etiology, pathogenesis, and treatment. Vol. 28, Allergo Journal International. Springer Medizin;2019.311–26.
11. Rajput R, Sagari S, Durgavanshi A, Kanwar A. Paracetamol induced Steven-Johnson syndrome: A rare case report. Contemp Clin Dent. 2015;6:S278–81.
12. Ueta M. Results of Detailed Investigations Into Stevens-Johnson Syndrome With Severe Ocular Complications. Invest Ophthalmol Vis Sci. 2018;59(14):DES183–91.
13. Génin E, Schumacher M, Roujeau JC, Naldi L, Liss Y, Kazma R, et al. Genome-wide association study of Stevens-Johnson syndrome and Toxic Epidermal Necrolysis in Europe. Orphanet J Rare Dis. 2011;6(1).
14. Chen Z, Boehnke M, Wen X, Mukherjee B. Revisiting the genome-wide significance threshold for common variant GWAS. G3: Genes, Genomes, Genetics. 2021;11(2).

15. Siegel RJ, Bridges SL, Ahmed S. HLA-C: An Accomplice in Rheumatic Diseases. ACR Open Rheumatol. 2019;1(9):571–9.

16. Fitriany J, Alratisda F. Stevens Johnson Syndrome. Jurnal Averrous. 2019;5(1):1–22.

17. Mckinley BJ, Allen ME, Michels N. Photodistributed Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis: A Systematic Review and Proposal for a New Diagnostic Classication. 2022. available at Research Square [https://doi.org/10.21203/rs.3.rs-1748665/v1].

18. Kawai Y, Hitomi Y, Ueta M, Khor SS, Nakatani K, Sotozono C, et al. Mapping of susceptible variants for cold medicine-related Stevens-Johnson syndrome by whole-genome resequencing. Genomic Med . 2021;1–8.

19. Tamehiro N, Mujawar Z, Zhou S, Zhuang DZ, Hornemann T, Von Eckardstein A, et al. Cell Polarity Factor Par3 Binds SPTLC1 and Modulates Monocyte Serine Palmitoyltransferase Activity and Chemotaxis. 2009;284(37):24881–90.

20. Ueta M, Kinoshita S. Ocular surface inflammation is regulated by innate immunity. Prog Retin Eye Res. 2012 Nov 1;31(6):551–75.

21. Ueta M, Sawai H, Sotozono C, Hitomi Y, Kaniwa N, Kim MK, et al. IKZF1, a new susceptibility gene for cold medicine-related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement. J Allergy Clin Immunol.2015;135(6):1538-1545.e17.

22. Irham LM, Adikusuma W, Lolita L, Puspitaningrum AN, Afief AR, Sarasmita MA, et al. Investigation of susceptibility genes for chickenpox disease across multiple continents. Biochem Biophys Rep. 2023;33:101419

23. Fonseca DJ, Morel A, Llinás-Caballero K, Bolívar-Salazar D, Laissue P. Whole-exome sequencing in patients affected by Stevens-Johnson syndrome and toxic epidermal necrolysis reveals new variants potentially contributing to the phenotype. Pharmgenomics Pers Med. 2021;14:287–99.

24. Gough SCL, Simmonds MJ. The HLA Region and Autoimmune Disease: Associations and Mechanisms of Action. Curr Genomics. 2007;8:453–65.

25. Khor AHP, Lim KS, Tan CT, Kwan Z, Tan WC, Wu DBC, et al. HLA-A*31: 01 and HLA-B*15:02 association with Stevens-Johnson syndrome and toxic epidermal necrolysis to carbamazepine in a multiethnic Malaysian population. Pharmacogenet Genomics.2017;27(7):275–8.

26. Nakatani K, Ueta M, Khor SS, Hitomi Y, Okudaira Y, Masuya A, et al. Identification of HLA-A*02:06:01 as the primary disease susceptibility HLA allele in cold medicine-related Stevens-Johnson syndrome with severe ocular complications by high-resolution NGS-based HLA typing. Sci Rep. 2019 Dec 1;9(1).

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A bioinformatic approach to identify pathogenic variants for Stevens-Johnson Syndrome

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Stevens-Johnson syndrome (SJS) produces a severe hypersensitivity reaction caused by Herpes simplex virus or mycoplasma infection, vaccination, systemic disease, or other agents. Several studies have investigated the genetic susceptibility involved in SJS. To provide further genetic insights into the pathogenesis of SJS, this study prioritized high-impact, SJS-associated pathogenic variants through integrating bioinformatic and population genetic data. First, we identified SJS-associated single nucleotide polymorphisms from the genome-wide association studies catalog, followed by genome annotation with HaploReg and variant validation with Ensembl. Subsequently, quantitative trait locus expression analysis (eQTL) from GTEx identified human genetic variants with differential gene expression across human tissues. Our results indicate that two variants, namely rs2074494 and rs5010528, which are encoded by the *HLA-C* gene, were found to be differentially expressed in skin. The allele frequencies for rs2074494 and rs5010528 also appear to significantly differ across continents. We highlight the utility of these population-specific *HLA-C* genetic variants for genetic association studies, and aid in early prognosis and disease treatment of SJS.

Keywords: bioinformatics, genetic variation, genomic, pathogenic variants, Stevens-Johnson syndrome

Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are potentially life-threatening diseases [1]. In particular, SJS is a syndrome resulting from severe hypersensitivity reactions caused by infection with the herpes simplex or mycoplasma viruses, vaccinations, systemic diseases, certain agents, food, and drugs [2]. SJS occurs in the skin and mucous membranes in the orifices and eyes in mild to severe conditions, with abnormalities in the skin in the form of an erythema, vesicles, or bullae accompanied by purpura [3].

SJS/TEN is widespread in the mucocutaneous immune region, causing exfoliation of the skin on the mucosal surface [4]. The incidence of SJS, SJS/TEN, and TEN in the United States reported 9.2, 1.6, and 1.9 cases from 2009–2012 [5]. The incidence of SJS cases that occur in Indonesia is around 12 cases per year, with different causes [6]. The

incidence in the United Kingdom from 1995–2013 there were 5.76 cases of SJS/TEN per million people per year [7]. In Korea, it is reported that the incidence rate of SJS/TEN from 2009–2013 was 3.96–5.03 and 0.94–1.45 per million people per year [8]. Events caused by SJS, SJS-TEN, and TEN have an average of 5.3, 0.8, and 0.4 cases per million children each year [9]. The mortality rate of 4.8-9% in SJS, 19.4–29% in the SJS/TEN case, and 14.8–48% in TEN [10]. SJS can appear with non-specific fever symptoms that cause malaise, headache, cough, and rhinorrhea. On the skin, patients suffering from SJS can have polymorphic lesions and mucous membranes with marked skin blisters and erosion [11].

Therefore, primary prevention is the best mitigation for SJS. SJS is categorized as a severe cutaneous adverse reaction (SCAR), and several drugs have been implicated in disease pathogenesis. Non-steroidal anti-inflammatory drugs and other multi-ingredient formulations are widely used to relieve the symptoms. Several studies are reporting adverse skin drug reactions that are often SJS-associated with severe ocular complications [12]. Prevention is possible if patients who are susceptible to this SCAR when prescribed certain drugs are identified. Besides, the genomic variants are known to have an important role in SJS progression. However, little information revealed the specific variant as a biological risk gene in SJS. A previous study revealed associated variants in SJS progression (rs2844665, rs3815087, rs3130931, rs3130501, rs3094188, and rs9469003). In allopurinol-induced SJS and TEN, rs9469003 can detect an effect similar to that seen with an allele frequency risk of 15% and an associated risk of 99% [13]. Even though several studies have been exploited previously, a limited number of variants summarized the variants associated with expression in tissue-related SJS. This study aims to investigate the variants associated with SJS through a bioinformatic-based approach and further prioritize the biological risk variants. Besides, the pattern of gene expression profiles and population allele frequencies of genetic variants were assessed using various databases. Here, The results will enable future studies to assess whether these variants may be associated with various infectious risks for SJS/TEN, as well as SJSprogression and disease susceptibility.

Methods

Genomic information not only can be leveraged to identify the variant-associated disease, but it can also be translated into actionable knowledge for the disease. SJS is one of the severe skin reaction due to genomic risk factors. In this study, we used a bioinformatic-based approach to prioritize the pathogenic variants that potentially trigger the SJS. Detailed information regarding the study design has been depicted in Fig. 1. We used the keyword "Stevens-Johnson syndrome (SJS)" to derive SJS associated from the genome-wide association studies (GWAS) National Human Genome Research Institute (NHGRI) Catalog database http://www.ebi.ac.uk/gwas (accessed December 19, 2022). SJS associated with 74 single nucleotide polymorphisms (SNPs) were obtained and further analysis was carried out using HaploReg (version 4.1). Further analysis yielded a total of 41 SNPs with a significance value of p-value < 10^{-8}. This value is used to account for several tests in the GWAS catalog. These values are widely used to identify associations between variants and shared genetic traits with adjacent gene expression [14]. Furthermore, an evaluation was carried out between the relationships of various genetic variants and gene expression profiles using quantitative expression trait loci (eQTL) using the GTEx Portal database http://www.gtexportal.org/home/ (accessed December 19, 2022), which was found by gene expression from various networks. The *HLA-C* (human leukocyte antigen C) genetic variant is present in human skin and tissue (lower extremities) exposed to sunlight obtained from the GTEx Portal database. Then confirm the variant using the Ensembl Genome Browser https:// www.ensembl.org/index.html (accessed December 19, 2022). Furthermore, the allele frequencies of variants associated SJS were evaluated in different populations including African, American, East Asian, European, and Southeast Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia). Then, to find out the function of the various gene variants, an evaluation was carried out using the SNP Nexus database https://www.snp-nexus.org (accessed December 19, 2022).

Results and Discussion

Identificationof genomicvariantsfor SJS

We first identified SNPs associated with SJS from the GWAS database, resulting in 74 SNPs associated with SJS. We identified 41 unique SNPs associated with SJS after removing all SNPs duplication (Table 1). Based on the number of SNPs obtained, candidate SNPs were further constrained and prioritized using HaploReg version 4.1, with a p-value of <10^{-8}. Based on the findings presented in Table 2, we focused on two genomic variants from the same gene that qualify as the biological risk SNPs for SJS from this study.

