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Heritability of a resting heart rate in a 20-year follow-up family cohort with GWAS data: Insights from the STANISLAS cohort

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Abstract

Background: The association between resting heart rate (HR) and cardiovascular outcomes, especially heart failure, is now well established. However, whether HR is mainly an integrated marker of risk associated with other features, or rather a genetic origin risk marker, is still a matter for debate. Previous studies reported a heritability ranging from 14% to 65%.
Design: We assessed HR heritability in the STANISLAS family-study, based on the data of four visits performed over a 20-year period, and adjusted for most known confounding effects.
Methods: These analyses were conducted using a linear mixed model, adjusted on age, sex, tea or coffee consumption, beta-blocker use, physical activity, tobacco use, and alcohol consumption to estimate the variance captured by additive genetic effects, via average information restricted maximum likelihood analysis, with both self-reported pedigree and genetic relatedness matrix (GRM) calculated from genome-wide association study data.
Results: Based on the data of all visits, the HR heritability (h^2) estimate was 23.2% with GRM and 24.5% with pedigree. However, we found a large heterogeneity of HR heritability estimations when restricting the analysis to each of the four visits (h^2 from 19% to 39% using pedigree, and from 14% to 32% using GRM). Moreover, only a little part of variance was explained by the common household effect (<5%), and half of the variance remained unexplained.
Conclusion: Using a comprehensive analysis based on a family cohort, including the data of multiple visits and GRM, we found that HR variability is about 25% from genetic origin, 25% from repeated measures and 50% remains unexplained.

Keywords

Heart rate, heritability, genetic relatedness matrix, family study, cardiovascular diseases

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Introduction

Heart rate (HR) is an easy to measure phenotypic quantitative trait that is routinely used for risk prediction in the field of cardiovascular medicine. The association of resting HR with cardiovascular outcomes, especially heart failure, is well established.[1–5] From an epidemiological standpoint, determining the genetic part of this trait could further inform researchers of the inherited nature of this important clinical factor. Indeed, even if HR is ubiquitously perceived as a major risk marker in clinical medicine, whether HR is

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mainly an integrated marker of risk associated with other features, or rather a genetic origin risk marker, is still a matter of debate.

As for many other continuous phenotypic traits, variation of HR among individuals is influenced by genetic determinants, lifestyle, and environmental factors.[3] The heritability of a quantitative trait is the proportion of variance due to genetics.[6–9] More specifically, we used here the ''narrow sense'' heritability (h^2), which refers to the proportion of phenotypic variance attributable to the additive genetic factors only.[6,9–12]

The heritability of resting HR in healthy subjects varies significantly in the literature, ranging from 14% to 34% in family-based cohort studies, and from 53% to 65% in twin studies.[1,13–18] This high variability in heritability estimates is likely to be explained by study design, the way a shared environment is taken into account in a family study and the residual confounding effects of lifestyle factors.[3,14] In addition, the estimation of heritability in a family study has been traditionally performed using pedigree analysis, but the accuracy of estimations can largely be improved by the recent use of the genetic relatedness matrix (GRM), especially for siblings.[17,19] As a result, given the large variability of estimates in the literature, HR heritability estimated in a populational/familial setting, using GRM, that is based on the data of multiple time points and adjusted on most known confounding effects, could provide a more accurate estimate of HR heritability.

The purpose of the present study is, first, to estimate resting HR heritability using both self-reported pedigree and the GRM calculated from genome-wide association study (GWAS) data and, second, to evaluate the contribution of longitudinal data in these former estimations of HR heritability.

Methods

Study population

The design of the STANISLAS cohort has been previously reported.[20,21] In brief, the STANISLAS cohort study is a family-based longitudinal cohort, initially including 4295 healthy individuals of French origin from 1006 families living in the Lorraine region (north-eastern France). They were first recruited from 1994 to 1995 during a medical examination and were re-examined about every five years. At each visit, participants underwent a medical examination, gave a blood sample, and were interviewed by trained nurses using a structured questionnaire, including items on socio-demographic, medical and family history, smoking status, lifestyle, diet (including alcohol consumption), and anthropometric data. Moreover, the 1705 participants who took part in the fourth visit were genotyped using the GSA chip, designed by Illumina®. An Ethics Committee approved the study and informed consent was obtained from all participants.

