Do Genetic Factors Explain the Association Between Poor Oral Health and Cardiovascular Disease? A Prospective Study Among Swedish Twins

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Epidemiologic studies suggest positive associations between poor oral health and cardiovascular disease. The authors undertook a prospective study among 15,273 Swedish twins (1963–2000) to examine whether genetic factors underlying the 2 diseases could explain previous associations. They estimated hazard ratios and 95% confidence intervals controlling for individual factors and stratifying on twin pairs to control for familial effects. Quantitative genetic analyses estimated genetic correlations between oral diseases and cardiovascular disease outcomes. Tooth loss (hazard ratio (HR) = 1.2, 95% confidence interval (CI): 1.1, 1.4) and periodontal disease (HR = 1.3, 95% CI: 1.0, 1.4) were associated with small excess risks of cardiovascular disease; periodontal disease was also associated with coronary heart disease (HR = 1.4, 95% CI: 1.1, 1.6). Adjustment for genetic factors in co-twin analyses did not appreciably change estimates. In contrast, tooth loss was more strongly associated with coronary heart disease in twin models (HR = 2.1, 95% CI: 1.2, 3.8) compared with adjusting for individual factors alone (HR = 1.3, 95% CI: 1.1, 1.4). There was evidence of shared genetic factors between cardiovascular disease and tooth loss ($r_g = 0.18$) and periodontal disease ($r_g = 0.29$). Oral disease was associated with excess cardiovascular disease risk, independent of genetic factors. There appear to be common pathogenetic mechanisms between poor oral health and cardiovascular disease.

Abbreviations: $h^2$, heritability; ICD-9, International Classification of Diseases, Ninth Revision; IL, interleukin.
factors of relevance for both oral health and cardiovascular disease remain unknown at this time. Thus, it is impossible to control for confounding by genetic factors in any classic epidemiologic approach.

Because twins share genes to a known extent, studies within a twin cohort can be used to overcome this limitation. To this end, we undertook a co-twin analysis in a population-based cohort of more than 15,000 twins from the nationwide Swedish Twin Registry to examine whether tooth loss and periodontal disease increase risk of cardiovascular disease, controlling for genetic factors, familial exposures, and individual-level risk factors. In addition, we used quantitative genetic analysis to examine the extent to which genetic factors are shared between oral and cardiovascular diseases.

MATERIALS AND METHODS

Study population

The nationwide Swedish Twin Registry (18–21) is currently the largest population-based twin registry in the world, registering all twins born in Sweden since 1886. The registry was assembled in the 1950s and 1970s, and twins have been followed prospectively through questionnaires and interviews, as well as through national registers, by using a unique national registration number assigned to each Swedish resident. Zygosity of the twins is based on questionnaire data, where twins responded whether they were “as similar as peas in a pod” (monozygotic) or “no more alike than siblings in general” (dizygotic). Validation studies of the Swedish Twin Registry show high validity (>95% of pairs) in determining zygosity using questionnaires (18).

The present investigation is nested among the “old cohort,” comprising like-sexed twin pairs born in 1886–1925. In 1963, a written questionnaire was mailed to the old cohort if both twins in the pair were living and were residing in Sweden. The questionnaire collected information on health outcomes, risk factors, and preventive behaviors, including information on oral health. Questionnaire data were available from 18,536 twins. We excluded 1,087 twins with prevalent cardiovascular disease in 1963, 61 twins with erroneous or missing follow-up data, and 2,115 twins with missing information on smoking or other covariates. The final sample size was 15,273.

Oral health

Oral disease was based on self-report. All twins were asked, How many of your own teeth did you still have at 35 years of age? with the following 3 response categories: no teeth left or just a few, about half the teeth left, nearly all or all teeth left. To assess periodontal disease, the participants were asked, Have you noticed that some of your own teeth have come loose or even fallen out on their own? (yes/no). If they responded affirmatively, then they were asked whether this event happened for just a few teeth, for about half the teeth, or for all or nearly all the teeth. Twins were classified as having periodontal disease if at least half of their teeth had mobility, a sign of advanced periodontal disease. Someone with a few loose teeth was categorized as having minor disease. Respondents with only dentures, whether due to periodontal disease or other causes, were considered as a separate category.

Cardiovascular disease endpoints

Twins were followed prospectively for cardiovascular disease incidence and mortality through linkage with national databases by using the national registration number. Incidence data were gleaned from the Swedish Inpatient Register, which contains information on hospital discharges beginning in January 1964, when information was compiled from selected inpatient medical institutions as part of the Inpatient Register. Coverage in 1965 included 6 of 24 counties in Sweden, covered the most populous counties by the mid-1970s, and was 100% complete beginning in 1987. Provided medical services are, in effect, population based. Each record in the register corresponds to one hospitalization and includes the date of hospitalization and discharge, surgical procedures, and up to 8 medical conditions coded according to the International Classification of Diseases, Ninth Revision (ICD-9).

