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# Osteoarthritis of the Hands, Hips and Knees in an Australian Twin Sample — Evidence of Association with the Aggrecan VNTR Polymorphism

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Age-related changes in the composition of the cartilage matrix may be associated with the development of osteoarthritis, a relatively late-onset disease characterised by the destruction of joint cartilage. In order to investigate whether differences in the VNTR polymorphic region of aggrecan affect cartilage functionality and therefore the development of osteoarthritis, we examined the aggrecan polymorphic genotypes of a sample of 134 Australian twins aged over 50 (including 34 monozygotic and 27 dizygotic twin pairs). Clinical measures of hand, hip and knee osteoarthritis, as well as self-reported bone and joint pain, were tested for association with the aggrecan polymorphism. The results were consistent with either a deleterious effect of allele 27, or a protective effect of alleles 25 and 28, providing some additional evidence for an association between the aggrecan VNTR polymorphism and osteoarthritis of the hands, hips and knees.

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Osteoarthritis is a complex relatively late-onset disease characterized by degeneration of joint cartilage, often accompanied by abnormal bone growth, pain and loss of joint function. Many factors influence the onset and severity of the disease, including a strong genetic component (Loughlin, 2001). One of the hallmarks of the disease is destruction of joint cartilage by a number of matrix metalloproteinases, including the recently discovered family of ADAMTS/aggrecanases (Smith, 1999; Sandy et al., 2000). It is thought that the proteolytic cascade may be initiated by changes in cartilage matrix functionality, which may in turn be caused by abnormal wear, trauma, joint instability, metabolic insufficiency, or other unknown causes.

Age is a major determinant of onset of OA, and there are changes in the composition of cartilage matrix that occur with age. One such change is the depletion of aggrecan in the matrix, along with a reduction of the size and change in sulfation pattern of glycosaminoglycan chains attached to aggrecan (Grushko et al., 1989; Hardingham & Bayliss, 1990; Martin & Buckwalter, 2001). These aggrecan-associated changes lower the fixed charge density of the tissue, and result in a less resilient and less functional cartilage. Factors that influence the depletion of cartilage aggrecan and chondroitin sulfate with age would hypothetically prevent

or promote the onset of OA. One such potential factor has recently been described, the VNTR polymorphic region of aggrecan (Doege et al., 1997). This polymorphism results in differing numbers of repeating sequences in the chondroitin sulfate attachment domain of aggrecan, with 13 alleles thus far described, ranging between 13 and 33 repeats of a 19-amino acid motif. Such variation in potential numbers of chondroitin sulfate chains on each aggrecan molecule may affect cartilage functionality in different individuals, and lead to altered risk of OA. A previous report has shown association of the most common allele, allele 27, with bilateral hand OA, in an unrelated population (Horton et al., 1998). An increased risk of lumbar disc disease has also been associated with the shorter alleles of aggrecan (Kawaguchi et al., 1999). We have examined the aggrecan polymorphic genotypes of a population of twins in relation to incidence of hand, hip and knee OA, and the results are consistent with either a deleterious effect of allele 27, or a protective effect of alleles 25 and 28.

## Method

As part of a study designed to cover a wide range of health issues affecting older people (Kirk et al., 1998, 1999, 2002), self-report information on bone and joint pain and injuries was obtained from 3116 twins aged over 50 and listed with the Australian Twin Registry (1279 complete twin pairs and 558 singles). Subjects were questioned about ever having experienced pain, swelling or stiffness in any joints; prior diagnosis of osteoarthritis or degenerative arthritis, rheumatoid arthritis, and other forms of arthritis or rheumatism; prior bone fracture or joint injury; radiographs taken of hands, hips or knees in the last 5 years. They were also asked to indicate on a homunculus any

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joints currently affected by pain or swelling. Self report of pain and/or swelling in the joints of the hands, hips and knees were used as indicators of potential OA, excluding those joints indicated to have sustained prior injury. Data for the left and right sides of the body were combined. For the hands, pain or swelling in any of the DIP, PIP, MCP or CMC joints (excluding prior injury) was used as the indicator for potential osteoarthritis. Subjects indicating a history of rheumatoid arthritis were excluded from the study.