Through our integrative bioinformatics approach, two variants with a missense mutation (rs2074494, rs5010528) that encoded the *HLA-C* genes were prioritized as the biological risk SNPs for

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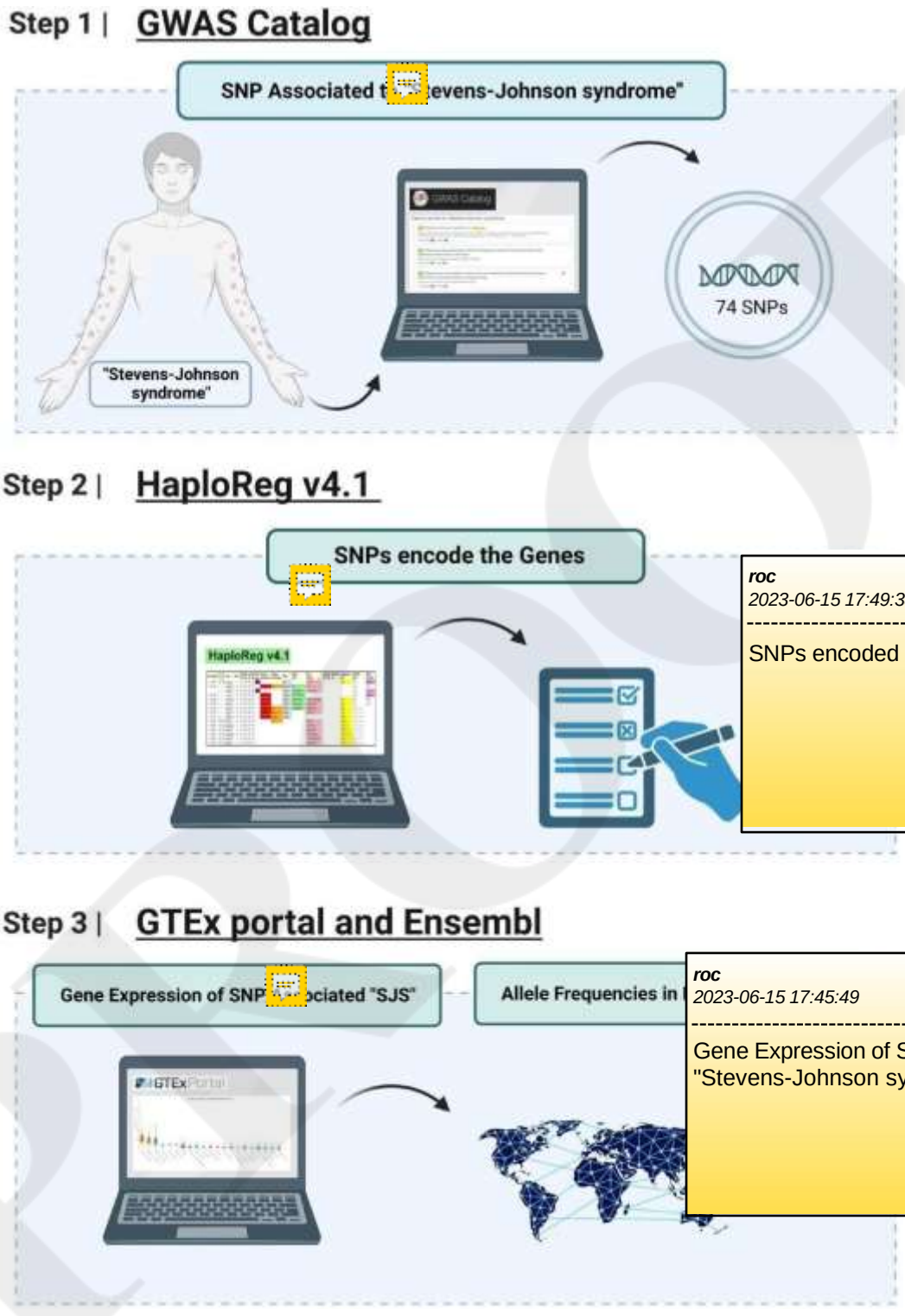

Fig. 1. Bioinformatics workflow for the identification of genetic variants associated with Stevens-Johnson syndrome. GWAS, genome-wide association studies; SNP, single nucleotide polymorphism.

Table 1. SNPs from the GWAS catalog with a significance of p-value < 10-8

Variant and risk allele	p-value
rs6457109	3×10^{-16}
rs7760545	4×10^{-16}
rs35835721	8×10^{-15}
rs137899365	3×10^{-14}
rs60581484	2×10^{-13}
rs1131151	4×10^{-12}
rs2074494	5×10^{-12}
rs4917014	8×10^{-11}
rs1562468327	6×10^{-10}
rs199755581	7×10^{-10}
rs11509487	1×10^{-9}
rs9469003	2×10^{-9}
rs4471527	2×10^{-9}
rs28381346	5×10^{-9}
rs6500265	6×10^{-9}
rs199755581	6×10^{-9}
rs114908185	7×10^{-9}
rs1297852527	9×10^{-9}
rs1371146120	1×10^{-8}
rs77491650	1×10^{-8}
rs4471527	1×10^{-8}
rs77542827	1×10^{-8}
rs3130501	2×10^{-8}
rs536142737	2×10^{-8}
rs16957893	2×10^{-8}
rs2734583	2×10^{-8}
rs150289893	2×10^{-8}
rs1286845082	2×10^{-8}
rs548089948	2×10^{-8}
rs3094188	3×10^{-8}
rs55765602	3×10^{-8}
rs77542827	3×10^{-8}
rs778096762	3×10^{-8}
rs1597607761	3×10^{-8}
rs1391213386	3×10^{-8}
rs374138762	4×10^{-8}
rs879656274	4×10^{-8}
rs1211926109	4×10^{-8}
rs116953913	5×10^{-8}
rs1263106470	6×10^{-8}
rs5010528	8×10^{-8}

SNP, single nucleotide polymorphism; GWAS, genome-wide association studies.

SJS. SJS is characterized by necrosis and shedding of the epidermis, known as the triad of desquamation of the vesiculobullous skin, orificemucosa, andeyes, accompanied by severegeneral symptoms[6]. It was also reported that the *HLA-C* gene has an important role in protecting against cancer and viruses. However, the *HLA-C* gene may also be involved in allograft rejection, the state of preeclampsia, and is also present in autoimmune diseases [15]. The diagnosis of SJS/TEN is a blistering autoimmune disease. which is included in linear IgA dermatosis and paraneoplastic pemphigus but is also present in pemphigus vulgaris and bullous pemphigoid, acute generalized exanthematous pustulosis, and later disseminated drug persistently erupting bullosa and staphylococcal scalded skin syndrome (SSSS) [16].

HLA-C gene expression across 16 human tissues

To evaluate *HLA-C* gene expression in human tissues, we used the GTEx Portal database (http://www.gtexportal.org/), which contains gene expression levels in various tissues. eQTL annotation comprises the most apparent functional consequences of genetic variation. Whole blood, spleen, lung, and lymphocyte cells demonstrate the highest *HLA-C* gene expression across the 16 human tissues analyzed from GTEx in Fig. 2. Furthermore, we have found that the ID SNPs rs2074494 and rs5010528 have similar gene expression variation in the Sun-Exposed Skin (Lower Leg). SJS is acute and can cause death occurs, therefore this disease is an emergency disease on the skin [16]. One Sun-Exposed Skin that is often exposed is exposure to ultraviolet radiation (UVR). In general, exposure to UVR has been reported as a risk factor for SJS and TEN [17].

HLA-C geneexpression in thesun-exposedskin

Human leukocyte antigen (HLA) class I genes, including *HLA-A*, *HLA-B*, and *HLA-C*, have been reported as the loci most strongly associated with susceptibility to all types of SJS and TEN, including cold medicine-related (CM-SJS) and TEN with severe ocular complications (SOC). Although non-synonymous substitutions affecting peptide binding or *HLA* molecular conformation have been considered significant factors in the pathogenesis of immunological diseases, indeed, different *HLA-C* expression levels have been reported for the different alleles, with higher HLA-C expression leading to increased Tc (*Trypanosoma cruzi*) responses and adverse effects in Crohn's disease. It was also reported that genetic variation in *HLA-A* and other autosomal genes has been identified as a risk factor for SJS/TEN associated with flu drugs with SOC such as CM-SJS or TEN with SOC [18].

When individuals with a genetic background containing SJS/

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Table 2. Stevens-Johnson syndrome variant and risk allele that codes for prioritized SNPs

Variant and risk allele	Variants near risk allele ($r^2 > 0.8$)	p-value	Gencode	Allele type
rs2074494	rs1050276	5×10^{-12}	*HLA-C*	Missense
rs5010528	rs1050409	8×10^{-8}	*HLA-C*	Missense

SNP, single nucleotide polymorphism.

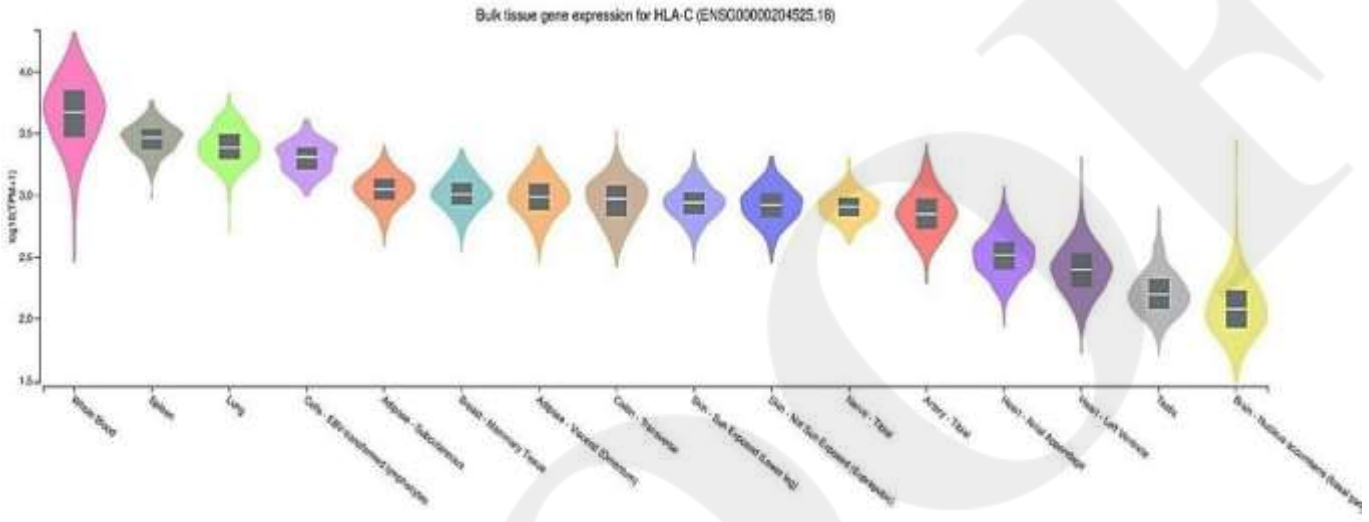

Fig. 2. *HLA-C* gene expression-associated with Stevens-Johnson syndrome in several human tissues from the GTEx Portal.

TEN with SOC susceptibility factors are infected by some viral or microbial infection, they develop abnormal immune responses [19]. It is reasonable to presume that there is an interaction between HLA multiplication and susceptibility genes such as *HLA-A* and *TLR3* [20], *HLA-A* and *REC14-32*, and *HLA-A* and *PTGER3* [21]. Several susceptibility genes for SJS/TEN CM associated with SOC may be involved in the formation of functional networks. An imbalance in this gene can trigger the mucocutaneous inflammation seen in patients with SJS/TEN associated with CM with SOC. SJS/TEN with SOC in the acute stage shows inflammation of the skin and ocular surface and oral mucosal erosions and paronychia [12].

Relationship between *HLA-C* gene and eQTLs from the GTEx database

Gene expression of SJS in human tissue was evaluated via the GTEx Portal database. This aims to determine the gene expression level in various tissues including in the skin tissue. We identified genomic variations from *HLA-C* gene expression using the GWAS catalog database and found 74 SNPs. From these analyses, we determined top 10 SNPs with the highest p-values. We were further processed with the variant annotation tool SNPnexus to determine the annotation of prioritized SNP variations. After these analyses, two statistically significant SNPs were obtained and prioritized. In this case, we prioritized 2 SNPs at risk for SJS based on an analysis of the number of SNPs expanded using HaploReg ver. 4.1 and a p-value of $< 10^{-8}$ to determine the functional annotation of the SNPs. The results of genetic variation are shown in Table 3.

Table 3 shows the two identified variants (rs2074494 and rs5010528) encoded the *HLA-C* gene in differential tissue expression in the human skin. By using the GTEx portal (http://www.gtexportal.org/home/), we further emphasized that the variants of rs2074494 and rs5010528 encoded the *HLA-C* genes were a higherexpression in the skin tissue to Table 3 and Fig. 3.

