GENETIC BACKGROUND OF TRAITS CONTRIBUTING TO REDUCED CORONARY BLOOD FLOW IN TWINS

PhD thesis booklet

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1. Introduction

Acute ischemic event originating from atherosclerosis is currently thought to be the most common cause of death world-wide.

Several mechanisms are known to contribute to the chronic disturbances of coronary arterial blood supply, including reduction of coronary blood flow reserve capacity, direct narrowing of the arteries due to stenotic plaques, reduced distensibility of both the coronary arteries and the aorta – which later infers the loss of facilited coronary blood flow during diastole due to premature wave reflection. Any of the above conditions predispose patients to a higher risk of cardiovascular morbidity and mortality – indeed, both the aortic stiffness parameter pulse wave velocity (PWV) and coronary calficication score (CACS) are well-established independent predictors of cardiovascular risk.

Currently there is no unified consensus on which one atherosclerosis burden (ATB) marker should we choose to express general atherosclerotic state, which would also be beneficial for risk stratification of patients. Although it is known that additional markers such as PWV

or CACS improve patient risk compared to Framingham risk score alone, there is still a limited role of these parameters in the current practice.

Evidence shows that atherosclerosis is not merely a modern disease induced by sedimentary lifestyle and other unhealthy habits. Recent CT scans of ancient people showed extensive calcifiation in their arteries. It is also known that atherosclerosis has a polygenic background. However, despite the increasing knowledge in this field, we still face difficulties in finding significant, replicable genetic pathways.

Just like in the case of ATB selection for cardiovascular risk survey, there is a missing unified approach on which atherosclerotic phenotypes should be used in genetic studies. Some studies focus on the involvement of one arterial segment failing to see the 'bigger picture', some studies use atherosclerosis 'surrogate' markers – however both generalizations from subspecific parameters and using imprecise global phenotypes might be misleading.

Twin studies are *quantitative* genetic studies where we can assess the magnitude of genetic effects on the

development of given phenotypical trait without the exact specification of these genes. Twin studies can help understand the initiation and development of diseases, clarify phenotypic and genotypic variation – which is also crucial in establishing the frames of reproducibility in genetic studies.

2. Objectives

The aim of this research was two-fold:

1) First specific aim

First, we sought to investigate the longitudinal patterns of aortic stiffness parameters such as aortic PWV and aortic augmentation index (AIx) – to gain inshight into the role of genetics or environmental factors on the long run, examining an international twin study population.

2) Second specific aim

Second, we aimed to develop an extracoronary ATB score (which is also radiologically more easily accessed) and hypothesized that this trait would correlate with the coronary burden Agatson score. Finally, we used the twin study design to determine if this correlation was mainly due to common genetic or environmental factors.

Additionally, we examined the relationship between aortic PWV (proposed to be a marker of generalized atherosclerosis) and CACS. Furthermore, we assessed predicting power of our model containing extracoronary ATB markers in assessing the presence of coronary calcification on CT.

3. Methods

1) *First specific aim*

In the first part of our research 148 Hungarian and 220 Italian twins were examined with a mean 4.4 years follow-up time. The longitudinal arterial stiffness and blood pressure measurements were conducted by the same experts (A.D.T, D.L.T), using the same non-invasive oscillometric device (Tensiomed Arteriograph, Medexpert Ltd, Budapest, Hungary). Zigosity assessment was based on self-reported questionnaire. All participants filled another questionnaire regarding lifestyle habits, medications, and chronic diseases. Body mass index was measured by a validated equipment (Omron Healthcare Ltd., Kyoto, Japan).

Aortic stiffness parameters and central blood pressure values were calculated automatically by the oscillometric device using the following equations:

$$aPWV\left(\frac{m}{s}\right) = \frac{Jug - Sy(m)}{RT/2(s)}$$

where Jug-sy is the jugulum-symphysis distance, RT is the return time of the systolic wave (time interval between first and reflected peak systolic values) and

$$Aix(\%) = \frac{P_2 - P_1}{PP} x100$$

Intercooled STATA for Windows (version 11.2; StataCorp, College Station, Texas, USA) was used for the descriptives statistics. The Mx software was used for the furhter calculations. Pearson's correlations on the longitudinal data from the two waves were assessed using bivariate saturated models. We determined the following correlations for each trait: within-individual/cross wave correlations, cross-twin/within-wave correlations and cross-twin/cross-wave correlation.