#### Clinical Evaluation

From a combination of self-reported OA and involvement of target joints for OA without prior history of joint trauma, twins potentially affected by OA were identified. In contrast, those twins not identifying joint problems were categorized as being unaffected by osteoarthritis. The clinical phase of the study required the examination of study subjects, along with taking radiographs and obtaining blood samples. Given age and condition, it was reasoned that participants would be unlikely to travel more than 50 kilometers from home. Consequently, 118 twin pairs residing in the vicinity of Brisbane or Melbourne were invited to participate: 63 pairs with at least one member potentially affected by OA (41 discordant and 22 concordant pairs) and an additional 55 unaffected pairs. On the day of study, subjects were examined independently by two consultant rheumatologists, blood was taken by venepuncture, a skin mold was made and radiographs taken of hands, knees and hips. Not all twins attended in pairs although many did. They were not examined in any set order and clinical examinations were carried out in separate rooms. No discussion was allowed regarding individual examinations.

**Rheumatologist assessment — standard homunculus.** A standard homunculus was completed by the rheumatologist for each subject to indicate whether there was evidence of OA in each of 68 peripheral joints. In order to make that decision, the rheumatologists were permitted to perform any clinical assessment, or combination of assessments, that they normally used in routine clinical practice, without reference to radiographs or laboratory test results (Bellamy et al., 1999a).

**Rheumatologist assessment — ACR criteria.** Each rheumatologist also determined the presence or absence of osteoarthritis in the hands, hips and knees of each subject according to the clinical criteria developed by The American College of Rheumatology (Altman et al., 1986, 1990, 1991; Bellamy et al., 1999b). Pain, morning stiffness, crepitus and bony enlargement were elicited using standard clinical techniques. Range of movement was measured using a Baseline long-arm goniometer, applied in a standard fashion. No radiographic or laboratory data were available at the time of these clinical assessments. While radiographic and laboratory information were later available (Bellamy, 1999c), allowing assessment to be made according to ACR clinical and radiological criteria, previous modelling of this data set has demonstrated little difference between models of OA created using the two types of ACR criteria (Kirk et al., 2002). Consequently, this present study does not consider separately the results obtained using the ACR clinical and radiological criteria.

#### Zygoty Determination

Zygoty of twin pairs participating in the clinical evaluation was determined by molecular analysis of 9 standard markers (short tandem repeat loci D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820, plus a segment of the amelogenin gene indicating sex) using a commercial kit (AmpFISTR Profiler Plus Amplification Kit, ABI). These results were cross-checked with blood group results for the ABO, MNS and Rh systems (Australian Red Cross Blood Service) and phenotypic data (hair, eye and skin colour).

#### Genotyping — Aggrecan Polymorphism

Aggrecan alleles were identified using a PCR assay of genomic DNA as previously described (Doege et al., 1997; Horton et al., 1998). Primers were from positions 2888–2911 (sense) and 4115–4092 (antisense) of the human aggrecan cDNA sequence (Doege et al., 1991) (Genbank U76615). Two hundred ng of genomic DNA was amplified in a 20 µl volume containing 10 pmoles of each primer, 1.5 mM MgCl<sub>2</sub>, 0.25 mM dNTPs, 0.125% Triton X-100, 0.5 U Taq polymerase, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, using an annealing temperature of 66 degrees C, and 29 cycles. Reaction products were resolved by electrophoresis on 1% agarose gels, visualized by ethidium bromide staining/UV illumination, and identified by comparison to markers and known standards.

#### Statistical Methods

Inspection of the allele frequencies for the sample (see Table 1) indicated that only four alleles (repeat numbers 25, 26, 27 and 28) were sufficiently represented in our small sample to allow for significance testing. Consequently, individuals with at least one allele not in this list were excluded from the analysis, resulting in a sample of 34 MZ twin pairs, 27 DZ twin pairs, and 12 unpaired individuals whose co-twins did not participate.

