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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.