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## Association of the *DVWA* and *GDF5* Polymorphisms with Osteoarthritis in UK Populations.

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## Abstract

**Objective:** Variants in the growth differentiation factor-5 (*GDF5*) and in the double von Willebrand factor A (*DVWA*) have been recently reported to be associated with osteoarthritis (OA) in Asian populations. The aim of the current study was to assess the role of such variants in OA susceptibility in two independent UK samples of Caucasian origin.

**Methods:** Polymorphisms rs11718863 and rs7639618 (*DVWA*) and rs143383 (*GDF5*) were genotyped in 999 knee OA cases, 843 hip OA cases, and 1166 controls from two UK studies from Nottingham and Chingford.

**Results:** In agreement with previous reports, the major allele at rs143383 (*GDF5*) was associated with higher risk of knee OA in our samples ( $OR_{MH} = 1.29$  95% CI 1.14-1.47  $p = 8 \times 10^{-5}$ ). Conversely, the major allele at the *DVWA* SNP rs7639618, which increased risk in Asians, was not associated with risk of knee OA, ( $OR_{MH} = 0.88$  95% CI 0.74-1.03;  $p = 0.12$ ). A meta-analysis of Asian and UK knee OA data indicated highly significant heterogeneity ( $I^2 = 92\%$   $Q = 48.5$   $p = 7 \times 10^{-10}$ ) and no significant association with knee OA using a random effects meta-analysis ( $OR_{DL} = 1.18$  95%CI 0.86-1.63;  $p = 0.309$ ).

**Conclusions:** Our data confirm that the *GDF5* variant is consistently associated with risk of knee OA. We found considerable ethnic variation in allele frequencies at the *DVWA* gene and no significant association in UK samples nor combining UK to Asian samples. Our results suggest that the effect that *DVWA* amino acid changes have on tubulin binding is unlikely to influence risk of OA in Caucasians.

## Introduction

Osteoarthritis (OA) is the most prevalent form of arthritis in the elderly. Primary OA is an idiopathic phenomenon, occurring in previously intact joints, with no apparent initiating factor such as joint injury or developmental abnormalities. Several lines of work have shown a role of genetics in OA including familial aggregation studies,[1-2] twin studies, linkage analysis and genetic association studies.[3-4]

Recent studies in Japanese and Chinese populations have reported associations of various loci with knee or hip OA. The growth and differentiation factor 5 (*GDF5*) is known to be involved in the development and maintenance of bone and cartilage.[5] In addition genetic variation at this locus has been shown to influence height in humans.[6] The common, T-allele of the rs143383 single nucleotide polymorphism (SNP) is associated with higher risk of knee and hip OA in Asian populations (OR =1.55,  $p < 1 \times 10^{-6}$ ) and luciferase reporter assays have revealed that the same allele mediates a significant reduction in the activity of the *GDF5* promoter.[7] Replication in European samples of the *GDF5* association showed a weaker effect of this variant than the one reported in Asians, statistically significant for knee OA (OR=1.13) but weaker for hip OA (OR=1.04 n.s.). [8] However, other variants, such as an intronic polymorphism in the *CALM1* gene which showed a strong association in the Japanese population [9] have been found not to have any effect on risk of OA in European studies.[10-11]

More recently a locus which had been until then been considered only a hypothetical gene (*LOC344875* GeneID= 344875) theoretically encoding a 1504 amino acid protein was found to be a real gene which actually encodes a 276 amino acid peptide and it was called by the authors double von Willebrand factor A (*DVWA*).[12] The common variants at two non-synonymous polymorphisms in the *DVWA* gene, rs11718863 and rs7639618, were reported in Japanese and Chinese patients to be very strongly associated with risk of knee OA (OR 1.43  $p < 7 \times 10^{-11}$ ) and to influence the binding of the DVWA protein to beta-tubulin. The authors concluded that the weaker tubulin binding by the wild-type protein was the likely molecular mechanism by which genetic variation in this gene was affecting risk of knee OA and that therefore tubulins and microtubules could be protective factors in the pathogenesis of osteoarthritis.