Heritability of the traits were calculated at both waves using a pathway model where latent variables:

additive genetic effects (A), common environmental effects (C) and unique environmental effects (E) contribute to the manifested (or phenotypical) variables (univariate model). Model fitting started with the testing of the full ACE model including all three latent variants. Sub-models (AE, CE, E) were then created, factor loadings were iteratively assessed and the submodels were compared to the full model (using the likelihood-ratio chi-square test). The best fitting model based on the lowest Akaike Information Criterion.

In bivariate Cholesky modelling the similar approach was used, however the expected covariance matrices were fitted to the observed covariance matrices allowing us the genetic vs. environmental decomposition of longitudinal variance for each trait.

2) Second specific aim

In the second part of our study 190 Hungarian twins were enrolled from the Hungarian Twin Registry. Opposite-sex dizygotic (DZ) twins were excluded from this study. We obtainment anthropometric data (weight, height/BMI) and participants filled a self-reported questionnaire about lifestyle and medical history.

Each participants underwent non-enhanced electrocardiogram-triggered cardiac CT (Brilliance CT, Philips HealthTech, Best, The Netherlands). Per os β-blocker (metoprolol, 100 mg max. dose) was given 1 hour prior to CT scan in case of rapid heart beat (> 65/min). Our protocol included 2.0 mm slice thickness, 120 kVp tube voltage and 20-50 mAs tube current depending on BMI. We evaluated CACS using commercially available software (Extended Brilliance Workspace; Philips Healthcare) - expressed in Agatston score [95].

Two experienced radiologists (A.D.T, D.L.T) performed carotid and femoral ultrasound examinations using high frequency (5-10 MHz) linear transducers (Philips HD15, Philips Healthcare, Best, The Netherlands). Carotid arteries were scanned bilaterally including the common carotid arteries and the proximal proximal 2-3 cm segments of the internal and external carotid arteries. Femoral arteries were visualized similarly: common femoral arteries and proximal parts of the deep and superficial femoral arteries were scanned.). Each plaque (≥1.5 mm endoluminal protrusion or >50% focal thickening relative to adjacent arterial wall) was

registered, and categorized based on their echogenicity type. We used three categories: hypo-, hyper- and mixed plaque types. Histologic specimens correlated to these categories showed that increasing echogenicity meant increasing amount of calcification.

To assess extracoronary atherosclerotic burden based on the ultrasound findings, we developed 4-segment scores. Plaque dissemination status was assessed by summing the number of affected arteries regarding (4S_hypo, 4S_mixed, 4S_hyper) or not regarding (4S_PL) plaque type.

Demographic data and phenotypic Spearman correlation were analyzed using the SPSS statistical program (SPSS Statistics 17). For the twin statistics we used OpenMx library of the R statistical program. We used age and sex adjustment for each variable. Agatston score was converted to ordinary values. Heritability of traits were calculated fitting univariate ACE models using a liability-threshold model. The submodels weres compared to the full model and the most parsimonious one was chosen using the Akaike Information Criterion.

Polychoric phenotypic correlations between CACS and 4S_hyper were assessed in monozygotic (MZ), DZ and all twins. Bivariate correlated factors model was used for the genetic vs. environmental decomposition of phenotypical resemblance using liability-threshold structural equation models. Model selection was similar to the univariate statistics. We then further categorized twin pairs regarding similarity in plaque localizations to assess wether MZ twins are more similar than DZ twins.

Additionally, we calculated the phenotypic correaltion between aortic PWV and CACS using SPSS Statistics 17.

Logistic regression models were used to assess significant determinants of CACS (including 4S scores and aortic PWV). The algebraic equation of the logistic regression is the basis of the model which can predict CAC. We used machine learning methods to assess performance of the model: binary classification was used, the test and training set were randomly separated (ratio: 0.7:0.3) and cross-validated.

4. Results

1) First specific aim

Patient demographic data showed no significant differences between MZ and DZ twins. However, Italian participants were slightly older, significantly more people reported to be current or former smoker and less people had medically treated hypertension.

