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Investigation of the association of Vogt–Koyanagi–Harada syndrome with IL23R-C1orf141 in Han Chinese Singaporean and ADO-ZNF365-EGR2 in Thai

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ABSTRACT

Background We performed a multistage genome-wide association study of Vogt–Koyanagi–Harada (VKH) syndrome in a Han Chinese population and identified two novel non-human leukocyte antigen candidate regions previously. The aim of the study was to replicate the association of *IL23R-C1orf141* and *ADO-ZNF365-EGR2* with VKH syndrome in four sets of multinational populations in Asia.

Method We conducted a candidate genes association study involving 185 patients with VKH syndrome and 287 normal controls from Han Chinese Singaporeans, non-Han Chinese, Thais and Koreans. Genotyping of 16 single nucleotide polymorphisms (SNPs) within *IL23R-C1orf141* and *ADO-ZNF365-EGR2* loci was performed using the Sequenom MassARRAY system or by Taqman SNP assays.

Results Eight SNPs in *IL23R-Clorf141* showed an association with VKH syndrome only in Han Chinese Singaporeans ($p=8.49\times10^{-5}$ to 1.02×10^{-3}, $p_{correction}=1.69\times10^{-4}$ to 2.04×10^{-3}) but not in the other groups tested. One SNP rs1884444 in *IL23R-Clorf141* was found to be weakly associated with VKH syndrome in the Han Chinese Singaporeans, but significance was lost following Bonferroni correction for multiple comparisons. Five SNPs in *ADO-ZNF365-EGR2* were found to be associated with VKH syndrome in Thai patients with VKH ($p=0.014$, $p_c=0.028$) but not in the other three ethnic groups tested.

Conclusions This study confirmed the genetic associations between SNPs in *IL23R-C1orf141* and VKH syndrome in Han Chinese Singaporeans but not in other Asian populations. In addition, we also successfully replicated the association of VKH syndrome with *ADO-ZNF365-EGR2* in a Thai population.

Vogt–Koyanagi–Harada (VKH) syndrome is a multisystemic autoimmune disorder characterised by bilateral granulomatous panuveitis frequently associated with alopecia, vitiligo, poliosis and central nervous system and auditory signs.[1 2] It frequently affects certain ethnic populations, such as Chinese, Japanese and native Americans.[3] It is one of the most common seen and severe sight-threatening uveitis entities in China. Although the precise aetiology and pathogenesis of VKH syndrome remains unknown, numerous studies have shown an association between *HLA-DR4* and *HLA-DR53* with VKH syndrome in various ethnic populations.[4–6] Additionally, a variety of non-human leukocyte antigen (HLA) genes including *CTLA-4*, *MIF*, *MCP-1*, *IL23A* and *C4A* were found to be associated with VKH syndrome.[7–11]

Recently, we performed the first multistage genome-wide association study (GWAS) of VKH syndrome in a Han Chinese population to investigate additional genetic variants, and identified two novel non-HLA candidate regions.[12] These candidate loci include several genes such as *IL23R-C1orf141* and *ADO-ZNF365-EGR2*. However, the association was only tested in Han Chinese and has not yet been confirmed in other ethnic populations. We therefore decided to repeat our study in a multinational project including Han Chinese Singaporeans from Singapore, non-Han Chinese patients with VKH from South-West China, Thais from Thailand and Koreans from South Korea.

A total of 16 single nucleotide polymorphisms (SNPs) in the two new loci including *IL23R-C1orf141* at 1p31.2 and *ADO-ZNF365-EGR2* at 10q21.3 were genotyped to investigate the association between the identified risk genes/loci and VKH syndrome in these other ethnic populations. The study confirmed our earlier findings and showed that *IL23R-C1orf141* was associated with Han Chinese Singaporeans but not in other Asian populations. In addition, we also found the association between *ADO-ZNF365-EGR2* with VKH syndrome in Thais.

