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Heritability assessment of cartilage metabolism. A twin study on circulating procollagen IIA N-terminal propeptide (PIIANP)

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SUMMARY

Objective: The aim of this investigation was to estimate the heritability of circulating collagen IIA N-terminal propeptide (PIIANP) by studying mono- and dizygotic healthy twin pairs at different age and both genders.

Design: 598 monozygotic (MZ) and dizygotic (DZ) twin individuals aged 18–59 years were recruited from the Danish Twin Registry. PIIANP was measured by competitive ELISA. The similarity of circulating PIIANP among MZ and DZ twins was assessed by intraclass correlations according to traits. The heritability was estimated by variance component analysis accounting for additive and dominant genetic factors as well as shared and non-shared environment but ignoring epistasis (genetic inter-locus interaction) and gene–environment interaction.

Results: The intraclass correlation of PIIANP in MZ and DZ twins was 0.69 (0.60–0.76) and 0.46 (0.34–0.58) respectively indicating a significant genetic impact on PIIANP in serum. Additive genetic effects explained 45% (21–70%), shared environment 24% (7–53%) and non-shared environment 31% (24–39%) of the total variance. The heritability estimate did not differ across ages and between genders.

Conclusions: The study shows that approximately 45% of the collagen IIA synthesis as assessed by the collagen IIA N-terminal propeptide in serum is attributable to genetic effectors while individual and shared environment account for 24% and 31% respectively. The heritability does not differ between genders or according to age.

Introduction

Collagen is produced and secreted as a precursor molecule with C- and N-terminal extensions termed procollagen peptides[1]. In the extracellular space these are cleaved off by specific C- and N-propeptidases after which collagen can participate in fibril formation[2–4]. Since procollagen propeptides are released from the parent molecule in a stoichiometric manner, the concentration of these peptides provides an opportunity to assess the current biosynthetic activity[5]. Type II A procollagen, which is particularly prevalent during embryogenesis, is re-expressed in adulthood, probably representing ongoing cartilage renewal and repair[6]. Low levels of PIIANP have been reported in patients with knee osteoarthritis (OA), However gradually increasing during follow-up for 5 years particularly among progressors[7–9]. Rousseau *et al.* were first to demonstrate, that PIIANP is decreased in rheumatoid arthritis (RA) of long duration[8]. More recently, we reported, that PIIANP is also decreased in newly diagnosed, anti-CCP positive RA and inversely associated with the anti-CCP titer indicating a chondrocyte suppressive effect by these antibodies[10].

The introduction of new biomarkers requires careful validation, including studies on pre-analytic and analytic variation. Previously,

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the heritability of circulating PIIANP has been assessed in OA multiplex families. Thus, Meulenbelt *et al.* investigated PIIANP in the GARP cohort and reported that the fraction of the total variance explained by shared genetic and environmental factors amounted to 70%[11]. In the CARRIAGE OA family cohort, the PIIANP heritability was 57%[12]. Based on classic twin methodology using healthy female twin volunteers Williams *et al.* estimated the heritability of circulating Cartilage Oligomeric Matrix Protein (COMP) to 40% with shared environment accounting for 28%[13]. Maintenance of the structural integrity of articular cartilage requires a delicate balance between formation and degradation of matrix constituents[14]. Therefore, we hypothesized, by analogy with COMP, that there is a major genetic influence on collagen II homeostasis as assessed by the serum level of PIIANP in a healthy study population. Therefore, in the present study we aimed to provide a heritability estimate on circulating PIIANP by studying healthy mono- and dizygotic male and female twin pairs.

Materials and methods

Study population

The twins were recruited from the GEMINAKAR–project, a study on components of the metabolic syndrome for which subjects were identified through the population-based Danish Twin Registry[15–17], which is among the largest and most comprehensive worldwide[18]. Participants in the GEMINAKAR study were recruited by means of a health assessment questionnaire which was mailed to a total of 2099 same sexed mono- and dizygotic twin pairs. Among these, both twins in 880 pairs consented to participate. One hundred and sixteen pairs were excluded due to morbidities, e.g., diabetes mellitus, cardiovascular diseases, pregnancy and breast-feeding and conditions rendering a progressive maximal bicycle test impossible. The twin pairs were stratified according to age and gender, yielding a total of 621 pairs. Among these we selected 299 consecutive pairs aged 18–59 years comprising 77 monozygotic (MZ) male pairs, 72 MZ female pairs and 150 same sexed, dizygotic (DZ) pairs. The demographic features available included sex, age, height, weight, BMI and smoking habits.

Blood sample preparation

Non-fasting blood samples were collected at 9 AM in plain tubes containing separation gel. They were allowed to clot on ice for max. 2 h and then centrifuged for 10 min at 3.500 rpm. Serum samples were frozen at −70°C degrees. The interval between blood drawing and freezing was max. 3 h.