In this study we investigated if the above mentioned genetic variants were associated with OA of the knee and the hip in two independent United Kingdom studies.

## Subjects and Methods

### Study subjects:

**1-Nottingham case-control study:** Individuals affected by knee or hip OA were recruited in Nottingham both from families with a history of OA and from clinic populations. All research participants gave written informed consent to take part. Approval for recruitment of index knee and hip OA cases and siblings of index hip OA cases was obtained from the research ethics committees of Nottingham City Hospital and North Nottinghamshire.

Hip and knee OA cases were recruited from hospital orthopaedic surgery lists (current and for the previous 5 years). All had been referred to hospital with symptomatic, clinically severe hip or knee OA and the majority had undergone unilateral or bilateral total hip replacement (THR) or total knee replacement (TKR) within the previous 5 years. Subjects were excluded from the study if they had other major arthropathy (e.g. rheumatoid arthritis, ankylosing spondylitis); Paget's disease of bone affecting the pelvis or femur; overt childhood hip disease (e.g. Legg-Calve-Perthe's disease, slipped femoral epiphysis, severe acetabular dysplasia); THR due to trauma or avascular necrosis of the femoral head; or terminal illness. We only included patients if they had a diagnostic code of primary hip osteoarthritis, and we excluded patients aged over 90 or those who had had surgery at under 40 years of age. Cases were further characterized by enquiry, examination and investigation. Height and weight were measured to calculate BMI. Controls were age matched individuals from the same hospital catchment area. Unaffected sibs of joint replacement probands, free from radiographic OA and over the age of 55 were also used as

controls. At most one affected and one unaffected sib per family were included and before including sibs in the genetic association analyses we confirmed that there were no differences in allele frequencies between unrelated controls and sib controls. For the *GDF5* SNP the minor allele frequencies (MAFs) were 37.1% in unaffected sibs and 38.4% in unrelated controls,  $\chi^2_{(1 \text{ df})} = 0.23$   $p < 0.63$  n.s. For the *DVWA* SNPs the MAF in unrelated controls was 14.9% and 15.4% in sib controls ( $\chi^2_{(1 \text{ df})} = 0.05$   $p < 0.82$ ). Hence we concluded that unaffected sibs were equivalent to unrelated controls in terms of allele frequencies at the polymorphisms studied. Pre-operative knee or pelvis radiographs of index knee or hip OA cases were examined to confirm the diagnosis and to grade for changes of OA. For siblings of index hip OA cases, new antero-posterior pelvis radiographs were obtained. Siblings of knee cases were assessed for radiographic knee OA. All pelvis and knee radiographs were scored for features of OA by a single observer using the Kellgren and Lawrence grade for the tibio-femoral compartments of each knee and each hip.[13] Self-reported ethnicity was assessed by a nurse administered questionnaire and only individuals of European descent were included in the genetic study. Additional details on these samples can be found in [1-2].

**2- The Chingford Study** is a prospective population-based longitudinal cohort of Caucasian women (assessed by questionnaire) derived from the age/sex register of a large general practice in North London, representative of the general UK population in terms of weight, height and smoking characteristics. The study design and rationale are described elsewhere in detail.[14] The Guys & St Thomas' Trust and Waltham Forest Trust ethics committees approved the study protocol. After study procedures were explained to participants, they gave written consent. The Kellgren and Lawrence (KL) grade was scored for the tibio-femoral compartment of each knee.[13] Radiographic knee OA was defined as a KL score  $\geq 2$  of one or both joints. Radiographic hip OA was defined definite as joint space narrowing and a  $KL \geq 2$  at either the right or left hip.

#### Laboratory methods:

For both Nottingham and Chingford study participants, genomic DNA was extracted from peripheral blood leukocytes of affected individuals and controls using standard protocols. Genotyping was carried out by Kbioscience Ltd, Hertfordshire UK. SNPs were genotyped using the KASPar chemistry, which is a competitive allele-specific PCR SNP genotyping system using FRET quencher cassette oligos. Genotyping accuracy, as determined from the genotype concordance between duplicate samples was 99.6%. All three polymorphisms were in Hardy-Weinberg equilibrium in controls ( $p > 0.05$ ).

