RESEARCH ARTICLE

Variants in IL23R-C1orf141 and ADO-ZNF365-EGR2 are associated with susceptibility to Vogt-Koyanagi-Harada disease in Japanese population

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Abstract

Vogt-Koyanagi-Harada (VKH) disease is a systemic inflammatory disorder that affects pigment cell-containing organs such as the eye (e.g., chronic and/or recurrent granulomatous panuveitis). While the exact etiology and pathogenic mechanism of VKH disease are unclear, HLA-DR4 alleles have been documented to be strongly associated with VKH disease in various ethnic groups. Recently, a genome-wide association study (GWAS) found two new genetic risk factors (IL23R-C1orf141 and ADO-ZNF365-EGR2) in a non-HLA region from a Han Chinese population. In this study, we replicated these GWAS findings in a Japanese population. A total of 1,643 Japanese samples (380 cases with VKH disease and 1,263 healthy controls) were recruited. We assessed four single nucleotide polymorphisms (SNPs) shown in previous GWAS: rs78377598 and rs117633859 in IL23R-C1orf141, and rs442309 and rs224058 in ADO-ZNF365-EGR2. A significant allelic association with VKH disease was observed for all of the four SNPs (rs78377598: \( \text{p}_c = 0.0057 \); rs117633859: \( \text{p}_c = 0.0017 \); rs442309: \( \text{p}_c = 0.021 \); rs224058: \( \text{p}_c = 0.035 \)). In genotypic association analysis, the minor alleles of IL23R-C1orf141 rs78377598 and rs117633859 had the strongest association with disease susceptibility under the additive model (\( \text{p}_c = 0.0075 \) and \( \text{p}_c = 0.0026 \), respectively.)
respectively). The minor alleles of \textit{ADO-ZNF365-TEGR2}\( rs442309 \) and \( rs224058 \) were most strongly associated with disease susceptibility under the dominant model (\( p_c = 0.00099 \) and \( p_c = 0.0023 \), respectively). The meta-analysis of the current and previous studies found that all of the four SNPs exhibited a significantly strong association with VKH disease (meta-\( p < 0.00001 \): \( rs78377598 \), meta-odds ratio (OR) = 1.69; \( rs1176338 \), meta-OR = 1.82; \( rs442309 \), meta-OR = 1.34; \( rs224058 \), meta-OR = 1.33). In summary, our study replicated significant associations with VKH disease susceptibility reported in a previous GWAS. Thus, the \textit{IL23R-C1orf141} and \textit{ADO-ZNF365-TEGR2} loci may play important roles in the development of VKH disease through genetic polymorphisms.

**Introduction**

VKH disease is a systemic polymorphic autoimmune disorder that targets organs with melanocytes such as the eye, meninges, inner ear, skin, and hair [1]. VKH disease, along with sarcoidosis and Behcet’s disease, is one of the causes of endogenous uveitis and an ophthalmological condition that is most common in the Japanese population [2,3]. VKH disease in the acute stage is characterized by the development of bilateral uveitis associated with multifocal exudative retinal detachment (RD) in the posterior pole and inflammation signs often observed in the anterior ocular, such as mutton-fat keratic precipitates, iris nodules, and shallow anterior chamber. Early-phase of fluorescein angiography (FA) in the acute stage shows multiple focal areas of leakage at the level of retinal pigment epithelium, and late-phase of FA shows dye pooling within subretinal fluid (SRF). In the chronic stage of VKH disease, sunset glow fundus characterized by orange-red discoloration due to depigmentation of the choroid is found [4–6]. The incidence of VKH disease varies worldwide. The disease occurs more frequently among people with dark skin pigmentation, as well as in those of Asian descent, Native Americans, and Hispanics compared to Caucasians [4,7]. In Japan, VKH disease accounts for about 7% of all uveitis patients [3]. In contrast, VKH disease patients represent only about 1% to 4% of all uveitis cases in the United States [1].