PIIANP measurement

PIIANP was measured in duplicate by a competitive ELISA (Millipore/LINCO Research, Billerica, MA, USA). This assay is based on a polyclonal antibody raised against recombinant GST-human type II procollagen 2 fusion protein, which is specific for the N-propeptide of type IIA collagen[19]. Lowest detection limit was 30.0 ng/ml. Serum samples were diluted 1:2 using assay buffer. The analyses were conducted according to the instructions by the manufacturer. Intra-assay coefficients of variation were 10.4% at low (148–198 ng/ml) and 3.5% at high (504–551 ng/ml) concentrations respectively. Inter-assay coefficients of variation were 29.9% at low (43–144 ng/ml) and 12.6% for high (360–748 ng/ml) concentrations assayed on freeze-dried control samples provided by the manufacturer.

Analyses of twin similarity

The similarity of circulating PIIANP among MZ and DZ twins was assessed by means of intraclass correlations. Classic twin study methodology is based on the fact that MZ twins have identical segregating genes, whereas DZ twins, like ordinary siblings, share, on average, half of their segregating genes. Thus, a higher phenotypic similarity in MZ than in DZ twins is anticipated if there is a significant genetic influence on the trait studied[20].

To approximate a bivariate normal distribution, the PIIANP level was transformed into a logarithmic scale. The influence of potential confounding factors like sex, age, height, weight, BMI and smoking habits on the serum level of PIIANP was assessed by regression methods.

Heritability estimate

Heritability is defined as the proportion of total variance in a population due to genetic variation[21]. The extent to which variation in a trait is attributable to genetics was estimated through variance component analysis. The phenotypic variance (P) can be separated into four variance components: variance due to additive genetic effects (A), genetic dominance (D), shared (family) environment (C) and non-shared (individual) environment (E), i.e., $P = A + D + C + E$. A maximum of three of the variance components can be estimated simultaneously. A full variance component model is the ACE model, reduced variance component models include AE and CE models, where individual variance components are set at zero. Nonadditive genetic effects such as genetic dominance (D) may also be important, so the ADE model was tested as well. In agreement with standard practice gene–environment and gene-gene interaction as well as gene–environment correlations were not calculated[22]. The method for selecting the best model among submodels followed standard procedures (structural-equation analyses) using the statistical tool (twinlm) in the Analysis of Multivariate Events *mets*-package in R (ver.0.1-12)[23] according to the following criteria: (1) a nonsignificant *P* value in the χ^2 goodness of fit test, and (2) minimizing the Akaike Information Criterion [AIC = $\chi^2 - 2 \times 2$ degrees of freedom (d.f.)].

Results

Table I shows the demographics of the cohorts. MZ and DZ twins were comparable according to sex, age, height, weight and BMI but differed slightly regarding smoking habits.

In multiple regression analyses, the PIIANP level in individual twins was close to being significantly associated with sex, but not with age, height, weight, BMI and smoking. The regression coefficient for sex was 0.06 ($P = 0.08$). Correlation diagrams for the logarithmic value of PIIANP in MZ and DZ twins are presented in Fig. 1. Each dot represents the serum level of PIIANP in a pair of

Table I
Characteristics of twins according to zygosity

	MZ	95%CI	DZ	95%CI
Number	298		300	
Female (%)	48%		51%	
Age (years) (*r*)	35.5 (17–55)	32.4–34.7	35.9 (18–59)	34.9–37.0
Height (cm)	173.1	172.0–174.7	173.6	172.6–174.7
Weight (kg)	72.2	70.8–73.6	74.0	72.3–75.6
BMI (kg/m^2)	24.0	23.6–24.3	24.6	24.0–24.9
Smoker (%)	24%		35%	
PIIANP (ng/mL)	650	623–677	624	599–649

r: range.