#### Statistical Analysis

Allele odds ratios were calculated by comparing the number of alleles among cases and controls and the p-value was computed using a Pearson's chi-square. Only an additive model was tested for alleles. For the *DVWA* SNPs only the allelic tests were carried out as this is the only model reported in the literature. Because a previous meta-analysis reported both additive and dominant models for the *GDF5* polymorphism, we also tested different genetic models. A logistic regression was carried out with disease status being the dependent variable, genotype status as the independent variable 0=CC, 1=CT+TT in the dominant model and 0=CC+CT, 1=TT in the recessive model (including age, BMI and sex as covariates in the model. Only the best fitting model is reported.

*Statistical Power:* the statistical power given the minor allele frequencies (MAFs) of the *GDF5* and *DVWA* variants in our European samples given a specified odds ratio, for hip and knee OA was calculated using a log-additive (co-dominant) genetic model with an  $\alpha = 0.05$ . The Quanto Software v 1.2. (University of Southern California, CA, USA <http://hydra.usc.edu/gxe>) was used

*Fixed effect meta-analyses:* In the absence of inter-study heterogeneity within samples we constructed a Mantel-Haenszel meta-analysis of data from the samples to assess the overall evidence of association. The Mantel-Haenszel chi-squared test and the Mantel-Haenszel estimate of the odds ratio ( $OR_{MH}$ ) [15] were used to provide a summary test and odds ratio.

*Random effects meta-analysis:* In the DerSimonian and Laird method, studies are considered as a random sample from a population of studies. The random effect model incorporates the heterogeneity of

the studies. The overall treatment effect is estimated by a weighted average of the individual effects with weights inversely proportional to the variance of the observed effects. We tested the assumption of heterogeneity for each planned analysis using the method of DerSimonian and Laird based on work first presented by Cochran.[15] The statistical significance of the combined effect for the DerSimonian–Laird odds ratio ( $OR_{DL}$ ) was estimated using the Z-statistic which is the ratio of the point estimate to its standard error.

## Results:

The descriptive characteristics of cases and controls in the Chingford and Nottingham samples are presented in table 1.

**Table 1.** Descriptive characteristics of the study cohorts

Osteoarthritis Status	Origin of Study	n	Sex F/M (F%)	BMI kg/m <sup>2</sup>	(SD)	age yrs	(SD)
Unaffected controls	Chingford	512	512/0 (100%)	26.15	(4.26)	63.00	(5.80)
radiographic knee OA (KL $\geq$ 2) *	Chingford	264	264/0 (100%)	28.08	(5.29)	66.28	(6.23)
radiographic hip OA (KL $\geq$ 2 and JSN>0) *	Chingford	50	50/0 (100%)	25.46	(4.72)	67.42	(6.71)
Unaffected controls	Nottingham	654	371/383 (57%)	26.47	(3.91)	66.89	(10.81)
total knee replacement	Nottingham	735	428/308 (58%)	29.54	(5.13)	68.48	(8.52)
total hip replacement	Nottingham	793	532/261 (67%)	26.99	(4.41)	66.06	(9.41)

\* KL= Kellgren and Lawrence radiographic grade, JSN= joint space narrowing grade, 14 individuals were affected at both the hip and the knee by this definition.

We computed the statistical power available to us and assessed that, given the sample size available combining both studies and the allele frequencies in controls we had 80% statistical power to detect a minimum odds ratio of 1.21 for either knee OA or hip OA for the *GDF5* risk allele using a co-dominant (additive) genetic model,  $\alpha=0.05$ . For the *DVWA* variants, the major allele frequency is 85% in the UK controls, so the combined studies had 90.87% statistical power to detect an association with knee OA with an odds ratio of 1.34 ( $\alpha=0.05$ ) for the major allele which was the average odds ratio reported by Miyamoto and co-workers [12] in their replication samples (Japan set C + Chinese). No previous data exist on hip OA and *DVWA*, but the current sample size would allow us to detect an odds ratio of 1.30 or higher for the major allele with 80% power and  $\alpha=0.05$ . Hence, the current study is sufficiently powered to detect the size of genetic effects previously reported with knee OA for *DVWA* in replication studies.