Allele frequencies of SJS candidate variants across continents

Once we identified the candidate *HLA-C* expression–associated variants, we set out to determine the allele frequencies across transcontinental populations as shown in Table 4. The allele frequencies for both variants were evaluated in different population including African, American, East Asian, European, and Southeast

Table 3. *HLA-C* expression quantitative trait loci (eQTL) analysis from the GTEx Portal database

SNP ID	Gencode ID	Gene Symbol	p-value	Effect size	Tissue	Expression level
rs2074494	204525.16	*HLA-C*	7.2×10^{-10}	-0.48	Artery-Tibial	CC>CT>TT
	204525.16	*HLA-C*	13×10^{-6}	-0.50	Muscle-Skeletal	CC>CT>TT
	204525.16	*HLA-C*	15×10^{-6}	-0.78	Brain-Nucleus accumbens (basal ganglia)	CC > CT > TT
	204525.16	*HLA-C*	17×10^{-6}	-0.51	Heart-Left ventricle	CC > CT > TT
	204525.16	*HLA-C*	46×10^{-6}	-0.58	Testis	CC > CT > TT
	204525.16	*HLA-C*	36×10^{-5}	-0.39	Skin-Sun exposed (lower leg)	CC>CT>TT
rs5010528	204525.16	*HLA-C*	5.4×10^{-26}	0.56	Adipose-Subcutaneous	AA> AG>GG
	204525.16	*HLA-C*	1×10^{-21}	0.41	Whole blood	AA> AG>GG
	204525.16	*HLA-C*	1.4×10^{-14}	0.51	Adipose-Visceral	AA> AG>GG
	204525.16	*HLA-C*	4.1×10^{-14}	0.49	Lung	AA> AG>GG
	204525.16	*HLA-C*	1.1×10^{-10}	0.68	Spleen	AA> AG>GG
	204525.16	*HLA-C*	2.1×10^{-9}	0.51	Heart-Atrial appendage	AA> AG>GG
	204525.16	*HLA-C*	5.7×10^{-8}	0.28	Colon-Transverse	AA> AG>GG
	204525.16	*HLA-C*	9.9×10^{-8}	0.38	Skin-Not sun exposed (suprapublic)	AA> AG>GG
	204525.16	*HLA-C*	5.1×10^{-7}	0.72	Cells-EBV-transformed lymphocytes	AA> AG>GG
	204525.16	*HLA-C*	7.0×10^{-7}	-0.25	Nerve-Tibial	AA> AG>GG
	204525.16	*HLA-C*	7.7×10^{-7}	0.31	Nerve-Tibial	AA> AG>GG
	204525.16	*HLA-C*	1.6×10^{-6}	0.34	Breast-Mammary tissue	AA> AG>GG
	204525.16	*HLA-C*	$1,8 \times 10^{-6}$	0.32	Skin-Sun exposed (lower leg)	AA>AG>GG

Source: Expression Quantitative Trait Loci (eQTL) obtained from the GTEx Portal.

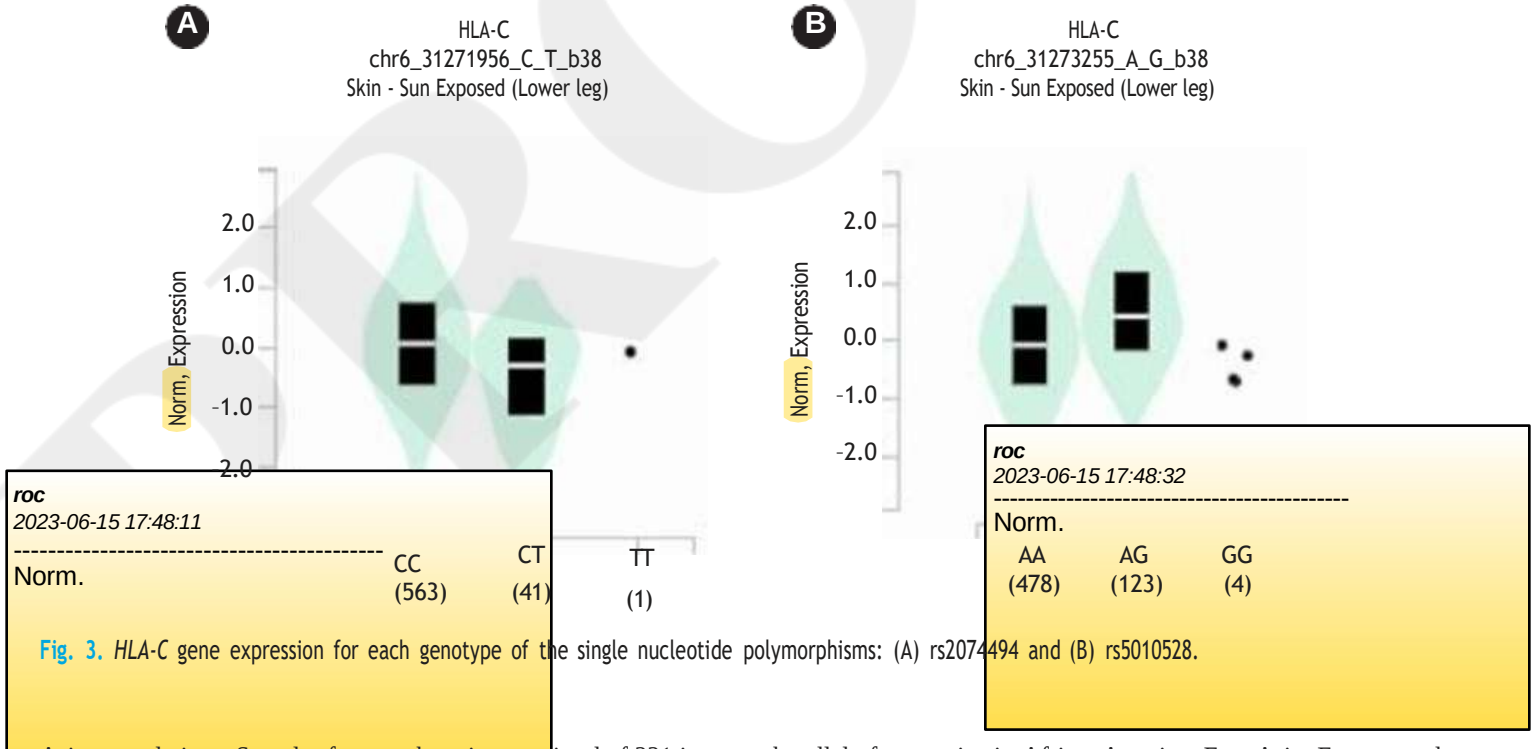

Fig. 3. *HLA-C* gene expression for each genotype of the single nucleotide polymorphisms: (A) rs2074494 and (B) rs5010528.

Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia). We extracted the allele frequencies in Africa, America, East Asia, Europe, and Southeast Asia from the Ensemble Genome Browser (http://www.ensembl.org). Allele frequencies across populations differ for each

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Table 4. Analysis of allele frequencies for the *HLA-C* gene from SNPnexus variant annotation

SNP ID	Position (hg38)	Gene symbol	Location	Allele		Allele frequency (n)				
				Ref	Alt	AFR	AMR	EAS	EUR	SAS
rs2074494	Chr6:31271956	*HLA-C*	Missense	C	T	T: 0.008 (10)	T: 0.099 (69)	T: 0.189 (191)	T: 0.037 (37)	T: 0.040 (39)
rs5010528	Chr6:31273255	*HLA-C*	Missense	A	G	G: 0.243 (321)	G: 0.187 (130)	G: 0.056 (56)	G: 0.138 (139)	G: 0.099 (97)

SNP, single nucleotide polymorphism; Ref, Reference; Alt, slternate; AFR, Africa; AMR, America; EAS, East Asia; EUR, Europe; SAS, Southeast Asia.

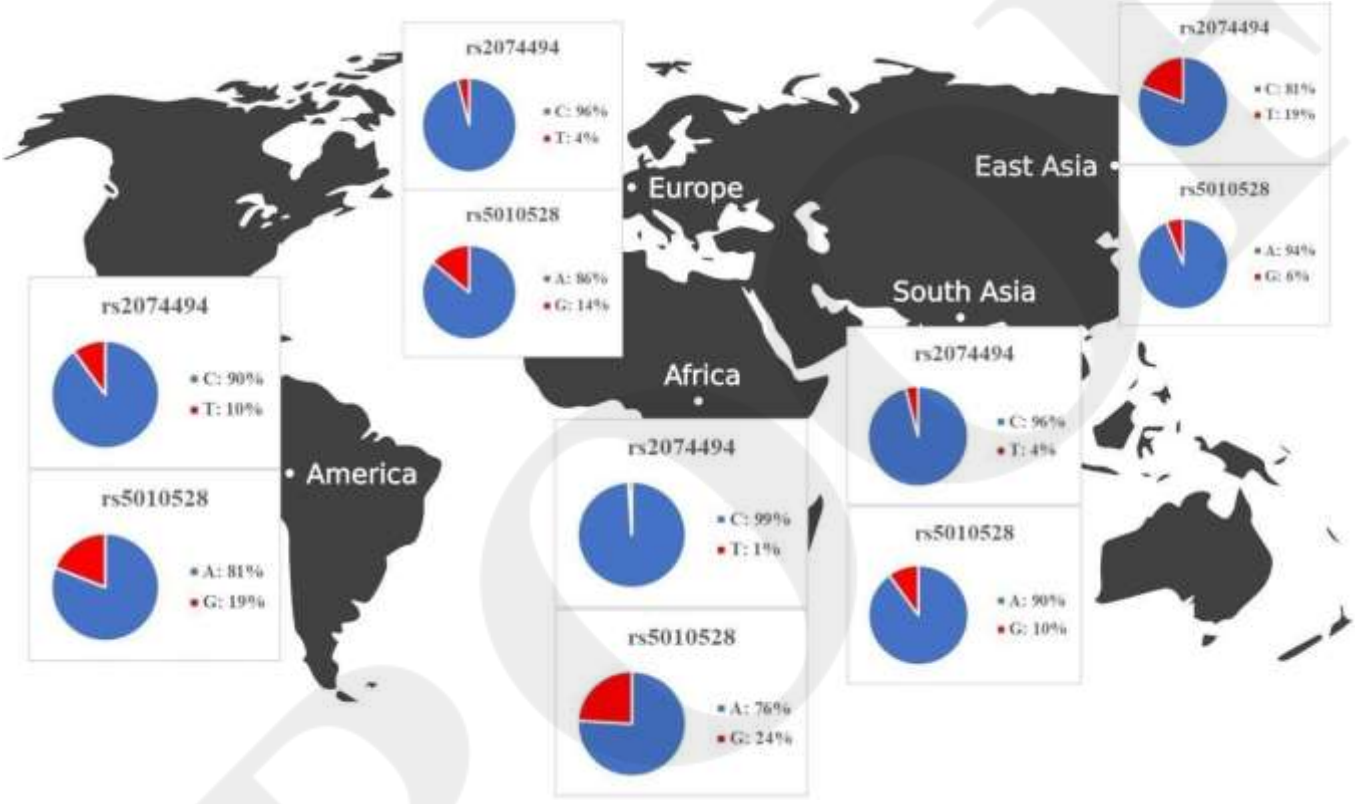

Fig. 4. Summary of allele frequency analysis on *HLA-C* gene expression in Africa, America, East Asia, Europe, and Southeast Asia.

HLA-C variant. Table 4 and Fig. 4 show gene expression levels at higher frequencies of the rs5010528 associated (G) allele and rs2074494 associated (T) allele. At population frequencies of the rs5010528 (G) allele, the Asian population (East Asia and Southeast Asia) is expressed at a much lower level than that from the populations of Africa, America, and Europe.

The allele frequency of the "rs2074494" T allele in the African population is expressed at a much lower level than that of the populations of America, Europe, and Southeast Asia. Taken together, the allele frequencies of the variants "rs2074494" and "rs5010528" indicated the contribution of differential variant prevalence for *HLA-C* gene expression.

