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## Genomewide Linkage Scan of Hand Osteoarthritis in Female Twin Pairs Showing Replication of Quantitative Trait Loci on Chromosome 2 and 19

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**Abstract**

**Objective.** Until recently there has been little agreement between conflicting OA linkage results. The purpose of this study was to conduct a whole-genome linkage scan to identify susceptibility loci for idiopathic hand OA in a large, population-based sample of females.

**Methods.** Two OA-related radiographic phenotypes: DIP-OA (distal interphalangeal joints) and Tot-KL (Kellgren-Lawrence score for both hands) chosen *a priori* were examined on 592 (296 pairs) monozygous (MZ) and 1292 (509 pairs) Dizygous (DZ) females. A genomewide scan using microsatellite markers spaced every 10 cM was performed on 1018 DZ twins. First, heritability of the two OA phenotypes was estimated. Next, multipoint linkage analysis was conducted using a modified version of the Haseman-Elston method in a generalized linear model.

**Results.** Heritability for DIP-OA and Tot-KL were found to be 47.6% and 67.4%, respectively. A genomewide scan produced reliable evidence of significant linkage of DIP-OA on chromosome 2 at 90cM (LOD=2.90) and for Tot-KL on chromosome 19 at 65cM (LOD=4.26). These results are in agreement with data previously published. Several other significant linkage peaks were observed, e.g. on chromosomes 1 at 250cM and 3 at 30cM, but were less reliably confirmed.

**Conclusion.** This is one of the largest OA linkage studies performed to date and provides clear evidence for linkage at two quantitative trait loci (on Ch 2 at 90cM and Ch 19 at 65cM). Since the results were robust and replicated in previous smaller studies, the fine mapping of these regions is a logical next step to pinpoint potential susceptibility gene(s) of interest.

**Key words:** Kellgren-Lawrence scores, distal interphalangeal joints, heritability, multipoint linkage analysis, Haseman-Elston method, permutation.

## Introduction

It is well established that idiopathic osteoarthritis (OA), and in particular hand OA is a heterogeneous and multifactorial process of joint degeneration, which appears with exceptionally high prevalence in aged populations. A number of community based studies showed that the majority of adults over 55 years of age have radiographic evidence of hand OA (1). Determining definitive etiologic factors, however, poses methodological and other problems. Different factors are known to be associated with OA, including age, gender, biomechanics, ethnic background, genetics and others (2).

After age, genetic factors play a major role, and in the majority of studies have explained 30%-60% of the residual variation (2-5). Several genome-wide scans with hand OA have been reported, but the results have been inconsistent, often because of small sample size and low power. Despite some reasonable sized studies, results of only marginal significance have been obtained (3,4,6). Such conflicting results might reflect differences in sample ascertainment, age and cohort effects, false positive results due to multiple testing problems or methodological problems such as in disease definitions. This is particularly complex in hand OA, where numerous and small joints are examined, which may have different age related rate of OA progression (7,8).

In an attempt to clarify this situation we have used a large community-based sample of DZ twin pairs to search for susceptibility loci by whole-genome linkage screening.

## Material and Methods

### *Sample and Phenotypes.*

The data examined in the present study were from the TwinsUK Adult Twin Registry (described in detail elsewhere 9). All participants gave written informed consent before entering the study and the St. Thomas' Hospital research ethics committee approved the project. Twins have been shown to be similar to age-matched singletons for a range of health and lifestyle variables (10).

