Single Nucleotide Polymorphisms and Osteoarthritis
An Overview and a Meta-Analysis

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Abstract: Osteoarthritis (OA) is a complex disorder characterized by degenerative articular cartilage and is largely attributed to genetic risk factors. Single nucleotide polymorphisms (SNPs) are common DNA variants that have shown promising and efficiency, compared with positional cloning, to map candidate genes of complex diseases, including OA.

In this study, we aim to provide an overview of multiple SNPs from a number of genes that have recently been linked to OA susceptibility. We also performed a comprehensive meta-analysis to evaluate the association of SNP rs7639618 with double von Willebrand factor A domains (DVWA) gene with OA susceptibility.

A systematic search of studies on the association of SNPs with susceptibility to OA was conducted in PubMed and Google scholar. Studies subjected to meta-analysis include human and case-control studies that met the Hardy–Weinberg equilibrium model and provide sufficient data to calculate an odds ratio (OR). A total of 9500 OA cases and 9365 controls in 7 case-control studies relating to SNP rs7639618 were included in this study, where the ORs with 95% confidence intervals (CIs) were calculated.

Over 50 SNPs from different genes have been shown to be associated with either hip (23), or knee (20), or both (13) OA. The ORs of these SNPs for OA and the subtypes are not consistent. As to SNP rs7639618 of DVWA, increased knee OA risk was observed in all genetic models analyzed. Specifically, people from Asian with G-allele showed significantly increased risk of knee OA (A versus G: OR = 1.28, 95% CI 1.13–1.46; AA versus GG: OR = 1.60, 95% CI 1.25–2.05; GA versus GG: OR = 1.31, 95% CI 1.18–1.44; AA versus GA+GG: OR = 1.34, 95% CI 1.12–1.61; AA+GA versus GG: OR = 1.40, 95% CI 1.19–1.64), but not in Caucasians or with hip OA.

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease worldwide, affecting approximately 10% of men and 18% of women over 60 years of age.1,2 Multiple factors, including advanced age, excess body weight, repeated trauma or surgery to the joint structures, abnormal joints at birth, gout, diabetes, and hormone-related disorders, have been demonstrated to contribute to increased risk of OA.3,4 Previous epidemiological studies from twin-pair and family-based segregation analyses have provided clear evidence of a heritable component in susceptibility of OA. However, the specific genetic factors that lead to OA are currently largely unknown. It therefore remains a challenge to identify candidate genes or risk alleles that contribute to OA pathogenesis.

Identification of candidate genes responsible for numerous monogenic disorders has been successful over the past decades with the technology of positional cloning.5,6 Similar strategy has been used to target many complex diseases, including asthma, heart disease, cancer, and OA.5,7,8 However, due to lack of suitable genetic markers, not many candidate genes have been identified that show a clear OA etiology. Single nucleotide polymorphisms (SNPs) are common genomic DNA variations within a population. SNPs, in combination with genome-wide association studies (GWAS), have significantly accelerated complex disease gene localization.5,8,9 SNPs of genes COL1-A1, VEGF, GDF5, and IL-8, etc., have been associated with OA. Given the heterogeneity and complexity of OA, it is not surprising that these gene polymorphisms show different levels of association with increased risk of OA. Some polymorphisms may be specific to OA subtypes (hip-, knee-, or hand-OA) and ethnic groups, but the results are with a wide range of discrepancy.

Recently, multiple studies have indicated an association of double von Willebrand factor A domains (DVWA) gene with susceptibility to OA. DVWA gene variants encode 2 protein isoforms, the long (L-DVWA, 385 amino acids) and short (S-DVWA, 276 amino acids) proteins. DVWA protein is predicted to have 2 domains homologous to the VWA domain,
which typically is involved in cell adhesion and protein–protein interactions.\textsuperscript{10,11} Interestingly, L-DVWA and S-DVWA were mainly expressed in articular cartilage, suggesting a potential function of these isoforms in OA.\textsuperscript{12} Indeed, DVWA has been associated with susceptibility to knee OA and linked to OA etiology, possibly by interacting with β-tubulin. Data from later replication study and meta-analyses of one of the DVWA polymorphisms further confirmed its association with OA in European and Asian populations.\textsuperscript{13} However, no significant association between DVWA and OA was observed in Europeans.\textsuperscript{14} In addition, there are multiple SNPs in DVWA gene with allelic difference. The association between DVWA and OA has been inconsistent due to allelic effect and effect size of case-controlled studies analyzed.\textsuperscript{14}

In this study, we summarized and analyzed the SNPs that have recently been associated with OA and calculated their ORs with OA susceptibility. We also provided an updated and comprehensive meta-analysis to evaluate the association of SNP rs7639618 of DVWA with OA susceptibility with consideration of publication bias and the source of heterogeneity.