METHODS

Recruitment of patients and normal controls

One hundred eighty-five patients with VKH syndrome and 287 normal controls were recruited for this project. The first set included 32 Han Chinese Singaporean patients with VKH and 94 controls from Singapore. These were generally the descendants of immigrants from the South-Eastern coast of China in the provinces of Fujian and Guangdong during the 19th and early half of the 20th century. The second set included 38 patients and 60 controls from a non-Han Chinese ethnic population from South-West China. China is a united multi-ethnic country since ancient times. Fifty-five ethnic groups other than the Han population are referred to as minorities and include ethnic groups such as Manchu, Mongolian, Hui, Tibetan, Uygur, Kazak, Miao, Yi and Zhuang. The third set included 81 patients and 33 controls from Thailand. The last set included 34 Korean patients with VKH and 100 healthy controls. All patients strictly fulfilled the First International Workshop criteria for VKH syndrome.[13] Cases were excluded from the study if the diagnosis was uncertain. All cases were diagnosed by senior ophthalmologists. DNA was extracted from peripheral blood using standard techniques as described below. The study protocols were

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approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, Singapore National Eye Centre, Ramathibodi Hospital, Bangkok and Sensory Organs Institute, Medical Research Center, Seoul. All procedures followed the tenets of the Declaration of Helsinki and informed written consent was obtained from every participant. All experiments were performed in accordance with the approved guidelines and regulations.

SNPs' selection

To increase the reliability of the results, 16 SNPs were chosen as candidates. SNPs enrolled in this study within the *IL23R-C1orf141* and *ADO-ZNF365-EGR2* loci showed strong genetic associations with VKH syndrome in our earlier GWAS. These SNPs are located within the gene *IL23R-C1orf141* on chromosome 1p31.2 including rs77258390, rs3762318, rs78597810, rs12568393, rs12561798, rs76436269, rs78377598, rs117633859, rs1884444 (these nine SNPs are located in the same linkage disequilibrium (LD) block) (figure 1) and SNPs located within the gene *ADO-ZNF365-EGR2* on chromosome 10q21.3 including rs10995276, rs442309, rs224048, rs224052, rs224057, rs224058 and rs10995307 (these seven SNPs are located in the same LD block) (figure 2). LD block was estimated and showed that the selected SNPs were in strong LD (*IL23R-C1orf141* r^2=0.92–0.95 and *ADO-ZNF365-EGR2* r^2=0.76–0.92) (figures 1 and 2). As these SNPs are located in two loci, the p value of Bonferroni correction (p_c) is corrected by multiplication with n=2.

DNA extraction and SNP genotyping

Genomic DNA samples were extracted from the peripheral blood of patients with VKH syndrome and healthy controls using QIAGEN QIAamp DNA Mini Blood Kit (Hilden, Germany) according to the manufacturer's instructions. Fifteen SNPs, except rs117633859, were performed using the Sequenom MassARRAY system (Sequenom) according to the manufacturer's instructions. DNA samples were genotyped

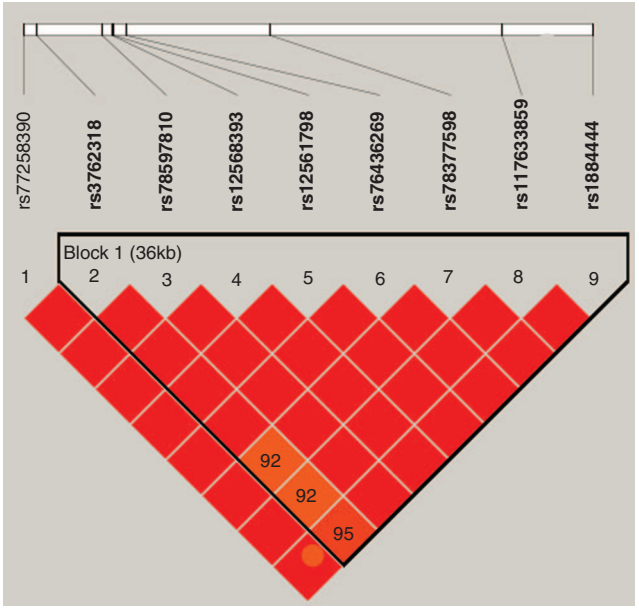

Figure 1 Linkage disequilibrium (LD) block was estimated for nine single nucleotide polymorphisms of *IL23R-C1orf141* using our data. Numbers in the squares indicate correlation coefficient (r^2) values.