Plain posterior-anterior hand radiographs with both hands placed flat were obtained from each study participant. The films from twins were not read paired nor necessarily close in time. The radiographic features of hand OA: 1) the presence of osteophytes (OSP) and 2) joint space narrowing (JSN) were examined at each of 15 joints, on each hand. OSP and JSN were separately evaluated and graded from 0 to 3 for increasing severity using a standardized atlas (11). In addition, the summary grade for each joint of the hand joints (30, in total) was evaluated according to the Kellgren and Lawrence (KL) grading system (0-4) and in accordance to the original atlas. The intra-observer reproducibility for each site/trait was estimated using a weighted kappa statistic and found to be satisfactory ( $>0.78$ ) for different joints and features. For the present genetic analysis we 'a priori' selected two clearly defined OA phenotypes, used in other studies: 1. DIP-OA, obtained as a sum of OSP and JSN scores of 10 distal interphalangeal joints, and 2. Tot-KL, the sum of KL scores for all 30 joints, on both hands. The main reasons for selecting these phenotypes were that they had the highest reproducibility and highest prevalence in the sample, of all the OA phenotypes available. They showed the closest correlation with age, and after adjustment were the closest to a normal distribution. Finally, these traits had the strongest heritability of all hand joint combination studied.

**Genotyping.**

Genome-wide linkage analysis used 737 highly polymorphic DNA markers spaced approximately every 10 cM using standard fluorescence-based genotyping methodologies, and are described in full detail elsewhere (12). The estimated genotyping error rate was <1%.

**Statistical analysis.**

First, all 538 MZ and 1256 DZ twins having complete radiological data available for all joints, were included in analysis of the OA-phenotypes distribution, adjustment for age and model fitting analysis to estimate heritability of the chosen phenotypes (13). Next, 1028 DZ twins genotyped at each DNA marker were subjected to multipoint linkage analysis (MPL). This was conducted using generalized linear modelling (GLM) in STATA (StataCorp, version 9, 2005). This technique is based on optimal Haseman and Elston methods (14). This method is algebraically equivalent to other likelihood techniques but has the advantage of being robust to deviations in multivariate normality by freely estimating the coefficient of variation (i.e., the mean and variance-corrected residual error) and by utilizing a robust Huber estimate of variance (14). Regression diagnostics were used to check the reliability of model fit, including an iterative regression (IR) routine applied to GLM by use of the Huber estimate, followed by biweight iterations that removes the gross outliers prior to calculating starting values and then performs the iterations. On a genome-wide basis, any observed divergence between GLM and GLM<sub>IR</sub> results indicates potentially poor-fitting models for specific regions. The prioritised regions were selected if the LOD score obtained were >2.5 and consistent between GLM and GLM<sub>IR</sub> results. The final confirmation of the positive results were made by computing the genome-wide significance level (empirical p-value) using a permutation approach, which is not affected by violation of the normality assumption, as implemented in STATA package.

**Results**

Both OA phenotypes correlated significantly ( $p < 0.001$ ) with age and age squared. Some 12.0% and 26.4% of DIP-OA and Tot-KL variation was attributable to age effects. Likelihood ratio test of the patterns of the traits inheritance revealed that the most parsimonious model included contributions from additive genetic effects and random environment, with the heritability estimates 0.48 (95% CI: 0.40 – 0.54) for DIP-OA and 0.67 (95% CI: 0.610 - 0.728) for Tot-KL respectively. In agreement with this were intra-class correlation coefficients in the DZ cohort, for both DIP-OA and Tot-KL (0.24 and 0.25,  $p < 0.001$ , for both variables). The phenotypic correlation between the adjusted Tot-KL and DIP-OA was 0.67 ( $p < 0.001$ ), suggesting that a substantial part of the variation (~55%) of each variable may be governed by independent factors.

Figure 1 depicts the evidence for linkage for the entire genome scan, for DIP-OA and Tot-KL. The figure shows all nominal LOD-scores estimated by using the freely estimating coefficient of variation option that is robust to phenotypic departure from normality assumption. By our rigid criteria of  $\text{LOD} > 2.5$ , five chromosomal areas were identified both for DIP-OA and for Tot-KL. The corresponding LOD scores and their respective chromosomal locations are summarized in Table 1. These results were checked using the iterative robust routine. For DIP-OA we obtained good confirmation of the results on chromosome 2, where both multipoint linkage (MPL) peaks coincided (Fig 2A). A marginal confirmation was observed also on chromosome 3, where the LOD score obtained in robust analysis reached 3.92, however, the corresponding linkage peak was located at 45cM position (Fig 2A). For Tot-KL we obtained clear correspondence in the results on

chromosome 1 ( $LOD_{IR} = 2.67$ ) and chromosome 19, where the respective IR peak was even higher, achieving value 5.19 (Fig 2B). The empirical p-values for corresponding linkage peaks were estimated as 0.005 for Tot-KL on chromosome 1, and 0.002 on chromosome 19, and 0.023 for DIP-OA on chromosome 2.