Sample

In total, four individuals from the fourth visit were excluded from the analyses because they had atrial fibrillation. Characteristics of all included subjects are listed in Table 1 (a) and (b). A total of 10,142 observations were included in the present analysis, 4928 of which had GWAS data. The distribution of subjects who attempted one or more visits (up to four) is described in Table 2.

Outcome

At visit four, the resting HR was determined after 10 minutes of rest, using a standard 12-lead electrocardiogram. For the three other visits, HR was assessed using clinical measures performed at rest.

Covariates

In the following analysis, we used the following as categorical covariates: sex, current smoker status, use of beta-blockers, consumption of tea or coffee, and practice of physical activities. As the relationship between age and HR was not linear, age was modeled using restricted cubic splines, with five knots at fixed percentiles (5%, 27.5%, 50%, 72.5%, and 95%) of the distribution.[22]

Household effect definition

In this family-based study, defining the common environment component of the total variance represents the most challenging part of the work. At recruitment time, only nuclear families with young children were included. However, first the children grew up between visits, and second new family members, such as a daughter-in-law or a son-in-law, were subsequently included in the cohort, creating new households. In order to best fit with the common household effect for family members, we arbitrarily decided that children aged more than 20 years old were taken out from their first nuclear family and were attributed a new family ID. The distribution of the number of members belonging to the same household effect at each visit is reported in Figure 1.

Statistical methods

As a first step, we described heritability using parent–offspring correlations. However, these correlations do not account for covariates and household effect.

Table 1. Participant characteristics: (a) all participants; (b) participants with available genotype.

(a)	Visit 1 ($n = 4252$)	Visit 2 ($n = 2934$)	Visit 3 ($n = 1319$)	Visit 4 ($n = 1637$)	All visits ($n = 10,142$)
Number of families, *n*	1213	1118	736	1177	2106
Parents, *n* (%)	1992 (46.8%)	1491 (50.8%)	792 (60.0%)	1011 (61.8%)	5286 (52.1%)
Children, *n* (%)	2260 (53.2%)	1443 (49.2%)	527 (40.0%)	626 (38.2%)	4856 (47.9%)
Male, *n* (%)	2137 (50.3%)	1460 (49.8%)	665 (50.4%)	799 (48.8%)	5061 (49.9%)
Age (yrs), mean ± sd	27.0 ± 14.3	32.0 ± 15.3	38.6 ± 15.6	49.5 ± 14.1	33.6 ± 16.7
Age parents (yrs), mean ± sd	41.4 ± 5.0	46.2 ± 5.6	49.4 ± 8.5	59.6 ± 6.0	47.5 ± 8.9
Age children (yrs), mean ± sd	14.3 ± 3.9	17.4 ± 4.5	21.9 ± 5.9	33.2 ± 5.1	18.4 ± 7.6
Heart Rate (bpm), mean ± sd	70.1 ± 12.2	68.2 ± 12.1	64.6 ± 11.0	62.4 ± 9.6	67.6 ± 12.0
Covariates					
Beta-blockers, *n* (%)	21 (0.5%)	38 (1.3%)	17 (1.3%)	136 (8.3%)	212 (2.1%)
Coffee/Tea, *n* (%)	1962 (46.1%)	1717 (58.5%)	1012 (76.7%)	1528 (93.3%)	6219 (61.3%)
Sport, *n* (%)	2598 (61.1%)	1167 (39.8%)	398 (30.2%)	842 (51.4%)	5005 (49.3%)
Tobacco, *n* (%)	786 (18.5%)	637 (21.7%)	291 (22.1%)	377 (23.0%)	2091 (20.6%)
Alcohol, *n* (%)	1808 (42.5%)	1373 (46.8%)	511 (38.7%)	1382 (84.4%)	5074 (50.0%)