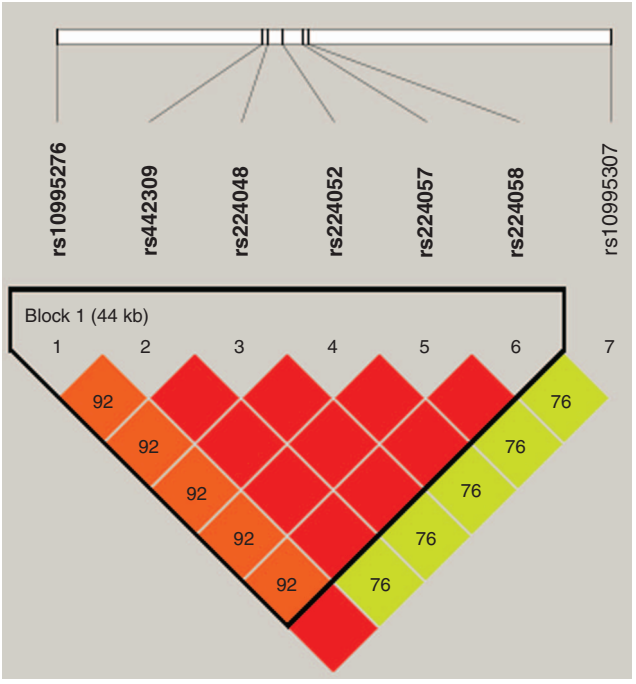

Figure 2 Linkage disequilibrium (LD) block was estimated for seven single nucleotide polymorphisms of *ADO-ZNF365-EGR2* using our data. Numbers in the squares indicate correlation coefficient (r^2) values.

using TaqMan SNP genotyping assays for rs117633859 (assay ID: AHVJKBP; Applied Biosystems, Foster City, California, USA) on the Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's instructions.

Statistical analysis

The real-time PCR data were analysed by manufacturer supplied software, V2.0.6 (Applied Biosystems, Foster City, California, USA). The χ^2 test was applied for the evaluation of the Hardy–Weinberg equilibrium. Genotype frequencies were estimated by direct counting. The χ^2 test or Fisher's exact test using SPSS V.17.0 (SPSS, Chicago, Illinois, USA) was used for the comparison of genotype and allele frequencies between patients and healthy controls. ORs and 95% CIs were calculated using SPSS V.17.0 to estimate disease risk. LD was measured with Haploview V.4.0. A Bonferroni correction was applied to correct for multiple comparisons and a statistical significant difference was considered with a p_c value ≤0.05. A power analysis was performed using the Quanto software to verify the association between VKH syndrome and the tested SNPs.

RESULTS

The study was performed using 185 patients and 287 controls. The detailed clinical findings of patients with VKH and controls are summarised in tables 1 and 2.

We had previously reported two new non-HLA susceptibility loci for VKH syndrome including *IL23R-C1orf141* at 1p31.2 and *ADO-ZNF365-EGR2* at 10q21.3 in Han Chinese using a GWAS approach.[12] In the current study, we genotyped 16 SNPs with the two loci associated with VKH syndrome in patients and controls originating from four different multiethnic Asian populations to confirm the association of these two loci with this disease.

