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Genetic contribution to cartilage volume in women: a classical twin study

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***Objective*. A classical twin study was performed to assess the relative contribution of genetic and environmental factors to cartilage volume.**
***Methods*. The subjects were 136 adult female twins: 31 monozygotic and 37 dizygotic twin pairs. The subjects had a T2-weighted fat-saturated sagittal gradient echo MRI performed of their right knee. Femoral, tibial and patella cartilage volumes were measured using 3D Slicer, a piece of software that facilitates semi-automatic segmentation, generation of three-dimensional surface models and quantitative analysis. The intraclass correlations were calculated, and maximum-likelihood model fitting was used to estimate genetic and environmental variance components. All variables were adjusted for age, BMI and femoral condyle size.**
***Results*. The intraclass correlations for all of the cartilage volumes assessed were higher in monozygotic than dizygotic twin pairs. The heritabilities (95% confidence intervals) obtained from model fitting were: femoral, 61% (36–77%); tibial, 76% (56–87%); patella, 66% (47–79%); and total cartilage volume, 73% (51–85%).**
***Conclusion*. This study provides evidence for the importance of genetic factors in determining cartilage volume. Identifying heritability is the first step on the way to finding specific genes, which may improve our insight in the pathophysiology of cartilage disorders including the etiology of complex diseases such as osteoarthritis.**

KEY WORDS: Cartilage volume, Genetics, MRI, Twin study.

Osteoarthritis is the most common form of arthritis and represents a major contributor to functional impairment and reduced independence in older adults [1–3]. The measurement of knee cartilage volume by magnetic resonance imaging (MRI) is being developed as a possible outcome measure in osteoarthritis (OA) [4, 5]. A number of studies have been, and are being, conducted to ascertain the determinants of cartilage volume both in subjects with and without OA [6–9]. However, we are unaware of studies that have investigated the extent to which the variance of cartilage volume is explained by genetic factors.

There have been a number of investigations exploring the genetic influence on OA. For many years a genetic component to certain forms of OA has been suspected to be present, particularly in women. Early studies by Stecher [10] demonstrated that Heberden's nodes were three times as common in the sisters of 64 affected subjects as in the general population. Previous twin studies have suggested that genetic factors appear important at the hand and knee, explaining between 39 and 65% of the variance in radiological OA [11]. These studies relied upon plain radiography to assess the genetic contribution to knee OA. This is a two-dimensional technique that is sensitive to artefacts resulting from mal-positioning [12] or meniscal subluxation [13]. Several authors have reported poor agreement between standard radiography and the actual cartilage thickness and/or state of the articular surface in arthroscopy [14–18]. Moreover, radiography is unable to differentiate between femoral and tibial cartilage loss, is less sensitive to focal than to general cartilage lesions

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and cannot demonstrate the pattern of cartilage destruction throughout the joint surface. Due to these limitations, measurements of the joint space width in radiographs are not ideal for reliably evaluating cartilage alterations.

MRI has many advantages, including providing direct visualization of cartilage and covering the whole joint in one examination. Recently researchers have used cartilage volume and thickness measured from MRI of the knee to examine both normal and arthritic cartilage in patients [19–23]. Using segmentation techniques, researchers have the potential to reproducibly obtain cartilage volume measurements [24]. MRI knee cartilage volume correlates with radiographic knee OA [25], animal studies have shown that cartilage volume is inversely related to the risk of OA [26], and comparisons of MR parameters of articular cartilage morphology with histologic measures have shown a good correlation between these two modalities [27].

Given the importance of understanding cartilage volume both in health and disease, we undertook this study. The main aim of the study was to determine the proportion of the variance of cartilage volume (semi-automated measure) explained by genetic and environmental factors in a sample of unselected twins.

Patients and methods

Study population

The subjects were 68 female twin pairs (age range 48–80), previously unaware of the purpose of the study, recruited from the Australian National Health and Medical Research Council (NHMRC) registry and a volunteer sample recruited through a local media campaign. Subjects were recruited on the basis that they were post- or peri-menopausal. Zygosity was determined routinely by standardized questionnaire, and multiplex DNA fingerprinting with variable number tandem repeats was used for confirmation where zygosity was uncertain following administration of the questionnaire [28].

Research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by the hospital's Human Research Ethics Committee. Consent was obtained from each subject after full explanation of the purpose, nature and risk of all procedures used.