**MATERIALS AND METHODS**

**Literature Search**

A systematic search of studies on the association of SNPs with susceptibility to OA was conducted in PubMed and Google scholar. “Single Nucleotide Polymorphism”, “SNP”, and “Osteoarthritis” were used as key words for the searching. The odds ratios (ORs) with 95% confidence intervals were calculated on the basis of data provided in the literature. For meta-analysis, the PubMed was searched for eligible articles up to the end of June 2015. Following keywords were used for the searching “polymorphism”, “SNP”, “rs7639618”, “von Willebrand factor A domains”, “DVWA”, “Osteoarthritis”, “OA.” Google academic searching was also performed to obtain additional information.\textsuperscript{15}

**Study Selection**

Studies subjected to meta-analysis were selected according to the following inclusion criteria: human studies; studies investigating the association between the SNP rs7639618 and osteoarthritis (OA); case-control studies providing sufficient data on genotypes to calculate an OR; the genotype distribution of the control population met the Hardy–Weinberg equilibrium (HWE) model. Two reviewers were assigned to independently assess the studies using the inclusion criteria and disagreement was subjected to discussion with a third reviewer for a consensus agreement.

**Data Extraction**

The following data were collected from each eligible study: name of first author, years of publication, ethnicity, OA sites, HWE, number of cases and controls, genotype frequency in cases and controls. Different ethnicity descents were classified as Caucasian and Asian. When HWE in the controls was not reported, an online program (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to test the HWE by \( \chi^2 \) test for goodness of fit.\textsuperscript{16}

**Evaluation of the Study Quality**

The quality of the studies was evaluated by 2 reviewers according to the predefined scale for quality assessment, which was modified from previous meta-analysis.\textsuperscript{16,17} In this scale, 5 items, including the representativeness of cases, source of controls, sample size, quality control of genotyping methods, HWE, were carefully checked. Total scores were recorded with a range from 0 to 10 and a higher score indicated a better quality of the study. Any disagreement of the score was modified based on discussion between the 2 reviewers.

**Statistical Analysis**

Crude ORs together with their corresponding 95% CIs were used to assess the strength of association between the rs7639618 SNP and the risk of OA. The pooled ORs were calculated for allelic comparison (A versus G), heterozygote model (GA versus GG), dominant model (AA+GA versus GG), recessive model (AA versus GA+GG), respectively. Heterogeneity among the trials was analyzed in this study using the Q statistic (significance level of \( P \) value <0.10) and the \( I^2 \) test (greater than 50% as evidence of significant inconsistency). When significant heterogeneity (\( P < 0.10 \) or \( I^2 > 50\% \)) was achieved, the random effect model was used to combine the effect sizes of the included studies. If no significant heterogeneity was found, fixed effect was selected to pool the data. Meta-regression analysis was performed to detect the source of heterogeneity. The variance (\( r^2 \)) between studies was used to quantify the degree of heterogeneity and the percentage of \( r^2 \) was used to describe the extent of heterogeneity.\textsuperscript{17,18} Sensitivity analyses were performed to identify individual study effect on pooled results and test the reliability of results.\textsuperscript{17} In addition, subgroup analyses were stratified by ethnicity. Potential publication bias was estimated using the Begg test and a forest plot was used to analyze and display the results. All calculations were measured and analyzed using the STATA (version 11.0).

**Ethical Statement**

No ethical approval was needed for this manuscript, as the data used in this review and the meta-analyses have all been published.

**RESULTS**

**Multiple SNPs From a Number of Genes are Associated with OA**

Based on the quality of the studies and the selection criteria as described above, we summarized most of the SNPs that have recently been linked to OA susceptibility. As illustrated in Figure 1, there are 56 SNPs from 50 genes or gene loci that have been associated with OA or OA subtypes. Specifically, 23 SNPs from 21 genes, including COL11A1 (rs1241164, rs4907986, rs2615977),\textsuperscript{19,20} VEGF (rs833058), and IGF1 (rs2195239) etc., are associated with hip OA; 20 SNPs from 17 genes, including GDF5 (rs143383), ADAMTS14 (rs4774096), and DVWA (rs7639618, rs17113836, and rs3773472), etc., are frequently seen in knee OA; while individuals containing 13 SNPs of 12 genes, including IL8 (rs4073, rs2227306), TGFβ1 (2227306), and SMAD3 (rs12901499), etc., tend to develop both hip and knee OA. These results demonstrate involvement of multiple SNPs in susceptibility to OA and its subtypes.

**Odds Ratios of Candidate SNPs Associated With OA**

To evaluate the strength of association with OA, we have summarized the crude ORs of each of the candidate SNPs with 95% confidence intervals (CIs). Listed in Table 1 are detailed
Multiple polymorphisms are associated with the risk of OA. Illustrated are 56 SNPs from 50 genes that have been linked to OA risk. Among these, 23 SNPs from 21 genes have been associated with hip OA (pink ones); 20 SNPs from 17 genes are frequently altered in knee OA (green ones); while other 13 SNPs of 12 genes are reportedly associated with both knee- and hip-OA (yellow ones).