Table 1 Constitution of patients with Vogt–Koyanagi–Harada syndrome and normal control subjects in this study

	Case			Control		
	Sample size	Mean age	Male/female	Sample size	Mean age	Male/female
Chinese Singaporean	32	55.40±16.13	18/14	94	39.43±10.07	62/32
Non-Han Chinese	38	35.52±12.16	15/23	60	34.30±9.69	34/26
Thai	81	45.34±17.03	39/42	33	42.34±13.08	8/25
Korean	34	50.79±16.33	12/22	100	68.23±9.73	39/61

Association of the nine gene polymorphisms with susceptibility to VKH syndrome of *IL23R-C1orf141* at 1p31.2

Nine SNPs in *IL23R-C1orf141* at the 1p31.2 locus, that is, rs77258390, rs3762318, rs78597810, rs12568393, rs12561798, rs76436269, rs78377598, rs117633859 and rs1884444 were genotyped in Han Chinese Singaporeans, non-Han Chinese, Thais and Korean patients with VKH. Our results showed that eight out of nine SNPs in *IL23R-C1orf141* showed an association with VKH syndrome in Han Chinese Singaporeans ($p=8.49\times10^{-5}$ to 1.02×10^{-3}, $p_{correction}=1.69\times10^{-4}$ to 2.04×10^{-3}) (table 3). A stratification analysis study on gender showed a significant association for several SNPs of *IL23R-C1orf141* in Han Chinese Singaporean males ($p_c=6.4\times10^{-4}$ to 2.0×10^{-3}) but not in females (see online supplementary table S1).

Replication of suggestive associations ($p=0.028$ to 0.056, $p_{correction}=0.056$ to 0.112) was sought in 38 non-Han Chinese descent participants with VKH and 60 healthy controls, but significance was lost following Bonferroni correction for multiple comparisons. No association was found for *IL23R-C1orf141* and VKH syndrome in the Korean or Thai group (table 3).

Association of the seven gene polymorphisms with susceptibility to VKH syndrome of *ADO-ZNF365-EGR2* at 10q21.3

ADO-ZNF365-EGR2 has been identified as a risk factor for VKH syndrome in a Han Chinese population.[12] The seven candidate SNPs, including rs10995276, rs442309, rs224048, rs224052, rs224057, rs224058 and rs10995307, were genotyped in Han Chinese Singaporeans, non-Han Chinese, Thai and Korean patients (185 patients and 287 controls). The results revealed that five SNPs in *ADO-ZNF365-EGR2* were found to be associated with VKH syndrome in the Thai patients following Bonferroni correction ($p=0.014$, $p_c=0.028$). Nevertheless, the results revealed that these candidate SNPs in *ADO-ZNF365-EGR2* were not associated with VKH syndrome in the other three ethnic populations tested (table 3). As shown in online supplementary table S1, the suggestive association was lost in both male and female Thai patients after performing a stratified analysis by gender.

Table 2 Clinical features of the included patients with Vogt–Koyanagi–Harada (VKH) syndrome

Clinical features	Patients with VKH Total, n=185 (%)	Chinese Singaporean n=32	Non-Han Chinese n=38	Thai n=81	Korean n=34
Male	84 (45.41)	18	15	39	12
Female	101 (54.59)	14	23	42	22
Uveitis	185 (100)	32	38	81	34
Nuchal rigidity	31 (16.76)	7	7	13	4
Headache	70 (37.84)	8	18	38	6
Scalp sensitivity	22 (11.89)	7	8	5	2
Tinnitus	60 (32.43)	13	19	24	4
Dysacusia	35 (18.92)	4	12	17	2
Alopecia	34 (18.38)	8	17	7	2
Poliosis	42 (22.70)	2	13	25	2
Vitiligo	22 (11.89)	8	4	8	2

DISCUSSION

In this study, the reported association of *IL23R-C1orf141* at 1p31.2 with VKH in Chinese Han, as shown in our earlier GWAS,[12] was confirmed in Han Chinese Singaporean patients with VKH, but could not be replicated in patients from South-West non-Han Chinese, Korea or Thailand. Furthermore, the study replicated the association with VKH syndrome and *ADO-ZNF365-EGR2* in a Thai population but failed to replicate the association in the other three sets of Asian patients.

