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Architecture of cortical bone determines in part its remodelling and structural decay

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ABSTRACT

Bone remodelling accelerates and becomes unbalanced after menopause; less bone is deposited than resorbed from the surface of canals traversing the cortex. The canals enlarge so the intracortical surface area enlarges. We hypothesized that cortical bone with a larger internal surface area, due to more or larger canals, is more liable to being remodelled, further enlarging the internal surface area and facilitating more remodelling and structural deterioration. For 95 monozygotic twin pairs aged 40–61 years, we measured internal cortical surface areas and structure of the distal tibia using high resolution peripheral computed tomography, and three circulating bone remodelling markers. Using principal component (PC) analyses, we identified one summary measure of intracortical and endocortical bone surface areas, cortical porosity and volumetric bone mineral density (structure PC), and one summary measure of bone remodelling markers (remodelling PC). We applied a twin regression analysis (Inference on Causation by Examination of Familial Confounding; ICE FALCON) to assess consistency with a causal component in the association between a predictor (X) and an outcome (Y) by testing if the regression coefficient for the X value of the co-twin decreases after adjusting for the X value of the twin herself. With Y = remodelling PC, the regression coefficient for structure PC in the co-twin was 0.29 ($p < 0.001$) before, and 0.18 ($p = 0.03$) after, adjusting for her own structure PC (40% lower; $p = 0.06$). With Y = structure PC, the regression coefficient for remodelling PC in the co-twin was 0.17 ($p = 0.01$) before, and 0.20 ($p < 0.001$) after, adjusting for her own remodelling PC (22% higher; $p = 0.7$). The structure of bone, its surface area to bone matrix volume configuration, might contribute in part to its own remodelling and deterioration, but not vice versa.

Introduction

The cellular machinery of bone modelling and remodelling is the final common pathway expressing all genetic and environmental factors influencing bone structure [1]. During young adulthood, remodelling is balanced; a volume of old or damaged mineralised bone matrix is removed and replaced by an equal volume of new bone matrix and no permanent structural decay occurs. After menopause, remodelling accelerates; there are more basic multicellular units (BMUs) initiated upon each of the three (intracortical, endocortical and trabecular) components of the bone's inner (endosteal) surface. In addition, remodelling by each of the BMUs becomes unbalanced; each time a volume of mineralised bone matrix is removed by teams of osteoclasts, less bone matrix is deposited by the teams of osteoblasts of a BMU. This produces focal structural decay characterized by increased intracortical porosity, cortical thinning, trabecular thinning and loss of complete trabecular plates [2].

As remodelling is surface dependent, variation in remodelling intensity might be partly explained by differences in the surface area available to facilitate remodelling [3,4]. Bone fashioned with greater surface area per unit mineralised bone matrix volume could be more accessible to being remodelled and therefore decayed. For example, from studying female twin pairs [5] we found positive associations between the intracortical and endocortical surface areas and bone remodelling markers and suggested that this was consistent with the notion that a larger internal surface area facilitates higher remodelling of cortical bone.

We recognised that the reverse is also plausible; higher remodelling upon cortical surfaces might result in larger surface because remodelling upon Haversian canals enlarges their cross-sectional diameter focally and so the perimeter and surface area increase at that location. Similarly, resorption upon the endocortical surface lining the medullary canal produces a concavity which might also enlarge the endocortical surface area focally. Therefore, remodelling might be self-perpetuating; a larger

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surface area might provide more locations for the receipt of signals from bone matrix in need of remodelling. If more remodelling further increases the surface area, a viscous cycle of accelerated deterioration of cortical bone would proceed.

Because our previous study was cross-sectional, it was not possible to test these alternative pathways using conventional analyses. This is a twin study and there are correlations between bone structure and remodelling within a twin, and bone structure in one twin and bone remodelling markers in the co-twin. Therefore we can use a recently developed twin regression analysis for studying evidence consistent with causation to test these hypotheses which we call Inference on Causation from Examination of Familial Confounding (ICE FALCON) [6–8]. This approach has been applied to predictors of mammographic density, a risk factor for breast cancer [6,7] and to eczema in infancy as a predictor of childhood asthma and hay fever [8].

To see how the argument works, consider sister pairs and *BRCA1* germline mutation status (X) as a predictor of breast cancer (Y); see Fig. 1. The sister of a mutation carrier is at increased risk of the disease because she has a 50% chance of having also inherited the mutation. Therefore, there is a 'cross-trait cross-pair' regression coefficient for the association of a woman's breast cancer status (e.g. Y_1) with her sister's mutation status (X_2). However, it is confounded by the mutation status of the woman herself (X_1), because once a woman's mutation status is known, then in terms of that woman's breast cancer risk the mutation status of her sister becomes irrelevant. If Y_1 is regressed against both X_1 and X_2, the cross-trait cross-pair regression coefficient becomes zero.