**Table 1. Significant multipoint LOD scores obtained at the first stage of analysis**  
(\* shows results confirmed by  $GLM_{IR}$ ).

Chromosome	Location (cM)	Closest marker	Marker Location (cM)	Nominal LOD
<b>Phenotype: DIP-OA</b>				
2p13.2-2p14*	90	D2S285	86.2 - 89.9	2.9
3p25.1-3p25.2*	30	D3S1263	30.4 - 36.1	2.8
9q34.2-9q34.3	155	D9S164-	147.9 - 148.1	4.5
		D9S1826	159.6 - 160.2	
12q21.33-12q22	100	D12S351-	95.6 - 97.1	3.93
		D12S346	104.6 - 106.1	
19q13.41	90	D19S571-	84.1 - 87.7	3.99
		D19S418	92.6 - 97.5	
<b>Phenotype: Tot-KL</b>				
1q42.12-1q42.13*	250	D1S425-	231.1 - 235.3	3.02
		D1S2842	273.5 - 277.3	
2p.12-2p13.3	95	D2S2110	90.82 - 95.1	3.97
4q32.3	170	D4S1597	169.1 - 169.4	3.84
6q11.2-6q12	80	D6S257	79.92 - 80.0	3.05
19q13.2*	65	D19S5420	70.14	4.26

## Discussion

There are no clear-cut or universally accepted criteria for the selection of the primary phenotype, which has resulted in substantial heterogeneity in the epidemiologic and genetic literature. However, regardless of the method employed all previous studies have invariably reported a significant familial component in variation of OA, after adjusting for age (2-6).

In the present linkage study we used two phenotypes selected a priori on the basis of their distribution, n, substantial interindividual variation, significant familial aggregation and similarity to phenotypes examined in previous studies. The estimates of heritability obtained in this study were highly significant and were in accord with previously published data. The major findings were the three reliable multipoint linkage (MPL) signals observed for our two OA phenotypes. The genomic regions with multipoint LOD scores greater than 2.5 were observed on chromosome 2 at 90cM for DIP-OA; on chromosome 1 at 250cM and on chromosome 19 at 65cM for Tot-KL. These relatively strong linkage signals were obtained by both linkage methods (Fig 2). The reliability and significance of all three peaks were confirmed by the estimated empirical p-values. The additional significant linkage ( $LOD = 2.80$ ) for DIP-OA on chromosome 3 (30cM from *p* terminus) was not fully supported by the robust routine. Despite a high LOD score (3.92) the peak of the MPL plots from the free and robust analyses did not overlap (30cM vs 45cM). The positive linkage data for this chromosome is therefore less reliable than the data generated for chromosome 1, 2, and 19.

The same is true with respect to chromosome 6 at 80cM, where LOD=3.05 was initially observed with Tot-KL, and also coincided with a lower peak for DIP. Note also that DIP-OA and Tot-KL linkage peaks well overlapped on some chromosomes, namely, 2, 6, 19 (Fig 1) although were not confirmed by our strict statistical criteria. These regions are nevertheless of interest since they may reflect common genetic effects shared by two phenotypes, and thus at least partly explain the significant phenotypic correlation between them. Of particular interest is chromosome 19q13 (Table 1) where both peaks were reliably significant: LOD=3.99 and 4.46, for DIP-OA and TOT-KL. Although the regions didn't exactly coincide, 90cM vs. 65cM respectively, the 95% confidence intervals (CI) for the locations of QTLs obtained in model free linkage analyses are very wide and often defined as 1.5 - 2 LOD score drop (16). Even with more the conservative estimate recently proposed by Manichaikul et al. (17), if the markers spacing is every 10cM (as was the case in our study): one needs a drop of ~1.2 in LOD. This produces a 95%CI of approximately 30cM, which overlap with the empirical MPL location of the peaks suggesting a possible common QTL.

