ORIGINAL ARTICLE

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Identification of novel variants for complicating cardiac disease in the scrub typhus infection using whole genome sequencing

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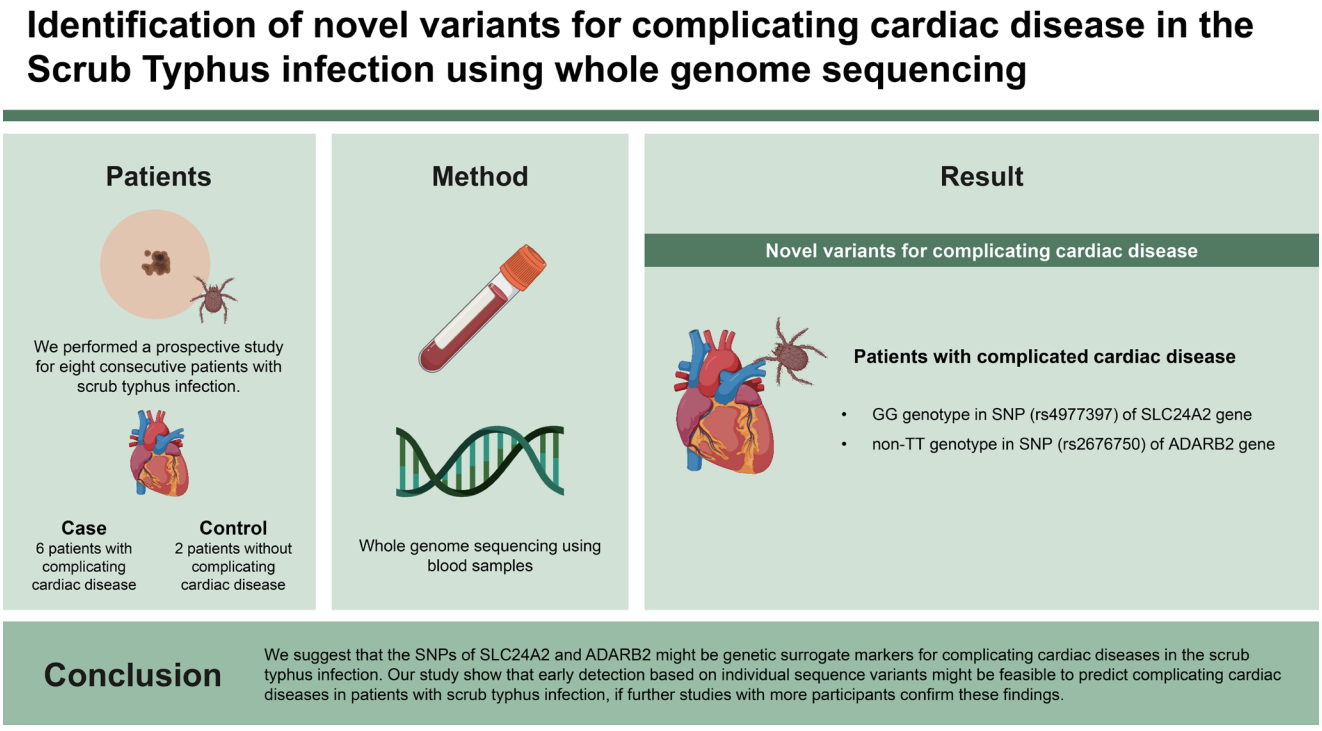

Background/Aims: Scrub typhus infection has been known to complicate cardiovascular diseases mainly attributing to high mortality. Genetic susceptibility loci for complicating cardiac diseases such as atrial fibrillation, heart failure, and ischemic heart disease identified by genomic study have been limited in scrub typhus infection. Therefore, we investigated the genetic novel variants predicting complicating cardiac diseases in patients with confirmed scrub typhus infection using whole genome sequencing.