Information on deaths due to cardiovascular disease was available from the National Cause of Death Registry, which includes dates of death from specific causes obtained from death certificates and coded according to ICD-9 standards. Medical certification is carried out by the attending physician or coroner using both clinical records and autopsy reports (22). This registry, established in 1961, maintains cause of death for more than 99% of the Swedish population who died after that year.

We used ICD-9 classifications to categorize an individual as having cardiovascular disease based on incidence or mortality. We evaluated total cardiovascular disease (ICD-9 codes 401–448) as well as coronary heart disease (ICD-9 codes 410–414) and stroke (ICD-9 codes 430–438), which comprise the most common cardiovascular disease entities. Observation time was calculated from date of entry into the cohort (January 1, 1963) until occurrence of any primary cardiovascular disease event or death, censoring on account of other death, or end of the observation period (December 31, 2000).

Statistical analysis

The relation between poor oral health and cardiovascular disease outcomes was assessed by using time-to-event analyses. Twins were followed from date of return of the questionnaire (in 1963) to development of cardiovascular disease outcome, or they were censored at time of death from other causes or end of follow-up (December 31, 2000).

Kaplan-Meier survival estimates for the association of tooth loss and periodontal disease with cardiovascular disease outcomes were compared by using the Mantel-Haenszel log-rank statistic. Multivariate proportional hazards models (Proc PHREG, SAS software; SAS Institute, Inc., Cary, North Carolina) were used to estimate hazard ratios and 95% confidence interval with 3 approaches. First, we
adjusted for the following individual-level risk factors for oral conditions and cardiovascular diseases: age (continuous), sex, education (university, gymnasium, vocational, compulsory, elementary, other), marital status (partner, yes/no), number of siblings (ordinal), smoking (current, <1 pack/day, former), body mass index (<20.0, 20–21.9, 22–23.9, 24–25.9, 26–29.9, >30 kg/m²), diabetes (yes/no), and hypertension (yes/no). All twins’ data were used for this analysis, and 95% confidence intervals were calculated by using a robust sandwich estimator to account for potential correlation of data within twin pairs.

Second, we used a co-twin design and conditioned on twin pair using stratified proportional hazards models. Twin pairs are inherently matched on familial effects, including genetic factors and shared environmental exposures. Third, because monozygotic twins share 100% of their genes whereas dizygotic twins share only 50%, we also conducted a co-twin analysis among monozygotic pairs alone, which additionally controls for unmeasured confounding by genetic effects. We compared the results from the co-twin analysis with the model controlling for only individual-level factors. Differences in the relative risks for the co-twin and unstratified analyses would suggest that familial effects contribute to the correlation of data within twin pairs.

We took advantage of the twin design to estimate the extent to which shared genetic factors explain the correlation of poor oral health and cardiovascular diseases. Our multivariate twin modeling used a Cholesky decomposition model and the Mx software program (23). The modeling approach estimates the heritability ($h^2$) of the individual traits, which is interpreted as the proportion of variation in the second. For example, if twin A and twin B in a pair each had periodontal disease and cardiovascular disease, the pair would be characterized as concordant. If genetic influences are important for the covariation between 2 traits, the cross-correlation among monozygotic pairs would be larger than the cross-correlation among dizygotic pairs. To simplify the modeling strategy, the models were fit directly to contingency tables, that is, whether oral and cardiovascular diseases were present/absent in twin A versus twin B, separately for monozygotic and dizygotic twins. The range of the genetic correlations is −1.0 to 1.0.

The study protocol complied with the principles of the Declaration of Helsinki. The Karolinska Institutet ethical committee approved the research protocol for the Swedish Twin Registry, and informed consent was obtained from all participating twins.

RESULTS

Almost 40% of the twins had experienced some tooth loss, with 14% having few or no teeth remaining in 1963. The prevalence of periodontal disease at baseline was 14%, with 3% of twins reporting severe tooth mobility. Baseline demographic and risk factor characteristics of the 15,273 twins are presented in Table 1 according to tooth loss and periodontal disease status. Participants with substantial tooth loss (few or no teeth remaining) at age 35 years tended to be less educated, had a higher prevalence of hypertension, and had a larger sibship size compared with those with the majority of their teeth. Participants with periodontal disease were on average older and less educated and had a higher prevalence of hypertension.

During more than 35 years of follow-up, the participants contributed almost 410,000 person-years to the study base, and 8,148 cases of cardiovascular disease occurred. The incidence rates per 1,000 person-years were 20.0 for cardiovascular disease, 7.3 for coronary heart disease, and 2.8 for stroke.