Variants within the *IL23R* gene have been reported to be associated with chronic inflammatory diseases, such as ankylosing spondylitis, psoriasis, inflammatory bowel disease (IBD) and Behcet's disease.[14–17] *IL23R* has also been found to be involved in the development of ankylosing spondylitis in Iranian and Chinese Han patients.[18–20] A report from Japan suggested that the *IL23R* gene was associated with autoimmune thyroid diseases (AITDs), but that this was restricted to a specific ethnic group.[21] Previous studies have shown a stronger association of *IL23R* with Behcet's disease in patients from Turkey[22] as compared with cases from Algeria.[23] Two SNPs located in the second haplotype block of the *IL23R* gene showed a marginal association with the risk of polyarticular psoriatic arthritis ($p=0.05$) in a Romanian case–control cohort.[24] Why patients from Han Chinese descent show an association with *IL23R-C1orf141* with VKH syndrome whereas other Asian populations do not is not yet clear. Although the clinical features of the patients included in our study are generally similar, it is possible that VKH in non-Han Asian patients may represent a subentity of VKH syndrome. It is also possible that the association with *IL23R-C1orf141* is weaker in the other non-Han Asian populations tested and that the sample size was too small to obtain statistically significant findings. Larger sample sizes are needed to address this explanation due to the power value of less than 80%. A gender-based stratification analysis of the *IL23R-C1orf141* association with VKH showed a significant effect in male Han Chinese Singaporeans but not in the female population, which suggests that *IL23R-C1orf141* may have a gender skewed impact on VKH in Han Chinese Singaporeans. We found that five SNPs on *IL23R-C1orf141*, including SNP rs3762318, rs12568393, rs78377598, rs117633859 and rs1884444, showed no significant association with VKH in the