Although the exact etiology of VKH disease remains unclear, genetic factors may play an important role in disease development. A strong association of VKH disease with human leukocyte antigen (HLA)-\( DR4 \) has been reported by some ethnic groups [8–11]. The pathogenesis of VKH disease may be implicated by multifactorial factors through environmental triggers and susceptibility genes such as \( HLA \) and non-\( HLA \) [12,13].

The characteristic clinical findings of tissue depigmentation in VKH disease point to the possible involvement of melanocytes in the pathogenesis. The tyrosinase gene family (e.g., \textit{tyrosinase}, \textit{tyrosinase-related protein (TRP) 1}, \textit{TRP2} and \textit{dopachrome tautomerase}) is expressed specifically in melanocytes and involve in pigmentation. In earlier studies, \textit{TRP1} and \textit{TRP2} induced an experimental autoimmune disease in Lewis rats. The clinical course and histological findings resembled human VKH disease [14]. It is also reported that human VKH-like disease is induced in Akita dogs by immunizing them with \textit{TRP1} [15]. Further, lymphocytes obtained from VKH disease patients were reactive to peptides derived from tyrosinase gene family [16]. These studies suggest that tyrosinase gene family may be responsible for human VKH disease. However, the association of VKH disease with genes in the tyrosinase gene family has been showed negative results in Japanese patients with VKH disease [13].

A recent genome-wide association study (GWAS) of patients with VKH disease from a Han Chinese population identified two new non-\( HLA \) candidate regions, namely interleukin 23
receptor (IL23R)-chromosome 1 open reading frame 141 (C1orf141) on 1p31.2 and 2-ami-noethanethiol dioxygenase (ADO)-zinc finger protein 365 (ZNF365)-early growth response 2 (EGR2) on 10q21.3 [17]. These two new loci were also assessed in replication studies that included the Han Chinese in Singapore, a non-Han Chinese population in southwestern China, and patients with VKH disease from Thailand and Korea. In these studies, IL23R-C1orf141 on 1q31.2 was associated with VKH disease among patients of Han Chinese descent in Singapore but not in those of other Asian ethnicities. The association between ADO-ZNF365-EGR2 on 10q21.3 and VKH disease has only been confirmed in a Thai population [18].

To further explore these issues, we conducted a replication study in Japanese patients with VKH disease. We investigated an association between VKH disease and four single nucleotide polymorphisms (SNPs), namely rs78377598 and rs117633859 on 1p31.2 and rs1142309 and rs224058 on 10q21.3, which have been previously reported [17,18]. In addition, we performed a random-effects meta-analysis of the odds ratios (ORs) of four SNPs in Japanese and other Asian populations.

Materials and methods

Participants

We recruited 380 unrelated Japanese patients with VKH disease (41.6% male, mean age 51.3 ± 14.7 years [range 21 to 81 years]) and 1,263 unrelated Japanese healthy controls (46.8% male, mean age 54.6 ± 14.3 years [range 20 to 87 years]) (Table 1). The patients were diagnosed between 2003 and 2015 according to the "Revised Diagnostic Criteria for VKH Disease" at the Uveitis Survey Clinic of Yokohama City University, Hokkaido University, Kagoshima University, Jikei University, Kyorin University, Kyusyu University, Japan Community Healthcare Organization Osaka Hospital, and Saga University. All patients met the criteria established by the 2001 First International Workshop on Vogt-Koyanagi-Harada Disease [9]. The details of criteria are (i) no history of penetrating ocular trauma / surgery before uveitis onset, (ii) no clinical / laboratory evidence of other ocular disease, (iii) bilateral ocular involvement: diffuse choroiditis (focal regions of SRF, bullous serous RD) and FA (focal areas of delay in choroidal perfusion, multifocal areas of hyperfluorescence, pooling within SRF, and optic nerve staining), (iv) cerebrospinal fluid pleocytosis, and (v) integumentary findings (alopecia, poliosis and vitiligo) [19]. Clinical presentation showed little variation among patients. The control subjects were all healthy volunteers of similar ethnic origin as the patients, and were not related.

