Heritability and Linkage Study on Heart Rates in a Mongolian Population

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ABSTRACT

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Elevated heart rate has been proposed as an independent risk factor for cardiovascular diseases, mortality and all cause mortality, but their interrelationships are not well understood. In this study, we performed a genome-wide linkage scan in 1002 individuals (mean age 30.6 years, 54.5 % women) from 95 extended families of Mongolia and determined quantitative trait loci that influence heart rate. The DNA samples were genotyped using deCODE 1000 microsatellite markers for 3 cM density genome-wide linkage scan. Correlation analysis was carried out to evaluate the correlation of the covariates and the heart rate. T-tests of the heart rate were also performed on sex, smoking and alcohol intake. Consequently, this model was used in a nonparametric genome-wide linkage analysis using variance component model to create a multipoint
logarithm of odds (LOD) score and a corresponding $P$ value. In the adjusted model, the heritability of heart rate was estimated as 0.32 ($p < .0001$) and a maximum multipoint LOD score of 2.03 was observed in 77 cM region at chromosome 18. The second largest LOD score of 1.52 was seen on chromosome 5 at 216 cM. Genes located on the specified locations in chromosomes 5 and 18 may be involved in the regulation of heart rate.

Key words: genome-wide linkage scan; heart rate; heritability
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I. INTRODUCTION

1. Epidemiological Evidence

Epidemiological and clinical studies suggest that elevated heart rate is a potential risk factor for a variety of cardiovascular diseases including atherosclerosis, coronary artery disease, myocardial infarction, arterial hypertension and heart failure. 2, 5,9,12,14,25,29

The elevated heart rate is closely associated with age, high blood pressure, body mass index, smoking, alcohol consumption, high cholesterol level, and physical inactivity. But, even after adjusting for other potential risk factors, elevated heart rate remains significantly associated with cardiovascular disease, mortality and all cause mortality in patients with cardiovascular disease as well as general population. 7, 9-11, 13, 15, 16, 26,28, 36

The normal weight individuals with
lower resting heart rate have lower levels of cardiovascular disease risk factor and mortality.\(^{42}\)

In the latter study those 5,713 working men were participated and followed up for 23 years. The sudden death and total mortality was increased with high heart rate, and this association was still significant after adjusting for potential confounding factors such as blood pressure, age, and physical inactivity.\(^{45}\) Moreover, in a study those 125,000 men and 96,000 women were participated, a high heart rate was related with cardiovascular mortality and the hazard ration was 1.59.\(^{46}\)

The prognostic power of high heart rate for cardiovascular disease was clear not only within the general population but also among patients with acute coronary syndrome, diabetes mellitus, high blood pressure, acute myocardial infarction and heart failure. In a recent survey of 10,267 patients with acute coronary syndromes, a higher initial and delayed heart rate highly predictive of higher short- and long-term mortality in patients with acute coronary syndromes.\(^{43}\)

In the Coronary Artery Surgery Study which enrolled 24,913 subjects who suspected cardiovascular diseases and followed up for 14 years. In this study, a total mortality and cardiovascular mortality were significantly associated with high heart rate in patients with coronary artery disease.\(^{7}\) Moreover, in the Framingham heart study which
enrolled patients with high blood pressure and followed up for 36 –
years. In this study, after adjusting for age, systolic blood pressure,
smoking, body mass index and other potential confounding factors,
odds ratios for all cause mortality was 2.18 and 2.14 respectively, in
men and women. Overall, these data provide that the significant
association between high heart rate and cardiovascular disease, and
mortality.

2. Genetic Evidence

A number of twin and family studies have reported that genetic
factors influence the regulation of heart rate. There is a
genic component in heart rate generation and heart rate variability in
monozygotic and dizygotic twin pairs. A significant genetic regions
contributing to heart rate variability has been identified on chromosome
15 at 62 cM and chromosome 2 at 153 cM. In a twin study, a gender
difference in heart rate variability between men and women is
demonstrated. Women have greater heart rate variability than men even
after controlling for a large number of potential confounders such as
age, oral contraceptive use and menopausal status.

In the recent meta-analysis of genome-wide scans for study
networks that enrolled Caucasians and African-Americans, the
replication between various ethnic groups as well as the study networks with low heterogeneity has been identified on chromosome 5p13-14.\textsuperscript{18} Moreover, a polymorphism in the B1 adrenergic receptor was determined to be significantly related to heart rate in a hypertensive cohort.\textsuperscript{47}

In the Hypertension Genetic Epidemiology Network (HyerGen) study, which included hypertensive subjects, 195.06 cM region of chromosome 4 is seemingly related to heart rate variability in both African Americans and Caucasian.\textsuperscript{39} In the Framingham Heart Study, which 1345 participants from largest 310 pedigrees, there was a replicated association of chromosome 9p21 with major cardiovascular disease.\textsuperscript{19}