Methods: We performed a prospective study for eight consecutive patients with scrub typhus infection. During follow-up, six cases were clinically diagnosed with complicating cardiac diseases and two controls without complicating cardiac diseases. The whole genomes of the all patients were sequenced, and the individual sequence variants were compared between

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case and control patients. Variant genotypes were compared and identified as a single nucleotide polymorphism (SNP) of the different genotype distributions between six cases and two controls.

Results: The GG genotype in SNP (rs4977397) of solute carrier 24 family member 2 (SLC24A2) gene and non-TT genotype in SNP (rs2676750) of adenosine deaminase, RNA specific, B2 (ADARB2) gene were distinctively found in the case patients with complicated cardiac disease, compared with control patents in the scrub typhus infection.

Conclusions: We suggest that the SNPs of SLC24A2 and ADARB2 might be genetic surrogate markers for complicating cardiac diseases in the scrub typhus infection. Our study show that early detection based on individual sequence variants might be feasible to predict complicating cardiac diseases in patients with scrub typhus infection, if further studies with more participants confirm these findings.

Keywords: Scrub typhus; Cardiac diseases; Single nucleotide polymorphism; Solute carrier 24 family member 2; Adenosine deaminase RNA specific B2

INTRODUCTION

Scrub typhus is a well-known endemic seasonal infectious disorder confined to Southeastern Asia and Western Pacific rim [1-3] and complicating cardiovascular adverse outcomes remain still under-recognized [4,5]. Even though the most of patients with scrub typhus infection can be cured with conventionally infectious control [6], as regard to complicated cardiovascular disease in the scrub typhus infection, the overall mortality rate has been reported from 16.7% to 30% which might be attributed to cardiovascular complication [7]. In addition, scrub typhus infection-induced cardiovascular complication has been recently reported as poor prognosis factor which have been recognized atrial fibrillation (AF) as clinical surrogate marker and associated with cardiovascular complication [8,9]. In addition, Genome-wide association studies (GWAS) have been utilized to assess the pathologically genetic connection of cardiovascular diseases and genetic susceptibility in the development of cardiovascular complication [2,3]. A recent GWAS was mostly performed and identified approximately several common genetic susceptibility loci for AF, heart failure (HF), and ischemic heart disease (IHD). Genetic susceptibility loci for complicating cardiac diseases such as AF, HF, and IHD identified by genomic study have been limited in scrub typhus infection. Therefore, we investigated the genetic novel variant/marker predicting complicating cardiac diseases such as AF, HF, and IHD in patients with confirmed scrub typhus infection using whole genome sequencing.

METHODS

Blood samples preparation for study

Consecutive eight patients with scrub typhus infection participated in this study. This study were approved by the Institutional Review Board in Chung-Ang University Hospital (2109-002-476) and Eulji University Hospital (EMC 2017-10-006) and adhered to the principles of the Declaration of Helsinki. The whole blood samples were corrected in ethylene-diamine-tetraacetic acid (EDTA) tube for genomic DNA extraction.

DNA extraction and quality check

Genomic DNA was extracted from eight whole blood samples with Exgene™ Blood SV kit (GeneAll, Seoul, Korea), following the manufacturer's protocol (Blood SV mini exgene). Genomic DNA was analyzed by Qubit fluorometer dsDNA assay Kit (Invitrogen, Carlsbad, CA, USA) as well as Infinite F200 Pro NanoQuant (TECAN, Männedorf, Switzerland) to verify the quality (O.D. 260/280 ratio is 1.8–2.0 and O.D. 260/230 ratio greater than 1.6) and quantity (1 μg for library construction).