Another study revealed that *HLA-S* genewhich were reported to be potential associated in chickenpox disease [22]. In this study, we investigated the skin tissue expression of the *HLA-C* gene, which has been linked to SJS and can lead to SCAR infection. Notably, the variants associated with *HLA-C* expression have not been reported for SJS. Basic research on the genetics of SJS and TEN to date has focused on HLA, the system associated with the presence of specific receptors, cytotoxic proteins, and the part of immunocytes during disease pathogenesis [23]. Considering the global impact of SJS, examining the distribution of *HLA-C* variants may be an essential quest that allows further understanding of global disease susceptibility.

The *HLA* gene encodes several molecules that are crucial to the immunesystem. With that in mind, astrong relationshipbetween

HLA genes and autoimmune diseases has been demonstrated for more than half a century [24]. Findings that most patients with carbamazepine-induced SJS and CBZ-SJS/TEN toxic epidermal necrolyses have an associated *HLA-B*15:02* in an Asian population. In contrast, the association with *HLA-A**31:01 was only reported in Japan and Europe. and has a novel association between *HLA-A*31:01* and CBZ-SJS/TEN in Indians [25]. The association with various *HLA* genes can then be analyzed using publicly available databases. We used publicly available databases like the GTEx Portal, SNPnexus, and Ensembl. We identified genetic variants associated with *HLA-C* expression in skin tissue, the leading site of SCAR infection in SJS disease. It has previously been reported that specific *HLA* genotypes have been associated with the occurrence of severe skin disease due to drug-induced side effects (SCARs), which cases are included in SJS/TEN [26].

The allele frequencies in all populations differ for each SNP, as shown in Fig. 4. In general, it is known that the G and A allele frequencies for rs2074494 and rs5010528 were also seen to have a lower frequency in Southeast Asia (rs2074494, 4%, and rs5010528, 14%),Europe(rs2074494, 4%, and rs5010528, 16%), and East Asia (rs2074494, 19%, and rs5010528, 6%), compared with American (rs2074494, 10%, and rs5010528, 24%) and African (rs5010528, 24%) populations. In conclusion, by leveraging a bioinformatic-based approach it is revealed the pathogenic variants that are potentially associated with SJS. We propose that these variants could be used for further study to identify the SJS diagnostic biomarker as well as for prognosis. However, we acknowledged that there are limitations to the bioinformatic-based approach used to investigate the genetic variants associated with SJS. One of the main limitations is that not all the variants necessarily have genes that encode them (i.e., non-coding variants), and even if they do, these genes or genetic variants may not be suitable drug targets. Nonetheless, clinical validation is recommended as a next step to confirm our findings and gain a better understanding of the underlyingetiology and functionaleffect of the SJSdisease.

Conclusion

In this study, we conducted a comprehensive bioinformatic analysis of SJS from genomic databases, revealing differential tissue expression of the *HLA-C* gene across 16 human tissues. Even though *HLA-C* is highly expressed in whole blood, spleen, lung, and lymphocyte cells, the relevant disease variants (rs2074494 and rs5010528) are differentially expressed in the skin tissue. Overall, alleles for rs2074494 and rs5010528 have lower frequencies in Southeast Asia (rs2074494, 4%, and rs5010528, 14%), Europe (rs2074494, 4%, and rs5010528, 16%), and East Asia (rs2074494, 19%, and rs5010528, 6%), as compared to the American population (rs2074494, 10%, and rs5010528, 24%) and the African (rs5010528, 24%) population.

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Authors' Contribution

Conceptualization: MM, JCF, MRM, LMI. Data curation: MM, JCF, MRM, LMI, WA, RC. Formal analysis: LMI, WA, RC. Methodology: MM, JCF, MRM. Writing - original draft: MM, JCF, MRM, LMI. Writing - review & editing: MM, JCF, MRM, LMI, NS, WA, RC, AWS.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Wang L, Varghese S, Bassir F, Lo YC, Ortega CA, Shah S, et al. Stevens-Johnson syndrome and toxic epidermal necrolysis: a systematic review of PubMed/MEDLINE case reports from 1980 to 2020. FrontMed(Lausanne) 2022;9:949520.
2. Deore SS, Dandekar RC, Mahajan AM, Shiledar VV. Drug induced-Stevens Johnson syndrome: a case report. Int J Sci Stud 2014;2:84-87.
3. Menaldi SL, Bramono K, Indriatmi W. Ilmu Penyakit Kulit dan Kelamin [Dermatology and Venereology]. 7th ed. Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia, 2016.
4. Shanbhag SS, Chodosh J, Fathy C, Goverman J, Mitchell C, Saeed HN. Multidisciplinary care in Stevens-Johnson syndrome. Ther Adv Chronic Dis 2020;11:2040622319894469.
5. Hsu DY, Brieva J, Silverberg NB, Silverberg JI. Morbidity and

mortality of Stevens-Johnson syndrome and toxic epidermal necrolysis in United States adults. J Invest Dermatol 2016;136:1387-1397.
6. Putri ND, Mutiara H, Sibero HT, Sukohar A. Steven-Johnson syndrometcausaparacetamol.JMedulaUnila2016;6:101-107.
7. Frey N, Jossi J, Bodmer M, Bircher A, Jick SS, Meier CR, et al. The epidemiology of Stevens-Johnson syndrome and toxic epidermal necrolysis in the UK. J Invest Dermatol 2017;137:1240-1247.
8. Yang MS, Lee JY, Kim J, Kim GW, Kim BK, Kim JY, et al. Incidence of Stevens-Johnson syndrome and toxic epidermal necrolysis: a nationwide population-based study using National Health Insurance Database in Korea. PLoS One 2016;11:e0165933.
9. Hsu DY, Brieva J, Silverberg NB, Paller AS, Silverberg JI. Pediatric Stevens-Johnson syndrome and toxic epidermal necrolysis in the UnitedStates.JAmAcadDermatol2017;76:811-817.
10. Paulmann M, Mockenhaupt M. Severe skin reactions: clinical picture, epidemiology, etiology, pathogenesis, and treatment. Allergo JInt2019;28:311-326.
11. Rajput R, Sagari S, Durgavanshi A, Kanwar A. Paracetamol induced Steven-Johnson syndrome: a rare case report. Contemp Clin Dent2015;6:S278-S281.
12. Ueta M. Results of Detailed investigations Into Stevens-Johnson syndrome with severe ocular complications. Invest Ophthalmol VisSci2018;59:DES183-DES191.
13. Genin E, Schumacher M, Roujeau JC, Naldi L, Liss Y, Kazma R, et al. Genome-wide association study of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe. Orphanet J Rare Dis 2011;6:52.
14. Chen Z, Boehnke M, Wen X, Mukherjee B. Revisiting the genome-wide significance threshold for common variant GWAS. G3(Bethesda)2021;11:jkaa056.
15. Siegel RJ, Bridges SL Jr, Ahmed S. HLA-C: an accomplice in rheumaticdiseases.ACROpenRheumatol2019;1:571-579.
16. Fitriany J, Alratisda F. Stevens Johnson syndrome. J Averrous 2019;5:94-115.
17. Mckinley BJ, Allen ME, Michels N. Photodistributed Stevens-Johnson syndrome and toxic epidermal necrolysis: a systematic review and proposal for a new diagnostic classification. Preprint at https://doi.org/10.21203/rs.3.rs-1748665/v1 (2022).
18. Kawai Y, Hitomi Y, Ueta M, Khor SS, Nakatani K, Sotozono C, et al. Mapping of susceptible variants for cold medicine-related Stevens-Johnson syndrome by whole-genome resequencing. NPJ Genom Med 2021;6:9.
19. Tamehiro N, Mujawar Z, Zhou S, Zhuang DZ, Hornemann T, von Eckardstein A, et al. Cell polarity factor Par3 binds SPTLC1 and modulates monocyte serine palmitoyltransferase activity and chemotaxis. JBiolChem2009;284:24881-24890.
20. Ueta M, Kinoshita S. Ocular surface inflammation is regulated by innateimmunity.ProgRetinEyeRes2012;31:551-575.
21. Ueta M, Sawai H, Sotozono C, Hitomi Y, Kaniwa N, Kim MK, et al. IKZF1, a new susceptibility gene for cold medicine-related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement. J Allergy Clin Immunol 2015;135:1538-1545.
22. Irham LM, Adikusuma W, Lolita L, Puspitaningrum AN, Afief AR, Sarasmita MA, et al. Investigation of susceptibility genes for chickenpox disease across multiple continents. Biochem Biophys Rep 2023;33:101419.
23. Fonseca DJ, Morel A, Llinas-Caballero K, Bolivar-Salazar D, Laissue P. Whole-exome sequencing in patients affected by Stevens-Johnson syndrome and toxic epidermal necrolysis reveals new variants potentially contributing to the phenotype. Pharmgenomics Pers Med 2021;14:287-299.
24. Gough SC, Simmonds MJ. The HLA region and autoimmune disease: associations and mechanisms of action. Curr Genomics 2007;8:453-465.
25. Khor AH, Lim KS, Tan CT, Kwan Z, Tan WC, Wu DB, et al. HLA-A*31: 01 and HLA-B*15:02 association with Stevens-Johnson syndrome and toxic epidermal necrolysis to carbamazepine in a multiethnic Malaysian population. Pharmacogenet Genomics2017;27:275-278.
26. Nakatani K, Ueta M, Khor SS, Hitomi Y, Okudaira Y, Masuya A, et al. Identification of HLA-A*02:06:01 as the primary disease susceptibility HLA allele in cold medicine-related Stevens-Johnson syndrome with severe ocular complications by high-resolutionNGS-basedHLAtyping.SciRep2019;9:16240.

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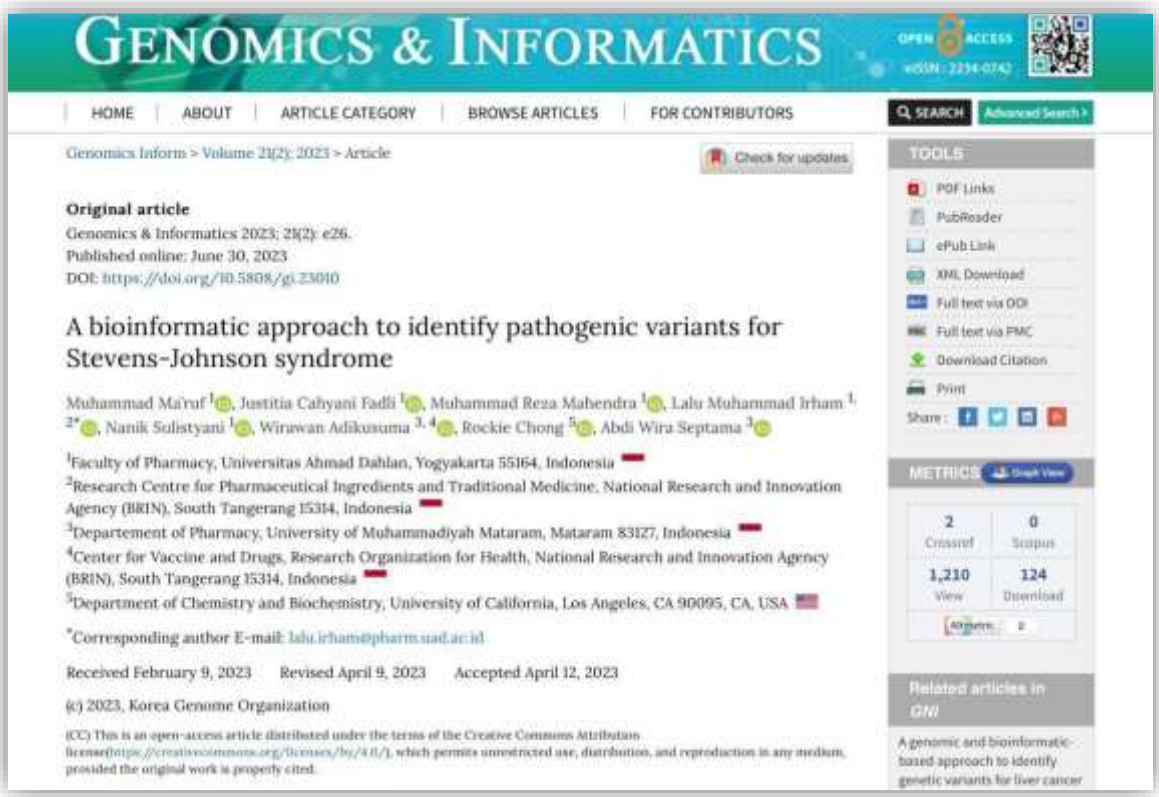