**TABLE 1. List of Genes and SNPs Associated With OA**

| Genes    | Associated Variant | Nucleotide Change | Diseased Region | OR (95% CI) | Reference          |
|----------|--------------------|-------------------|----------------|-------------|--------------------|
| ANP32A   | rs7165403          | T/C               | Hip OA         | 0.67 (0.53–0.84) | Valdes et al13 |
| ASTN2    | rs4836732          | C/T               | Hip OA         | 1.19 (1.1–1.28)  | Zeggini et al14 |
| CALM2    | rs10153674         | A/G               | Hip OA         | 1.65 (1.03–2.65) | Mototani et al15 |
| CAMK2B   | rs3757837           | A/G              | Hip OA         | 1.27 (1.15–1.41) | Evangelou et al16 |
| CHST11   | rs835487            | G/A               | Hip OA         | 1.15 (1.08–1.22) | Zeggini et al14 |
| COL11A1  | rs1241164           | C/T               | Hip OA         | 0.82 (0.74–0.89) | Rodriguez et al17 |
| COL11A1  | rs4907986           | C/T               | Hip OA         | 1.12 (1.06–1.17) | Rodriguez et al17 |
| COL11A1  | rs2615977           | G/T               | Hip OA         | 1.10 (1.05–1.15) | Panoutsopoulou et al19 |
| COL9A2   | rs7533252           | A/G               | Hip OA         | 2.1 (1.66–2.67)  | Nakki et al18 |
| DP1102   | rs225014            | A/G               | Hip OA         | 1.79 (1.37–2.34) | Meulenbelt et al19 |
| DNAS110  | rs10773046          | A/G               | Hip OA         | 1.07 (1.03–1.11) | Evangelou et al16 |
| DOT1L    | rs12982744          | C/G               | Hip OA         | 0.88 (0.82–0.94) | Castano et al20 |
| DYSK2    | rs10878630          | A/G               | Hip OA         | 1.09 (1.04–1.14) | Evangelou et al16 |
| FILIP1   | rs9350591           | C/T               | Hip OA         | 1.2 (1.11–1.3)   | Evangelou et al16 |
| HMGN3    | rs1577792           | A/G               | Hip OA         | 1.07 (1.04–1.11) | Zeggini et al14 |
| IFRD1    | rs5009270           | A/G               | Hip OA         | 1.1 (1.06–1.14)  | Evangelou et al16 |
| IGIF1    | rs2195239           | C/G               | Hip OA         | 1.18 (1.01–1.37) | Limer et al21 |
| IGFBP3   | rs788748            | A/G               | Hip OA         | 0.92 (0.86–0.99) | Evans et al22 |
| KLHDC5   | rs10492367           | G/T               | Hip OA         | 1.18 (1.11–1.27) | Zeggini et al14 |
| NACA2    | rs1761018            | A/G              | Hip OA         | 1.12 (1.06–1.18) | Evangelou et al16 |
| NCOA3    | rs6094710            | A/G              | Hip OA         | 1.28 (1.18–1.39) | Evangelou et al16 |
| PHF2     | rs12551314           | A/C               | Hip OA         | 1.16 (1.07–1.25) | Evangelou et al16 |
| Genes                  | Associated Variant | Nucleotide Change | Diseased Region | OR (95% CI) | Reference                  |
|-----------------------|--------------------|-------------------|-----------------|-------------|----------------------------|
| VEGF                  | rs833058           | C/T               | Hip OA          | 0.92 (0.88–0.97) | Rodriguez et al[^17]        |
| ADAM12                | rs1871054          | C/T               | Knee OA         | 1.84 (1.57–2.23) | Wang et al[^43]             |
| ADAMTS14              | rs4747096          | A/G/T             | Knee OA         | 1.25 (1.05–1.5)  | Rodriguez et al[^44]        |
| ASPN                  | rs13301537         | A/G               | Knee OA         | 1.61 (1.32–1.97) | Liang et al[^45]            |
| COL9A3 (1740C>T)      | rs61734651         | C/T               | Knee OA         | 1.31 (1.05–1.63) | Ikeda et al[^46]            |
| DUS4L                 | rs4730250          | A/G               | Knee OA         | 1.17 (1.11–1.24) | Evangelou et al[^47]        |
| DVWA                  | rs7639618          | C/T               | Knee OA         | 1.29 (1.15–1.45) | Miyamoto et al[^22]         |
| DVWA                  | rs11173836         | A/G               | Knee OA         | 1.37 (1.16–1.63) | Miyamoto et al[^22]         |
| DVWA                  | rs3773472          | C/G               | Knee OA         | 1.44 (1.22–1.7)  | Miyamoto et al[^22]         |
| ER-β                  | rs9340799          | A/G               | Knee OA         | 1.47 (1.08–1.99) | Ren et al[^48]              |
| ER-β                  | rs1256049          | G/A               | Knee OA         | 1.68 (1.16–2.44) | Lee et al[^49]              |
| GDF5                  | rs143383           | C/T               | Knee OA         | 1.18 (1.1–1.27)  | Zhang et al[^50]            |
| GPR22                 | rs3815148          | A/C               | Knee OA         | 1.14 (1.09–1.19) | Kerkhof et al[^51]          |
| HIF1A                 | rs11549465         | C/T               | Knee OA         | 5.03 (1.53–18.2) | Fernandez et al[^52]        |
| IL-16                 | rs11556218         | G/T               | Knee OA         | 0.77 (0.66–0.9)  | Luo et al[^53]              |
| IL-16                 | rs4072111          | A/G               | Knee OA         | 0.69 (0.58–0.81) | Luo et al[^53]              |
| IL-17A G-197A         | rs2275913          | A/G               | Knee OA         | 0.71 (0.53–0.96) | Han et al[^54]              |
| ILIRN                 | rs315920           | C/T               | Knee OA         | 1.18 (1–1.39)    | Limer et al[^41]            |
| IL-6                  | rs1800796          | G/C               | Knee OA         | 0.51 (0.32–0.8)  | Fernandes et al[^55]        |
| MMP8                  | rs1940475           | C/T               | Knee OA         | 0.72 (0.57–0.90) | Nakki et al[^56]            |
| SMAD3                 | rs12901499         | A/G               | Hip OA          | 1.22 (1.09–1.36) | Valdes et al[^57]           |
| SMAD3                 | rs12901499         | A/G               | Hip OA          | 1.22 (1.12–1.34) | Valdes et al[^57]           |
| TP63                  | rs12107036         | A/G               | Knee OA         | 1.23 (1.13–1.35) | Zeggini et al[^58]          |
| BA66                  | rs3117582          | C/A               | OA              | 0.63 (0.45–0.89) | Etokebe et al[^58]          |
| BA66                  | rs3117582          | A/C               | OA              | 1.58 (1.12–2.23) | Etokebe et al[^58]          |
| C9ORF3                | rs4385527          | C/T               | OA              | 0.62 (0.52–0.75) | Cui et al[^59]              |
| COX-2                 | rs20417            | C/G               | OA              | 0.57 (0.43–0.75) | Schneider et al[^60]        |
| GNL3                  | rs11177            | C/T               | OA              | 1.16 (1.11–1.22) | Zeggini et al[^34]          |
| IL-8                  | rs4073             | A/T               | OA              | 1.41 (1.02–1.94) | Yu et al[^61]               |
| IL-8                  | rs2227306          | C/T               | OA              | 1.48 (1.02–2.14) | Yu et al[^61]               |
| INSR                  | rs2059807          | C/T               | OA              | 1.15 (1.01–1.31) | Cui et al[^59]              |
| LHCGR                 | rs1340572          | A/G               | OA              | 0.82 (0.7–0.95)  | Cui et al[^59]              |
| LRCH1                 | rs912428           | C/T               | OA              | 1.42 (1.07–1.89) | Spector et al[^32]          |
| VDR Apal              | NA                 | NA                | OA              | 1.16 (1.02–1.32) | Zhu et al[^33]              |
| TGF-β1                | rs1982073          | C/G/T             | OA              | 1.26 (1.13–1.41) | Cong et al[^64]             |