Whole genome sequencing

Genomic DNA for input into the TruSeq Nano DNA protocol (Truseq Nano DNA Library Prep Reference Guide) was quantified and diluted to 2 ng/μL. Twenty-five microlitres of genomic DNA were sheared using an S2 Ultrasonicator (Covaris, Woburn, MA, USA) using the settings as for the TruSeq DNA protocol (Truseq Nano DNA Library Prep Reference Guide). Library preparation was performed accord-

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ing to the manufacturer's instructions. Adaptor enrichment was performed using eight cycles of enrich DNA fragments (polymerase chain reaction) according to the manufacturer's instructions. The final products were quantified using the Agilent TapeStation 4200 HSD1000 screen tapes (Agilent Technologies, Santa Clara, CA, USA) and KAPA Library Quantification Kit (KK4824; Kapa Biosystems, Vienna, Austria). The individual samples were pooled and sequenced on the Illumina NovaSeq6000 (Illumina, San Diego, CA, USA) with 150 bp paired-end by following the manufacturer's protocols. Image analysis were performed using the NovaSeq6000 control Software (version 1.3.1; Illumina) and the output data was demultiplexed with bcl2fastq (version 2.20; Illumina).

Data analysis of sequence alignment

The quality of the reads was checked using fastQC (version 0.11.7; Illumina), helping the understanding for the basic quality for sequence quality score, GC contents, N contents, length distribution, and duplication level. After checking the read quality, the low-quality base below Q20 (accuracy 99%) were trimmed using Trimmomatic (version 0.36). High quality reads were then aligned to the human reference genome hg19 using BWA (version 0.7.17) [10] with minimum seed length of 45. After the alignment of the reads to reference genome, the duplicated reads were further removed using MarkDuplicates in GATK (version 4.0.11.0)

Variant call and annotation

For the read alignment, whole chromosome regions were split for each chromosome using samtools (version 1.8) [11,12]. Then, Base Quality Score Recalibration (BQSR) process was done to adjust the quality score using BaseRecalibrator in GATK (version 4.0.11.0) [13]. For the realigned and recalibrated reads, variants were called using HaplotypeCaller GATK (version 4.0. 11.0) [12,14].

Scrub typhus induced cardiac complication

Regardless of stroke, we classified the control group as patients without cardiac disease and the case group as patients with cardiac disease. Scrub typhus induced cardiac complication was defined as new-onset AF, acute HF and IHD. New-onset AF was defined with a diagnosis code of ICD-10 I48 (paroxysmal AF) within 30 days of the index date without previous history of AF. New-onset acute HF was defined with a diagnosis code of ICD-10 I40 (acute myocarditis), I30 (acute pericarditis), or I50 (HF) within 30 days of the index date. New-onset IHD was defined 1) with a diagnosis code of ICD-10 I21 (acute myocardial infarction) or I20 (angina pectoris) within 30 days of the index date, and 2) treatment with coronary bypass graft surgery, primary coronary intervention, or thrombolytic agents.

RESULTS

Whole genome sequencing results

A total of eight patients with scrub typhus infection (see Table 1) were subjected to Whole Genome Sequencing analysis. The amount of sequence generated from each sample was about 96 to 148 Gb, producing a sequence that is 30× or more of the total genomic DNA amount. Compared to the human reference genome sequence, a total of 4.28 million to 4.39 million variant sequences were detected. Among them, about 3.4 million single nucleotide polymorphisms (SNPs) were detected, and about 35,000 mutations

Table 1. Clinical baseline characteristics in all patients with scrub typhus infection

Patients	Age, yr	Sex	Admission days	Underlying disease	Cardiac complication	Intensive care
Control 1	60	Female	6	Hypertension	No	No
Control 2	62	Male	4	Old stroke	No	No
Case 1	70	Female	8	Diabetes	Stroke, AF	Yes
Case 2	58	Male	22	Hypertension	Acute coronary syndrome, AF	Yes
Case 3	72	Female	14	Dyslipidemia	Heart failure, AF	Yes
Case 4	75	Female	14	Hypertension and diabetes	Heart failure, AF	Yes
Case 5	67	Female	18	Dyslipidemia	AF	No
Case 6	71	Male	19	None	AF	No

AF, atrial fibrillation.

were located in the protein coding regions. In this study, among these SNPs, SNPs that have been reported to be related to cardiovascular disease through previous studies (see Table 2) were extracted and analyzed to see if there was a