Table 3 The association of IL23R/C1orf141 and ADO/ZNF365/EGR2 with VKH syndrome

Chr	Gene	SNP	Allele	Stage	MAF (Case/control)	p Value	p_c Value	OR (95% CI)
1	IL23R/C1orf141	rs77258390	A	Chinese Singaporean	18.8/3.7	8.49×10^{-5}	1.69×10^{-4}	5.9 (2.2 to 15.9)
				Non-Han Chinese	25.0/14.2	0.056	NS	2.0 (0.9 to 4.1)
				Thai	6.2/3.0	0.335	NS	2.1 (0.4 to 9.8)
				Korean	5.9/4.5	0.665	NS	1.2 (0.4 to 4.0)
1	IL23R/C1orf141	rs3762318	C	Chinese Singaporean	20.3/4.8	1.46×10^{-4}	2.92×10^{-4}	5.0 (2.0 to 12.5)
				Non-Han Chinese	27.6/15.8	0.045	NS	2.0 (1.0 to 4.1)
				Thai	7.4/4.5	0.429	NS	1.6 (0.4 to 6.1)
				Korean	5.9/4.5	0.665	NS	1.2 (0.4 to 4.0)
1	IL23R/C1orf141	rs78597810	C	Chinese Singaporean	18.8/3.7	8.49×10^{-5}	1.69×10^{-4}	5.9 (2.2 to 15.9)
				Non-Han Chinese	25.7/14.2	0.045	NS	2.0 (1.0 to 4.3)
				Thai	6.2/4.5	0.631	NS	1.3 (0.3 to 5.1)
				Korean	5.9/4.5	0.665	NS	1.2 (0.4 to 4.0)
1	IL23R/C1orf141	rs12568393	A	Chinese Singaporean	18.8/3.7	8.49×10^{-5}	1.69×10^{-4}	5.9 (2.2 to 15.9)
				Non-Han Chinese	25.0/13.8	0.049	NS	2.0 (0.9 to 4.3)
				Thai	6.2/4.5	0.631	NS	1.3 (0.3 to 5.1)
				Korean	5.9/4.5	0.665	NS	1.2 (0.4 to 4.0)
1	IL23R/C1orf141	rs12561798	C	Chinese Singaporean	18.8/3.7	8.49×10^{-5}	1.69×10^{-4}	5.9 (2.2 to 15.9)
				Non-Han Chinese	25.0/14.2	0.056	NS	2.0 (0.9 to 4.1)
				Thai	6.2/4.5	0.631	NS	1.3 (0.3 to 5.1)
				Korean	5.9/4.5	0.665	NS	1.2 (0.4 to 4.0)
1	IL23R/C1orf141	rs76436269	A	Chinese Singaporean	18.8/3.7	8.49×10^{-5}	1.69×10^{-4}	5.9 (2.2 to 15.9)
				Non-Han Chinese	25.0/14.2	0.056	NS	2.0 (0.9 to 4.1)
				Thai	6.2/4.5	0.631	NS	1.3 (0.3 to 5.1)
				Korean	5.9/4.5	0.665	NS	1.2 (0.4 to 4.0)
1	IL23R/C1orf141	rs78377598	T	Chinese Singaporean	18.8/5.3	1.02×10^{-3}	2.04×10^{-3}	4.1 (1.6 to 10.1)
				Non-Han Chinese	26.3/14.2	0.034	NS	2.1 (1.0 to 4.4)
				Thai	6.2/4.5	0.631	NS	1.3 (0.3 to 5.1)
				Korean	5.9/4.5	0.665	NS	1.2 (0.4 to 4.0)
1	IL23R/C1orf141	rs117633859	G	Chinese Singaporean	21.7/5.9	3.50×10^{-4}	7.00×10^{-4}	4.4 (1.9 to 10.4)
				Non-Han Chinese	27.1/13.9	0.028	NS	2.3 (1.1 to 4.9)
				Thai	6.3/4.7	0.65	NS	1.4 (0.4 to 5.1)
				Korean	7.4/5.2	0.50	NS	1.5 (0.5 to 4.4)
1	IL23R/C1orf141	rs1884444	G	Chinese Singaporean	43.8/30.3	0.049	NS	1.7 (0.9 to 3.2)
				Non-Han Chinese	56.6/45.8	0.143	NS	1.5 (0.8 to 2.7)
				Thai	36.4/30.3	0.379	NS	1.3 (0.7 to 2.4)
				Korean	35.3/40.0	0.504	NS	0.8 (0.4 to 1.4)
10	ADO/ZNF365/EGR2	rs10995276	A	Chinese Singaporean	12.5/9.6	0.506	NS	1.3 (0.5 to 3.2)
				Non-Han Chinese	21.1/14.2	0.209	NS	1.6 (0.7 to 3.4)
				Thai	17.9/7.6	0.047	NS	2.6 (0.9 to 7.2)
				Korean	16.2/14.5	0.738	NS	1.1 (0.5 to 2.4)
10	ADO/ZNF365/EGR2	rs442309	T	Chinese Singaporean	20.3/22.3	0.734	NS	0.8 (0.4 to 1.7)
				Non-Han Chinese	31.6/30.8	0.913	NS	1.0 (0.5 to 1.9)
				Thai	34.6/18.2	0.014	0.028	2.3 (1.1 to 4.8)
				Korean	39.7/33.0	0.282	NS	1.4 (0.7 to 2.6)
10	ADO/ZNF365/EGR2	rs224048	T	Chinese Singaporean	20.3/22.3	0.734	NS	0.8 (0.4 to 1.7)
				Non-Han Chinese	31.6/30.5	0.875	NS	1.0 (0.5 to 1.9)
				Thai	34.6/18.2	0.014	0.028	2.3 (1.1 to 4.8)
				Korean	39.7/33.0	0.282	NS	1.4 (0.7 to 2.6)
10	ADO/ZNF365/EGR2	rs224052	A	Chinese Singaporean	20.3/22.3	0.734	NS	0.8 (0.4 to 1.7)
				Non-Han Chinese	31.6/30.5	0.875	NS	1.0 (0.5 to 1.9)
				Thai	34.6/18.2	0.014	0.028	2.3 (1.1 to 4.8)
				Korean	39.7/33.0	0.282	NS	1.4 (0.7 to 2.6)
10	ADO/ZNF365/EGR2	rs224057	A	Chinese Singaporean	20.3/22.3	0.734	NS	0.8 (0.4 to 1.7)
				Non-Han Chinese	31.6/30.5	0.875	NS	1.0 (0.5 to 1.9)
				Thai	34.6/18.2	0.014	0.028	2.3 (1.1 to 4.8)
				Korean	39.7/33.0	0.282	NS	1.4 (0.7 to 2.6)
10	ADO/ZNF365/EGR2	rs224058	T	Chinese Singaporean	20.3/22.3	0.734	NS	0.8 (0.4 to 1.7)
				Non-Han Chinese	31.6/30.8	0.913	NS	1.0 (0.5 to 1.9)

