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Insight into genetic determinants of resting heart rate

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ABSTRACT

Background: Recent studies suggested that resting heart rate (RHR) might be an independent predictor of cardiovascular mortality and morbidity. Nonetheless, the interrelation between RHR and cardiovascular diseases is not clear. In order to resolve this puzzle, the importance of genetic determinants of RHR has been recently suggested, but it needs to be further investigated.
Objective: The aim of this study was to estimate the contribution of common genetic variations on RHR using Genome Wide Association Study.
Methods: We performed a Genome Wide Association Study in an isolated population cohort of 1737 individuals, the Italian Network on Genetic Isolates — Friuli Venezia Giulia (INGI-FVG). Moreover, a haplotype analysis was performed. A regression tree analysis was run to highlight the effect of each haplotype combination on the phenotype.
Results: A significant level of association ($p < 5 \times 10^{-8}$) was detected for Single Nucleotide Polymorphisms (SNPs) in two genes expressed in the heart: *MAML1* and *CANX*. Founding that the three different variants of the haplotype, which encompass both genes, yielded a phenotypic correlation. Indeed, a haplotype in homozygosity is significantly associated with the lower quartile of RHR (RHR ≤ 58 bpm). Moreover no significant association was found between cardiovascular risk factors and the different haplotype combinations.
Conclusion: Mastermind-like 1 and Calnexin were found to be associated with RHR. We demonstrated a relation between a haplotype and the lower quartile of RHR in our populations. Our findings highlight that genetic determinants of RHR may be implicated in determining cardiovascular diseases and could allow a better risk stratification.

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1. Introduction

Several epidemiological studies reported a significant association between resting heart rate (RHR) and cardiovascular mortality (Greenland et al., 1999; Tverdal et al., 2008). Renewed interest in this clinical variable has been elicited by recent observations, suggesting that high RHR is an independent predictor of cardiovascular mortality and morbidity (Diaz et al., 2005; Jouven et al., 2005). Moreover, high RHR has been highlighted as a possible risk factor for cancer and sudden death (Fox et al., 2007; Jouven et al., 2005). Despite this evidence, the complex interrelation between RHR and cardiovascular disease is not completely understood. In particular, it is unclear whether high RHR is a true risk factor or it is merely an epiphenomenon of a common defect underlying a complex clinical condition (Singh et al., 1999). RHR is likely to be a complex trait, depending on hereditary and environmental factors (Martin et al., 2004). The importance of the genetic background in RHR has been recently suggested (Eijgelsheim et al., 2010). Several studies based on candidate-gene approach highlighted a significant genetic component for RHR; moreover genome-wide association studies (GWAS) have recently identified common variations associated with RHR (den Hoed et al., 2013; Wilk et al., 2006). The clinical impact of these findings remains unknown; nevertheless the identification of the genetic background of RHR could allow a better risk estimation in cardiovascular disease.

The use of isolated populations reduces heterogeneity of complex and/or quantitative traits, and proved to be very useful in identifying polymorphisms with relevant clinical impact (Arcos-Burgos and Muenke, 2002). These communities, owing to their origin by few founders, their isolation, a high rate of inbreeding and low immigration, have preserved homogeneous genomes over the centuries (Kristjansson et al., 2002; Thorgeirsson et al., 2003). Therefore, the general reduction of genetic and environmental variation, the availability of well-documented

Abbreviations: *CANX*, Calnexin; *MAML1*, mastermind like 1; BMI, bone mass index; RHR, resting heart rate; bpm, beats per minute; SNP, single nucleotide polymorphism; ECG, electrocardiogram; maf, minor allele frequency; *SERCA*, sarco(endo)plasmic reticulum Ca^{2+} ATPase; FVG, Friuli Venezia Giulia.

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extended pedigrees and the opportunity to map genetic disease loci using linkage disequilibrium analysis provide an increase in statistical power to identify genes (den Hoed et al., 2013; Peltonen, 2000; Urbanek et al., 2010). We combined the advantages derived from the study of isolated populations with the statistical power of GWAS to analyze the genetic background of RHR in Italian isolated populations.

2. Methods

2.1. Samples

The biological samples and the phenotype used for the analysis belong to the Italian Network on Genetic Isolates — Friuli Venezia Giulia (INGI-FVG) project. 1737 people living in these villages voluntarily participated to a medical screening and blood samples were collected for genetic analysis.

Each individual underwent a complete cardiological evaluation with physical examination, electrocardiogram (ECG) and echocardiogram. Exclusion criteria were: a) lack of ECG data, b) age ≤ 15 years, c) heart failure, d) implanted peacemaker, e) atrial fibrillation, and f) use of antiarrhythmic drugs (including beta-blockers and digitalis).

2.2. Genotyping

DNA of 1376 samples was extracted from peripheral blood by automated purification method using Qiagen EZ1 workstation (http://www.quiagen.com) and quantified using NanoDrop (http://www.nanodrop.com). All samples were genotyped with Illumina 370k arrays (http://www.illumina.com) and then imputed using MACH (http://www.sph.umich.edu/csg/abecasis/MACH/) to a common set of ~2.5 million autosomal SNPs based on linkage disequilibrium (LD) patterns observed in Hap Map release 22 CEU samples (http://www.hapmap.org/). Imputed allele dosage and genotyped SNPs were used to analyze the quantitative genetic trait of RHR.