Table 2. Previously reported SNPs related with cardiovascular diseases

Chr	SNP ID	Gene symbol	HGVS	Ref	Alt	Control patients		Case patients					
						1	2	1	2	3	4	5	6
1	rs660240	CELSR2	c.*1167T>C	T	C	CC	CC	TC	CC	CC	CC	CC	CC
1	rs608930	GORAB	n.170617306G>T	G	T	GT	GT	GT	GT	TT	GT	GT	GT
1	rs503706	PRRX1	c.241+1484T>C	T	C	TC	TC	TC	CC	CC	TC	TC	TC
2	rs515135	APOB	n.21286057T>C	T	C	CC	CC	CC	CC	TC	CC	CC	CC
2	rs6544713	ABCG8	c.322+431T>C	T	C	CC	CC	CC	CC	CC	CC	CC	CC
2	rs2252641	TEX41	n.464-26926T>C	T	C	CC	TC	CC	TC	CC	CC	CC	CC
2	rs840616	CALCRL	n.188196469T>C	T	C	CC	CC	CC	CC	CC	CC	CC	CC
2	rs3820888	SPATS2L	c.18+6210T>C	T	C	TC	TC	TC	TC	CC	CC	TC	TC
3	rs6790396	SCN10A	c.2281-1533G>C	C	G	GG	GG	GG	GG	GG	GG	CG	GG
3	rs17005647	FRMD4B	c.162+28798G>A	C	T	TT	CT	CT	CT	CT	TT	TT	TT
3	rs667920	STAG1	c.3066-1267C>A	G	T	TT	TT	TT	TT	TT	TT	GT	TT
3	rs4266144	CCNL1	n.156852592C>G	C	G	GG	CG	CG	CG	GG	CG	GG	CG
3	rs60902112	XXYLT1	c.786-10013G>A	C	T	CT	CT	TT	CT	CT	CT	TT	CT
4	rs3960788	SLC9B1	c.-1-2749A>G	T	C	CC	TC	TC	TC	TC	TC	TC	TC
4	rs6847935	PITX2	n.111696651A>T	A	T	AT	AT	TT	TT	AT	AT	AT	TT
5	rs6596717	EFNA5	n.106427609C>A	C	A	CA	CA	CA	AA	CA	CA	CA	AA
5	rs2012809	SLC27A6	n.128190363A>G	A	G	GG	GG	GG	GG	GG	GG	GG	GG
6	rs1307274	NUDT3	n.34240576T>G	T	G	TG	GG	GG	GG	GG	GG	GG	TG
6	rs6905288	VEGFA	c.*6574G>A	G	A	GA	GA	AA	GA	AA	GA	AA	AA
6	rs1591805	CENPW	n.126717064A>G	A	G	GG	GG	GG	GG	GG	GG	GG	GG
7	rs11509880	TMEM106B	c.282-1941G>A	G	A	AA	GA	GA	AA	AA	GA	GA	AA
7	rs6462078	CREB5	n.28413187C>A	C	A	AA	AA	AA	AA	AA	AA	AA	AA
9	rs4977397	SLC24A2[a]	n.20235004A>G	A	G	AG	AG	GG	GG	GG	GG	GG	GG
10	rs61848342	CAMK1D	n.12303813T>C	T	C	CC	TC	CC	TC	TC	TC	TC	TC
10	rs7096385	SIRT1	c.943-1666T>C	T	C	CC	TC	CC	TC	TC	TC	CC	CC
10	rs10749053	RBM20	c.2551-3135T>C	T	C	CC	CC	CC	TC	CC	CC	CC	CC
11	rs949078	SORL1	n.121629007C>T	C	T	TT	CT	TT	TT	TT	CT	TT	CT
12	rs4766578	ATXN2	c.3317-1852A>T	T	A	AA	AA	AA	AA	AA	AA	AA	AA
12	rs6560886	FBRSL1	c.1585-695T>C	T	C	CC	CC	CC	CC	CC	CC	CC	TC
14	rs2738413	SYNE2	c.19056+237A>G	A	G	AG	GG	GG	AG	AG	AG	GG	GG
14	rs1152591	SYNE2	c.19057-64A>G	A	G	AG	GG	GG	AG	AG	AG	GG	GG
14	rs10873299	IRF2BPL	n.77426711A>G	A	G	GG	AG	AG	AG	AG	GG	GG	AG
16	rs2286466	RPS2	c.261T>C	A	G	GG	GG	GG	GG	GG	GG	AG	GG
19	rs12976411	ZNF507	c.*8031A>T	A	T	AT	AT	AT	TT	AT	TT	AT	TT