ADAM12 = ADAM metalloproteinase domain 12; ADAMTS14 = ADAM metalloproteinase with thrombospondin type 1 motif 14; ANP32A = acidic nuclear phosphoprotein 32 family member A; ASPN = aspin; ASTN2 = astrotactin 2; BA66 = BCL2 associated athanogene 6; C9ORF3 = chromosome 9 open reading frame 3; CALM2 = calmodulin 2; CAMK2B = calcium/calmodulin-dependent protein kinase II beta; CHST11 = carbohydrate (chondroitin 4) sulfotransferase 11; COL9A2 = collagen, type IX, alpha 3; COL9A3 = collagen, type IX, alpha 2; DIO2 = diiodinase, iodothyronine, type II; DIO2 = diiodinase, iodothyronine, type II; DIO2 = diiodinase, iodothyronine, type II; DIO2 = diiodinase, iodothyronine, type II; DIO2 = diiodinase, iodothyronine, type II; DIO2 = diiodinase, iodothyronine, type II; DVWA = double von Willebrand factor A domains; DYRK2 = dual specificity tyrosine-(Y)-phosphorylation regulated kinase 2; ER-α = estrogen receptor 1 (alpha); ER-β = estrogen receptor 2 (ER beta); FHL1 = filament A interacting protein 1; GDF5 = growth differentiation factor 5; GNL3 = guanine nucleotide binding protein-like 3; GPR22 = G protein-coupled receptor 22; HIF1A = hypoxia inducible factor 1, alpha subunit; HMGN3 = high mobility group nucleosomal binding domain 3; IFRD1 = interferon-related developmental regulator 1; IFGBP3 = insulin like growth factor binding protein 3; IL16 = interleukin 16; IL-17A G-197A = interleukin-17A; ILIRN = interleukin 1 receptor antagonist; INSR = insulin receptor; LHCGR = Luteinizing hormone/choriogonadotropin receptor; LRCH1 = leucine-rich repeats and calponin homology (CH) domain containing 1; MMP8 = matrix metalloproteinase 8; NA = not available; NACA2 = nascent polypeptide-associated complex alpha subunit 2; NCOA3 = nuclear receptor coactivator 3; OR = odds ratios; PHF2 = PHD finger protein 2; SMAD3 = SMAD family member 3; TGF-β1 = transforming growth factor, beta 1; TP63 = tumor protein p63; VDR Apal = vitamin D (1,25-dihydroxyvitamin D3) receptor Apal; VEGF = vascular endothelial growth factor.