Intra-individual longitudinal correlation of aortic PWV was moderate (r=0.35, 95% confidence interval: CI: 0.25-0.45), but stronger in case of aortic AIx (r=0.60, 95% CI: 0.52-0.67). Cross-twin/within wave correlations were significantly higher in MZ (r_{MZ} between 0.35 and 0.6) than in DZ twins (r_{DZ} between 0.15 and 0.48) both at wave 1 and wave 2, indicating a strong genetic influence Cross-twin/cross-wave correlations were weak-moderate in MZ twins (r= 0.34 for aortic PWV and 0.32 for aortic AIx), however these were hiher than the correlations observed in DZ twins.

Bivariate Cholesky decomposition allowed the assessment of model parameters explaining the longitudinal covariations. Best-fitting model was the AE model, Our results showed that genetic factors contributed

in a high percentage (cov_g=0.88, 95%CI: 0.61-1.00) to the longitudinal covariance of aortic PWV phenotype. On the other hand, genetic factors explained also substantial but lesser proportion of longitudinal covariance in the case of aortic AIx (cov_g=0.55, 95% CI 0.35-0.70). Environmental covariation and correlation also appeared as a contributors of intermediate importance (cov_e= 0.45, 95% CI 0.30-0.65; r_e= 0.52, 95% CI 0.38-0.64) in the case of AIx.

2) Second specific aim

DZ twins in the Hungarian twin population were somewhat older and had relatively more frequent femoral atherosclerosis. No other characteristics were found to be significantly different in the two groups.

Spearman correlation between CACS and four-segment plaque scores showed that the distribution state of any calcification in the carotid/femoral arteries (4S_hyper/mixed) correlated with CACS best (0.604, $p<0.01$). However, 4S_hyper was chosen for further analysis as it showed better correlation than 4S_mixed (0.551 vs 0.444, $p<0.001$). 4S_PL showed similar magnitude of correlation (0.557, $p <0.01$), however

4S_hypo showed weak correlation with CACS (0.289, p <0.01).

We found moderate heritability for both CACS (h=0.67, 95%CI: 0.35-1) and 4S_hyper (0.69, 95% CI: 0.38-1). Dissemination of hypoechoic plaques was however mainly influenced by unique environmental factors (E=1). The 4-segment score of mixed plaque type is influenced both by genetic and unique environmental factors (h=0.49, 95% CI: 0-0.76; E= 0.50, 95%CI: 0.24-1). The highly heritable and phenotypically correlating CACS and 4S_hyper was decomposed in the following bivariate genetic analysis and we found substantial genetic correlation between the two traits (0.86, 95%CI: 0.42-1).

Categorizing MZ and DZ twin pairs regarding similarities or contradictions in their plaque localizations, we found higher similarity in MZ twin

The phenotypic correlation between aortic PWV and CACS was 0.394 (p<0.01) – less, than the correlation between 4S_hyper and CACS. Logistic regression showed that age sex, 4S_hyper and 4S_mixed were significant determinators of CACS (aortic PWV

did not reach significance thus was left out from the model). The diagnostic values of the model in predicting CACS were good (area under curve was 0.84,.with highest sensitivity (0.81) and specificity (0.84) at threshold value of 0.4, overall accuracy: 0.83 (CI: 0.70-0.91).

5. Conclusions

In our international and national twin studies we investigated the longitudinal and cross-sectional heritability and genetic association patterns of four important and promising CV risk factors, also potentially describing atherosclerosis burden (ATB): aortic PWV and aortic AIx (rigidity markers of the aorta), CACS and four-segment scores derived from carotid-femoral ultrasound. Our most prominent and novel findings are the following:

(1) Both aortic PWV and aortic AIx longitudinal varations are substantially genetically determined during a 4.4-year follow-up period.

(2) The variance components analyses showed that aortic AIx - although mainly genetically determined – is partly

more sensitive to environmental factors. Therefore, therapeutics aiming to reduce aortic AIx might be more effective than in the case of aortic PWV.

(3) CACS phenotypically correlated well with both four-segment plaque score (4S_PL) and four-segment score of hyperechoic plaques (4S_hyper).

(4) Calcified plaque score of carotid/femoral arteries and CACS showed substantial overlap in the genetic factors that contribute to them – therefore, having a high 4S_hyper score makes people genetically more prone to have a higher CACS.

(5) This genetic correlation may be partly due to genetic predisposition to atherosclerosis 'dissemination route', - however, further larger and longitudinal studies are needed to support this idea.

(6) We repeated the earlier finding, that combination of carotid and femoral ultrasound could improve coronary calcification prediction. Our results also higlight the importance of genetic factors in the etiology of atherosclerosis.

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