Data ascertainment

Demographic information was obtained by a standardized investigator-administered questionnaire. The following data were collected: personal and lifestyle factors; physical activity; usual and past use of tobacco; menstrual cycle patterns; previous use of oral contraceptives and reproductive history; occupational history; past knee injury; trauma and activity history. In addition, qualitative data relating to symptoms, stiffness, pain and physical dysfunction using the WOMAC [29] was recorded. Finally, clinical assessment was performed. This included a medical history concentrating on joint, bone, major organ disease, medication use, height, weight and clinical examination of the knees, hips and hands.

MRI

The subjects' right knees were imaged on a 1.5-T whole body magnetic resonance unit (Signa Advantage GE Medical Systems, Milwaukee, WI) with use of a commercial transmit-receive extremity coil. The following image sequence was performed:

Three-dimensional (3D) spoiled gradient-echo (SPGR) images; flip angle 40°; repetition time 30 ms; echo time 7 ms; field of view 13 cm; bandwidth 16 kHz; 256 × 256 matrix; slice thickness 1.2 mm; acquisition time 5 min 42 s; one acquisition. This method of acquisition has been previously validated for cartilage volume measurements [30, 31].

Volume measurement

Cartilage volumes were measured using 3D Slicer, a software that facilitates semi-automatic segmentation, generation of 3D surface models and quantitative analysis [32]. The measurement time to assess cartilage volume for each subject was ~20 min. The process incorporated intensity-based segmentation, with manual separation of femoral, tibial and patella cartilages.

Reproducibility

Reproducibility of this method was previously examined on a sample of 10 subjects [33]. Three observers trained in interpretation of musculoskeletal MRI held three standardization sessions to minimize inconsistencies, and independently measured cartilage volumes on all of the subjects blinded to patient details. One observer repeated these measures a fortnight later. The intraclass correlation (ICC) was computed from the analysis of variance on the variation in cartilage volume explainable by different observers. The intra-observer ICCs were 0.99 (95% CI 0.98–1.00) for tibial, patella and femoral cartilage volumes, whereas the ICCs were 0.96 (95% CI 0.92–1.00) for femoral cartilage volume and 0.97 (95% CI 0.94–1.00) for tibial and patella cartilage volumes. The mean (range) cartilage volumes for the 10 subjects were: patella 2.1 ml (1.0–3.4), tibia 3.5 ml (1.7–6.4) and femoral 8.9 ml (5.4–14.2).

Statistical analysis

Background to twin analysis

The classical twin study makes use of the fact that monozygotic (MZ) twins share identical genotypes, whereas dizygotic (DZ) twins are no more alike genetically than siblings, sharing on average 50% of their segregating genes. If MZ twins show a larger resemblance for a specific trait than DZ twins this is likely to be due to genetic factors. A higher MZ than DZ ICC provides a first indication of genetic influence. Structural equation modelling allows a more extensive separation and quantification of the observed phenotypic variance into its genetic and environmental components: additive genetic variance (A), dominance genetic variance (D), shared (or common) environmental variance (C) and specific (or unique) environmental variance (E), which also contains measurement error. Heritability (h^2) can be defined as the ratio of additive genetic variance to total phenotypic variance.

Analytical approach

Preliminary data analysis was performed and ICCs were estimated using Stata [34]. We estimated genetic and environmental influences on all variables, both before and after adjustment for age, BMI and femoral condyle size. Femoral condyle size was used as an index of joint size. Adjusted estimates were obtained by model fitting to trait residuals after removal of the effect of age and BMI by linear regression [35].

Model fitting procedure

The significance of A, C and D was tested by removing them sequentially in specific submodels, eventually leading to a model that gives the most parsimonious fit to the data. In this 'best fitting' model, the pattern of variances and covariance is explained by as few parameters as possible. We report parameter estimates such as the heritabilities from these best fitting models. Submodels were compared with the full model by hierarchic χ^2 tests. The difference between a submodel and that of the full model itself is distributed as χ^2, with degrees of freedom equal to the difference of the number of estimated parameters in the full model and the number of estimated parameters in the submodel. Akaike's information criterion ($AIC = \chi^2 - 2df$) was also used to evaluate the fit of the genetic models. The model with the lowest AIC reflects the best balance between goodness-of-fit and parsimony. Estimates of variance components and their 95% confidence intervals (CIs) were obtained from the best fitting model. All quantitative genetic model fitting was carried out with the statistical modelling package Mx [36].