Information about the names of associated genes, the SNP numbers, locations of OA (knee- or hip-OA), OR values, and literature source. Most of the SNPs associated with hip OA showed a value of odds ratio greater than 1.0, with SNP rs225014 of DIO2 showing the highest value of 1.79 (1.37–2.34, Figure 2A, Table 1). The odds ratios of SNPs that are associated with knee OA were also calculated and most are above the value of 1.2 with SNP rs4747096 of ADAM12 showing the highest value of 1.84 (1.57–2.23, Figure 2B, Table 1). Similar results were observed for SNPs of genes that are associated with both knee and hip OA (Figure 2C, Table 1), although some SNPs showed an OR value less than 1.0 as seen in groups of hip- or knee-OA (Figure 2, Table 1). Together, these results suggest that multiple SNPs from a variety of genes showed different levels of association with knee and/or hip OA.
Eligible Studies Selected for Meta-Analysis of DVWA and OA

By searching the PubMed and Google scholar database using above key words (SNP, rs7639618, DVWA, and OA), we have identified approximately 50 relevant papers, including 38 studies that potentially show an association between DVWA and OA. After exclusion of duplicated studies and improper studies determined by reading the title and abstracts, 9 studies meet the selection criteria. Due to lack of genotype frequency in 2 of the studies, 7 studies were eventually subjected to meta-analysis of the association between SNP rs7639618 of DVWA and OA. The detailed process of literature screening is outlined in Figure 3. In these studies, 9500 OA cases and 9365 controls were identified according to the inclusion and exclusion criteria. Six knee-OA-related studies contained information from 6807 knee OA cases and 7785 controls,13,14,21–23 while 3 studies involving hip OA include 2693 hip OA cases and 1580 controls.13,14,25 The HWE of genotype distribution in the controls was tested in all studies and they were all in consistent with HWE. Detailed information about the OA types, the year and countries the studies conducted, allelic difference, power of HWE, and the quality score of the studies is provided in Table 2.

SNP rs7639618 is Significantly Associated with Knee OA

Through meta-analysis of SNP rs7639618 of DVWA, we observed a significantly increased risk of knee OA susceptibility in allelic comparison in Asians (A versus G: OR = 1.16, 95% CI 1.04–1.30 Figure 4). The OR values for each of the allelic models are listed in Table 2 and illustrated in Figure 5A (homozygote model, AA versus GG: OR = 1.39, 95% CI 1.10–1.76), Figure 5B (heterozygote model, GA versus GG: OR = 1.20, 95% CI 1.11–1.30), Figure 6A (homozygote model, AA versus GG: OR = 1.26, 95% CI 1.05–1.50), and Figure 6B (dominant model, AA+GA versus GG: OR = 1.20, 95% CI 1.04–1.39). As shown in Table 3, there was significant heterogeneity between studies, ranging from 0 to 0.029. We therefore performed subgroup analysis according to ethnicity. No heterogeneity was shown in heterozygote model and thus, a fixed model was applied for its pooled OR. For other allelic models that show significant heterogeneity, a randomized effect model was used. The results showed that there was a statistically increased knee OA risk in all allelic models (A versus G: OR = 1.28, 95% CI 1.13–1.46, Figure 4; AA versus GG: OR = 1.60, 95% CI 1.25–2.05, Figure 5A; GA versus GG: OR = 1.31, 95% CI 1.18–1.44, Figure 5B; AA versus GA+GG: OR = 1.26, 95% CI 1.05–1.50), and Figure 6B (dominant model, AA+GA versus GG: OR = 1.40, 95% CI 1.19–1.64, Figure 6B and Table 3). The results in Asians were similar to that of overall comparisons of pooled eligible researches (Table 3), while in Caucasians, even with increased sample size, there is no significant association in any allelic models compared as illustrated in Figure 4 (A versus G: OR = 1.00, 95% CI 0.87–1.16), Figure 5A (AA versus GG: OR = 0.96, 95% CI 0.61–1.51), Figure 5B (GA versus GG: OR = 1.04, 95% CI 0.92–1.19), Figure 6A (AA versus GA+GG: OR = 0.95, 95% CI 0.62–1.46), Figure 6B (AA+GA versus GG: OR = 1.00, 95% CI 0.82–1.22), and Table 3. Together, these results support that SNP rs7639618 of DVWA was only associated with an increased risk of knee OA in Asians.