Continued

Table 3 Continued

Chr	Gene	SNP	Allele	Stage	MAF (Case/control)	p Value	p_c Value	OR (95% CI)
				Thai	34.6/18.2	0.014	0.028	2.3 (1.1 to 4.8)
				Korean	39.7/33.0	0.282	NS	1.4 (0.7 to 2.6)
10	ADO/ZNF365/EGR2	rs10995307	C	Chinese Singaporean	21.9/26.1	0.504	NS	0.7 (0.4 to 1.5)
				Non-Han Chinese	30.3/30.2	0.989	NS	1.0 (0.5 to 1.8)
				Thai	32.7/25.8	0.302	NS	1.4 (0.7 to 2.6)
				Korean	36.8/30.5	0.342	NS	1.3 (0.7 to 2.3)

Chr, chromosome; NS, no significance; MAF, frequency of minor allele; p_c value, p Value after Bonferroni correction; OR: OR for minor allele; SNP, single nucleotide polymorphism; VKH, Vogt–Koyanagi–Harada.

overall non-Han Chinese group. However, these five SNPs did show a significant difference in females (p_c=0.02 to 0.04) (see online supplementary table S1). It should be noted that the statistical power of the gender analysis was low and should therefore be interpreted cautiously (see online supplementary table S2).

The study replicated the association with VKH syndrome and *ADO-ZNF365-EGR2* in a Thai population but failed to replicate the association in the other three sets of Asian patients. Previous studies showed that genetic polymorphisms of *ZNF365* are associated with immune-related diseases in both Asia and Latin America.[25 26] *ZNF365* was reported to be associated with atopic dermatitis in a Japanese population.[25] Variants of *ZNF365* have also been identified to be associated with susceptibility to breast cancer.[27] Associations were observed with SNPs in the *ZNF365* gene and breast cancer risk in Korean women and other ethnic populations.[27–34] An Italian cohort showed an association with IBD both in adult and paediatric cohort of patients, with a small influence on subphenotypes.[35] Allelic association analysis (two tailed) showed that *ZNF365* was significantly associated with overall susceptibility for Crohn's disease in Canadian children.[26] The Oregon Sudden Unexpected Death Study (Oregon-SUDS) suggested that the *ZNF365* region contains relevant candidate genes for sudden cardiac death.[36] The early growth response gene-2 (*EGR2*) was characteristically expressed by the iTregs, which played a major role in controlling immune responses.[37] Moreover, in the Thai group, *ADO-ZNF365-EGR2* showed a significant association with VKH while this was lost after performing a gender-based stratified analysis. The inconsistent result may due to the significant gender frequencies between patients and controls and the low statistical power of the comparisons (see online supplementary table S2).

The various pathogenesis and mechanism behind many ethnic groups base on the interaction of genes and environment. The difference of the associations among the nationalities may be partly explained by the population genetic heterogeneity. Hapmap data shows that the frequencies of SNPs in *IL23R-C1orf141* and *ADO-ZNF365-EGR2* are different between CEU (Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain collection) and CHB (Han Chinese in Beijing) (see online supplementary table S3). Large size of samples is need to be collect in confirming study of European population on these two loci in our study. In addition to the SNPs we selected, another SNPs in *IL23R-C1orf141* and *ADO-ZNF365-EGR2* may inflict pathogenesis and mechanism of VKH syndrome in CEU patients.