We found that the major allele at the *GDF5* variant was significantly associated with knee OA both in the Chingford and Nottingham studies (Table 2). A combined analysis of the T allele using an additive model on knee OA resulted in an odds ratio  $OR_{MH}$  1.29 (95%CI 1.14-1.47,  $p=8 \times 10^{-5}$ ) and of  $OR_{MH}$  1.12 (95% CI 0.97-1.29  $p<0.12$ ) for hip OA. There was no evidence of heterogeneity between the two studies ( $I^2=0\%$  for both knee OA and hip OA). The genetic model found to give the smallest p-value in these samples was a recessive one, i.e. the TT genotype vs. the CC +CT genotypes, and the *GDF5* genotype was significantly associated with knee OA in both studies and also with THR in the Nottingham study after adjustment for sex, age and BMI (Table 2).

**Table 2.** Association between the GDF5 SNP rs143383 and osteoarthritis of the knee and the hip.

study		MAF (minor C/ major T)	risk allele = major (T) OR risk allele (95% CI)	P-value	Genotypes			OR recessive	
					CC	CT	TT	adj for age, BMI, sex	P-value
<i>Chingford</i>	controls	40.5% (412/606)			84 (16.5%)	244 (47.9%)	181 (35.9%)		
	knee ROA	32.4% (168/350)	<b>1.42 (1.13-1.76)</b>	<b>0.0021</b>	35 (12.6%)	98 (39.7%)	126 (47.5%)	<b>1.95 (1.40-2.71)</b>	<b>8x10<sup>-5</sup></b>
	hip ROA	41.00% (41/59)	0.97 (0.68-1.37)	0.88	18 (24.0%)	27 (34.0%)	32 (42.0%)	1.33 (0.72-2.46)	0.36
<i>Nottingham</i>	controls	37.69% (487/805)			79 (12.2%)	329 (50.9%)	238 (36.8%)		
	TKR	32.85% (483/987)	<b>1.23 (1.05-1.44)</b>	<b>0.0079</b>	85 (11.5%)	313 (42.5%)	337 (45.8%)	<b>1.54 (1.22-1.94)</b>	<b>2x10<sup>-4</sup></b>
	THR	34.62% (545/1029)	1.14 (0.98-1.33)	0.086	103 (13.0%)	339 (43.0%)	345 (43.8%)	<b>1.34 (1.08-1.66)</b>	<b>0.007</b>

\* adjusted for age, sex and BMI

MAF= minor allele frequency ROA= radiographic OA TKR= total knee replacement THR=total hip replacement

Figure 1A shows that combining the present hip OA data with the European hip OA data from Chapman et al [8] did not yield a significant result overall (OR=1.07 95%CI 0.98-1.16 p=0.13). For knee OA data, on the other hand, a statistically significant odds ratio was seen combining the European samples reported by Chapman et al [8] with our data (figure 1B). Therefore our data increase the support in the literature for a role of the *GDF5* promoter variant in risk to knee OA.

The two SNPs in the *DVWA* gene reported to be associated with knee OA and tubulin binding in Asian populations are rs11718863 and rs7639618. rs11718863 encodes a Tyr to Asn substitution and rs7639618 encodes a Cys to Tyr change. The minor allele frequencies for these SNPs are much lower in European than in Asian samples as also seen in public domain data ([http://www.hapmap.org/cgi-perl/gbrowse/hapmap\\_B36/](http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B36/)). We found that in UK controls from the present study the minor allele frequencies of these SNPs were 15.33% for rs7639618 and 15.37% for rs11718863, yet were 48.3% and 48.0% respectively in the Japanese samples and over 50% in the Chinese samples studied by Miyamoto and coworkers.[12]

In the UK samples studied here these two SNPs are in almost complete linkage disequilibrium (genotypes are identical for all except one of the individuals genotyped resulting in  $r^2=0.988$   $p<1\times 10^{-250}$  in controls). Hence we only show the association data for rs7639618 (Table 3).