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A bioinformatic approach to identify pathogenic variants for Stevens-Johnson syndrome

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A bioinformatic approach to identify pathogenic variants for Stevens-Johnson syndrome

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Stevens-Johnson syndrome (SJS) produces a severe hypersensitivity reaction caused by Herpes simplex virus or mycoplasma infection, vaccination, systemic disease, or other agents. Several studies have investigated the genetic susceptibility involved in SJS. To provide further genetic insights into the pathogenesis of SJS, this study prioritized high-impact, SJS-associated pathogenic variants through integrating bioinformatic and population genetic data. First, we identified SJS-associated single nucleotide polymorphisms from the genome-wide association studies catalog, followed by genome annotation with HaploReg and variant validation with Ensembl. Subsequently, expression quantitative trait locus (eQTL) from GTEx identified human genetic variants with differential gene expression across human tissues. Our results indicate that two variants, namely rs2074494 and rs5010528, which are encoded by the *HLA-C* (human leukocyte antigen C) gene, were found to be differentially expressed in skin. The allele frequencies for rs2074494 and rs5010528 also appear to significantly differ across continents. We highlight the utility of these population-specific *HLA-C* genetic variants for genetic association studies, and aid in early prognosis and disease treatment of SJS.

Keywords: bioinformatics, genetic variation, genomic, pathogenic variants, Stevens-Johnson syndrome

Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are potentially life-threatening diseases [1]. In particular, SJS is a syndrome resulting from severe hypersensitivity reactions caused by infection with the herpes simplex or mycoplasma viruses, vaccinations, systemic diseases, certain agents, food, and drugs [2]. SJS occurs in the skin and mucous membranes in the orifices and eyes in mild to severe conditions, with abnormalities in theskin in theform of an erythema, vesicles, or bullaeaccompanied by purpura[3].

SJS/TEN is widespread in the mucocutaneous immune region, causing exfoliation of the skin on the mucosal surface [4]. The incidence of SJS, SJS/TEN, and TEN in the United States reported 9.2, 1.6, and 1.9 cases from 2009–2012 [5]. The incidence of SJS

cases that occur in Indonesia is around 12 cases per year, with different causes [6]. The incidence in the United Kingdom from 1995–2013 there were 5.76 cases of SJS/TEN per million people per year [7]. In Korea, it is reported that the incidence rate of SJS/TEN from 2009–2013 was 3.96–5.03 and 0.94–1.45 per million people per year [8]. Events caused by SJS, SJS-TEN, and TEN have an average of 5.3, 0.8, and 0.4 cases per million children each year [9]. The mortality rate of 4.8-9% in SJS, 19.4–29% in the SJS/TEN case, and 14.8–48% in TEN [10]. SJS can appear with non-specific fever symptoms that cause malaise, headache, cough, and rhinorrhea. On the skin, patients suffering from SJS can have polymorphic lesions and mucous membranes with marked skin blisters and erosion [11].

Therefore, primary prevention is the best mitigation for SJS. SJS is categorized as a severe cutaneous adverse reaction (SCAR), and several drugs have been implicated in disease pathogenesis. Non-steroidal anti-inflammatory drugs and other multi-ingredient formulations are widely used to relieve the symptoms. Several studies are reporting adverse skin drug reactions that are often SJS-associated with severe ocular complications [12]. Prevention is possible if patients who are susceptible to this SCAR when prescribed certain drugs are identified. Besides, the genomic variants are known to have an important role in SJS progression. However, little information revealed the specific variant as a biological risk gene in SJS. A previous study revealed associated variants in SJS progression (rs2844665, rs3815087, rs3130931, rs3130501, rs3094188, and rs9469003). In allopurinol-induced SJS and TEN, rs9469003 can detect an effect similar to that seen with an allele frequency risk of 15% and an associated risk of 99% [13]. Even though several studies have been exploited previously, a limited number of variants summarized the variants associated with expression in tissue-related SJS. This study aims to investigate the variants associated with SJS through a bioinformatic-based approach and further prioritize the biological risk variants. Besides, the pattern of gene expression profiles and population allele frequencies of genetic variants were assessed using various databases. Here, The results will enable future studies to assess whether these variants may be associated with various infectious risks for SJS/TEN, as well as SJS progression and disease susceptibility.

Methods

Genomic information not only can be leveraged to identify the variant-associated disease, but it can also be translated into actionable knowledge for the disease. SJS is one of the severe skin reaction due to genomic risk factors. In this study, we used a bioinformatic-based approach to prioritize the pathogenic variants that potentially trigger the SJS. Detailed information regarding the study design has been depicted in Fig. 1. We used the keyword "Stevens-Johnson syndrome (SJS)" to derive SJS associated from the genome-wide association studies (GWAS) National Human Genome Research Institute (NHGRI) Catalog database (http://www.ebi.ac.uk/gwas; accessed December 19, 2022). SJS associated with 74 single nucleotide polymorphisms (SNPs) were obtained and further analysis was carried out using HaploReg (version 4.1). Further analysis yielded a total of 41 SNPs with a significance value of p-value < 10^{-8}. This value is used to account for several tests in the GWAS catalog. These values are widely used to identify associations between variants and shared genetic traits with adjacent gene expression [14]. Furthermore, an evaluation was carried out between the relationships of various genetic variants and gene expression profiles using expression quantitative trait locus (eQTL) using the GTEx Portal database (http://www.gtexportal.org/home/; accessed December 19, 2022), which was found by gene expression from various networks. The *HLA-C* (human leukocyte antigen C) genetic variant is present in human skin and tissue (lower extremities) exposed to sunlight obtained from the GTEx Portal database. Then confirm the variant using the Ensembl Genome Browser (https://www.ensembl.org/index.html; accessed December 19, 2022). Furthermore, the allele frequencies of variants associated SJS were evaluated in different populations including African, American, East Asian, European, and Southeast Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia). Then, to find out the function of the various gene variants, an evaluation was carried out using the SNP Nexus database (https://www.snp-nexus.org; accessed December 19, 2022).

Results and Discussion

Identification of genomic variants for SJS

We first identified SNPs associated with SJS from the GWAS database, resulting in 74 SNPs associated with SJS. We identified 41 unique SNPs associated with SJS after removing all SNPs duplication (Table 1). Based on the number of SNPs obtained, candidate SNPs were further constrained and prioritized using HaploReg version 4.1, with a p-value of $<10^{-8}$. Based on the findings presented in Table 2, we focused on two genomic variants from the same gene that qualify as thebiological risk SNPsfor SJS fromthisstudy.

Through our integrative bioinformatics approach, two variants with a missense mutation (rs2074494, rs5010528) that encoded

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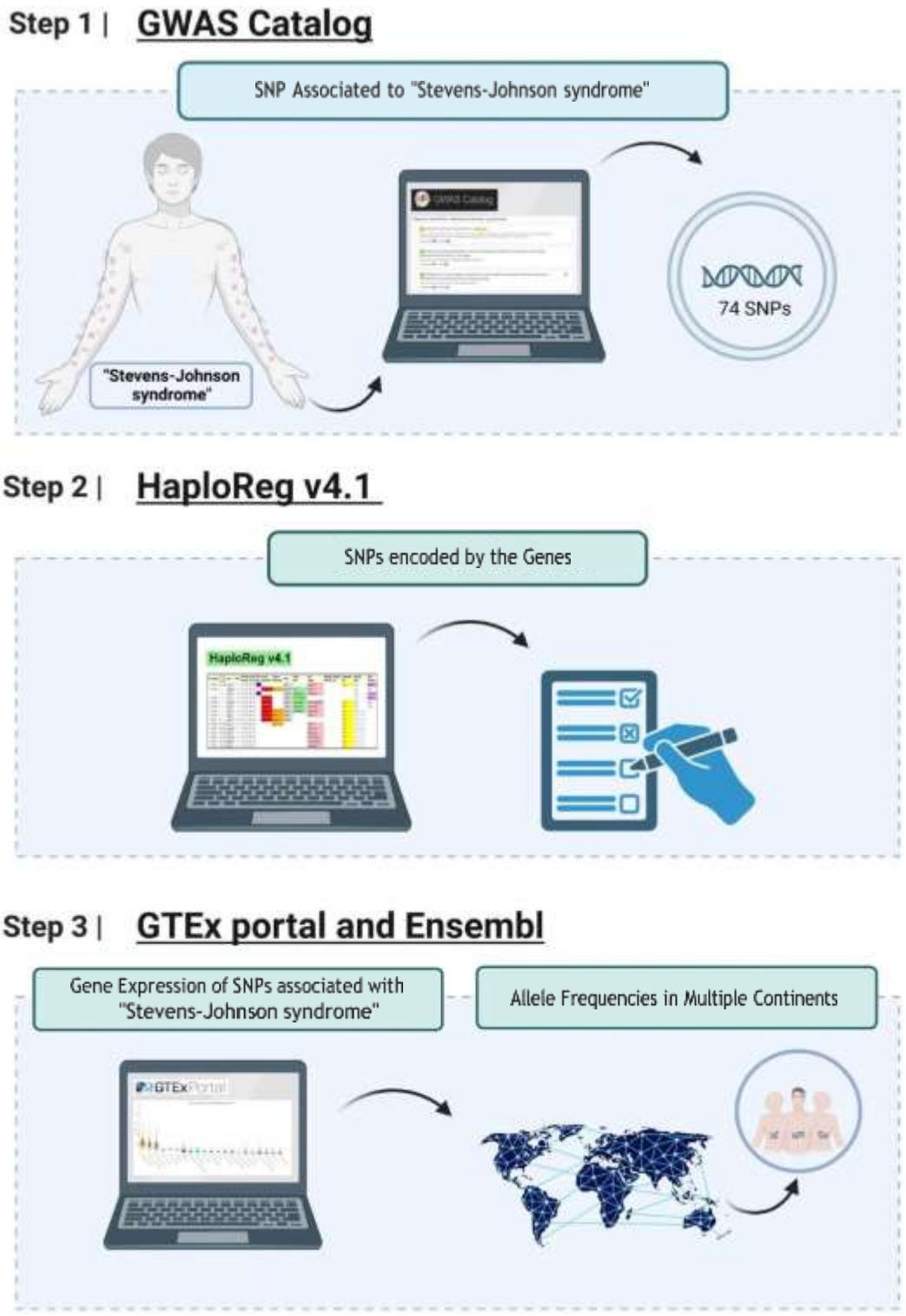

Fig. 1. Bioinformatics workflow for the identification of genetic variants associated with Stevens-Johnson syndrome. GWAS, genome-wide association studies; SNP, single nucleotide polymorphism.