Our three main linkage regions on chromosomes 1,2,19 have been reported in previous linkage scans for OA (Table 2). Thus for example, Hunter and colleagues (4) using the Framingham sample recently reported significant linkage of the first principal component derived from 96 variables (KL scores, OSP scores, JSN scores) for 32 joints with chromosome 1 at 218cM. Their signal was relatively close to our result for Tot-KL at 250cM. Interestingly, their original LOD of 2.02 increased to 3.03 when males were excluded from the analysis, indicating that the specific linkage signals may be sex-specific. This is a particular advantage of the present study, since we used a single-sex sample. The authors of this study also noted that most of the variation of their first principal component was caused by DIP scores, suggesting a more joint specific consideration of the observed linkage. Table 2 also indicates that our highest LOD score of 4.26 found at 65cM on chromosome 19 for Tot-KL concurs with the results of Demissie et al (6), who recorded significant association of the Tot-KL score in the Framingham cohort to D19S178 marker, located in almost precisely the same area, 68cM.

The association of DIP-OA to chromosome region 2 at 90cM, observed in this linkage analysis, also appears to be a promising finding. We found a LOD of 3.95 close by at 95cM for Tot-KL, although not confirmed in the robust method. Several independent genomewide scans have reported the linkage of this region to total hand scores and to a variety of DIP-OA phenotypes (e.g. 3, Table 2). The latter study used a large sample of Icelandic families with probands having severely affected DIPs. They initially estimated a LOD of 2.2, which almost completely coincided with our peak, at 94.8cM. However, when they added additional markers and altered the phenotype to include CMC1 joints, their linkage peak increased to 4.4, but moved to 48cM. Demissie et al (6) reported their total hand sum of JSN to be associated with this area, 51.5cM. Yet, at least two other studies suggested that DIP-OA can be linked to the chromosomal region observed in the present study (Table 2). Leppavuori's team (15) in a small family-based sample found a significant association of DIP to 116cM near the IL1R1 gene cluster. Greig and colleagues (18) using a modest sample of affected families, from a similar ethnic background to ours, found significant linkage to markers mapped to 103.7 position on this chromosome. As the confidence intervals are wide for model-free linkage methods (17), it is possible that these areas coincide- and represent a replication.

**Table 2. Summary of linkage literature on hand OA replicated in this study**

Chromosome # Map position (cM)	Nearest marker	LOD score	Phenotype	Sample	Reference
#1, 250	D1S425- D2S2842	3.02	Total- KL	Community, UK	Present data
#1, 218	Not available	2.02	DIP-OA	Community, US	4
#1, 202	Not available	3.03	DIP-OA	Community, US	4 (only females)
#1, 102	D1S1665	2.96	Total JSN score	Community, US	15
#19, 65	D19S414- D19S418	4.26	Total- KL	Community, UK	Present data
#19, 52	D19S433	1.82	Total JSN score	Community, US	15
#19, 68	D19S178	1.83	Total- KL	Community, US	15
#2, 90	D2S136- D2S139	2.87	DIP-OA	Community, UK	Present data
#2, 94.8	D2S1566	2.20	DIP-OA	Affected families; original mapping; Iceland	3
#2, 48	D2S2168	2.44	DIP-OA	Affected families; additional mapping; Iceland	3 (additional markers)
#2, 48	D2S2168	4.44	DIP-OA + CMC-1	Affected families; additional mapping; Iceland	3 (additional markers)
#2, 51.5	D2S405	2.23	Total JSN score	Community, US	15
#2, 103.7	Not available	1.04 (p=0.014)	DIP-OA	Affected families, UK	18
#2, 116	IL1R1 (q -shoulder)	p=0.0001	DIP-OA	Affected families, Finland	15