(b)	Visit 1 ($n = 1461$)	Visit 2 ($n = 1232$)	Visit 3 ($n = 718$)	Visit 4 ($n = 1517$)	All visits ($n = 4928$)
Number of families, *n*	667	618	442	1114	1200
Parents, *n* (%)	915 (62.6%)	794 (64.4%)	232 (32.3%)	935 (61.6%)	3130 (63.5%)
Children, *n* (%)	546 (37.4%)	438 (35.6%)	486 (67.7%)	582 (38.4%)	1798 (36.5%)
Male, *n* (%)	702 (48.0%)	604 (49.0%)	354 (49.3%)	743 (49.0%)	2403 (48.8%)
Age (yrs), mean ± sd	31.8 ± 13.9	36.3 ± 14.8	41.9 ± 14.5	49.4 ± 14.1	39.8 ± 16.0
Age parents (yrs), mean ± sd	41.9 ± 4.9	46.5 ± 5.6	50.9 ± 7.1	59.6 ± 6.2	49.7 ± 9.1
Age children (yrs), mean ± sd	14.8 ± 4.5	17.8 ± 5.0	23.2 ± 5.3	33.2 ± 5.1	22.6 ± 9.2
Heart Rate (bpm), mean ± sd	68.5 ± 11.5	67.2 ± 11.7	63.8 ± 10.3	62.5 ± 9.6	65.6 ± 11.1
Covariates					
Beta-blockers, *n* (%)	7 (0.5%)	15 (1.2%)	9 (1.3%)	126 (8.3%)	157 (3.2%)
Coffee/Tea, *n* (%)	862 (59.0%)	833 (67.6%)	573 (79.8%)	1412 (93.1%)	3680 (74.7%)
Sport, *n* (%)	1116 (76.4%)	468 (38.0%)	196 (27.3%)	775 (51.1%)	2555 (51.8%)
Tobacco, *n* (%)	234 (16.0%)	220 (17.9%)	113 (15.7%)	343 (22.6%)	910 (18.5%)
Alcohol, *n* (%)	694 (47.5%)	625 (50.7%)	306 (42.6%)	1288 (84.9%)	2913 (59.1%)

bpm: beats per minute; sd: standard deviation; yrs: years

Table 2. Distribution of subjects who attempted one or more visits (up to four).

		All subjects	Subset of subjects with genetic data
Number of visits in which subjects participated	1	1322	23
	2	1571	311
	3	1078	685
	4	611	557
	Total	10,142	4928

We used a dedicated linear mixed model, which allowed us to simultaneously include additive genetic effects across the genome, common environment effects shared by families and fixed effects. All analyses were performed using R (version 3.5.0).

Pedigree matrix. Traditionally, the kinship or pedigree matrix is derived from a carefully curated pedigree, joining together the individuals with phenotypes from their self-reported genealogical relationships. To this end, we used the R package Kinship2 (https://cran.r-project.org/web/packages/kinship2/index.html) to create the matrix

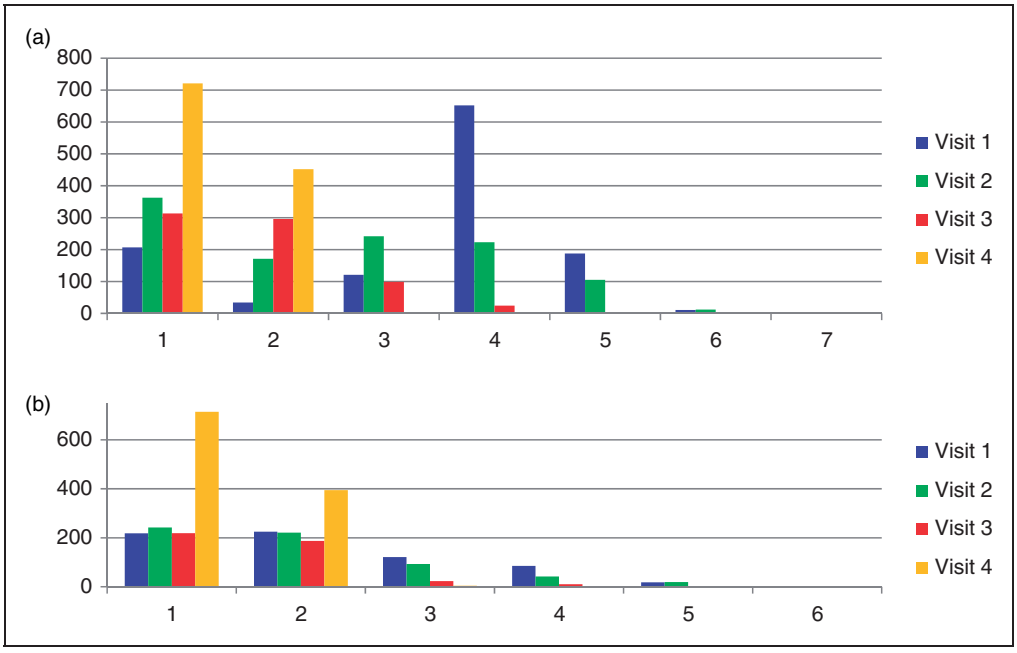

Figure 1. Histograms of the numbers of family members in the same household effect for each of the four visits: (a) for all participants; (b) for genotyped individuals only.

of family relationships between pairs of individuals (i.e. unrelated, 0; parents–offspring, 0.5; full-sibling, 0.5; half-sibling, 0.25; etc.).