2.3. Phenotype collection

RHR was derived by ECG recordings (Mortara instrument ELI 250 was used to obtain ECG measurements). RHR was calculated as number of beats per minute (bpm). In addition, we categorized the level of RHR into 4 classes (low, normal, normal-high, high) according to the empirical distribution in our samples. Blood pressure was calculated on the mean of three blood pressure estimations. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mm Hg and diastolic blood pressure (DBP) ≥ 90 mmHg and/or use of anti-hypertensive medications. ECG and blood pressure measurements were obtained in supine position, after at least 5 min of rest. According to the World Health Organization, anemia is defined as level of hemoglobin of less than 13 g/dL in men and less than 12 g/dL women.

Moreover, all participants were told to avoid caffeine products and physical activity for 12 h prior to examination. Anthropometric variables and records about lifestyle and medical history were obtained for all the subjects. We defined the parameter "physical activity" as follows: physical activity has a score that can be 1 or 0. An individual has a score of 1 if the said individual practices at least 3 h/week of sporting activity otherwise it is 0.

The final total number of recruited, analyzed and directly genotyped individuals was 855.

2.4. Statistical analysis

Because of the presence of close relatives in our dataset, statistical analyses were performed using a mixed model regression where the kinship matrix is the random effect, as implemented in GenABEL and ProbABEL. As statistical test we used a method of mixed model (GRAMMAR) (Aulchenko et al., 2007; Thompson and Shaw, 1990) and we performed an additive genetic model using sex, age, hypertension, physical activity and bone mass index (BMI) as covariates.

GWAS results were plotted using LocusZoom (Pruim et al., 2010).

The haplotypes for each individual on genes obtained from GWAS were phased using PLINK v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/) (Purcell et al., 2007) using the genotyped SNP present in Illumina 370k arrays. Using a partitioning analysis using R package party (Hothorn et al., 2006), a regression tree was built in order to check the effect on the phenotype of each haplotype combination. The subjects were subsequently grouped according to the haplotypes and we analyzed the differences in mean of RHR; furthermore the test of Mann–Whitney was performed to assess the p-value of each comparison.

For each population a genetic distance matrix was created, based on Fst values (Reynolds et al., 1983) using only the SNPs present into the haplotypes. Fst was calculated in R using the package 'adegenet' (Jombart, 2008). We used the genetic distance matrix to perform a PCO (Principal Coordinate analysis). This method starts with a similarity matrix or dissimilarity matrix (or distance matrix) and assigns each item a position in a dimensional space. Using PCO we can visualize group differences (Gower, 1966). We therefore compared the PCO graph with the levels of RHR at population level, in order to look for a possible population-specific gradient and a correlation between the genetic diversity at haplotypes and RHR differences at population level.

3. Results

3.1. Study population

The clinical features of our populations are summarized in Table 1. The estimated heritability of resting heart rate in FVG was very close to 27%.

According to the partition by quartile, we categorized RHR as "low" with values under the 25th percentile (RHR ≤ 58 bpm), "normal" with values between the 25th and the 50th percentiles (58 < RHR ≤ 64 bpm), "normal-high" with values between the 50th and the 75th percentiles (64 < RHR ≤ 73 bpm) and "high" over the 75th percentile (RHR > 73 bpm), as reported in Table 2.

3.2. Genome Wide Association Study of RHR

After performing the default filtering (call rate ≥ 97%, Rsq ≥ 0.3, Hardy–Weinberg Equilibrium p-value (HWE) ≥ 1×10^{-6}, minor allele frequency (MAF) ≥ 0.05) a total of 2,058,182 genotyped autosomal SNPs and imputed SNPs passed quality controls and 855 individuals.

In order to detect common genetic variants influencing the variability of RHR trait, a genome wide analysis (GWA) was carried out (Supplementary Fig. S1) and the inflation factor (λ) was calculated in order to check for presence of stratifications in our populations. The inflation factor (λ) was 0.9999003 for INGI-FVG, indicating the absence of stratification.

A significant level of association ($p < 5 \times 10^{-8}$) was detected for more SNPs on 5q35 inside Mastermind-Like1 (*MAML1*, 5q35.3, expressed in the heart; OMIM: 605424) and Calnexin (*CANX*, 5q35.3; OMIM: 114217). Results were plotted using LocusZoom (Fig. 1); p-values are reported in Supplementary Table S1. The most associated SNP is rs6893300 (p-value = 9×10^{-9}) in the intron region of *CANX*.

3.3. Haplotype analysis

In order to investigate the effect of these genes on the phenotype, a haplotype analysis was performed, identifying one haplotype that encompasses both *MAML1* and *CANX*. Three different variants of this haplotype were found: H1 = TTAAA, H2 = CCAGG and H3 = CTCGG (see Supplementary Table S2 for the SNP present into the haplotypes and Supplementary Table S3 for haplotype frequencies).

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