Tests for allelic associations between the various osteoarthritis measures and the aggrecan polymorphism were performed using Mx 1.50 (Neale et al., 1999). Since all the measures of OA considered here are dichotomous (rheumatologist or self-report diagnosis), analyses were implemented using maximum likelihood methods for raw ordinal data. The models fitted to the data assume that underlying each variable is a continuum of liability which is normally distributed in the population. Dichotomous measures are obtained by imposing a threshold on this liability distribution, with affected individuals in the region of the distribution above the threshold (Neale & Cardon, 1992).

Univariate analyses were conducted on each type of measure (self-report, rheumatologist's assessment using standard homunculus, rheumatologist's assessment using ACR clinical criteria) for each of three sites: hands (any joint in either hand), hips (left and/or right affected) and knees (left and/or right affected). For each analysis, chi-square difference tests were conducted to determine whether the prevalence (as expressed by the threshold parameter) and age and sex effects could be equated across three groups: MZ (monozygotic) and DZ (dizygotic) twin pairs, and unpaired individuals, and then a similar test was performed to check whether allelic effects on prevalence

**Table 1**  
Distribution of Aggrecan VNTR Alleles in a Sample of 146 Australian Individuals, Predominantly of Northern European Ancestry

Observed allele repeat number	Frequency	%
19	1	0.3
22	8	2.7
23	1	0.3
25	22	7.5
26	60	20.5
27	128	43.8
28	69	23.6
29	3	1.0

estimates could also be equated. This results in models providing estimates of the prevalence at each site for each type of measure, and the effects of age, sex and different aggrecan VNTR alleles on that prevalence. Finally, a model with all allelic effects removed was compared to the previous model, to determine whether the observed allelic effects are statistically significant. Since only 4 different alleles are considered, and one of these (26 repeats) is arbitrarily chosen as a benchmark for comparison, this results in a chi-square difference test with three degrees of freedom.

Multivariate analyses were conducted on the basis that the various measures (self-report, rheumatologist’s assessments using the standard homunculus or ACR criteria) used to diagnose osteoarthritis at a given site are all measuring the same condition (e.g., OA of the hands) but with different levels of accuracy. If this assumption holds, then prevalence estimates, MZ and DZ twin pair correlations, and age, sex and allelic effects should all be able to be equated across the models fitted to the three different measures of osteoarthritis at a given site. Since the measurement errors in each type of measurement should be independent, this serves to reduce the impact of measurement errors on the results.

**Results**

**Univariate Analysis**

For each of the nine measures of osteoarthritis considered in this study (self-report, standard ACR clinical criteria for

each of hands, hips and knees), no significant differences in prevalence were observed between MZ and DZ twin pairs, or between complete twin pairs and unpaired individuals. There were also no differences in age or sex effects between the various groups for any of the nine variables.

The results of the univariate modelling of self-reported and rheumatologist-diagnosed osteoarthritis of the hands, hips and knees are presented in Table 2. These univariate results approach statistical significance for only two of the measures (ACR clinical criteria for osteoarthritis of the hands, and self-report for osteoarthritis of the knees). However, it can be seen from Table 2 that the threshold displacements for OA of the hands appear relatively consistent across the three diagnostic methods for both the 25-repeat and 28-repeat alleles. The threshold displacements for the 28-repeat allele for OA of the hips also demonstrate substantial consistency.

**Multivariate Analysis**

In order to increase statistical power and decrease the impact of measurement error, multivariate analysis techniques were used to combine the results of the various types of measurement of osteoarthritis at each site of interest. For measures of OA of the hips and knees, all three measures (self-report, and the rheumatologists’ assessments using the standard homunculus and ACR criteria) were able to be combined. However, the parameters obtained for the univariate model of hand OA using the standard homunculus

**Table 2**  
Tests for Association Between the Aggrecan VNTR Polymorphism and Univariate Measures of Osteoarthritis of the Hands, Hips and Knees

Site	Assessment	Threshold displacement (relative to 26-repeat allele) <sup>1</sup>			$\chi^2$ test for significance of allelic effects	
		25-repeat	27-repeat	28-repeat	$\Delta\chi^2_3$	<i>p</i>
Hands	Self-report	0.53	0.03	0.21	2.69	0.44
	Homunculus	0.77	0.35	0.36	5.87	0.12
	ACR	0.46	-0.42	0.42	8.43	0.04
Hips	Self-report	0.32	0.55	0.72	4.77	0.19
	Homunculus	-0.38	0.34	0.59	4.13	0.25
	ACR	-0.32	-0.25	0.39	3.18	0.37
Knees	Self-report	NA <sup>2</sup>	0.21	0.30	8.10	0.04
	Homunculus	0.13	-0.20	0.05	1.52	0.68
	ACR	NA <sup>2</sup>	0.02	0.09	2.86	0.41