Results

The data in Table 1 show the general characteristics (including the cartilage volumes) of the entire group of twin pairs studied (68 pairs). Whilst the DZ twins were heavier and had more injuries than MZ pairs, none of the characteristics were significantly different between MZ and DZ twins. Twenty-two subjects had knee OA by ACR clinical criteria [37].

The ICCs for MZ twins (rMZ) and DZ twins (rDZ) are presented in Table 2. For all variables, rMZ was greater than rDZ, implying an important genetic influence.

The genetic influence was subsequently confirmed by model fitting (Table 3). All models are adjusted for age, BMI and femoral condyle size. The heritabilities (95% CIs) obtained from model fitting were: femoral, 61% (36–77%); tibial, 76% (56–87%); patella, 66% (47–79%); and total cartilage volume, 73% (51–85%). There was no evidence of correlation with physical activity or history of knee injury so these were not included in the model. Model fitting on both the unadjusted values and residuals was extremely similar. In each case the AE model was the best fitting model. This means that A could not be excluded from the model without a significant worsening of the fit (ACE *vs* CE) in each of the four cases. On the other hand, D and C did not contribute significantly.

A comparison of the total cartilage volume for MZ and DZ twins is presented graphically in Fig. 1. In general these confirm the higher correlation between MZ than DZ co-twins. The scatterplots for other sites showed comparable correlation between MZ and DZ twins.

Table 1. Characteristics of MZ ($n = 31$) and DZ ($n = 38$) twin pairs

	MZ twins[a]	DZ twins[a]
Age (years)	60.6 (7.7)	60.1 (7.9)
Height (cm)	159.1 (7.1)	160.3 (5.4)
Weight (kg)	62.8 (9.8)	66.9 (13.2)
BMI (kg/m^2)	24.8 (3.4)	26.1 (5.1)
Current physical activity[b]	34.5 (5.0)	34.3 (6.7)
Previous knee injury (%)	29.0	37.8
Femoral cartilage volume (ml)	10.2 (2.2)	9.8 (1.9)
Tibial cartilage volume (ml)	4.6 (1.0)	4.4 (1.4)
Patella cartilage volume (ml)	2.9 (0.9)	2.8 (0.7)
Total cartilage volume (ml)	17.8 (3.5)	16.9 (3.6)

[a]Figures in parentheses indicate mean S.D.
[b]Framingham Physical Activity Index [46].

Table 2. ICCs (95% CI) for femoral, tibial, patella and total cartilage volume

	rMZ	rDZ
Femoral cartilage volume (ml)	0.67 (0.48–0.87)	0.11 (0.00–0.43)
Tibial cartilage volume (ml)	0.68 (0.49–0.87)	0.36 (0.07–0.64)
Patella cartilage volume (ml)	0.73 (0.56–0.89)	0.18 (0.00–0.50)
Total cartilage volume (ml)	0.73 (0.57–0.90)	0.22 (0.00–0.53)

Discussion

This is the first twin study to examine the genetic contribution to cartilage volume in the knee. Our data show the heritability of cartilage volume among women to be 61–76% depending on the compartment of interest. The genetic influence observed in this study is unlikely to be confounded by age differences, anatomic differences between the twins in terms of height or build, or differences in lifestyle variables such as physical activity or history of knee injury.

Plain radiography is unable to directly visualize cartilage, and measurement of joint space is used as a surrogate for cartilage volume change. A genetic contribution to joint space narrowing (JSN) at the knee has been suggested in a previous twin study [28]. This study suggested that radiological JSN (a surrogate for cartilage volume change) had a heritability of ~0.42. Our heritability estimate is greater, likely as a result of less measurement error in MRI technique than plain radiography. Our results underline that the genetic basis of cartilage volume is likely to be complex [38–40]. There was no evidence for a dominant gene effect at any of the sites studied; however, the sample size was relatively small, which would limit the ability to detect a dominant gene effect.

Table 3. Variance component estimates (95% CI) for the best fitting model

	Variance component	
	h^2 (95% CI)	e^2 (95% CI)
Femoral cartilage volume (ml)	0.61 (0.36–0.77)	0.39 (0.23–0.64)
Tibial cartilage volume (ml)	0.76 (0.56–0.87)	0.24 (0.13–0.44)
Patella cartilage volume (ml)	0.66 (0.47–0.79)	0.34 (0.21–0.53)
Total cartilage volume (ml)	0.73 (0.51–0.85)	0.27 (0.15–0.49)

All variables were adjusted for age, BMI and femoral condyle size.
h^2, heritability (additive genetic influence); e^2, specific environmental influence.