Genetic data quality control. The quality of the genotype data was checked with the R package Gaston.[23] Only polymorphic autosomal single nucleotide polymorphisms (SNPs) were kept; then SNPs with a call rate <99%, Hardy–Weinberg equilibrium with p value $< 10^{-8}$, or one of each duplicated SNP pair (same chromosome, same position) were excluded. Samples with a call rate <95% and those with aberrant heterozygosity (hz; mean hz $\pm$ 3 standard deviations) were also excluded. The sample homogeneity was assessed using principal component analysis on founders and no outlying individual was found. After quality control steps, 526,785 SNPs were available for 1590 individuals.

GRM. Traditional pedigree matrices are now often replaced by GRMs, which are computed using polymorphic SNPs. The GRM contains genotype correlations, which estimate genomic relatedness between pairs of individuals. For each pair of individuals, the genotype correlation is the sum of the products of standardized genotypes between two individuals.[9,16,24,25] The use of GRM allowed a better inference of relatedness between siblings, instead of an expected average.

Heritability estimation. Subsequently, the two matrices (pedigree and GRM) were used in a linear mixed model to estimate the variance captured by additive genetic effects via average information restricted maximum likelihood analysis, implemented in the R package Gaston[23]

$$Y = X\beta + \omega + \delta_H + \delta_{Ind} + \delta_V + \varepsilon$$

where Y is the vector of phenotypes (HR), X is the covariates matrix, β is a fixed effect vector of covariates, $\omega \sim N(0,\tau K)$ is the individual genetic random effect vector, where K is the GRM or the pedigree matrix, $\delta_i \sim N(0,\gamma_i \Sigma_i)$, $i \in \{H,\ Ind,\ V\}$, are potential random effect vectors used to correct household effects, individual effects and/or visit effects, respectively, Σ_i is the corresponding design matrix, and $\epsilon \sim N(0,\sigma^2 I)$ is the residual vector. Then, the heritability is usually estimated by

$$h^2 = \frac{\tau}{\tau + \gamma_H + \gamma_{Ind} + \gamma_V + \sigma^2}$$

For each time-point and for all visits taken together, three models were performed, with first all participants (using the pedigree matrix); second, the subset of genotyped individuals using GRM; and, third, the subset of genotyped individuals using pedigree matrix (in order to compare results from the pedigree matrix and GRM within the same group of individuals). All models were fitted with all fixed effects and with a random household effect. When all visits were gathered together, we added two random effects (γ_v for visit and γ_{ind} for

individual effect) to take into account the variance component resulting from repeated measures.

In the supplementary materials, three other models are presented in order to test the model for sensitivity and robustness: (a) with no fixed effect and no household effects; (b) with all covariates as fixed effects and no household effects; and (c) with age and sex as covariates and household effects. In addition, in the supplementary materials, gender stratified analyses have also been realized.

Results

Characteristics of the study population detailed by visit are presented in Table 1. Figure 1 represents the number of family members included in one household effect according to each visit. At the first visit, 54% of subjects belonged to families comprising four members, whereas for the fourth visit, 61% of subjects did not share any common environment (according to our definition of household effect). The subset of genotyped individuals corresponds to participants who attended the fourth visit (Table 1(b)) as the family structure is different. This is because only half of the initially included participants attended visit four, and the proportion of parents was higher in this last visit. Indeed, children from visit one (which were young adults at visit four) were less likely to attend visit four than their parents. Consequently, only 13% of genotyped subjects at visit one belonged to 4-member families.

Scatterplots and correlation tests between parent–offspring pairs showed that parental and offspring HR are significantly correlated (see Supplementary Figure 1) suggesting a heritable part of HR. Table 3 presents the percentages of variance decomposition of HR for each of the four visits and for all visits taken together. We showed that heritability estimations are sensitive to the time-point, showing large heterogeneity of variance decomposition, and then of HR heritability estimations depending on the visit studied (h^2 from 19% to 39% using the pedigree matrix and from 14% to 32% using GRM for visit one and for visit three, respectively).