No Significant Association Between SNP rs7639618 and Hip OA

The association between SNP rs7639618 of DVWA and the risk of hip OA was analyzed in 3 independent studies. Random-effects model was used in the dominant model and heterozygote model due to the presence of heterogeneity, while fixed-effects model was used in other models without significant heterogeneity. Overall, no significant association was identified in any of the allelic models analyzed (A versus G: OR = 0.98, 95% CI 0.89–1.08, AA versus GG: OR = 1.03, 95% CI 0.77–1.37, GA versus GG: OR = 0.89, 95% CI 0.74–1.07, Table 3).
AA versus GA+GG: OR = 1.10, 95% CI 0.85–1.41, and AA+GA versus GG: OR = 0.91, 95% CI 0.75–1.09, Figure 7). Five studies in 2 papers were carried out in Caucasian population. Subgroup analysis of Caucasian population was also assessed, and there is no significant association between SNP rs7639618 and hip OA in any allelic models analyzed (A versus G: OR = 1.00, 95% CI 0.89–1.12, AA versus GG: OR = 1.25, 95% CI 0.83–1.89, GA versus GG: OR = 0.91,

![FIGURE 3. Flow chart of study selection. Studies selected for current meta-analysis are as illustrated. Thirty-eight studies from 50 relevant papers show a potential association between DWVA and OA. Only 9 studies meet the selection criteria. Seven studies were eventually subjected to meta-analysis as 2 of the studies lack genotype frequency.](image)

| Studies and Subtypes | Year | Country | Case | Control | P for HWE | Quality |
|----------------------|------|---------|------|---------|-----------|---------|
| Knee OA              |      |         |      |         |           |         |
| Valdes14             | 2008 | Chingford | GG   | GA      | AA        |         |
| Valdes14             | 2008 | Nottingham | 505  | 201     | 27        | 475     | 169     | 11      | 0.350   | 6.5     |
| Miyamoto22           | 2008 | Japan    | 99   | 107     | 36        | 166     | 222     | 95      | 0.184   | 10      |
| Miyamoto22           | 2008 | China    | 145  | 187     | 85        | 106     | 192     | 115     | 0.156   | 10      |
| Meulenbelt13         | 2009 | UK       | 275  | 85      | 6         | 504     | 215     | 19      | 0.487   | 5       |
| Meulenbelt13         | 2009 | The Netherlands | 98   | 36      | 3         | 538     | 188     | 13      | 0.459   | 5       |
| Meulenbelt13         | 2009 | Spain    | 171  | 72      | 6         | 189     | 70      | 12      | 0.103   | 5       |
| Meulenbelt13         | 2009 | Greece   | 280  | 80      | 8         | 291     | 97      | 11      | 0.401   | 5       |
| Meulenbelt13         | 2009 | Japan 1  | 253  | 293     | 95        | 162     | 327     | 140     | 0.304   | 5       |
| Meulenbelt13         | 2009 | Japan 2  | 99   | 107     | 36        | 166     | 222     | 95      | 0.184   | 5       |
| Meulenbelt13         | 2009 | China    | 145  | 187     | 85        | 106     | 192     | 115     | 0.156   | 5       |
| Takahashi23          | 2010 | Japan    | 369  | 428     | 136       | 397     | 600     | 228     | 0.960   | 5       |
| Lee24                | 2013 | Korea    | 519  | 857     | 361       | 212     | 374     | 139     | 0.835   | 7       |
| Bravata21            | 2015 | Sicilian | 41   | 18      | 2         | 72      | 25      | 3       | 0.390   | 6       |
| Hip OA               |      |         |      |         |           |         |
| Valdes14             | 2008 | Chingford | 34   | 15      | 1         | 357     | 143     | 12      | 0.820   | 6.5     |
| Valdes14             | 2008 | Nottingham | 544  | 234     | 15        | 475     | 169     | 11      | 0.350   | 6.5     |
| Meulenbelt13         | 2009 | UK       | 808  | 288     | 20        | 504     | 215     | 19      | 0.487   | 5       |
| Meulenbelt13         | 2009 | The Netherlands | 67   | 31      | 1         | 538     | 188     | 13      | 0.459   | 5       |
| Meulenbelt13         | 2009 | Spain    | 175  | 84      | 8         | 189     | 70      | 12      | 0.103   | 5       |
| Zhu25                | 2010 | China    | 81   | 185     | 102       | 106     | 192     | 115     | 0.160   | 7       |
95% CI 0.73–1.13, AA versus GA+GG: OR = 1.27, 95% CI 0.84–1.92, and AA+GA versus GG: OR = 0.92, 95% CI 0.74–1.15, Figure 7).