Results concerning the association between poor oral health and cardiovascular outcomes are presented in Table 2 for the 3 analytic approaches: 1) controlling for only individual-level
risk factors; 2) performing co-twin analysis among all twin pairs, which inherently controls for genetic factors and shared early-life exposures; and 3) conducting co-twin analyses among monozygotic pairs only, which assesses residual confounding by genetic factors.

After adjustment for individual risk factors, the relative risk of total cardiovascular disease was 1.2 (95% confidence interval: 1.1, 1.4) for participants with major tooth loss compared with all of their teeth (Table 2). The co-twin analyses did not appreciably change this relative risk. For periodontal disease, the majority of confounding was due to established individual factors. Twins with severe tooth mobility were at a 30% (95% confidence interval: 1.0, 1.4) greater risk of developing cardiovascular disease than

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those with no tooth mobility. Results of the co-twin analysis were similar (relative risk = 1.3, 95% confidence interval: 1.0, 1.7).

The risk of coronary heart disease was 1.3-fold (95% confidence interval: 1.1, 1.4) higher for those with none or few teeth remaining compared with those with all teeth, adjusting for individual risk factors (Table 2). Although there was little change in this hazard ratio in the co-twin analysis among all pairs, the relative risk increased to 2.1 (95% confidence interval: 1.2, 3.8) when the analysis was limited to monozygotic twins. For periodontal disease, the relative risk of coronary heart disease was 1.4 (95% confidence interval: 1.1, 1.6), adjusting for individual risk factors. The results from the co-twin analyses were similar, such that periodontal disease was associated with a 50% greater risk of coronary heart disease after adjusting for individual and familial factors.

When we adjusted for individual confounders, the risk of stroke was 1.2 times (95% confidence interval: 1.0, 1.5) higher for those with substantial tooth loss compared with all of their teeth. Further adjustment for familial effects in co-twin analysis among all pairs appeared to strengthen this association; for monozygotic pairs alone, the relative risk was below 1, although the confidence intervals were wide. We found no effect of periodontal disease on stroke in unstratified models or in the co-twin analyses among all pairs. Wide confidence intervals in the monozygotic co-twin analyses make definitive conclusions difficult about whether genetic confounding or chance is involved.

The results of the Cholesky decomposition analyses among the 15,000 twins showed significant estimates of $h^2$ for the individual traits. The risk of tooth loss ($h^2 = 0.55$) and periodontal disease ($h^2 = 0.42$) individually showed strong genetic components. Genetic factors were also important for development of cardiovascular disease ($h^2 = 0.32$) and coronary heart disease ($h^2 = 0.20$), although estimates of $h^2$ were smaller than for the oral conditions. In examining the extent to which genes are shared between these outcomes, we found evidence of a positive correlation between baseline tooth loss ($r_G = 0.18$) and periodontal disease ($r_G = 0.29$) on future risk of total cardiovascular disease. For coronary heart disease, there was evidence of a substantial negative genetic correlation with tooth loss ($r_G = -0.30$), in line with co-twin analysis, and a positive genetic correlation with periodontal disease ($r_G = 0.33$). The genetic correlations suggest that unknown genetic factors represent 50%–75% of the phenotypic correlations between these diseases within twin pairs.

**DISCUSSION**

To our knowledge, this study is the first to examine whether genetic factors could explain the association between poor oral health and cardiovascular disease. Our data suggest that tooth loss and periodontal disease are associated with a small excess risk of cardiovascular disease and a greater excess risk of coronary heart disease. For cardiovascular disease, the lack of attenuation in relative risks seen in co-twin analyses indicates that confounding by familial effects did not lead to spurious associations. The majority of confounding may be due to individual-level factors. For coronary heart disease and tooth loss, an interesting epidemiologic pattern emerged. Strengthening of the relative risk among monozygotic pairs, if real, suggests that genetic factors were negative confounders of the association between tooth loss and coronary heart disease. Once confounding by these factors was taken into account, tooth loss appeared to be associated with a more than 2-fold greater risk of coronary heart disease.

To our knowledge, data from the multivariate twin models are also unique, and they suggest common pathogenetic links between oral health and cardiovascular disease. For coronary heart disease and tooth loss, there was a significant negative genetic correlation, in line with the co-twin analyses in which the hazard ratio increased among monozygotic pairs. Although this finding could be due to chance, these data also may suggest that a substantial proportion of the genes that increase risk of one disease would decrease risk of the other. In contrast, the positive genetic correlation between coronary heart disease and periodontal disease indicates that genes involved in one condition may also be involved in the other.