Chr, chromosome; SNP, single nucleotide polymorphism; HGVS, human genome variant sequence; Ref, reference sequence; Alt, alternative sequence.
[a]The genotype differed between control patients and case patients.

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difference between the case and the control group.

Association analyses

Table 1 shows the clinical characteristics of all enrolled patients with scrub typhus infection. Case patients developed new-onset AF, HF or acute coronary syndrome in longer stay of intensive care units, compared with control patients. Table 2 shows the previously reported SNPs related with cardiovascular diseases. Among these SNPs, the rs4977397 (chromosome 9, n.202350004A>G), solute carrier 24 family member 2 (SLC24A2) gene was distinctively found in the case patients with complicated cardiac disease, compared with control patents in the scrub typhus infection. Table 3 shows SNP in case patients with scrub typhus infection among previously reported SNPs not related with cardiovascular disease. The rs2676750 (chromosome 10, c.101-99601A>G), non-TT genotype, adenosine deaminase, RNA specific, B2 (ADARB2) gene was distinctively found in the case patients with complicated cardiac disease in the scrub typhus infection. Finally, the GG genotype in SNP (rs4977397) of SLC24A2 and non-TT genotype in SNP (rs2676750) of ADARB2 in patients with cardiac complications after scrub typhus infection is illustrated in Table 4.

DISCUSSION

This study presents genetic surrogate markers for cardiac complications associated with the scrub typhus infection and it is the first trial conducting a risk-analysis of the occurrence of cardiac adverse events after the infection. In the present study, GG genotype in rs4977397 of SLC24A2 and non-TT genotype in rs2676750 of ADARB2 were distinctively found in the patients with complicating cardiac disease compared with patients without complicating cardiac disease in the scrub typhus infection. This is therefore the first study depicting the variants of SLC24A2 and ADARB2 genes implicated in cardiac complications due to scrub typhus infection.

In the present study, newly developed clinical AF was observed in patients under case group, with some combination of HF and IHD compared with control patients (Table 1), which was also demonstrated in the Korean national data analysis in the scrub typhus infection [9]. It is known that new-onset AF is significantly associated with all-cause death in critically ill patients with other common infectious diseases and has the incidence risk of 2.2-fold for 1-year death compared to those without new-onset AF [15]. The Korean national data also shows that new-onset AF occurred in the 7.7% of all patients in the intensive care unit and has an incidence risk of 4.5-fold for 3-month mortality compared

Table 3. SNP in case patients with scrub typhus infection among previously reported SNPs not related with cardiovascular disease

Chr	SNP ID	Gene symbol	HGVS	Ref	Alt	Control patients		Case patients					
						1	2	1	2	3	4	5	6
10	rs2676750	ADARB2	c.101-99601A>G	T	C	TT	TT	CC	CC	CC	CC	TC	CC

Chr, chromosome; SNP, single nucleotide polymorphism; HGVS, human genome variant sequence; Ref, reference sequence; Alt, alternative sequence.