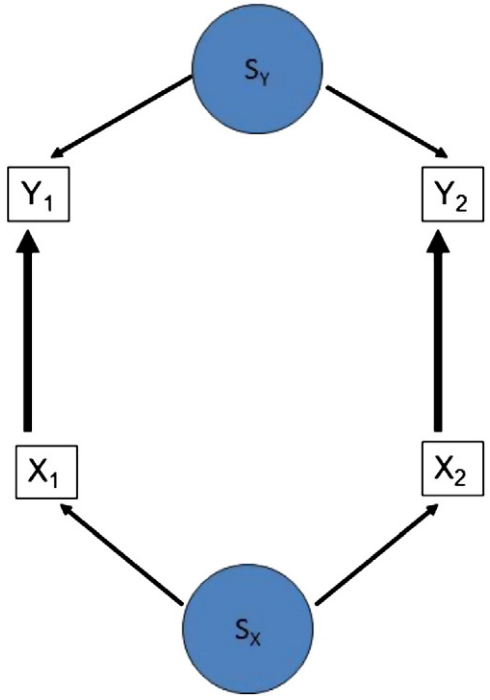

Fig. 1. A path diagram representing the casual association between *BRCA1* mutation status and breast cancer for pairs of sisters. Squares represent measured variables, circles represent unmeasured variables, and the arrows indicate the direction of causation between variables. Let Y_1 and Y_2 represent the breast cancer status of sisters 1 and 2, respectively, within the same pair, and X_1 and X_2 represent their corresponding mutation status for the breast cancer susceptibility gene, *BRCA1*. S_Y represents the risk factors for breast cancer, other than *BRCA1* mutation status, that are shared by sisters; i.e. the causes of Y shared by the sisters. S_X represents the cause of X shared by the sisters, namely their shared parenthood. Causal pathways, and therefore correlations or associations, between two variables are established by proceeding backwards along causal arrow(s), then forwards along causal arrow(s). Consider going from Y_1 to X_2. There is a causal pathway from Y_1 to X_1 to S_X to X_2. Note, however, that if X_2 is known, this pathway from Y_1 to X_2 is 'blocked'. While there is a causal pathway from Y_1 to S_Y to Y_2, it stops there (one cannot reverse direction more than once) so there is no connection between Y_1 and X_2 through this route. Therefore, given knowledge of X_1, there is no association between Y_1 and X_2; see text.

There is an analogy between this new twin regression analysis and the classic twin model, in that both methods address issues of causation by framing a one-tailed hypothesis test. The classic twin model makes inference about the existence of unmeasured genetic causes on a single measured trait by considering the null hypothesis of no difference in correlation coefficient between monozygotic pairs and dizygotic pairs versus the alternate hypothesis of a reduced correlation in dizygotic pairs. ICE FALCON makes inference about one measured familial trait having a causal effect on another measured trait by testing the null hypothesis of no change in a regression coefficient versus the alternate hypothesis of a reduction in the regression coefficient. Neither method 'proves' causation; both seek evidence consistent with causation.

Here we have applied the twin regression analysis, first to the situation where the Y variable is a measure of bone remodelling for one twin and the X variables are the bone structure measures of one or both of the twins. We fitted a series of models in which first only one of the X variables was included, and then both X variables were included. We then examined whether there was any reduction in the cross-trait cross-pair regression coefficient after adjusting for the X variable of the self. Second, we tested the reverse, that bone remodelling determines bone structure, by letting the Y variable be a measure of bone structure and the bone remodelling measures of the twins be the X variables. To simplify matters, we conducted a principal components analysis to see if we could identify one or more major dimensions to each of the bone structure and bone remodelling data.

Material and methods

Subjects

From 2008 to 2009, through the Australian Twin Registry we recruited 113 monozygotic (MZ) female twin pairs aged 40–61 years living in Melbourne, Australia [5]. Using a questionnaire, we assessed zygosity (concordance with zygosity determined by molecular testing is about 97% [7]) and excluded twin pairs in which one or both had a hysterectomy before menopause, had an illness or used drug therapies that affect bone, or were using hormone replacement therapy, leaving 95 pairs. All subjects gave written informed consent and the study was approved by the Austin Health Ethics Committee.

High-resolution peripheral quantitative computed tomography (HR-pQCT) (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) with an isotropic resolution of 82 μm was used to quantify cortical volumetric bone mineral density (vBMD) and porosity [9]. Measurements were made at the non-dominant distal tibia. The in vivo precision was 0.7% to 4.4%. Daily quality control was carried out by scanning a reference phantom containing rods of hydroxyapatite (QRM Moehrendorf, Germany). Radiation exposure was ~ 5 μSv per measurement.

Intracortical and endocortical bone surface (BS) areas at the distal tibia were measured using marching cubes that create triangular models of the surfaces from 3D data [10]. Surface measures were validated in vitro using 20-micron scans of excised trabecular cubes of the radius. Bone surface area/bone volume (BS/BV) by XtremeCT correlated with BS/BV by microCT-40 ($r = 0.98$) but the absolute values were overestimated because segmentation overestimates trabecular thickness (BS/BV = 17.4 vs. 11.3 1/mm by microCT-40), mean BS =2201 vs. 1920 mm^2 by microCT-40. The intracortical and endocortical surfaces were expressed per unit cortical tissue volume (Cortical TV; cortical bone including its pores). Cortical TV (mm^3) was expressed as the cortical CSA (mm^2) times the length of each scan (104 slices × 0.082 mm thickness).