Notes: <sup>1</sup> a significant positive threshold displacement is equivalent to a decrease in prevalence (i.e., a protective effect relative to the 26-repeat allele)  
<sup>2</sup> cell size too small to estimate accurately

could not be equated to the parameters obtained from the univariate models of hand OA according to self-report and ACR criteria, and consequently the homunculus-based measure of hand osteoarthritis was not included in the multivariate model. Results of the combined analysis described above are shown in Table 3 for each of the sites of interest (hands, hips and knees). All three sets of results approach statistical significance, supporting the possibility of allelic association at each of the sites. The additive genetic variance in osteoarthritis at each site attributable to these allelic effects (Mather & Jinks, 1971) is estimated to be approximately 5–7% of the total variance in osteoarthritis.

Due to the increase in statistical power from using multivariate analysis, it was also possible to test specifically for differences between individual alleles (25, 27 and 28-repeat) and the 26-repeat allele, using chi-square tests with one degree of freedom. These tests are also presented in Table 3, as the confidence intervals accompanying each allelic threshold deviation value. It can now be seen specifically that the 28-repeat allele appears to be associated with a lower prevalence of osteoarthritis of the hips than the 26-repeat allele, and likewise the 25-repeat allele is associated with a lower prevalence of osteoarthritis of the knees than the 26-repeat allele.

## Discussion

Despite the general acceptance of a strong genetic component in the etiology of OA, there are many factors and apparently a number of genes that can contribute to this complex disease. Aggreacan is an essential gene for cartilage development (Watanabe et al., 1994), but also provides a unique degree of allelic variation in the functional domain of an essential matrix gene. These results provide weak evidence for an association between the aggreacan VNTR polymorphism and osteoarthritis of the hands, hips and knees. However, it should be noted that due to insufficient sample size, it was not possible to test for population stratification. The study by Horton et al. (1998) of OA as diagnosed by radiographs in 93 elderly white American

men suggested that the 27-repeat allele was associated with increased risk of hand (but not knee) osteoarthritis. By contrast, the current study indicates that the 27-repeat allele is not significantly associated with an increased risk of OA when compared to the 26-repeat allele, but that there is a small potential protective effect (5–7% of variance in OA) of the 25-repeat and/or 28-repeat alleles when compared to the 26-repeat allele. These results provide additional support for previous observations that different alleles of an essential matrix component, aggreacan, may influence susceptibility to forms of osteoarthritis.

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**Table 3**

Tests for Association Between the Aggreacan VNTR Polymorphism and Multivariate Measures of Osteoarthritis of the Hands, Hips and Knees. Statistically Significant Allelic Effects ( $p < 0.05$ ) are Highlighted in Bold Type

Site	Number of measures	Threshold displacement (relative to 26-repeat allele) <sup>a</sup>			$\chi^2$ test for significance of allelic effects		Percentage of variance in OA accounted for by allelic effects
		25-repeat	27-repeat	28-repeat	$\Delta\chi^2_3$	$p$	
Hands	2	0.42 (-0.17 – 1.09)	-0.15 (-0.49 – 0.18)	0.27 (-0.12 – 0.68)	<b>7.73</b>	<b>0.05</b>	6.1%
Hips	3	-0.06 (-0.55 – 0.47)	0.16 (-0.17 – 0.50)	0.57 (0.14 – 1.03)	8.10	0.04	7.1%
Knees	3	0.59 (0.09 – 1.15)	-0.04 (-0.31 – 0.24)	0.17 (-0.13 – 0.49)	7.47	0.06	5.2%

Note: <sup>a</sup> a significant positive threshold displacement is equivalent to a decrease in prevalence (i.e., a protective effect relative to the 26-repeat allele).

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