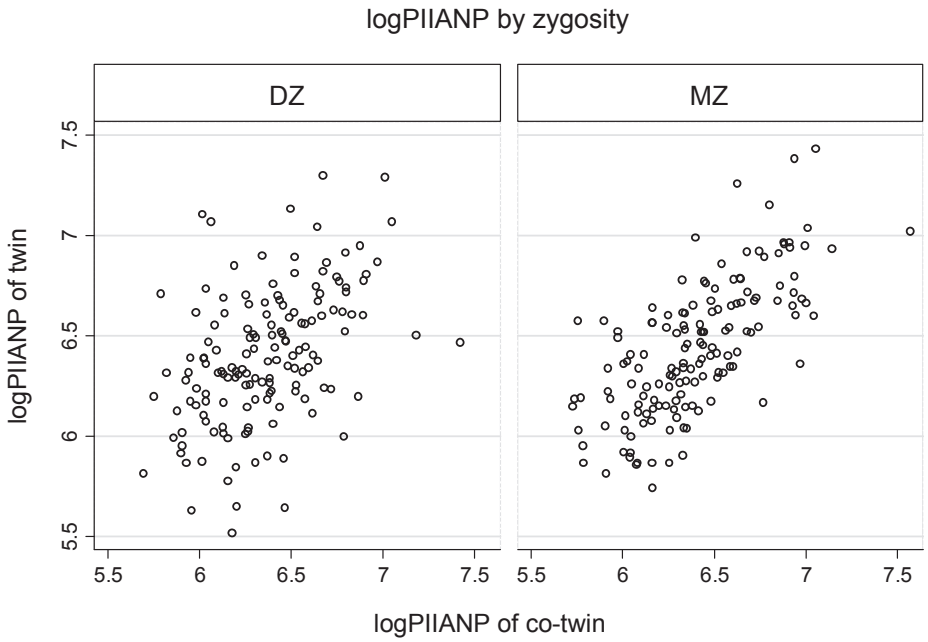

Fig. 1. Correlation diagrams on logPIIANP in MZ and DZ twins. Each dot represents the logarithm of the serum PIIANP level in a pair of twins, with one twin assigned to the abscissa and the other to the ordinate. Correlation coefficient for DZ: 0.46 and for MZ: 0.69.

twins, with one assigned to the abscissa and the other to the ordinate. The closer clustering of dots in MZ compared to DZ twins indicates that genes are important contributors to the variance in the serum level of PIIANP.

The within pair correlation was estimated by using intraclass correlation coefficient (ICC). ICC was higher for the MZ twins (0.69 (95%confidence interval (CI): 0.60–0.76)) than for DZ twins (0.46 (95%CI: 0.34–0.58)) (Table II).

In the variance component analysis, ACE gave the best fit by the AIC. In this model the additive genetic effect was estimated to 45% (95%CI: 21–70%), shared environment to 24% (95%CI: 7–53%) and non-shared environment to 31% (95%CI: 24–39%) of the total variance (Table II).

Heritability is population specific and may vary due to demographic characteristics. However, comparison of the PIIANP heritability between age-groups [Fig. 2] and genders revealed no differences. The heritability for females and males were 37% (95% CI: 11–71%) and 54% (95%CI: 17–88%) respectably ($P = 0.57$).

Discussion

Knowledge about the relative contribution of genetic and environmental modifiers of cartilage metabolism is important for the interpretation of biomarker studies in joint diseases, particularly those with a genetic background like OA, RA and the spondyloarthropathies[24–26]. This study shows that genes account for around 45% of the variation of PIIANP in serum among healthy, adult twins aged 18–59 years. Twenty four percent of the variation is determined by shared environment and 31% by non-shared environment. Importantly, this heritability estimate did not differ between genders or according to age. The participating twins were healthy as assessed by questionnaire and clinical examination. We therefore assume that the results can be extrapolated to similar healthy singleton populations[27].

The heritability of cartilage homeostasis can be assessed by static measures including imaging (e.g., X-ray, MRI), which reflect the net result of previous events or by dynamic measures (e.g., molecular markers) reflecting current metabolic processes. In a 5-yr longitudinal study on OA patients, the PIIANP level was higher in X-ray progressors than in non-progressors[9]. In addition, in a 2-year prospective study on 117 subjects with knee OA, the serum levels of PIIANP and COMP were associated with the rate of medial chamber cartilage loss as assessed by MRI[28]. However, such an association was not detected in a more recent study by Hunter *et al.*[29].A classical twin study based on MRI has estimated the heritability of the cartilage volume to 61–76% with specific environmental determinants accounting for the remaining influence[30]. The heritability of knee OA has been estimated to 39–44%[31] and the heritability of OA progression in the knee has been reported to 47–71%[32]. However, it should be borne in mind that the heritability

Table II
Biometrics of PIIANP serum levels

PIIANP	Corr MZ	Corr DZ	Heritability	Dominant genetic effect	Shared environment	Individual environment	Log lik.	AIC	*P*-value
Saturated	0.68 (0.52–0.80)	0.46 (0.30–0.60)							
ACE	0.69 (0.60–0.76)	0.46 (0.33–0.57)	0.45 (0.21–0.71)	0	0.24 (0.07–0.53)	0.31 (0.24–0.39)	−119.8	249.5	0.48*
ADE	0.69 (0.62–0.76)	0.35 (0.31–0.38)	0.70 (0.62–0.77)	0.00 (0.00–0.00)	0	0.30 (0.23–0.38)	−121.3	252.5	0.14*
AE	0.70 (0.62–0.76)	0.35 (0.31–0.38)	0.70 (0.62–0.77)	0	0	0.30 (0.23–0.38)	−121.2	250.5	0.08†
CE	0.58 (0.50–0.65)	0.58 (0.50–0.65)	0	0	0.58 (0.50–0.65)	0.42 (0.35–0.50)	−125.6	259.2	0.00†

Twin–twin correlation coefficients were adjusted for sex. Results from the variance component analysis are presented, showing that the ACE model gave the best fit by the AIC.
* Compared with the saturated model.
† Compared with the ACE model.