Test of Heterogeneity and Sensitivity

As significant heterogeneity was shown between studies in each comparison, we therefore investigated the source of heterogeneity by ethnicity, year of report, and sample size using meta-regression analysis and subjected to allelic comparison (A versus G). Studies containing more than 1000 participants were categorized as “large,” while studies with less than 1000 participants were categorized as “small.” Meanwhile, group of cases with an average age greater than 65 were categorized as “high-risk,” while cases younger than 65 were assigned to “low-risk” group. Group of “mixed” cases indicate that no data of age are available. The results suggested that ethnicity (P = 0.002), group average age (P = 0.024), and the year the study conducted (P = 0.038), but not the sample size (P = 0.438), contributed to the source of heterogeneity. In addition, ethnicity, the average age, and year of study could explain 37% of the variance (τ²). We also performed sensitivity analysis to evaluate whether the present meta-analysis is stable and the results showed that no individual study affected the pooled OR (Figure 8A).

Publication Bias

A Begg funnel plot and a Begg test were used to assess for publication bias for all allelic models. The result showed no obvious asymmetry, indicating no publication bias (Figure 8B).

DISCUSSION

OA is characterized by progressive cartilage matrix degradation, subchondral bone sclerosis, and osteophyte formation. These are nonreversible processes due to the limited repair capacity of cartilage. With the disease advances, pain becomes the most prominent symptom which is almost unbearable and eventually leads to joint replacement. Thanks to the better life and medical conditions, people live longer nowadays, but it also raises the incidence and the population suffering from OA. Hence, OA is a major source of pain, disability, and a tremendous socioeconomic burden worldwide. Unfortunately, given the heterogeneity of multiple subtypes and the complexity of OA pathogenesis, it remains a challenge to find unanimous biomarkers that help with the early diagnosis and targeted therapy for all OA patients. Recently, personalized medicine becomes a hot topic in health care and may be achieved due to existence of SNPs, the most common genetic variations, within a population. There are many SNPs within different genes that have shown various associations with OA. Identification of these gene SNPs and their correlation with OA would further our understanding of the molecular mechanisms involved in the pathogenesis of OA, so as to develop better diagnostics and more targeted therapy at early stages of OA.

In this effort, we provided an overview of multiple SNPs in association with OA susceptibility. We identified more than 50 SNPs from a number of genes that have been linked to either hip (COL11A1, VEGF, etc.), or knee (COL9A3, ASPN, GDF5, etc.), or both (IL-8, TGF-B1, etc.) OA. Among these genes, GDF5, which encodes the growth differentiation factor 5, is a
member of the transforming growth factor-β superfamily. GDF5 has previously been shown to play a role in development and maintenance of bone and cartilage.\textsuperscript{14} Notably, SNP rs143383 of GDF5 is known to be associated with high risk of OA.\textsuperscript{14} The difference of GDF5 SNPs in relationship with OA subtypes and ethnic groups was also observed.\textsuperscript{29} In a recent GWAS associated meta-analysis of OA candidate genes, \textit{COL11A1} and \textit{VEGF} were significantly associated with OA. Interestingly, SNPs rs4907986 and rs1241164 of \textit{COL11A1} and SNP rs833058 of \textit{VEGF} all showed association with hip OA.\textsuperscript{14} It was previously shown that mutation in \textit{Coll11a1} may cause deposition of degraded type II collagen in articular cartilage and

FIGURE 5. Meta-analysis of the association rs7639618 and knee OA. A, Forest plot of homozygote model for overall comparison (AA versus GG: OR: 1.39, 95% CI 1.10–1.76). B, Forest plot of heterozygote model for overall comparison (GA versus GG: OR: 1.20, 95% CI 1.11–1.30).
eventually lead to OA.\textsuperscript{30} While it is relatively established for a role of GDF5, COL11A1, and VEGF in OA pathogenesis, the association between SNPs of these genes with OA susceptibility is not always consistent. SNP rs2615977 of COL11A1 was also shown to be associated with OA. However, another COL11A1 SNP rs1676486, which has been linked to lumbar disc herniation (LDH, also a degenerative musculoskeletal disease), was found not associated with OA.\textsuperscript{20} In our analysis, we tried to summarize most of the gene SNPs that have shown some correlation with OA or its subtypes. The OR of many of these SNPs/genes are not always consistent, further meta-analysis of multiple studies may overcome the limited sample size and