Table 1. Characteristics of the study populations.

| Characteristic          | Cases (n = 380) | Controls (n = 1,263) |
|------------------------|----------------|---------------------|
| Male                   | 41.6%          | 46.8%               |
| Mean age [SD; range], years | 51.3 [14.7; 21–81] | 54.6 [14.3; 20–87] |
| Uveitis                | 100.0%         |                     |
| Nuchal rigidity        | 10.9%          |                     |
| Headache               | 58.4%          |                     |
| Scalp allergy          | 11.9%          |                     |
| Tinnitus               | 32.2%          |                     |
| Dysacusia              | 22.3%          |                     |
| Alopecia               | 6.9%           |                     |
| Poliosis               | 10.4%          |                     |
| Vitiligo               | 6.4%           |                     |

SD, standard deviation.
to each other or to the VKH disease patients. All controls had no clinical manifestations or family history of any type of immune-related diseases. All participants gave their written informed consent. The study was approved by the ethics committees of Yokohama City University, Hokkaido University, Kagoshima University, Jikei University, Kyorin University, Kyushu University, Japan Community Healthcare Organization Osaka Hospital, and Saga University and conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

**SNP genotyping within IL23R-C1orf141 and ADO-ZNF365-EGFR2 genes**

We assessed the four SNPs that showed a strong association with VKH disease in a previous GWAS: rs78377598 and rs117633859 in IL23R-C1orf141 on the 1p31.2 locus, and rs442309 and rs224058 in ADO-ZNF365-EGFR2 on the 10q21.3 locus [17]. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Standardized conditions were used to prevent variation in DNA quality. SNP genotyping was performed using the TaqMan 5’ exonuclease assay with primers supplied by Applied Biosystems (Foster City, CA, USA). Polymerase chain reaction (PCR) was performed in a 10 µL reaction mixture containing 1x TaqMan Universal PCR Master Mix (Applied Biosystems), 24 nm of each primer-probe set, and 3 ng genomic DNA. The PCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. The probe’s fluorescence signal was detected using the StepOne-Plus Real-Time PCR System (Applied Biosystems).

**Statistical analysis**

We performed allelic and genotypic association analyses, and calculated Hardy-Weinberg equilibrium using SNP and Variation Suite 8.4.0 software (Golden Helix, Inc., Bozeman, MT, USA, http://www.goldenhelix.com). For genotypic association analysis, we applied three different genetic models to assess each minor allele: additive (2/2 vs. 1/2 vs. 1/1), dominant (2/2 +1/2 vs. 1/1), and recessive (2/2 vs. 1/2+1/1) models (assuming that 2 is the minor allele and 1 is the major allele). Differences in allele and genotype frequencies between cases and controls were assessed by correlation/trend test. The p-values and ORs in genotype models were adjusted for age and sex. The obtained p-values were corrected for multiple testing using Bonferroni’s method based on the number of tested SNPs (n = 4). A corrected p-value (pc) < 0.05 was considered significant.

**Random-effects meta-analysis**

We conducted a random-effects meta-analysis of the current and previous studies using the generic inverse variance method and logarized OR. The pooled OR corresponding to one risk allele increase in allelic model for each SNP was calculated [20–22]. The heterogeneity was estimated using I² statistics as follows: 0%: indicates no heterogeneity; 0% to 30%: might not be important; 30% to 50%: may represent moderate heterogeneity; 50% to 75%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity [22]. We used Review Manager ver. 5.3 (Cochrane Collaboration, Oxford, UK) to perform meta-analysis.