It is also important to note that variance decompositions, calculated from GRM or the pedigree matrix, were very similar for the subset of genotyped

Table 3. Percentages of variance decomposition for each visit and for all visits: (a) for all participants; (b) for genotyped participants.

	Heritability h^2 (%)	Household effect (%)	Repeated measures (%)	Residual variance (%)
(a)				
Visit 1 ($n = 4252$)	18.96	9.48		71.56
Visit 2 ($n = 2934$)	29.92	3.33		66.75
Visit 3 ($n = 1319$)	39.48	0.30		60.22
Visit 4 ($n = 1642$)	30.87	3.87		65.26
All visits ($n = 10,142$)	24.53	2.90	24.87	47.70
(b)				
Visit 1 ($n = 1461$)				
GRM	13.81	6.46		79.73
Kinship	11.36	7.30		81.34
Visit 2 ($n = 1232$)				
GRM	21.08	4.65		74.26
Kinship	21.72	4.22		74.06
Visit 3 ($n = 718$)				
GRM	22.78	0.00		77.22
Kinship	22.56	0.00		77.44
Visit 4 ($n = 1517$)				
GRM	31.72	4.02		64.25
Kinship	31.15	3.68		65.17
All visits ($n = 4928$)				
GRM	23.98	1.98	22.85	51.18
Kinship	23.23	1.98	23.61	51.18

GRM: genetic relatedness matrix

individuals. Conversely, when comparing results obtained for all individuals and for the subset of genotyped individuals, they were quite discordant, and heritability estimates were almost always lower for the subset of genotyped individuals, whether using GRM or the pedigree matrix (for example, at visit three, $h^2 = 39\%$ versus 22%, respectively, for all individuals and for the subset of genotyped individuals). When we considered all visits together, heritability estimates were homogeneous according to the model tested, based on either GRM or the pedigree matrix (from 23% to 25%, respectively).

In the supplementary material, we show the results for variance decompositions using three other models; first, no covariate and no household effect; second, all covariates and no household effect; and third, sex, age, and household effect. We did not find any substantial difference between results from the pedigree matrix and GRM (Supplementary Tables 1–3), but we showed differences in estimates according to the model and the structure of the dataset (i.e. the visit studied).

Gender stratified analysis for each visit (Supplementary Table 4) showed no substantial differences from the results obtained in the whole population. However, we found that household effect is more important for women than for men. Figure 2 shows histograms of variance decomposition for each visit and all visits taken together. We showed that only a small part of variance is explained by a common household effect (1.98% for the subset of genotyped subjects to 2.90% for all subjects when considering all visits), except for visit one, where almost all participants of a same family were gathered into the same household effect. An important part of the variance remained unexplained; after accounting for repeated measures, genetic and household effects, the proportion of the variance that remained unexplained for HR ranged from 48% (for all subjects) to 51% (for the subset of genotyped subjects).