**Table 3** Association between *DVWA* rs7639618 (rs11718863)\* SNP and osteoarthritis of the knee and the hip.

cohort		MAF	risk allele	P-value	Genotypes		
		(minor A /major G)	= major (G) § OR risk allele (95% CI)		GG	AG	AA
<b>Chingford</b>	controls	16.3% (167/857)			357 (69.7%)	143 (27.9%)	12 (2.34%)
	knee ROA	15.9% (85/444)	1.03 (0.77-1.37)	0.84	188 (71.2%)	68 (25.8%)	8 (3.03%)
	hip ROA	17.0% (17/83)	0.95 (0.55-1.64)	0.86	34 (68.0%)	15 (30.0%)	1 (2.00%)
<b>Nottingham</b>	controls	14.60% (191/1117)			474 (72.4%)	169 (25.8%)	11 (1.68%)
	TKR	17.39% (255/1211)	<b>0.81 (0.66-0.99)</b>	<b>0.046</b>	505 (68.8%)	201 (27.4%)	27 (3.68%)
	THR	16.64% (264/1322)	0.86 (0.70-1.05)	0.13	544 (68.6%)	234 (29.5%)	15 (1.89%)
<b>Japan 1¶</b>	controls	48.25%(607/651)					
	knee OA	37.68% (483/799)	<b>1.54 (1.32-1.81)</b>	<b>7.3 x 10<sup>-8</sup></b>			
<b>Japan 2¶</b>	controls	42.65% (412/554)					
	knee OA	36.98% (179/305)	<b>1.27 (1.01-1.59)</b>	<b>0.038</b>			
<b>Chinese¶</b>	controls	51.09% (422/404)					
	knee OA	42.81% (357/477)	<b>1.40 (1.15-1.69)</b>	<b>0.0007</b>			

\* given the almost complete LD between rs11718863 and rs7639618 allele and genotype counts for rs11718863 are identical to those shown for rs7639618 except for one individual (a control).

§ risk is reported for the major allele for consistency with the original report [12].

¶ Data from reference [12], Japan 1 refers to "set B" and Japan 2 to "set C" in reference [12].

The *DVWA* rs7639618 major allele, associated with increased risk of knee OA in Asians, was significantly associated with lower risk of total knee replacement and not with radiographic knee OA nor with hip OA (Table 3). Combining the two studies using TKR in the Nottingham cohort and radiographic OA in the Chingford we obtained a summary Mantel-Haenszel  $OR_{MH} = 0.88$  (95% CI 0.74-1.04  $p < 0.12$ ) for the association of allele G with knee OA and heterogeneity between the two UK studies did not achieve statistical significance ( $I^2 = 37%$ ,  $p < 0.21$ ). For hip OA the combined analysis resulted in  $OR_{MH} = 0.88$  (95% CI 0.72-1.05  $p < 0.14$ )

For comparison, the allele counts and frequencies in the Japanese and Chinese case-control studies previously reported [12] are also shown (Table 3). When we combined the current UK data with the published Japanese and Chinese data for rs7639618 we found very strong evidence of heterogeneity between studies ( $I^2 = 91.8%$   $p < 7 \times 10^{-10}$ ) therefore we used a random effects DerSimonian-Laird model. The odds ratios of individual studies and the meta-analysis for knee OA and the *DVWA* variant are summarized in figure 2. The random effects model resulted in  $OR_{DL} = 1.18$ , 95%CI 0.86-1.63;  $p = 0.309$  for knee OA for the combined Asian and European studies indicating no support for this variant being involved in risk of knee OA overall.