**Results**

We performed genotyping of four SNPs in the VKH disease patient and control groups. The genotype frequencies of all four SNPs were all in Hardy-Weinberg equilibrium for the cases and controls. **Table 2** shows the allelic association results for the four SNPs. Two SNPs
Table 2. Allelic association results for rs78377598 and rs117633859 in IL23R-C1orf141, and rs442309 and rs224058 in ADO-ZNF365-EGR2.

| SNP                | Chr. | Position (GRCh37) | Alleles (1>2) | Call Rate, % | Minor Allele Freq., % | Cases (n = 380) | Controls (n = 1,263) | P     | Pc    | OR (95% CI) |
|--------------------|------|-------------------|---------------|--------------|-----------------------|----------------|----------------------|-------|-------|--------------|
| rs78377598         | 1    | 67,612,502        | C>T           | 98.8         | 8.4                   | 5.3            | 0.0014               | 0.0057 | 1.65 (1.21–2.25) |
| rs117633859        | 1    | 67,627,828        | A>G           | 98.9         | 9.1                   | 5.5            | 0.00043              | 0.0017 | 1.71 (1.26–2.31) |
| ADO-ZNF365-EGR2    |      |                   |               |              |                       |                |                      |       |       |              |
| rs442309           | 10   | 64,490,495        | C>T           | 99.1         | 42.8                  | 37.2           | 0.0053               | 0.021  | 1.27 (1.07–1.49) |
| rs224058           | 10   | 64,498,865        | G>A           | 98.8         | 42.6                  | 37.3           | 0.0088               | 0.035  | 1.25 (1.06–1.47) |

1, major allele; 2, minor allele; OR, odds ratio; CI, confidence interval.

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(rs78377598 and rs117633859 in IL23R-C1orf141 were significantly associated with VKH disease in the Japanese population (p = 0.0014 and p = 0.00043, respectively). Statistical significance was kept following Bonferroni’s correction (rs78377598: \( p_c = 0.0057 \) and rs117633859: \( p_c = 0.0017 \)). The T allele of rs78377598 and the G allele of rs117633859 were more frequent alleles in patients with VKH disease than the control group, indicating that these alleles were susceptible to VKH disease (OR = 1.65 and OR = 1.71, respectively). The variants in the ADO-ZNF365-EGR2 locus also showed a significant association with the disease (rs442309: p = 0.0053, p = 0.021 and rs224058: p = 0.0088, p = 0.035). Both allele frequencies of the T allele of rs442309 and the A allele of rs224058 were higher in VKH disease patients compared to the controls (OR = 1.27 and OR = 1.25, respectively).

The results of genotypic association analysis for the four SNPs under different genetic models are presented in Table 3. The minor alleles of rs78377598 and rs117633859 in IL23R-C1orf141 had the strongest association with the risk of VKH disease under the additive model (rs78377598: p = 0.0019, p = 0.0075, OR = 1.64 and rs117633859: p = 0.0066, p = 0.0026, OR = 1.70). These alleles were also significantly associated with VKH disease under the dominant model (rs78377598: p = 0.0031, p = 0.012, OR = 1.68 and rs117633859: p = 0.0011, p = 0.0043, OR = 1.75). The minor alleles of rs442309 and rs224058 in ADO-ZNF365-EGR2 were most strongly associated with the risk of VKH disease under the dominant model (rs442309: p = 0.0025, p = 0.00099, OR = 1.58 and rs224058: p = 0.00057, p = 0.0023, OR = 1.53) and also showed a significant association with the disease under the additive model (rs442309:

Table 3. Genotypic association results for rs78377598 and rs117633859 in IL23R-C1orf141, and rs442309 and rs224058 in ADO-ZNF365-EGR2.