Most published studies on genetic influences on the variation of heart rate focused on Caucasian and not on Asian populations. In this study, we assessed genetic components involved in heart rate variation using data from the GENDISCAN (GENe DIScovery for Complex traits. in isolated large families of Asians of Northeast) study. Large extended families enable the full utilization of family design for linkage study.\textsuperscript{49}

The GENDISCAN study is committed to incorporating most of methodological issues of complex diseases using genetically
homogeneous population and emphasizing the quantitative phenotypes.\textsuperscript{49}

The purpose of this study was to assess the heritability of heart rate and heart rate variability and to identify susceptible loci for heart rate variability in an Asian population. Recognition of the genetic determinants of heart rate and heart rate variability may provide additional insight into the pathophysiology of the cardiovascular disease and mortality.
II. MATERIALS AND METHODS

1. Subjects

One thousand and two Mongolian individuals (54.5 % women) from 95 extended families from Dornod, Mongolia were genotyped. Informed consents to participate in the study were obtained from all subjects. The protocol for the GENDISCAN study was approved by the institutional review board (IRB) of Seoul National University of Korea (approval number, H-0307-105-002).

2. Heart Rate and Covariates

Four consecutive measurements of the heart rate and blood pressure were made on both arms of each participant while seated, employing a standard electronic sphygmomanometer. When the four blood pressure measurements differed by more than 10 mmHg, a fifth measurement was made and the lowest four were used in the analyses.

The larger means of each set of measurements were accepted as heart rate and the blood pressure respectively. Measurements in children from age 10 to 16 were made using appropriate cuffs and a mercury sphygmomanometer. Data on potentially confounding factors for heart rate such as age, sex, smoking, and alcohol consumption were collected through interviews performed by trained interviewers. The
standing height in centimeter (cm) and weight in kilograms (kg) were measured, and body mass index (BMI) was calculated in kg/m².

3. Genotyping

For those who agreed to be genotyped, genomic DNA was extracted from peripheral venous blood leukocytes using standard procedure and genotyped using deCODE 1,000 STR marker platform. Whole genome analyses have been performed. For 1,000 deCODE marker sets, optimized multiplex PCR reactions were set up on Zymark ALH 400, run on MJR Tetrad™ and pooled on Gilson Cyberlab C200 robots. The reaction volume was 5 µl, and for each PCR 20 ng of genomic DNA was amplified in the presence of 2 pmol of each primer, 0.25 U AmpliTaq Gold, 0.2 mM dNTPs and 2.5 mM MgCl2 (buffer was supplied by the manufacturer, Applera). Cycling conditions were as follows: 95°C for 10 min, followed by 37 cycles of 94°C for 15 s, annealing for 30 s at 55°C, and 1 min extension at 72°C. The PCR products were supplemented with the internal size standard GS500-LIZ, and the pools were separated and detected on 3730 Sequencers. Alleles were automatically called using DAC, an allele-calling program developed at deCODE genetics Inc. and the program deCODE GT was used to fractionate called genotypes, according to quality, and to edit when necessary.
4. Statistical Analysis

4.1 Correlation analysis

We carried out a correlation analysis to evaluate the correlation of covariates (age, sex, smoking, alcohol intake, brachial systolic pressure, brachial diastolic pressure, brachial mean arterial pressure and body mass index) and heart rate, and Pearson correlation coefficients were identified. T-tests of heart rate were also performed on the three categories, which are sex, smoking and alcohol intake, by using SAS statistical software version 9.1.

4.2 Pedigree and Genotype Data Cleaning, Single- and Multi-point Identity by Descent (IBD) Calculation

For the family relationship, nonpaternity was examined using PEDCHECK. Relationships other than paternity were checked using average identity by descent (IBD)-based method by PREST. After correcting pedigree error and mendelian errors, non-mendelian errors were examined and corrected using SimWalk. After pedigree and genotype errors were corrected, IBD matrices between every relationship pair were calculated. IBD matrix for single marker was calculated by SOLAR, and multipoint IBD (MIBD) matrices were computed on every 1 cM distance using Markov chain Monte Carlo
method by LOKI. We used Haldane’s mapping function to convert map distances into recombination fractions.

4.3 Heritability Estimation

Genetic components of selected phenotypes were estimated in terms of heritability. Narrow sense heritability, defined as the proportion of total phenotypic variation due to additive genetic effects, was calculated. Age-sex adjusted phenotype (adjusted for age, sex, age-square, product of age and sex, product of age-square and sex) was estimated by SOLAR for quantitative traits.