The power value of 0.85–0.94 for SNPs on *IL23R-C1orf141* showed the results reality of Han Chinese Singaporeans using a prevalence of VKH syndrome in Asian population of 0.001% represented by the prevalence of Japanese[38] because there was no other report of Asian population of VKH syndrome before (table 4). Especially, no risk was observed in *ADO-ZNF365-EGR2* in Han Chinese Singaporeans, which suggests that this study may lack power due to small sample size. We only collected samples from descendants of immigrants from the South-Eastern coast of China in the provinces of Fujian and Guangdong. However, the previous GWAS study included Han Chinese cohorts who not only originated from the Fujian and Guangdong Han population, but also included Han Chinese patients from other parts of China. The differences between the cohorts may partially explain the different results concerning *ADO-ZNF365-EGR2*. Additionally, the minor allele frequency of Han Chinese Singaporean on *ADO-ZNF365-EGR2* in Han Chinese Singaporean in the replication study did not reveal an association with VKH syndrome which may be due to the low statistical power of the comparison (power value 0.09–0.18; see online supplementary table S2) and a small size of samples used in this study. Concerning the validation study among non-Han Chinese, the small size of the tested samples might explain the false negative association results (power value 0.28–0.61) (see online supplementary table S2) even though the allele frequencies of *IL23R-C1orf141* in non-Han Chinese were higher than

Table 4 The power value of SNPs with VKH syndrome

Chr	SNP	Allele	Stage	MAF (Case/control)	OR	Power
1	rs77258390	A	Chinese Singaporean	18.8/3.7	5.9	0.94
	rs3762318	C		20.3/4.8	5.0	0.93
	rs78597810	C		18.8/3.7	5.9	0.94
	rs12568393	A		18.8/3.7	5.9	0.94
	rs12561798	C		18.8/3.7	5.9	0.94
	rs76436269	A		18.8/3.7	5.9	0.94
	rs78377598	T		18.8/5.3	4.1	0.85
	rs117633859	G		21.7/5.9	4.4	0.92
	rs1884444	G		43.8/30.3	1.7	0.42
10	rs10995276	A	Thai	34.6/18.2	2.3	0.67
	rs442309	T		17.9/7.6	2.6	0.53
	rs224048	T		34.6/18.2	2.3	0.67
	rs224052	A		34.6/18.2	2.3	0.67
	rs224057	A		34.6/18.2	2.3	0.67
	rs224058	T		34.6/18.2	2.3	0.67
	rs10995307	C		32.7/25.8	1.4	0.18

Power: we examined the power calculation using the Quanto program and assumed that the prevalence of VKH syndrome in Asian population is 0.001% according to the literature.
MAF, frequency of minor allele; SNP, single nucleotide polymorphism; VKH, Vogt–Koyanagi–Harada.

in the other groups (table 3). Further studies using a large size of samples from these populations are expected to validate our previous GWAS results.

It is worthwhile to point out that several possible limitations of the present study merit particular consideration. First, further research should be done to correlate the *IL23R-C1orf141* and *ADO-ZNF365-EGR2* gene polymorphisms with VKH syndrome in other populations in Asia to confirm the results. Furthermore, we have focused on common genetic variants and it is possible that the analysis of rare variants may provide further clues concerning the exact role of these genes in the pathogenesis of VKH syndrome. As suggested above, it is possible that the clinical features among patients from different Asian countries might represent subentities of the disease. Although we tried to match the controls for gender, there were significant gender differences with the groups of Han Chinese Singaporeans, non-Han Chinese and Thai patients with VKH. The statistical power of our study may be low due to the relatively small sample size and the results presented here therefore need to be confirmed in larger samples. Third, although molecular biological studies provide evidence for a role of *IL23R* polymorphisms, this result is suggestive and does not exactly explain how the genetic variant translates into physiologic processes and disease pathogenesis. Therefore, the association results presented here should be investigated further using functional experiments.

In summary, we confirmed the genetic associations between VKH syndrome and *IL23R-C1orf141* in Han Chinese Singaporeans but not in non-Han Asian patients. In addition, we also successfully replicated the association with VKH syndrome and *ADO-ZNF365-EGR2* in Thai patients with VKH.

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