A potential limitation of this study is the simple structure of the twin families that has some ambiguity in IBD status definition. This would have had the effect of reducing our chances of finding significant linkage. Having said this, it should be noted that the single sex twin pair design does have a number of advantages in genetic analysis of common age- and sex related traits. To strengthen the reliability of linkage results, we used a large, community-based sample, one of the largest that has been examined to date. We examined well-defined phenotypes, with very high intraobserver consistency of assessment, strong familial aggregation and we used techniques of linkage analysis robust to deviations from normality (14). In addition, to avoid possible false positive linkage signals, we validated our results with additional statistical tests. It should also be mentioned that the results of our analysis are unaffected by the potential sex-specific metabolic pathways and genetic influences, which have been observed in a number of genetic epidemiological studies, both in animal models (19) and in humans (20-22). The endocrine milieu and physiological background differ between male and female throughout life. Therefore, even in the absence of the sex-specific genes, sex could act as environmental factor that can influence disease risk and severity through sex-specific genotype interaction. We were unable to look separately for

symptomatic disease due to small numbers –but believe the results would be equally applicable to the correlations with severe disease and pain.

In summary, significant linkage peaks were observed on chromosomes 2 and 3 for DIP-OA, and on chromosomes 1 and 19 for Tot-KL. We found some overlap (Chr 2, 6 & 19) between the linkage peaks for the two phenotypes, which are certainly of interest but clearly require confirmation. However, our major peaks showed little overlap, which may suggest the potential influence of different metabolic pathways, governed by different genes, on different OA related phenotypes, as was recently suggested by Hunter et al. (4). Of our four significant linkage results, two (on chromosome 2, for DIP-OA and on chromosome 19, for Tot-KL) are supported further by results obtained by other teams using other samples. The fine mapping of these regions is now warranted and may well reveal gene(s) of interest in OA.

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The authors declare no conflicts of interest.

### **Legends to Figures**

Figure 1. Multipoint linkage analysis results of two OA-related phenotypes adjusted for age for all chromosomes. Suggestive linkages at the LOD score 2.5 shown by horizontal line. Solid line shows Tot-KL, interrupted – DIP-OA.

Figure 2. Significant multipoint linkage analysis results of two OA-related phenotypes adjusted for age. Fig 2A shows results for DIP-OA phenotype with chromosomes 2 and 3. Fig. 2B shows results for Tot-KL phenotype with chromosomes 1 and 19. The figure clearly demonstrates overlapping of peaks obtained by two methods of GLM technique observed on chromosomes 2 (fig 2A) and 1 & 19 (Fig 2B). Suggestive linkages at the LOD score 2.5 shown by horizontal line. Solid line shows LOD score estimate obtained with the total sample, interrupted line – LOD score obtained after removal the outlying individuals.