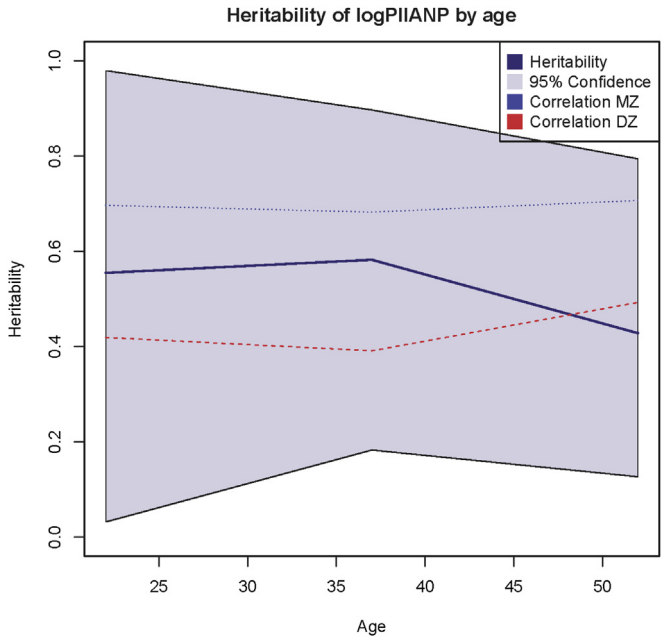

Fig. 2. The correlation coefficients and heritability with CI for MZ and DZ twins according to age. The twins were categorized into three groups, each spanning 15 years. Heritability (additive genetic effect) with 95%CI and correlations for MZ and DZ twins were estimated in each group.

of OA differs widely by anatomical site ranging from 28% in the hip to 68% in the CMC joints[24]. In these studies of heritability the best fitting model in the variance component analysis was an AE model, leaving non-shared environment as the only determinant beside heritability. Conceivably, genes which are implicated in the cartilage homeostasis do not differ between joints. By contrast, the environmental impact on individual joints or joint compartments may differ widely. The variance component analysis on PIIANP and COMP also includes the shared environment component (C), including current or previous shared determinants like physical activity or dietary habits. Meulenbelt *et al.* investigated PIIANP heritability in an OA family cohort and reported the contribution of shared genetic and environmental factors to account for 70% of the variance[11]. In our study we reported the A and C components separately, but by combining these components our estimate is virtually equal to that reported by Meulenbelt *et al.* In the study by Williams *et al.* the heritability of COMP in the circulation was estimated to 40% with a shared environment component at 28%[13]. However, while the study on COMP was based on females only, the present investigation included both male and female twin pairs, thereby providing an opportunity to evaluate potential differences between genders. In addition, our study demonstrates that the heritability estimate on PIIANP is largely independent on age [Fig. 2] and gender.

Some limitations should be addressed as well. Articular imaging data were not available. Although the participants had no self-declared musculo-skeletal complaints, subclinical OA cannot be entirely excluded, since radiographic OA occurs increasingly frequent with advancing age[33]. However with a mean age around 35 years and BMI at 24 in both zygosities, we believe that the prevalence of confounding OA or other joint diseases is low[33,34]. Finally, a heritability estimate based on a molecular marker provides an average estimate on hyaline cartilage metabolism at the level of the individual, but no information about potential differences at the level of individual joints.

In conclusion, the study shows that approximately 45% of the collagen IIA synthesis as assessed by the collagen IIA N-terminal propeptide in serum is attributable to genetic effectors, while individual and shared environment account for 31% and 24% respectably. The heritability estimate does not differ between genders or age.

Authors' contributions

All authors have made substantial contributions to (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Specific contributions are:

(1) The conception and design of the study: HM, PJ, AS, JH.
(2) Acquisition of data: GS, KK.
(3) Analysis and interpretation of data: HM, PJ, JH, AS.
(4) Drafting the article: HM, PJ.
(5) Revising the article critically for important intellectual content: HM, PJ, JH, AS, KK, GS.
(6) Final approval of the version submitted: HM, PJ, JH, AS, KK, GS.
(7) Statistical expertise: JH.
(8) Obtaining of funding: HM, PJ.
(9) Collection and assembly of data: HM.

HM takes responsibility for the integrity of the work as a whole, from inception to the final version of the article. HM was involved in analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding and collection and assembly of data.

Competing interest statement

The authors have no financial and personal relationships with other people or organizations that could potentially and inappropriately influence (bias) their work and conclusions.

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