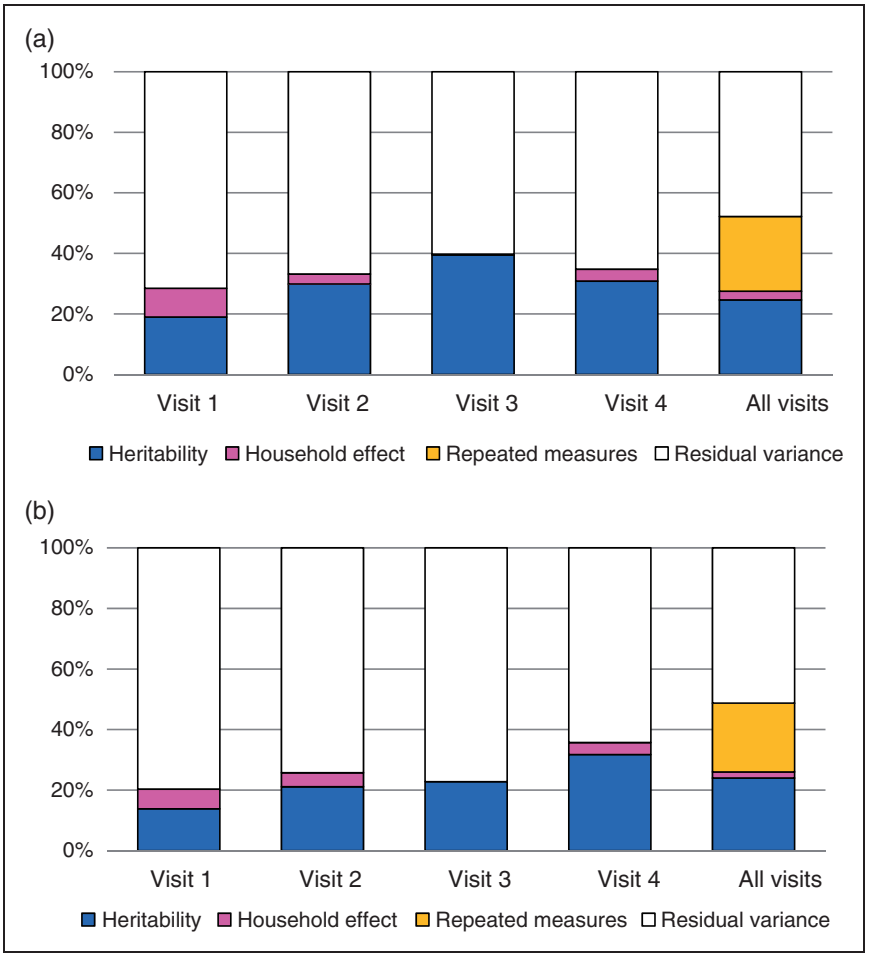

Figure 2. Histograms of variance decompositions according to the four visits and all visits: (a) for all participants (with pedigree matrix); (b) for individuals genotyped only (with genetic relatedness matrix (GRM)).

Fixed effects (from covariates) account for 12% (with GRM) and 17% (with the pedigree matrix) of the total variance, but the part of variance explained by these fixed effects has been removed from the total variance in our heritability estimate models (data not shown).

Discussion

Estimates of heritability of resting HR using multivariable-adjusted analysis of multiple-points suggests that about 25% of the observed HR variance can be attributed to additive genetic factors. This finding is in the lower range of previously published estimates from family studies,[1,14,16,18] and is largely lower than those from twin studies.[13,15,17] Common environmental effects do not seem to make a substantial contribution to variance in our study (less than 3%), whereas the intrinsic individual variability of HR was of a similar magnitude to the heritability (approximately 25% of individual effect assessed with repeated measures).

Finally, approximately 50% of the HR variance remained unexplained. This can be attributed to underlying interactions between genetic and environmental effects,[1] and to unaccounted genetic effects, such as dominance or epigenetic factors. Other unmeasured environmental factors may have been omitted in our study, even if our study design was especially dedicated to consider the majority of known cofactors in the estimation.

"Novelty" of the approach

In comparison with previously published results, our study presents the major advantage of estimating HR heritability from multiple time-point measures in a family cohort (i.e. with a wide age distribution). Moreover, some other studies used only a few covariates as adjustment variables, whereas in our study, we have tried to adjust the estimations for the majority of known confounding factors (including smoking status, physical activities, cardiovascular drugs intake, coffee, tea, and alcohol consumption). Lastly, in our study, we have used both GRM and the pedigree matrix with multiple time-point measures of HR; this approach has probably limited the misestimation of HR heritability.

Heritability of HR in a sample comprising a majority of children

Heritability is a population-based estimate, sensitive to the characteristics of the population in which it is estimated. Therefore, it could vary across populations, environments, and ages.[11,12] To the best of the authors' knowledge, our cohort is one of the first European cohorts with a sizeable proportion of young children (at the first visit). The low heritability estimate we observed at visit one suggests that factors other than genetic features affect HR in children, an aspect which has, to the best of the authors' knowledge, not been previously emphasized. It could also suggest that HR measurement variability/miscalculation in children could bias heritability estimates, even if we have used a complex method to take into account the HR variability according to the age of the subjects.

Validation of heritability estimates using the pedigree matrix or GRM

Heritability estimation may also be affected by the pedigree structure,[11] which is reflected in our study by the difference in heritability estimates according to the sample used (subset of genotyped subjects or all the cohort participants) and between visits. However, the results do not differ largely according to the sample when considering all visits together, which reinforces the robustness of our estimations (Table 3(b) and Supplementary Tables 1–3).