Table 1. SNPs from the GWAS catalog with a significance of p-value < 10^{-8}

Variant and risk allele	p-value
rs6457109	3×10^{-16}
rs7760545	4×10^{-16}
rs35835721	8×10^{-15}
rs137899365	3×10^{-14}
rs60581484	2×10^{-13}
rs1131151	4×10^{-12}
rs2074494	5×10^{-12}
rs4917014	8×10^{-11}
rs1562468327	6×10^{-10}
rs199755581	7×10^{-10}
rs11509487	1×10^{-9}
rs9469003	2×10^{-9}
rs4471527	2×10^{-9}
rs28381346	5×10^{-9}
rs6500265	6×10^{-9}
rs199755581	6×10^{-9}
rs114908185	7×10^{-9}
rs1297852527	9×10^{-9}
rs1371146120	1×10^{-8}
rs77491650	1×10^{-8}
rs4471527	1×10^{-8}
rs77542827	1×10^{-8}
rs3130501	2×10^{-8}
rs536142737	2×10^{-8}
rs16957893	2×10^{-8}
rs2734583	2×10^{-8}
rs150289893	2×10^{-8}
rs1286845082	2×10^{-8}
rs548089948	2×10^{-8}
rs3094188	3×10^{-8}
rs55765602	3×10^{-8}
rs77542827	3×10^{-8}
rs778096762	3×10^{-8}
rs1597607761	3×10^{-8}
rs1391213386	3×10^{-8}
rs374138762	4×10^{-8}
rs879656274	4×10^{-8}
rs1211926109	4×10^{-8}
rs116953913	5×10^{-8}
rs1263106470	6×10^{-8}
rs5010528	8×10^{-8}

SNP, single nucleotide polymorphism; GWAS, genome-wide association studies.

the *HLA-C* genes were prioritized as the biological risk SNPs for SJS. SJS is characterized by necrosis and shedding of the epidermis, known as the triad of disorders of the vesiculobullous skin, orifice mucosa, and eyes, accompanied by severe general symptoms [6]. It was also reported that the *HLA-C* gene has an important role in protecting against cancer and viruses. However, the *HLA-C* gene may also be involved in allograft rejection, the state of preeclampsia, and is also present in autoimmune diseases [15]. The diagnosis of SJS/TEN is a blistering autoimmune disease. which is included in linear IgA dermatosis and paraneoplastic pemphigus but is also present in pemphigus vulgaris and bullous pemphigoid, acute generalized exanthematous pustulosis, and later disseminated drug persistently erupting bullosa and staphylococcal scalded skin syndrome [16].

HLA-C gene expression across 16 human tissues

To evaluate *HLA-C* gene expression in human tissues, we used the GTEx Portal database (http://www.gtexportal.org/), which contains gene expression levels in various tissues. eQTL annotation comprises the most apparent functional consequences of genetic variation. Whole blood, spleen, lung, and lymphocyte cells demonstrate the highest *HLA-C* gene expression across the 16 human tissues analyzed from GTEx in Fig. 2. Furthermore, we have found that the ID SNPs rs2074494 and rs5010528 have similar gene expression variation in the Sun-Exposed Skin (Lower Leg). SJS is acute and can cause death occurs, therefore this disease is an emergency disease on the skin [16]. One Sun-Exposed Skin that is often exposed is exposure to ultraviolet radiation (UVR). In general, exposure to UVR has been reported as a risk factor for SJS and TEN [17].

HLA-C gene expression in the sun-exposed skin

Human leukocyte antigen (HLA) class I genes, including *HLA-A*, *HLA-B*, and *HLA-C*, have been reported as the loci most strongly associated with susceptibility to all types of SJS and TEN, including cold medicine-related (CM-SJS) and TEN with severe ocular complications (SOC). Although non-synonymous substitutions affecting peptide binding or *HLA* molecular conformation have been considered significant factors in the pathogenesis of immunological diseases, indeed, different *HLA-C* expression levels have been reported for the different alleles, with higher HLA-C expression leading to increased Tc (*Trypanosoma cruzi*) responses and adverse effects in Crohn's disease. It was also reported that genetic variation in *HLA-A* and other autosomal genes has been identified as a risk factor for SJS/TEN associated with flu drugs with SOC such as CM-SJS or TEN with SOC [18].

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Table 2. Stevens-Johnson syndrome variant and risk allele that codes for prioritized SNPs

Variant and risk allele	Variants near risk allele (r^2 > 0.8)	p-value	Gencode	Allele type
rs2074494	rs1050276	5×10^{-12}	*HLA-C*	Missense
rs5010528	rs1050409	8×10^{-8}	*HLA-C*	Missense

SNP, single nucleotide polymorphism; *HLA-C*, human leukocyte antigen C.

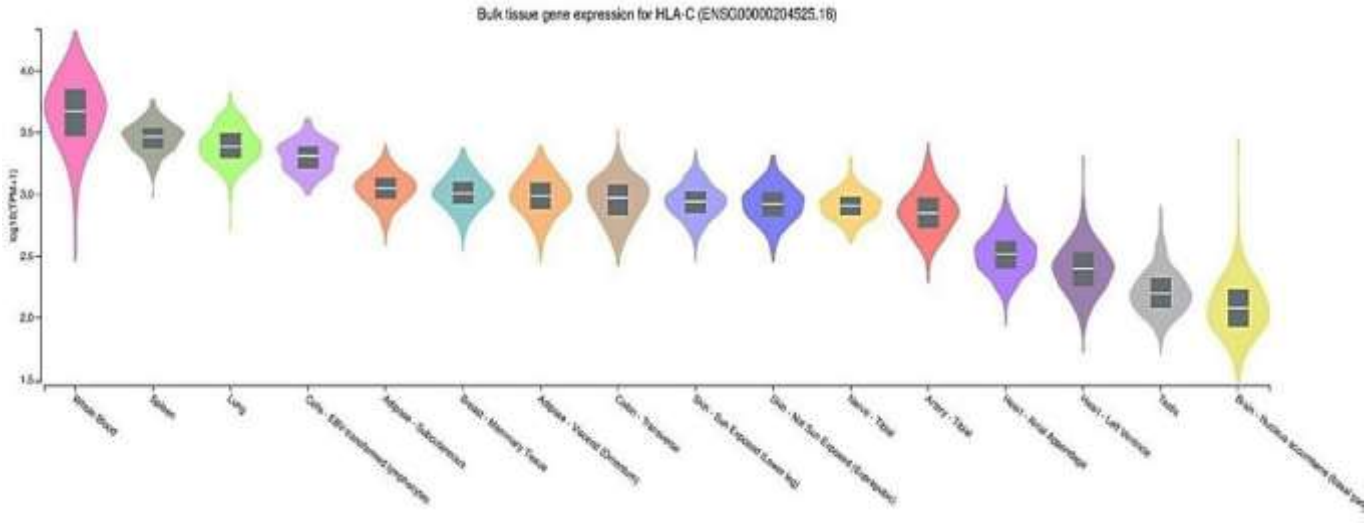

Fig. 2. Human leukocyte antigen C (*HLA-C*) gene expression-associated with Stevens-Johnson syndrome in several human tissues from the GTEx Portal.

When individuals with a genetic background containing SJS/TEN with SOC susceptibility factors are infected by some viral or microbial infection, they develop abnormal immune responses [19]. It is reasonable to presume that there is an interaction between HLA multiplication and susceptibility genes such as *HLA-A* and *TLR3* [20], *HLA-A* and *REC14-32*, and *HLA-A* and *PTGER3* [21]. Several susceptibility genes for SJS/TEN CM associated with SOC may be involved in the formation of functional networks. An imbalance in this gene can trigger the mucocutaneous inflammation seen in patients with SJS/TEN associated with CM with SOC. SJS/TEN with SOC in the acute stage shows inflammation of the skin and ocular surface and oral mucosal erosions and paronychia [12].

Relationship between *HLA-C* gene and eQTLs from the GTEx database

Gene expression of SJS in human tissue was evaluated via the GTEx Portal database. This aims to determine the gene expression level in various tissues including in the skin tissue. We identified genomic variations from *HLA-C* gene expression using the GWAS catalog database and found 74 SNPs. From these analyses, we determined top 10 SNPs with the highest p-values. We were further processed with the variant annotation tool SNPnexus to determine the annotation of prioritized SNP variations. After these analyses, two statistically significant SNPs were obtained and prioritized. In this case, we prioritized 2 SNPs at risk for SJS based on an analysis of the number of SNPs expanded using HaploReg version 4.1 and a p-value of $< 10^{-8}$ to determine the functional annotation of the SNPs. The results of genetic variation are shown in Table 3.

Table 3 shows the two identified variants (rs2074494 and rs5010528) encoded the *HLA-C* gene in differential tissue expression in the human skin. By using the GTEx portal (http://www.gtexportal.org/home/), we further emphasized that the variants of rs2074494 and rs5010528 encoded the *HLA-C* genes were a higher expression in the skin tissue to Table 3 and Fig. 3.

Allele frequencies of SJS candidate variants across continents

Once we identified the candidate *HLA-C* expression–associated variants, we set out to determine the allele frequencies across transcontinental populations as shown in Table 4. The allele fre-

Table 3. *HLA-C* eQTL analysis from the GTEx Portal database

SNP ID	Gencode ID (ENSG00000-)	Gene symbol	p-value	Effect size	Tissue	Expression level
rs2074494	204525.16	*HLA-C*	7.2×10^{-10}	-0.48	Artery-Tibial	CC > CT > TT
	204525.16	*HLA-C*	13×10^{-6}	-0.50	Muscle-Skeletal	CC > CT > TT
	204525.16	*HLA-C*	15×10^{-6}	-0.78	Brain-Nucleus accumbens (basal ganglia)	CC > CT > TT
	204525.16	*HLA-C*	17×10^{-6}	-0.51	Heart-Left ventricle	CC > CT > TT
	204525.16	*HLA-C*	46×10^{-6}	-0.58	Testis	CC > CT > TT
	204525.16	*HLA-C*	36×10^{-5}	-0.39	Skin-Sun exposed (lower leg)	CC > CT > TT
rs5010528	204525.16	*HLA-C*	5.4×10^{-26}	0.56	Adipose-Subcutaneous	AA> AG>GG
	204525.16	*HLA-C*	1×10^{-21}	0.41	Whole blood	AA> AG>GG
	204525.16	*HLA-C*	1.4×10^{-14}	0.51	Adipose-Visceral	AA> AG>GG
	204525.16	*HLA-C*	4.1×10^{-14}	0.49	Lung	AA> AG>GG
	204525.16	*HLA-C*	1.1×10^{-10}	0.68	Spleen	AA> AG>GG
	204525.16	*HLA-C*	2.1×10^{-9}	0.51	Heart-Atrial appendage	AA> AG>GG
	204525.16	*HLA-C*	5.7×10^{-8}	0.28	Colon-Transverse	AA> AG>GG
	204525.16	*HLA-C*	9.9×10^{-8}	0.38	Skin-Not sun exposed (suprapublic)	AA> AG>GG
	204525.16	*HLA-C*	5.1×10^{-7}	0.72	Cells-EBV-transformed lymphocytes	AA> AG>GG
	204525.16	*HLA-C*	7.0×10^{-7}	-0.25	Nerve-Tibial	AA> AG>GG
	204525.16	*HLA-C*	7.7×10^{-7}	0.31	Nerve-Tibial	AA> AG>GG
	204525.16	*HLA-C*	1.6×10^{-6}	0.34	Breast-Mammary tissue	AA> AG>GG
	204525.16	*HLA-C*	$1,8 \times 10^{-6}$	0.32	Skin-Sun exposed (lower leg)	AA> AG>GG

Source: Expression Quantitative Trait Loci (eQTL) obtained from the GTEx Portal.
HLA-C, human leukocyte antigen C; eQTL, expression quantitative trait loci; SNP, single nucleotide polymorphism.