## Discussion

In this study we have confirmed a role for the *GDF5* promoter variant in genetic susceptibility to knee OA in individuals of European descent from the UK. Consistent with previous data the association is stronger for knee OA than for hip OA, and we find that this association with knee OA in Europeans is found regardless of the genetic model used.

We also found that the major allele of non-synonymous polymorphisms in the *DVWA* gene, previously reported to be involved in tubulin binding and strongly associated with higher risk of knee OA in Chinese and Japanese patients, was, on the contrary, associated with lower risk of total knee replacement a UK study. This variant was not associated with the milder radiographic form of the disease nor with hip OA in UK samples, in spite of the fact that our study was sufficiently powered to detect genetic associations of the same magnitude or smaller than those originally reported for variants in the *DVWA* gene. We must note however that our control samples consisted both of unrelated controls and of age-matched unaffected sibs of OA patients which could potentially decrease our power to detect an association. Nevertheless, because the allele frequencies in unaffected sibs and in unrelated controls were extremely similar to each other this is unlikely to have affected our final conclusions.

The major difference between our data in UK samples and the study in Asians is that the rs7639618 G allele which was associated with higher risk of knee OA in the original report is associated with lower or no risk in our study. When Asian and UK data were combined we did not find any evidence for association between knee OA and this variant.

There are at least two different explanations for this discrepancy. First, both our TKR result and those in the original report in Asians could be false positives and the meta-analysis result would appear to favor that hypothesis. However, a second explanation could be that *DVWA* is really implicated in the pathogenesis of OA but that rs7639618 and the linked rs11718863 are not the true functional variants in the *DVWA* region involved in risk of OA. In such a hypothetical scenario it would be possible that a different SNP could be truly implicated in the pathogenesis of knee OA and such a theoretical SNP would need to be in positive LD in the UK cohorts studied here with the "A" allele in rs7639618 (encoding a Tyr) but in negative LD with the "A" allele and in positive LD with the "G" allele (encoding a Cys) reported in the Asian cases.[12]

The minor allele frequency of rs7639618 is three times lower among Caucasian than among Asian samples both in public domain data as well as in the present study. Further some notable differences in LD patterns can be seen for some of the SNPs in the same LD block studied by Miyamoto and coworkers.[12] For example, rs353093 is an intronic SNP in *DVWA* which was also associated with knee OA in that study with  $p < 6 \times 10^{-4}$ . LD between rs7639618 and rs353093 is  $r^2 = 0.53$  in Asians from HapMap and  $> 0.7$  in the Japanese samples studied in [12]. In Caucasians on the other hand, (CEPH data from HapMap release B36) LD is almost absent with an  $r^2 = 0.028$ . Hence, it is possible that differences in linkage disequilibrium in these two populations could account for the observed discrepancy.

The data at hand cannot definitively exclude that the discrepancies we find are due to LD and we must also note the lack of reproducibility among Caucasian patients of other very strong genetic associations with OA previously reported in Asians have been described. A recent meta-analysis of the *EDG2* gene [16] found no evidence for association of a promoter variant with OA in individuals of European descent, although it had been previously reported to be strongly associated with knee OA in Japanese patients. Similarly, the association of the *CALM1* promoter variant was not replicated in any of the Caucasian samples tested to date [8-9]. Such lack of reproducibility of Asian associations in Caucasian patients may have a number of explanations, including genetic or even environmental differences between Asians and Europeans with regards to their risk of OA.

The results from the present study indicate that the differences in tubulin binding by the *DVWA* protein due to the amino-acid changes encoded by rs7639618 and rs11718863 are unlikely to be responsible for any functional involvement of this gene in OA in Caucasians.

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**Competing interests:**

The authors declare no conflict of interest.

**Figure Legends.**

Figure 1: Forest plot of study-specific estimates and fixed effects summary odds ratio (OR) and 95% confidence interval (CI) for the association between rs143383 and (A) hip OA, (B) knee OA.

Figure 2: Forest plot of study-specific estimates and random effects summary OR and 95% confidence interval (CI) for the association between rs7639618 and knee OA in UK and Asian samples.

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