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Original Investigation

Fetal, Developmental, and Parental Influences on Cystatin C in Childhood: The Uppsala Family Study

Dorothea Nitsch, MD, MSc,[1] Johanna K. Sandling, MSc, PhD,[2] Liisa Byberg, PhD,[3] Anders Larsson, MD, PhD,[4] Torsten Tuvemo, MD, PhD,[5] Ann-Christine Syvänen, PhD,[2] Ilona Koupil, MD, DSc,[6] and David A. Leon, PhD[1]

Background: The aim was to identify determinants (biomedical and social characteristics of children and their parents) of cystatin C levels in healthy children drawn from a population sample.

Study Design: Cross-sectional study.

Setting & Participants: 425 pairs of consecutive full siblings born 1987-1995 in Uppsala were identified using the Swedish Medical Birth Registry and invited with their parents for examination in 2000-2001.

Outcome: Serum cystatin C level was log-transformed and analyzed using random-effects models.

Measurements: The examination in parents and children consisted of a nonfasting blood sample, anthropometry, and questionnaires about lifestyle and socioeconomic position. Tanner stage was used for assessment of pubertal status.

Results: In age-, height-, and body mass index–adjusted analyses, cystatin C level increased by 2.6% (95% CI, 0.3%-4.8%) higher in Tanner stage 2 vs 1 girls, and 1.6% (95%CI, 0.2%-3.1%) lower in boys than girls. For every 10% increase in maternal cystatin C level, offspring cystatin C level increased by 3.0% (95% CI, 2.2%-3.8%); the equivalent effect for paternal cystatin C level was 2.1% (95% CI, 1.3%-2.9%). Lower maternal education was associated with a 2.4% (95% CI, 0.3%-4.6%) higher cystatin C level in their offspring.

Limitations: Cross-sectional study design, missing cystatin C values for subset of parents, lack of urinary measurements, no gold-standard measurement of glomerular filtration rate.

Conclusions: There are intergenerational associations of cystatin C level in families in line with previous reports of heritability of kidney disease. Lower maternal education is associated with higher cystatin C levels in their children. Further studies of healthy children are needed to explore the biological mechanisms for these findings. If cystatin C is measured, these studies will need to record pubertal stages.

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INDEX WORDS: Birth weight; children; cystatin C; family study; intergenerational association; puberty.

Chronic kidney disease is associated with cardiovascular disease and all-cause mortality in the adult population.[1,2] A substantial body of literature suggests that in utero and postnatal growth, as well as maternal and social factors in early life, influence later blood pressure and cardiovascular disease.[3-14] Less is known about the influence of early-life factors on kidney function in childhood and adult life. A study of babies[15] and one of children[16] reported an association between birth weight and kidney volume, measured using ultrasound. Two US database studies reported an association of lower birth weights with a higher incidence of established kidney failure.[17,18] An additional body of research, largely using retrospectively collected data for both adults and children, attempted to confirm a positive association between lower birth weight and later kidney disease[19]; however, most of the current evidence may be affected by recall[20-23] and publication[22,24-29] bias. Studies have shown heritability of kidney function[30-37] and particular kidney diseases.[32,38,39] A recent study reported associations of cystatin C (a marker closely correlated with kidney function) levels in adolescents with pubertal stage, age, sex, and ethnicity.[40] In summary, little is known about how early-life factors affect kidney function (or closely correlated markers) in the general population in either adults or children.

The Uppsala Family Study was set up to investigate how early-life factors influence later cardiovascular risk factors in children from the general population and investigate the role of familial factors.[41] Cystatin C was measured in a subset of families (2 children and both parents). The aim of the present report was to use

From the [1]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK; and [2]Molecular Medicine, Department of Medical Sciences, [3]Orthopaedics, Department of Surgical Sciences, Uppsala Clinical Research Centre, [4]Clinical Chemistry, Department of Medical Sciences, [5]Department of Women's and Children's Health, Uppsala University, Uppsala; and [6]Centre for Health Equity Studies, Stockholm University/Karolinska Institutet, Stockholm, Sweden.

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Address correspondence to Dorothea Nitsch, MD, MSc, Department of Non Communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT, UK. E-mail: dorothea.nitsch@lshtm.ac.uk

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these data to describe the associations of cystatin C levels in childhood with parental characteristics and markers of fetal growth and pace of maturation.

METHODS

Study Design

Details of the Uppsala Family Study are reported elsewhere.[41] In brief, the Swedish Medical Birth Registry[42] enabled us to identify all women who gave birth in Uppsala Academic Hospital in 1987-1995. We focused on those who within 36 months had had 2 or more consecutive live births after 38-41 weeks of gestation.

The original objective of the study was to compare the effects of birth weight on childhood blood pressure within families to that between families. Because of this, a design was adopted that optimized the statistical power of the study to address this question. However, as shown here, when appropriate account is taken of the study design, these unique data provide a powerful resource for addressing other questions concerning the influence of familial and early-life factors on phenotypes in later childhood.