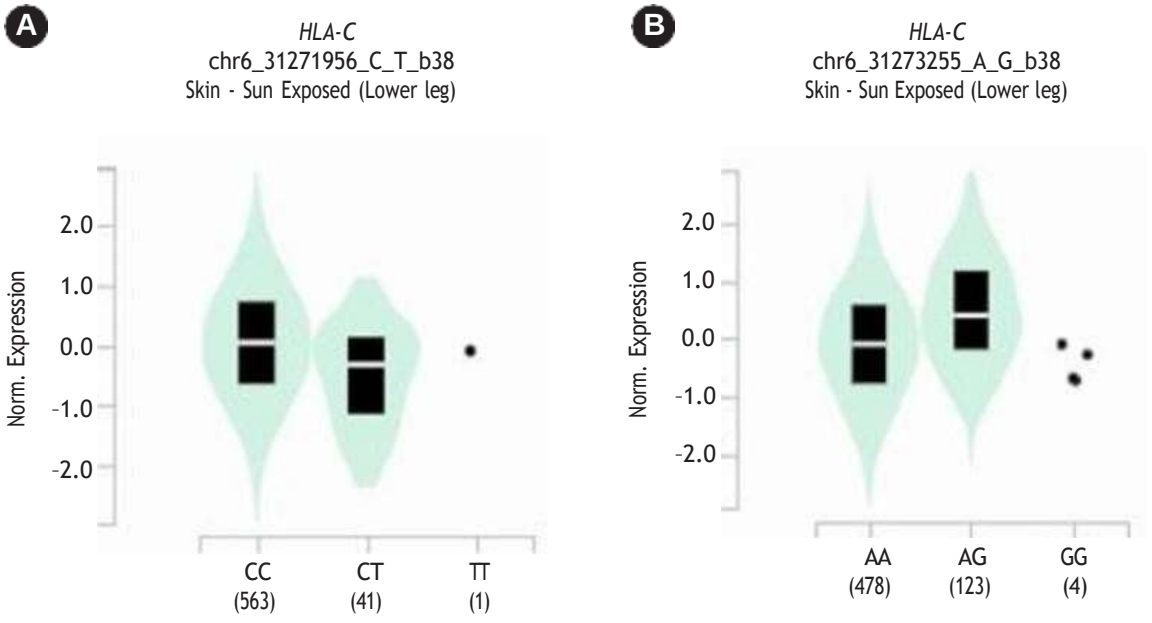

Fig. 3. Human leukocyte antigen C (*HLA-C*) gene expression for each genotype of the single nucleotide polymorphisms: (A) rs2074494 and (B) rs5010528.

quencies for both variants were evaluated in different population including African, American, East Asian, European, and Southeast Asian populations. Samples from each region consisted of 331 individuals (Africa), 199 individuals (America), 247 individuals (East Asia), and 136 individuals (Southeast Asia). We extracted the allele frequencies in Africa, America, East Asia, Europe, and

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Table 4. Analysis of allele frequencies for the *HLA-C* gene from SNPnexus variant annotation

SNP ID	Position (hg38)	Gene symbol	Location	Allele		Allele frequency (n)				
				Ref	Alt	AFR	AMR	EAS	EUR	SAS
rs2074494	Chr6:31271956	*HLA-C*	Missense	C	T	T: 0.008 (10)	T: 0.099 (69)	T: 0.189 (191)	T: 0.037 (37)	T: 0.040 (39)
rs5010528	Chr6:31273255	*HLA-C*	Missense	A	G	G: 0.243 (321)	G: 0.187 (130)	G: 0.056 (56)	G: 0.138 (139)	G: 0.099 (97)

HLA-C, human leukocyte antigen C; SNP, single nucleotide polymorphism; Ref, Reference; Alt, slternate; AFR, Africa; AMR, America; EAS, East Asia; EUR, Europe; SAS, Southeast Asia.

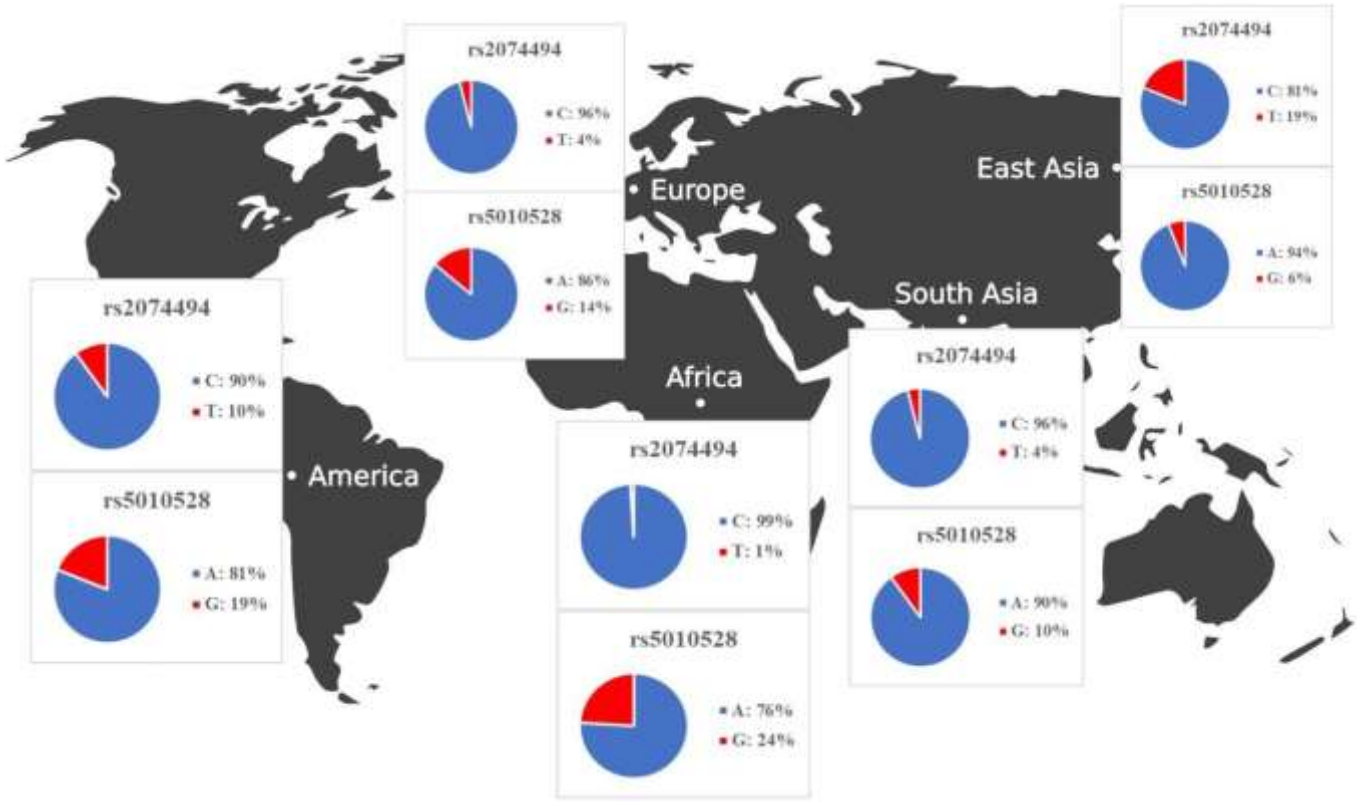

Fig. 4. Summary of allele frequency analysis on human leukocyte antigen C (*HLA-C*) gene expression in Africa, America, East Asia, Europe, and Southeast Asia.

Southeast Asia from the Ensemble Genome Browser (http://www.ensembl.org). Allele frequencies across populations differ for each *HLA-C* variant. Table 4 and Fig. 4 show gene expression levels at higher frequencies of the rs5010528 associated (G) allele and rs2074494 associated (T) allele. At population frequencies of the rs5010528 (G) allele, the Asian population (East Asia and Southeast Asia) is expressed at a much lower level than that from the populations of Africa, America, and Europe.

The allele frequency of the "rs2074494" T allele in the African population is expressed at a much lower level than that of the populations of America, Europe, and Southeast Asia. Taken together, the allele frequencies of the variants "rs2074494" and "rs5010528" indicated the contribution of differential variant prevalence for *HLA-C* gene expression.

Another study revealed that *HLA-S* gene which were reported to be potential associated in chickenpox disease [22]. In this study, we investigated the skin tissue expression of the *HLA-C* gene, which has been linked to SJS and can lead to SCAR infection. Notably, the variants associated with *HLA-C* expression have not been reported for SJS. Basic research on the genetics of SJS and TEN to date has focused on HLA, the system associated with the presence of specific receptors, cytotoxic proteins, and the part of immunocytes during disease pathogenesis [23]. Considering the global impact of SJS, examining the distribution of *HLA-C* variants

may be an essential quest that allows further understanding of global disease susceptibility.

The *HLA* gene encodes several molecules that are crucial to the immune system. With that in mind, a strong relationship between *HLA* genes and autoimmune diseases has been demonstrated for more than half a century [24]. Findings that most patients with carbamazepine-induced SJS and CBZ-SJS/TEN toxic epidermal necrolyses have an associated *HLA-B*15:02* in an Asian population. In contrast, the association with *HLA-A**31:01 was only reported in Japan and Europe. and has a novel association between *HLA-A*31:01* and CBZ-SJS/TEN in Indians [25]. The association with various *HLA* genes can then be analyzed using publicly available databases. We used publicly available databases like the GTEx Portal, SNPnexus, and Ensembl. We identified genetic variants associated with *HLA-C* expression in skin tissue, the leading site of SCAR infection in SJS disease. It has previously been reported that specific *HLA* genotypes have been associated with the occurrence of severe skin disease due to drug-induced side effects (SCARs), which cases are included in SJS/TEN [26].

The allele frequencies in all populations differ for each SNP, as shown in Fig. 4. In general, it is known that the G and A allele frequencies for rs2074494 and rs5010528 were also seen to have a lower frequency in Southeast Asia (rs2074494, 4% and rs5010528, 14%), Europe (rs2074494, 4% and rs5010528, 16%), and East Asia (rs2074494, 19% and rs5010528, 6%), compared with American (rs2074494, 10% and rs5010528, 24%) and African (rs5010528, 24%) populations. In conclusion, by leveraging a bioinformatic-based approach it is revealed the pathogenic variants that are potentially associated with SJS. We propose that these variants could be used for further study to identify the SJS diagnostic biomarker as well as for prognosis. However, we acknowledged that there are limitations to the bioinformatic-based approach used to investigate the genetic variants associated with SJS. One of the main limitations is that not all the variants necessarily have genes that encode them (i.e., non-coding variants), and even if they do, these genes or genetic variants may not be suitable drug targets. Nonetheless, clinical validation is recommended as a next step to confirm our findings and gain a better understanding of the underlying etiology and functional effect of the SJS disease.

Conclusion

In this study, we conducted a comprehensive bioinformatic analysis of SJS from genomic databases, revealing differential tissue expression of the *HLA-C* gene across 16 human tissues. Even though *HLA-C* is highly expressed in whole blood, spleen, lung, and lymphocyte cells, the relevant disease variants (rs2074494 and rs5010528) are differentially expressed in the skin tissue. Overall, alleles for rs2074494 and rs5010528 have lower frequencies in Southeast Asia (rs2074494, 4% and rs5010528, 14%), Europe (rs2074494, 4% and rs5010528, 16%), and East Asia (rs2074494, 19% and rs5010528, 6%), as compared to the American population (rs2074494, 10% and rs5010528, 24%) and the African (rs5010528, 24%) population.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Wang L, Varghese S, Bassir F, Lo YC, Ortega CA, Shah S, et al. Stevens-Johnson syndrome and toxic epidermal necrolysis: a systematic review of PubMed/MEDLINE case reports from 1980 to 2020.FrontMed(Lausanne)2022;9:949520.
2. Deore SS, Dandekar RC, Mahajan AM, Shiledar VV. Drug induced-Stevens Johnson syndrome: a case report. Int J Sci Stud 2014;2:84-87.