Our findings of a link between poor oral health and cardiovascular disease are in line with a number of prospective studies that have evaluated this association (1–8). Our data are also in line with a pilot study within the Swedish Twin Registry among 10 twin pairs discordant for coronary heart disease, whereby the twin with coronary disease exhibited greater bleeding and deeper pathologic pockets than his or her twin without coronary heart disease (24). The finding of a shared genetic component is intriguing, although data are limited on specific candidate genes involved in the etiology of oral and cardiovascular diseases (17). Systemic effects of severe periodontal disease may point to specific markers. For example, periodontal disease is associated with higher circulating levels of the inflammatory mediators C-reactive protein and tissue plasminogen activator as well as low density lipoprotein cholesterol (25).

One promising candidate gene is interleukin (IL)-1, involved in inflammation and comprising 3 genes in close proximity on chromosome 2q13: IL-1A, IL-1B, and IL-1ra (26). Polymorphisms in IL-1, such as IL-1A +4845 and IL-1B +3954, increase risk of periodontal disease, whereas different polymorphisms, for example, IL-1ra intron 2 variable number tandem repeat, may be associated with a higher risk of cardiovascular disease (27). Moreover, a small case-control study of coronary heart disease suggested that variants in IL-1R were associated with both conditions, although the sample size of the study was quite small (28).

The genetic factors shared between the 2 conditions may also act by influencing behavioral traits. For example, prior twin research suggests a strong genetic predisposition to tobacco use (29) and obesity (30); thus, part of the genetic correlation between oral and cardiovascular diseases may be explained through genetic determinants of these intermediate factors. Genome-wide association studies that contain data on both cardiovascular and oral health conditions will be useful to delineate the specific genetic pathways.
Our study has a number of strengths and limitations to consider. We applied a novel approach to assessing the association between oral and cardiovascular diseases, controlling for genetic and familial factors. With our prospective design and complete follow-up, selection and differential misclassification biases were minimized. Because the questionnaire collected extensive covariate data, we could adjust for several, but not all risk factors. Although co-twin analyses control for genetic and shared environmental factors, it is always possible that residual confounding by environmental factors may exist. On the other hand, if monozygotic twins, versus dizygotic twins, are more similar with respect to these environmental risk factors, we may attribute confounding by an environmental factor to a genetic factor. In the Cholesky decomposition models, we used binary outcomes (yes/no) for each of the diseases, and thus the modeling assumed a similar genetic correlation between the two conditions across time.

Although questionnaire data were based on self-report, validation studies have shown high validity for tooth loss (31, 32) and good validity for periodontal disease (33, 34). A reliability study of a younger cohort of Swedish twins showed excellent reliability among the twins regarding report of tooth loss (kappa = 86%) and periodontal disease (kappa = 89%). We also had only one measure of oral health status from 1963, so someone classified as unexposed in 1963 may have then developed disease. Such misclassification would likely underestimate the effect of oral and systemic diseases. However, Joshipura et al. (9) found that baseline tooth loss in 1986, and not more recent loss, was more strongly linked with development of stroke.

There are inherent advantages to using population-based registers to collect outcome measures, including minimizing loss to follow-up and maximizing study efficiency. These databases collect information on clinically relevant cardiovascular disease endpoints, and they rely on information gleaned from medical and autopsy records. Validation studies suggest that cardiovascular disease data from Swedish registers have good validity and that, in particular, coronary heart disease and stroke measures are reliable in the Swedish Twin Registry (35, 36). One limitation of the Inpatient Register is the incomplete nationwide coverage during 1964 and 1987. Some incident cardiovascular disease events may have been missed altogether or captured later as prevalent events when coverage was more complete or when cardiovascular disease death occurred. However, results limited to cardiovascular disease death, which were complete throughout study follow-up, were virtually identical.

In stratified proportional hazards models, both the number of events and the proportion of twins discordant on time to event contribute to the effective power of a study. Thus, it is likely that power was sufficient to detect effects in the co-twin analyses for cardiovascular disease and coronary heart disease. The power for analyses limited to the monozygotic pairs was lower, as shown by the wider confidence intervals.

Approximately 15% of adults in the United States and Europe suffer from advanced or destructive periodontal disease (37–39). In addition, cardiovascular disease is the leading cause of death in many developed countries (40). Because of its high prevalence, poor oral health could be a substantial source of morbidity and mortality if there were truly a causal relation between poor oral health and cardiovascular disease. This study provides additional insight into this question, suggesting that an association exists between oral and cardiovascular diseases, independent of genetic and familial effects. The findings address previous uncertainties concerning the relation between oral and cardiovascular disease, and they emphasize the need to search for underlying mechanisms, both genetic and biochemical. Moreover, these analyses highlight the opportunity to conduct epidemiologic studies among twins to explore shared pathogenic associations between exposures and disease. Although it is still unknown whether a common polymorphism influences both oral and cardiovascular diseases, we used the distinctive genetic relationship of twins to postulate directly that shared genetic factors are responsible for an association between the diseases.

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