| SNP                | Alleles (1>2) | Cases (n = 380) | Controls (n = 1,263) | Additive (2/2 vs. 1/2 vs. 1/1) | Dominant (2/2+1/2 vs. 1/1) | Recessive (2/2 vs. 1/2 +1/1) |
|--------------------|---------------|----------------|----------------------|-------------------------------|-----------------------------|-----------------------------|
|                    |               |                |                      | Frequency, %                | -Genetic Models*             |                             |
| IL23R-C1orf141     |               |                |                      |                              |                             |                             |
| rs78377598         | C>T           | 1.14/2.84.5    | 0.9/6.89.9           | 0.0019                       | 0.0075                      | 1.64 (1.21–2.23)           | 0.0031 | 0.012   | 1.68 (1.20–2.35) | 0.10 | 2.84 (0.85–9.42) |
| rs117633859        | A>G           | 1.3/15.5/83.2  | 0.5/10.1/89.4        | 0.00066                      | 0.0026                      | 1.70 (1.26–2.29)           | 0.0011 | 0.0043  | 1.75 (1.26–2.42) | 0.090 | 2.94 (0.88–9.80) |
| ADO-ZNF365-EGR2    |               |                |                      |                              |                             |                             |
| rs442309           | C>T           | 16.5/52.5/30.9 | 15.6/43.2/41.3       | 0.0061                       | 0.025                       | 1.26 (1.07–1.48)           | 0.00025 | 0.0099  | 1.58 (1.23–2.02) | 0.65 | 1.08 (0.78–1.47) |
| rs224058           | G>A           | 16.5/52.1/31.4 | 15.5/43.5/41.0       | 0.0092                       | 0.037                       | 1.24 (1.06–1.47)           | 0.00057 | 0.0023  | 1.53 (1.20–1.96) | 0.64 | 1.08 (0.79–1.48) |

1, major allele; 2, minor allele; OR, odds ratio; CI, confidence interval.
*p-values and ORs were adjusted for age and sex.

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p = 0.0061, p_c = 0.025, OR = 1.26 and rs224058: p = 0.0092, p_c = 0.037, OR = 1.24). No significant association was found for any of the four SNPs in the recessive model (p > 0.05).

The results of a random-effects meta-analysis of the current and previous studies are showed in Figs 1–4. The meta-analysis revealed that all of the tested SNPs exhibited a significantly strong association with the risk of VKH disease (meta-p < 0.00001, rs78377598: meta-OR = 1.69; rs117633859: meta-OR = 1.82; rs442309: meta-OR = 1.34; rs224058: meta-OR = 1.33). rs78377598 and rs1176338 in IL23R-C1orf141 had an increased risk of VKH disease in all ethnic populations (Figs 1 and 2). On the other hand, rs442309 and rs224058 in ADO-ZNF365-EGR2 did not always have an increased risk in all populations (OR ≤ 1.0 in the Han Chinese group in Singapore and the non-Han Chinese group) (Figs 3 and 4).

![Fig 1. Forest plot of the meta-analysis of the association of IL23R-C1orf141 rs78377598 and VKH disease.](https://doi.org/10.1371/journal.pone.0233464.g001)
Discussion

VKH disease is a systemic autoimmune inflammatory disorder. A number of studies have shown that genetic polymorphisms including HLA genes and non-HLA genes affect the susceptibility of VKH disease. Hou et al. identified new non-HLA candidate genes, namely IL23R-C1orf141 on 1p31.2 and ADO-ZNF365-EGR2 on 10q21.3, by GWAS targeting a group of Han Chinese patients with VKH disease [17]. In this study, we also found that these two genes are susceptibility genes involved in the pathogenesis of VKH disease in Japanese patients.

A previous report showed that the interleukin 23 receptor (IL23R) is expressed in the iris and ciliary bodies of healthy subjects, while C1orf141 is only expressed in the iris of healthy subjects [17]. Furthermore, genetic variants in IL23R are associated with multiple immune-
related diseases such as Behcet’s disease, Crohn’s disease, ulcerative colitis, psoriasis, and ankylosing spondylitis [23–31]. IL23R is expressed in type 17 helper T cells (Th17) cells, which are implicated in the pathogenesis of various immune-mediated diseases. IL23 signaling through the IL23R promotes the proliferation, maintenance, and activation of Th17 inducing neutrophil inflammation and autoimmune diseases [32–34]. Liang et al. reported that VKH disease patients with active uveitis had significantly higher percentages of Th17 and IL-23 as compared with inactive VKH disease patients and healthy controls [35]. C1orf141 is involved in psoriasis [36]; however, its function remains to be elucidated. These reports indicate that an activation of Th17 through the IL23R is involved in the pathogenesis of VKH disease. In the current study, we confirmed the significant association of rs78377598 and rs117633859 in
**Fig 4. Forest plot of the meta-analysis of the association of ADO-ZNF365-EGR2 rs224058 and VKH disease.** The lines with squares in the middle correspond to the study-specific 95% CI and OR. The central vertical solid line indicates the OR for the null hypothesis. The diamond represents the summary OR with its corresponding 95% CI.