3. Menaldi SL, Bramono K, Indriatmi W. Ilmu Penyakit Kulit dan Kelamin [Dermatology and Venereology]. 7th ed. Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia, 2016.
4. Shanbhag SS, Chodosh J, Fathy C, Goverman J, Mitchell C, Saeed HN. Multidisciplinary care in Stevens-Johnson syndrome. Ther AdvChronicDis2020;11:2040622319894469.
5. Hsu DY, Brieva J, Silverberg NB, Silverberg JI. Morbidity and mortality of Stevens-Johnson syndrome and toxic epidermal necrolysis in United States adults. J Invest Dermatol 2016;136: 1387-1397.
6. Putri ND, Mutiara H, Sibero HT, Sukohar A. Steven-Johnson syndrometcausaparacetamol.JMedulaUnila2016;6:101-107.
7. Frey N, Jossi J, Bodmer M, Bircher A, Jick SS, Meier CR, et al. The epidemiology of Stevens-Johnson syndrome and toxic epidermal necrolysis in the UK. J Invest Dermatol 2017;137:1240-1247.
8. Yang MS, Lee JY, Kim J, Kim GW, Kim BK, Kim JY, et al. Incidence of Stevens-Johnson syndrome and toxic epidermal necrolysis: a nationwide population-based study using National Health Insurance Database in Korea. PLoS One 2016;11: e0165933.
9. Hsu DY, Brieva J, Silverberg NB, Paller AS, Silverberg JI. Pediatric Stevens-Johnson syndrome and toxic epidermal necrolysis in the UnitedStates.JAmAcadDermatol2017;76:811-817.
10. Paulmann M, Mockenhaupt M. Severe skin reactions: clinical picture, epidemiology, etiology, pathogenesis, and treatment. Allergo JInt2019;28:311-326.
11. Rajput R, Sagari S, Durgavanshi A, Kanwar A. Paracetamol induced Steven-Johnson syndrome: a rare case report. Contemp ClinDent2015;6:S278-S281.
12. Ueta M. Results of detailed investigations into Stevens-Johnson syndrome with severe ocular complications. Invest Ophthalmol VisSci2018;59:DES183-DES191.
13. Genin E, Schumacher M, Roujeau JC, Naldi L, Liss Y, Kazma R, et al. Genome-wide association study of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe. Orphanet J Rare Dis 2011;6:52.
14. Chen Z, Boehnke M, Wen X, Mukherjee B. Revisiting the genome-wide significance threshold for common variant GWAS. G3(Bethesda)2021;11:jkaa056.
15. Siegel RJ, Bridges SL Jr, Ahmed S. HLA-C: an accomplice in rheumaticdiseases.ACROpenRheumatol2019;1:571-579.
16. Fitriany J, Alratisda F. Stevens Johnson syndrome. J Averrous 2019;5:94-115.
17. Mckinley BJ, Allen ME, Michels N. Photodistributed Stevens-Johnson syndrome and toxic epidermal necrolysis: a systematic review and proposal for a new diagnostic classification. Preprint at https://doi.org/10.21203/rs.3.rs-1748665/v1 (2022).
18. Kawai Y, Hitomi Y, Ueta M, Khor SS, Nakatani K, Sotozono C, et al. Mapping of susceptible variants for cold medicine-related Stevens-Johnson syndrome by whole-genome resequencing. NPJ GenomMed 2021;6:9.
19. Tamehiro N, Mujawar Z, Zhou S, Zhuang DZ, Homemann T, von Eckardstein A, et al. Cell polarity factor Par3 binds SPTLC1 and modulates monocyte serine palmitoyltransferase activity and chemotaxis.JBiolChem2009;284:24881-24890.
20. Ueta M, Kinoshita S. Ocular surface inflammation is regulated by innateimmunity.ProgRetinEyeRes2012;31:551-575.
21. Ueta M, Sawai H, Sotozono C, Hitomi Y, Kaniwa N, Kim MK, et al. *IKZF1*, a new susceptibility gene for cold medicine-related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement. J Allergy Clin Immunol 2015;135: 1538-1545.
22. Irham LM, Adikusuma W, Lolita L, Puspitaningrum AN, Afief AR, Sarasmita MA, et al. Investigation of susceptibility genes for chickenpox disease across multiple continents. Biochem Biophys Rep2023;33:101419.
23. Fonseca DJ, Morel A, Llinas-Caballero K, Bolivar-Salazar D, Laissue P. Whole-exome sequencing in patients affected by Stevens-Johnson syndrome and toxic epidermal necrolysis reveals new variants potentially contributing to the phenotype. PharmgenomicsPersMed 2021;14:287-299.
24. Gough SC, Simmonds MJ. The HLA region and autoimmune disease: associations and mechanisms of action. Curr Genomics 2007;8:453-465.
25. Khor AH, Lim KS, Tan CT, Kwan Z, Tan WC, Wu DB, et al. HLA-A*31: 01 and HLA-B*15:02 association with Stevens-Johnson syndrome and toxic epidermal necrolysis to carbamazepine in a multiethnic Malaysian population. Pharmacogenet Genomics 2017;27:275-278.
26. Nakatani K, Ueta M, Khor SS, Hitomi Y, Okudaira Y, Masuya A, et al. Identification of *HLA-A*02:06:01* as the primary disease susceptibility HLA allele in cold medicine-related Stevens-Johnson syndrome with severe ocular complications by high-resolution NGS-based HLAtyping.SciRep2019;9:16240.

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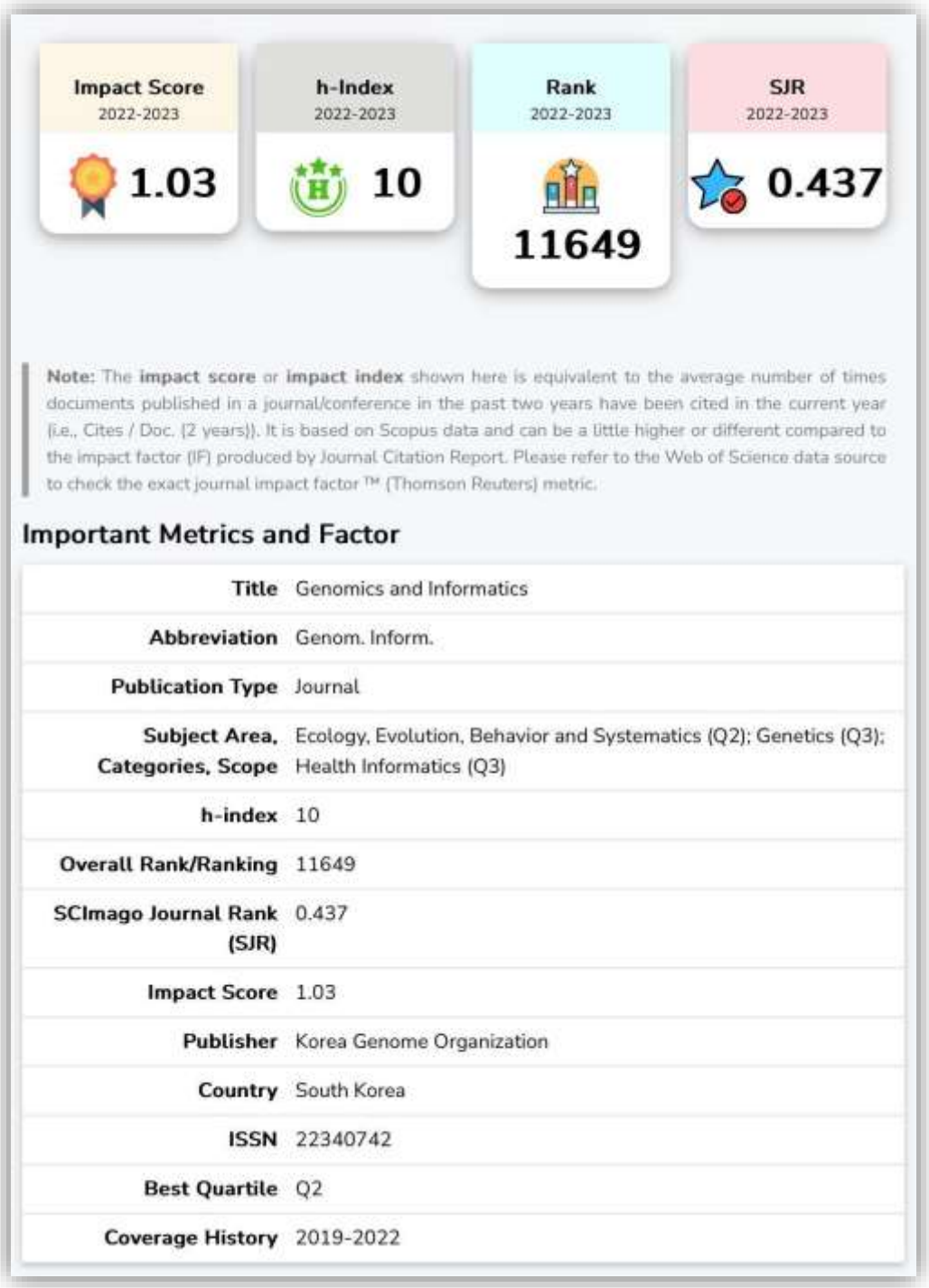
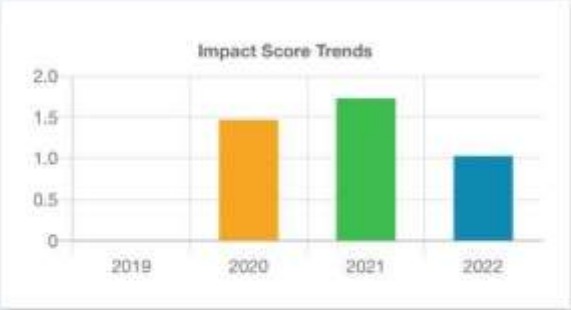


Genomics and Informatics Impact IF 2023 Prediction

Impact IF 2022 of **Genomics and Informatics** is **1.03**. If the same downward trend persists, Impact IF may **fall** in 2023 as well.

Impact IF Trend

Year wise Impact IF of Genomics and Informatics. Based on Scopus data.



Year	Impact IF
2023/2024	Coming Soon
2022	1.03
2021	1.73
2020	1.47
2019	0.00

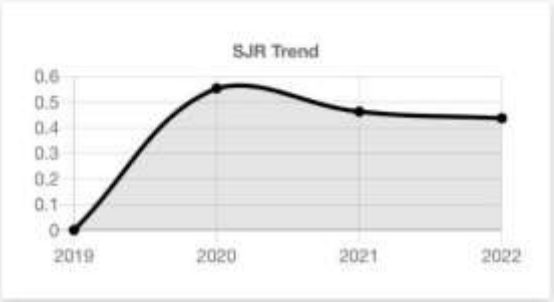
Genomics and Informatics h-index

Genomics and Informatics has an h-index of **10**. It means **10** articles of this **journal** have more than **10** number of citations. The h-index is a way of measuring the productivity and citation impact of the publications. The h-index is defined as the maximum value of h such that the given journal/author has published h papers that have each been cited at least h number of times.

Genomics and Informatics Rank and SCImago Journal Rank (SJR)

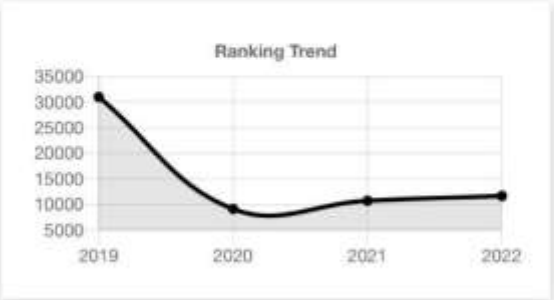
The overall rank of **Genomics and Informatics** is **11649**. According to SCImago Journal Rank (SJR), this journal is ranked **0.437**. SCImago Journal Rank is an indicator, which measures the scientific influence of journals. It considers the number of citations received by a journal and the importance of the journals from where these citations come.

SJR of Genomics and Informatics by Year



Year	SJR
2023/2024	Coming Soon
2022	0.437
2021	0.463
2020	0.554
2019	

Ranking of Genomics and Informatics by Year



Year	Ranking
2023/2024	Coming Soon
2022	11649
2021	10713
2020	9112
2019	30992