## References

- 1) Hart DJ, Spector TD. Definition and epidemiology of osteoarthritis of the hand: a review. *Osteoarthritis Cartilage* 2000; 8 Suppl A:S2-7.
- 2) Spector TD, MacGregor AJ. Risk factors for osteoarthritis: genetics. *Osteoarthritis Cartilage* 2004; 12 Suppl A:S39-44.
- 3) Stefansson SE, Jonsson H, Ingvarsson T, Manolescu I, Jonsson HH, Olafsdottir G, Palsdottir E, Stefansdottir G, Sveinbjornsdottir G, Frigge ML, Kong A, Gulcher JR, Stefansson K. Genomewide scan for hand osteoarthritis: a novel mutation in *matrilin-3*. *Am J Hum Genet* 2003;72:1448-59.
- 4) Hunter DJ, Demissie S, Cupples LA, Aliabadi P, Felson DT. A genome scan for joint-specific hand osteoarthritis susceptibility: The Framingham Study. *Arthritis Rheum* 2004;50:2489-96.
- 5) Suk EK, Malkin I, Dahm S, Kalichman L, Ruf N, Kobylansky E, Toliat M, Rutsch F, Nurnberg P, Livshits G. Association of *ENPP1* gene polymorphisms with hand osteoarthritis in a Chuvasha population. *Arthritis Res Ther* 2005;7:1082-90.
- 6) Demissie S, Cupples LA, Myers R, Aliabadi P, Levy D, Felson DT. Genome scan for quantity of hand osteoarthritis: the Framingham Study. *Arthritis Rheum*. 2002;46:946-52.
- 7) Egger P, Cooper C, Hart DJ, Doyle DV, Coggon D, Spector TD. Patterns of joint involvement in osteoarthritis of the hand: the Chingford Study. *J Rheumatol* 1995;22:1509-13.
- 8) Kalichman L, Cohen Z, Kobylansky E, Livshits G. Patterns of joint distribution in hand osteoarthritis: Contribution of sex, age and handedness. *Am J Hum Biol* 2004;16:125-134.
- 9) Spector TD, MacGregor A. The St Thomas' UK Adult Twin Registry. *Twin Research* 2002;5:440-443.
- 10) Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Research* 2001;4:464-77.
- 11) Burnett S, Hart DJ, Cooper C, Spector TD: A radiographic atlas of osteoarthritis. London: Springer-Verlag, 1994.
- 12) Wilson SG, Reed PW, Andrew T, Barber MJ, Lindersson M, Langdown M, Thompson D, Thompson E, Bailey M, Chiano M, Kleyn PW, Spector TD. A genome-screen of a large twin cohort reveals linkage for quantitative ultrasound of the calcaneus to 2q33-37 and 4q12-21. *J Bone Miner Res* 2004;19:270-7.
- 13) Snieder H, MacGregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *J Clin Endocrinol Metab* 1998;83:1875-80.
- 14) Barber MJ, Cordell HJ, MacGregor AJ, Andrew T. Gamma regression improves Haseman-Elston and variance components linkage analysis for sib-pairs. *Genet Epidemiol* 2004;26:97-107.
- 15) Leppavuori J, Kujala U, Kinnunen J, Kaprio J, Nissila M, Heliövaara M, Klinger N, Partanen J, Terwilliger JD, Peltonen L. Genome scan for predisposing loci for distal interphalangeal joint osteoarthritis: evidence for a locus on 2q. *Am J Hum Genet* 1999;65:1060-7.
- 16) Dupuis J and Siegmund D. Statistical methods for mapping quantitative trait loci from a dense set of markers. *Genetics* 1999;151: 373–86.

- 17) Manichaikul A, Dupuis J, Sen S and Broman KW. Poor performance of bootstrap confidence intervals for the location of a quantitative trait locus. *JHU Biostat* 2006: <http://www.bepress.com/jhubiostat/paper105>.
- 18) Greig C, Spreckley K, Aspinwall R, Gillaspay E, Grant M, Ollier W, John S, Doherty M, Wallis GA. Linkage to nodal osteoarthritis: Quantitative and qualitative analyses of data from a whole genome screen identifies trait dependent susceptibility loci. *Ann Rheum Dis* 2006; 27; [Epub ahead of print].
- 19) Orwoll, ES, Belknap JK, Klein RF. Gender specificity in the genetic determinants of peak bone mass. *J Bone Miner Res* 2001;16:1962–1971.
- 20) Ralston SH, Galwey N, MacKay I, Albagha OME, Cardon L, Compston JE, Cooper C, Duncan E, Keen R, Langdahl B, McLellan A, O'Riordan J, Pols H, Reid DM, Uitterlinden AG, Wass J and Bennett ST. Loci for regulation of bone mineral density in men and women identified by genome wide linkage scan: the FAMOS study. *Hum Mol Genet* 2005;14:943-51.
- 21) Peacock M, Koller DL, Lai D, Hui S, Foroud T, Econs MJ. Sex-specific quantitative trait loci contribute to normal variation in bone structure at the proximal femur in men. *Bone* 2005;37:467-73.
- 22) Weiss LA, Pan L, Abney M, Ober C. The sex specific genetic architecture of quantitative traits in humans. *Nat Genet* 2006;38:218-22.