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**FIGURE 6.** Meta-analysis of the association rs7639618 and knee OA. A, Forest plot of recessive model for overall comparison (AA versus GA+GG: OR: 1.26, 95% CI 1.05–1.50). B, Forest plot of dominant model for overall comparison (AA+GA versus GG: OR: 1.20, 95% CI 1.04–1.39).
inadequate statistical power of single case-control studies to provide more reliable results.31

DVWA gene contains multiple SNPs, including rs11718863, rs7639618, rs7651842, rs7639807, rs17040821, etc. These SNPs may cause protein functional changes or diseases, as described in studies associated with OA.21 However, as indicated above, the results of their association with OA are not consistent. In this study, we specifically analyzed the association of SNP rs7639618 of DVWA with OA through comprehensive meta-analysis. We have analyzed all available eligible studies that include 9500 OA cases and 9365 controls. Our results confirmed that SNP rs7639618 is associated with a significantly increased risk of knee OA, especially in Asian populations. Similar results were found in subgroup analysis by ethnicity. No evidence was found for the association between rs7639618 SNP and hip OA susceptibility in any genetic allelic models. We have performed heterogeneity analysis and the results showed that ethnicity, average age of case group, and the year of the study were the source of heterogeneity. When we restricted the ethnicity to Asian, there was also heterogeneity, suggesting that ethnicity was not the main source of heterogeneity. We have shown that ethnicity, age, and year of study account for 37% of the variance ($\tau^2$) by meta-regression analysis, while the sensitivity analysis demonstrated that our meta-analysis is stable. In addition, no limitation was made in the search, and the selection bias was well controlled as demonstrated by Begg funnel plot analysis, which showed no publication bias.

In summary, it should be noted that due to limited number of studies on SNP rs7639618 and OA, the relatively small sample size may affect the power and statistics of the meta-analysis.32

### TABLE 3. Results of Meta-Analysis

|                 | A/G   | $P_H$ | A/A+G/G | $P_H$ | G/A+G/G | $P_H$ | A/A+G+G/G | $P_H$ |
|-----------------|-------|-------|---------|-------|---------|-------|------------|-------|
| Knee OA Overall| 1.16  | 1.00  | 1.39    | 0.000 | 1.20    | 0.029 | 1.26       | 0.016 |
| Ethnicity       |       |       |         |       |         |       |            |       |
| Asian           | 1.28  | 0.000 | 1.60    | 0.001 | 1.31    | 0.150 | 1.34       | 0.019 |
| Caucasian       | 1.00  | 0.174 | 0.96    | 0.199 | 1.04    | 0.304 | 0.95       | 0.241 |
| Hip OA Overall  | 0.98  | 0.086 | 1.03    | 0.685 | 0.89    | 0.105 | 1.10       | 0.853 |
| Caucasian       | 1.00  | 0.118 | 1.25    | 0.833 | 0.91    | 0.084 | 1.27       | 0.878 |

OA = osteoarthritis; OR = odds ratio; $P_H$ = $P$ value for heterogeneity.

FIGURE 7. Forest plot of allele comparison of DVWA rs7639618 for overall comparison (A versus G, association of rs7639618 and hip OA). No significant association between SNP rs7639618 and hip OA in any allelic models was detected (A versus G: OR: 1.00, 95% CI 0.89–1.12, AA versus GG: OR: 1.25, 95% CI 0.83–1.89, GA versus GG: OR: 0.91, 95% CI 0.73–1.13, AA versus GA+GG: OR: 1.27, 95% CI 0.84–1.92, and AA+GA versus GG: OR: 0.92, 95% CI 0.74–1.15).
models also needs to be treated with caution when interpreting the results. The insufficient sample size in single pioneer or replication studies of multiple SNPs, including DVWA, did result in a wide range of values of the ORs. However, we have provided a comprehensive overview of most of the relevant SNPs in OA or its subtypes. We have also updated all available studies on DVWA SNPs, and our results further support an association of SNP rs7639618 with OA as recently indicated. In addition, we have performed multiple analyses including subgroup, heterogeneity, sensitivity, and meta-regression assessment. The results showed that our meta-analysis is stable and we have analyzed the sources of the heterogeneity as indicated above. Together, our results support that rs7639618 SNP is significantly associated with increased risk of knee OA in Asians. There was insufficient data to support an association between SNP rs7639618 and the risk of hip OA or OA in Caucasians, although further studies are required to validate this genetic epidemiology and to functionally characterize this DVWA variant with OA pathophysiology.

FIGURE 8. The result of sensitivity and Funnel plot analysis. A, Sensitivity analysis showed no individual study affected the pooled OR. B, Funnel plot analysis showed no potential publication bias.
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