Moreover, the increasing availability of dense genotyping array data and the use of GRM provide a real advantage for heritability estimation accuracy, and hence minimize the potential bias due to false genealogical data.[26] To the best of the authors' knowledge, very few studies on HR heritability have used GRM.[16]

Our study provides the advantage of using both GRM and the pedigree matrix in order to estimate HR heritability. In our study, the results suggest that GRM does not substantially add to the classical pedigree matrix approach, as estimates were very similar across methods (23.98% with GRM, versus 23.23% with pedigree matrix). This result shows that there are probably very few pedigree errors in our very well-characterized family-based population. However, the use of GRM provides three additional advantages: 1. it gives the opportunity to verify family declared relationship; 2. it provides a better estimation for sibling genetic relatedness; and 3. it takes into account the estimation of genetic relatedness between unrelated subjects.

Household effect

In our study, common household effect only has a small contribution to the HR variance, which is in accordance with previous findings.[1,27] However, estimating this effect is very challenging due to the definition of "common environment." We have chosen to consider one definition of "household effect" (i.e. children were considered as sharing the same environment as their parents if aged less than 20 years old). Using this

definition, our results suggest that a very small proportion of HR variability is explained by household effect; genetic and unknown factors appear as largely more important in explaining HR variability. Moreover, we identified a differential household effect according to gender, which is more pronounced in women than in men. However, this differential effect is difficult to ascertain as we necessarily lost the couple effect in the household effect in this analysis.

If we consider fixed effects in the estimation, the proportion of unexplained variance remain large (40–45% according to the model tested, data not shown).

Limitation

The size of the genotyped subset of participants is moderate when compared with other population studies. Some participants were lost to follow-up in the last visits, which has modified the familial structure of the sample through each visit. As already acknowledged, the misestimation of HR at the first visit may have been frequent in children, however, the difficulty to correctly assess HR in children is not limited to the scope of this study.

Because we have included visit and individual effects, an important part of the variance (about 25%) was attributed to repeated measures, which could be due to changes in methods for HR measurement during the 20 years' follow-up. It is possible that standardized HR measurements would have decreased variance explained by repeated measures and consequently increased heritability estimations.

Finally, this is a single-center study, recruiting participants from a limited geographic area during a routine examination at the Center for Preventive Medicine that investigates fairly homogenous participants. Yet, this has permitted the use of most of the genetic data, as outliers were infrequent.

Clinical and research implications

HR is central to the prediction of many cardiovascular events, including the occurrence of heart failure.[4] Our study suggests that HR heritability is only 25%, and that half of the variation of HR remains unexplained (even if fixed effects are included in the model), which could appear surprisingly high for one of the strongest cardiovascular risk markers. This result suggests that other unknown factors, not assessed in our study, are associated with HR differences across individuals. In other words, HR could be an integrated marker of risk that is neither the consequence of classical factors associated with cardiovascular risk as assessed in our analysis (activity level, smoking status, age, and gender), nor determined by inherited genetic features.

Our study strongly reinforces, using a comprehensive analysis, that we still do not fully understand the determinants of elevated HR. Variations in HR could be the consequence of subtle preclinical changes in cardiovascular function, subsequently associated with clinical cardiovascular events. Alternatively, HR could be a causal trigger of subsequent cardiovascular events. To date, no preventive trials specifically focusing on HR reduction have been undertaken, possibly because of our lack of understanding of its underlying biological determinants and our uncertainty in the causality between HR and subsequent cardiovascular outcome. To some extent, our study emphasizes the need to better understand the pathophysiology underlying higher resting HR, possibly to help tailor interventions targeting this important cardiovascular risk marker.

Conclusion

Using a comprehensive analysis based on a family cohort, including the data of multiple visits and GRM, we have found that HR variability is about 25% from genetic origin, 25% from repeated measures, and 50% remains unexplained.

Author contribution

CX, NG, JFD, PR, FZ, contributed to the conception or design of the work. CX, PdV, NG, CDR, DBD, ELF, JLM, JPF, JFD, PR contributed to the acquisition, analysis, or interpretation of data for the work. CX and NG drafted the manuscript. CDR, PdV, ELF, JPF, PR, JFD; JLM, FZ, DBD critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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