In a family-based design such as this, most statistical information about the association between birth weight and later outcomes within families comes from families whose offspring had discordant birth weights. In the same way, most information about the association between birth weight and outcomes between families came from families in which the offspring had very similar birth weights that are either toward the top or bottom ends of birthweight distribution. We therefore recruited only the 38% of all families that met 1 of the following inclusion criteria : (1) both siblings were in bottom one-fourth of sex-specific birth weight distribution (ie, $<$3.42 kg for boys and $<$3.30 kg for girls), (2) both siblings were in the top one-fourth of sex-specific birth weight distribution (ie, $>$3.98 kg for boys and $>$3.85 kg for girls), and (3) siblings were discordant in birth weight (ie, sex-adjusted difference $>$0.40 kg).

Birth-weight cutoff values were chosen to result in adequate numbers of families in each group. During an 18-month period, 1,967 families who fell into one of our sampling groups were invited to take part, and letters stated explicitly that only families in which the same biological father is living with his children are invited. After reminder letters and telephone contacts, 602 (31%) of the families invited were examined between May 2000 and November 2001, when the children were 5-14 years old. Participation rates were similar across sampling groups defined in terms of offspring birth weights. Signed informed consent of the mother (and father, if present) was obtained. The study received full approval from the Uppsala University ethics committee and the Swedish Data Inspection Board.

Demographic and Anthropometric Information

Details of the examinations are reported elsewhere.[41] For most families (95%), all family members were examined on the same occasion. The ambient temperature and season for the examination were recorded. Children's Tanner stage (pubic hair for boys and girls and breast development for girls) was assessed directly by visual inspection by trained pediatric nurses. Heights and weights were measured 3 times and the mean of the 3 measurements was used in the analysis. Weight and height quintiles were derived for all available data. Parents were given questionnaires that were completed and returned to us by 97% of participating families. These questionnaires covered the demographic and socioeconomic circumstances of family members and their lifestyle, health-related behaviors, and medical history. The highest levels of education obtained by both the mother and father were classified as university, secondary (minimum 10-11 years of full-time education), and lower (ie, less than secondary). The occupational social class of each parent was classified on the basis of the Swedish socioeconomic classification.[43] Birth weight and gestational age (based on the date of the last menstrual period or, when available, ultrasound) of the children and maternal smoking at the time of first booking were obtained from the Swedish Medical Birth Registry.[42] In this study of children with a wide range of ages extending into puberty, it is particularly important to take account of size relative to children of the same age. We therefore converted children's weight, height, birth weight (by week of gestation), and body mass index (BMI) at examination into standard deviation (SD) scores based on Swedish population reference values.[44,45] However, to make results directly comparable with the bulk of the existing literature, regression analyses reported in this article also used absolute birth weight (adjusted for gestational age).

Plasma Cystatin C Measurements

Blood was drawn from both parents and children for 425 families with 850 pairs of children, 420 mothers, and 401 fathers. Only data from these 425 families were used in the analyses reported here. Plasma cystatin C was measured with a particle-enhanced immunoassay (Gentian, www.gentian.com) using an Architect Ci8200 analyzer (Abbott, www.abbott.com) and reported in milligrams per liter. Total analytical imprecisions of the cystatin C method were 1.1% at 1.25 mg/L and 1.4% at 5.45 mg/L.

Statistical Methods

The distribution of serum cystatin C levels was skewed. However, log(cystatin C) was normally distributed across the whole range of values except for 2 outliers of 0.34 and 0.42 mg/L at the lower detection limit of the assay ($\sim$0.40 mg/L). Hence, we modeled log(cystatin C) using random-effects linear regression models and obtained combined coefficients as the weighted average of the within- and between-family effects, each coefficient weighted by the inverse of its variance.[46] There was no evidence of heterogeneity of between- and within-family effects, supporting our choice of analytic method.[47,48] Relative differences (ie, proportional changes in cystatin C level per unit change in the exposure x) are shown as percentage of difference in cystatin C level relative to the selected baseline group for each variable.

Of note, our results for associations of variables with log(cystatin C) are proportional to associations found for the Filler formula[49]: $\log(\text{GFR}) = 1.962 + [1.123 \times \log(1/\text{cystatin C})]$, where GFR is glomerular filtration rate. Because log(1/cystatin C) is equal to –log(cystatin C), the Filler formula implies that differences between children's log(estimated GFR) are proportional to changes in –log(cystatin C).

The sequence in which variables were added to successive models was guided by our assessment of the likely causal pathways and confounders that exist. Starting from our basic model (adjusted for age and sex at examination), we looked first at the effect of adding gestational age and concurrent height and weight SD scores. At this stage, we also looked at whether there was an indication of Tanner stage having an influence on log(cystatin C). This has been described before.[40,50]

For investigating intergenerational effects, we adjusted for season of examination to remove environmental effects that would explain familial correlations (eg, slightly higher cystatin C levels in families examined in summer as opposed to families examined in the winter). We also adjusted for current parental smoking status and anthropometry of family members to ensure that effects are not driven by passive smoking of the child or anthropometry.

Analyses were carried out using the statistical software package Stata10 (StataCorp; www.stata.com).