Table 4. Novel variants in patients with cardiac complication after scrub typhus infection

Chr	SNP ID	Gene symbol	HGVS	Ref	Alt	Control patients		Case patients					
						1	2	1	2	3	4	5	6
9	rs4977397	SLC24A2	n.20235004A>G	A	G	AG	AG	GG	GG	GG	GG	GG	GG
10	rs2676750	ADARB2	c.101-99601A>G	T	C	TT	TT	CC	CC	CC	CC	TC	CC

Chr, chromosome; SNP, single nucleotide polymorphism; HGVS, human genome variant sequence; Ref, reference sequence; Alt, alternative sequence.

with those without new-onset AF and cardiovascular complication has been developed within only few days of index diagnosis of scrub typhus infection [9]. The reason for complicating cardiovascular diseases could be explained by possible pathophysiology of scrub typhus infection itself which initiates at the site of skin inoculation, evolves into regional lymphadenopathy and spreads to vasculitis with subsequent target organ damage [16]. Subsequently, cardiovascular inflammation associated with the scrub typhus infection can lead to cardiovascular complications. Unlike other infectious diseases, evidence to date has shown a robust association between scrub typhus infection and cardiovascular complications.

The $Na_V1.5$, cardiac-type voltage-dependent Na^+ channel (SCN5A) and $K_V11.1$, delayed rectifier K^+ channels (KCNH2) have previously been implicated in the pathophysiology including AF and cardiac conduction disorders through international GWAS [17,18]. Another members of the $Na^+/Ca^{2+}/K^+$ exchangers gene family, cone/neuronal K^+-Dependent Na^+/Ca^{2+} Exchanger Isoform 2 (NCKX2), and rs10738554 of SLC24A2 gene, were previously associated with high blood pressure, which regulate to transport sodium, potassium, and calcium ions for homeostasis in the cardiovascular system [19,20]. However, SNP (rs4977397) of SLC24A2 has not been reported in patients with cardiac complications after scrub typhus infection. The rs4977397 SNP is located approximately 40 kb upstream of the SLC24A2 gene and has been reported to increase the risk of AF by approximately 1.04 times (p value = 9.0×10^{-9}) when carrying the A allele [18].

ADARB2 is a member of the double-stranded RNA adenosine deaminase family of RNA-editing enzymes [21]. To date, only a few association studies have investigated SNPs in the RNA editing genes ADARB1 and ADARB2. ADARB2 gene-smoking interactions affect hypertension [22]. ADARB2, rs2805533 is located in intron 7 of ADARB2 and rs2805533 induces the splicing abnormality of ADARB2 and modulates the function of ADARB2 [23]. However, the SNP (rs2676750) of ADARB2 associated with cardiac diseases has net been reported, yet. The rs2676750 SNP is located in intron 1 of the ADARB2 gene, and there has been no previous paper reported on the SNP.

Both SNPs (rs4977397 and rs2676750) are not located in the protein-coding region, so their effect on protein function is thought to be very small. Further research on the specific functions of SLC24A2 and ADARB2 in the heart is needed.

The present study is the first to demonstrate that scrub typhus infection could be genetically linked to cardiac complications through the co-segregation between genetic markers (SNPs of SCL24A2 and ADARB2) and cardiac complications. This extends our understanding of the biological connection between the genetic loci and cardiac complications in scrub typhus infection. However, a limitation of this study is that the number of patients studied is too small to conclude on the clinical significance. Further studies with more participants are required to confirm and validate these findings.

CONCLUSIONS

We suggest that GG genotype in SNP (rs4977397) of SLC24A2 gene and non-TT genotype in SNP (rs2676750) of ADARB2 gene might be genetic surrogate markers for complicating cardiac disease in the scrub typhus infection. Our study shows that early detection based on individual sequence variants might be feasible for predicting complicating cardiac diseases in patients with scrub typhus infection, if further studies with more participants confirm these findings.

KEY MESSAGE

1. SNP (rs4977397) of SLC24A2 gene and SNP (rs2676750) of ADARB2 gene might be novel variants for complicating cardiac disease in patients with scrub typhus infection.
2. The genetic novel variants using whole genome sequencing might predict complicating cardiac diseases in patients with confirmed scrub typhus infection.

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Conflicts of interest
The authors disclose no conflicts.

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