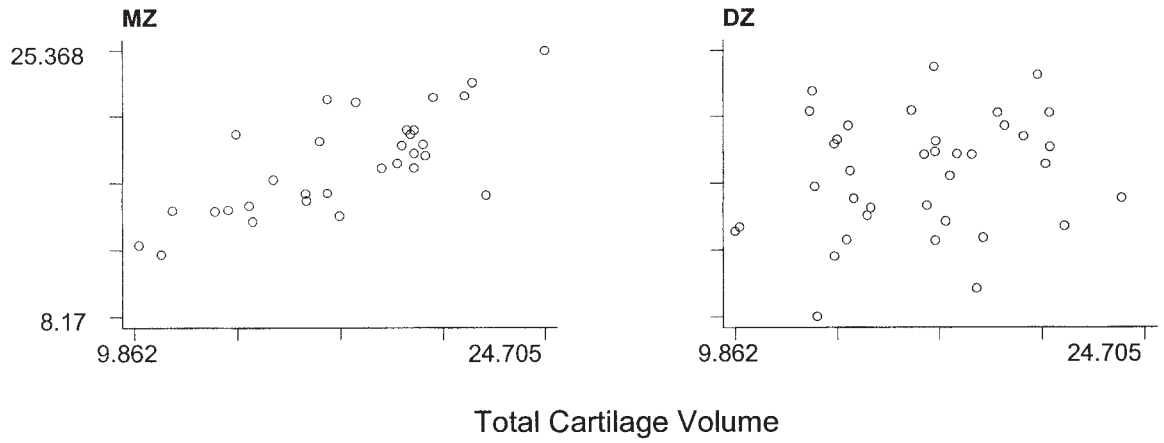

FIG. 1. Scatterplot of total cartilage volume for MZ and DZ twin pairs (plotted against the volume for their co-twin).

Determining heritability usually precedes further exploration by genetic linkage or association studies. Preliminary genetic linkage data in OA, where radiologic JSN was used as a surrogate for cartilage loss has implicated chromosomal regions encoding cartilage matrix components including fibronectin and the $\alpha 2$ chain of type V collagen [41–43]. The collagen type IIa1 gene, encoding the most abundant protein in cartilage, has been associated with JSN [44].

Twin studies have been subject to theoretical criticism in two main areas; the assumption of equal environmental sharing of the two zygosity groups and the generalizability of twins. In twin data exploring a wide range of common diseases and risk factors, we have never found any excess sharing of environment that was strongly associated with the trait sufficient to alter the heritability estimates [35]. The mean values of the characteristics of MZ and DZ twins in this study were very similar. Only weight and BMI showed slight differences between zygosities. Adjusting for age and BMI made no difference to the estimates of heritability in our analysis and provides reassurance that these estimates are a valid reflection of the genetic contribution to these measures.

The reported results are likely to be representative of the general population, because basic characteristics and disease prevalence of twins are similar to population-based, age-matched samples [45].

We have only studied women as the major focus of our research is on osteoporosis and OA, both of which are more prevalent in women. Our results cannot be directly extrapolated to males, although there is no clear reason to suggest major sex differences in heritability estimates.

As would be expected in an unselected population-based sample, although all grades of disease severity were represented, our principal findings relate to a definition of disease that encompasses mild changes of cartilage. Whilst 22 individuals had knee OA by ACR clinical criteria [37], the study was not designed specifically to examine more severe OA, nor to examine specific features of the disease or particular disease patterns, such as bilateral versus unilateral disease.

There is currently considerable work in progress with regard to isolation of the specific genes involved in cartilage pathophysiology and OA. Our data confirm that, as with JSN in the knee, knee cartilage volume variance in the population has an important genetic basis. Our heritability estimates indicate that 61–76% of the variance is explained by genetic factors in the female population and point to the likely success of linkage and association studies in identifying the genetic basis of cartilage volume. Currently, the major endpoint for association and linkage studies is plain radiographic evidence of OA. Our study demonstrates that cartilage volume is a useful endpoint for future gene searches.

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Conflict of interest

The authors have declared no conflicts of interest.

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