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IL23R-C1orf141 with VKH disease in the Japanese population. Taken together, IL23R are likely involved in the development of VKH disease through genetic variants of IL23R-C1orf141.

In this study, we succeeded in replicating previous GWAS findings showing that ADO-ZNF365-EGR2 is susceptibility locus involved in the development of VKH disease in the Japanese population. However, these findings were not reproduced in the Han Chinese Singaporeans and, the non-Han Chinese population from southwestern China [18]. This difference in results may be due to the small sample size in the previous replication study, which may increase the risk of false negative results, called type II errors. Our study recruited 380 Japanese patients with VKH disease. In contrast, the previous study was conducted with 32 cases from the Han Chinese Singaporeans, 38 cases from a non-Han Chinese population in southwestern China,
81 cases from Thai, and 34 cases from Koreans [18]. Obviously, these sample sizes were not enough to produce effective statistical results; hence, it is suggested that the previous study could not detect the association between VKH disease and ADO-ZNF365-EGR2 in the Han Chinese Singaporeans and the non-Han Chinese. In addition, it has been hypothesized that VKH disease may be triggered by virus infections, such as the Epstein-Barr virus and the cytomegalovirus [37,38]. This hypothesized factors may be dependent on environmental factors, Asian countries have different environmental factors [39]. Therefore, the etiology and disease mechanisms underlying VKH disease development may be elucidated by the effects of gene-environment interactions. ADO, ZNF365, and EGR2 are expressed in the iris and EGR2 is expressed in ciliary bodies and the choroid [18]. Moreover, these genes are reportedly associated with multiple immune-related diseases such as Behcet’s disease, Crohn’s disease, ulcerative colitis, atopic dermatitis, and systemic lupus erythematosus [25,26,40–44]. Thus, ADO-ZNF365-EGR2 may play suggestive effects in pathogenesis and mechanism of VKH disease in Japanese patients.

There were some differences in the disease-risk allele frequencies of IL23R-C1orf141 and ADO-ZNF365-EGR2 among control groups of Asian populations used in the current and previous studies (IL23R-C1orf141 rs78377598 and rs117633859: 4.5–5.9% in Japanese, Han Chinese Singaporeans [18], Thai [18], and Koreans [18], 9.5–9.7% in Han Chinese [17], 13.9–14.2% in non-Han Chinese from southwestern China [18]; ADO-ZNF365-EGR2 rs442309 and rs224058: 37.2–37.3% in Japanese, 25.6% in Han Chinese [17], 22.3% in Han Chinese Singaporeans [18], 30.8% in non-Han Chinese from southwestern China [18], 18.2% in Thai [18], 33.0% in Koreans [18]). However, the differences did not reflect the differences in association between VKH disease and the two loci among the Asian populations. The disease-risk allele frequencies of IL23R-C1orf141 are lower in Caucasians (2.5% in Caucasians from 1000 Genomes Project Phase 3 [45]) that have a low prevalence of VKH disease than in Asians, and those of ADO-ZNF365-EGR2 in Caucasians (52.0% [45]) are higher in Asians.

In conclusion, the current study confirmed a significant association between VKH disease and the two loci, IL23R-C1orf141 and ADO-ZNF365-EGR2, in the Japanese population, suggesting that genetic variants in these loci play important roles in disease development. To confirm and validate the correlation between VKH disease and these loci, future genetic studies with larger samples of Asian and other ethnic populations are needed.

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