4.4 Multipoint Variance Component-based Linkage Analysis for Heart rate (Genome-wide Linkage Analysis)

To reduce the type I error from deviated distribution, original values were normalized using Z-transformation. The genetic variance of heart rate was decomposed into specific additive genetic effects from specific markers (Quantitative trait loci, QTL) and non-QTL given by:
\[ \Omega_{ij} = \prod \sigma_{QTL}^2 + 2 \Phi_{ij} \sigma_{N-QTL}^2 + I e^2 \]

Where \( \Omega \) is the covariance matrix of the entire family, \( \pi \) is a matrix of the proportions of the specific QTL that the relative pairs share as IBD, \( \Phi \) is a kinship matrix, \( I \) is an identity matrix, \( \sigma_{QTL}^2 \) is specific QTL effects of the genetic markers, \( \sigma_{N-QTL}^2 \) is residual genetic effect, and \( e^2 \) is random environmental effects and errors. The likelihood that QTL effects can be estimated were compared with the likelihood of null hypothesis that specific QTL effect equals zero. The logarithm of odds (LOD) score between likelihood of null and alternative hypothesis were used to test the significance of linkage results. The multipoint linkage analyses were performed using SOLAR. In the genome-wide scans, age, sex, age\(^2\) and the interactions between them retained in the models as covariates at \( p < 0.10 \). Because variance composition method is sensitive to outliers, multivariate residual kurtosis in each analysis retained less than 0.8 thereby avoiding type 1 error.
III. RESULTS

Demographic and pedigree characteristics of the data set and the covariates are presented in table 1.

Table 1. Demographic and pedigree characteristics of the data set

| Characteristic                                      | Value          |
|-----------------------------------------------------|----------------|
| No. of families                                     | 95             |
| Mean ±SD no. of family members (range)              | 15.7 ± 12.4    |
| No. of genotyped subjects                           | 1002           |
| Mean ±SD age (in years)                             | 30.6 ± 15.4    |
| Percentage female (%)                               | 54.5           |
| Height (cm)                                         | 155.8 ± 11.1   |
| Weight (kg)                                         | 58.0 ± 15.6    |
| Body mass index (kg/m²)                             | 23.4 ± 4.5     |
| Brachial systolic pressure (mmHg)                   | 114.6 ± 16.3   |
| Brachial diastolic pressure (mmHg)                  | 67.3 ± 9.8     |
| Brachial Mean arterial pressure (mmHg)              | 83.1 ± 11.7    |
| Heart rate (bpm)                                    | 78.1 ± 10.9    |
| Current alcohol use (%)                             | 131(13.4)      |
| Smoking (%)                                         | 190(19.1)      |
| No. of study pairs of:                              |                |
| Parent-offspring                                    | 1812           |
| Full-sib pairs                                      | 734            |
| Sisters                                             | 198            |
| Brothers                                            | 167            |
| Brother and sister                                  | 369            |
| Half-sib pairs                                      | 395            |
| Grandparent-grandchild pairs                        | 1202           |
| Avuncular pairs                                     | 888            |
| First-cousin pairs                                  | 598            |
The data set of examined individuals included a large number of relative pair types as we have recruited extended families.

The data set included information on 2546 pairs of first-degree relatives (1812 parent-offspring pairs and 734 full-sib pairs), and 2485 pairs of their second-degree relatives (395 half-sibling pairs, 1202 grandparent-grandchild pairs). The other 888 and 598 pairs were avuncular and first-cousins, respectively.

Current alcohol use and smoking was reported by 13.4% and 19.1%, of the subjects, respectively. Mean age of the subjects was 30.6 yrs. Mean resting heart rate was 78.1 and the mean number of family members was 15.7. The mean body mass index (BMI) was 23.4 and the mean brachial systolic, diastolic, and arterial blood pressure was 114.6, 67.3, and 83.1, respectively.
Table 2 shows the relationships between baseline characteristics and heart rate. Age, brachial diastolic pressure, and body mass index (BMI) were significantly associated with mean heart rate. The adjusted heritability model includes age, gender, BMI, smoking and alcohol consumption.

Table 2. Relationships between baseline characteristic data and heart rate

|                           | Heart rate |
|---------------------------|------------|
| **Pearson correlation coefficients** |            |
| Age                       | - 0.21 (<.0001) |
| Brachial systolic pressure | - 0.01 (0.72) |
| Brachial diastolic pressure | 0.11 (0.0002) |
| Brachial Mean Arterial pressure | 0.06 (0.07) |
| Body mass index (kg/ m²)   | - 0.21 (<.0001) |
| **Mean values (SD)**      |            |
| Gender (t-test)            | 0.60       |
| Women                     | 78.3 ± 10.4 |
| Men                       | 77.0 ± 11.5 |
| Smoking (t-test)           | 0.35       |
| Yes                       | 77.5 ± 11.4 |
| No                        | 78.3 ± 10.7 |
| Alcohol (t-test)           | 0.53       |
| Yes                       | 77.5 ± 10.8 |
| No                        | 78.1 ± 10.9 |

*a P values reflect test of subgroup differences (women versus men).

b P value of smoking yes, no
c P value of alcohol yes, no
In the adjusted model, the heritability of heart rate was estimated to be 0.32 (p < .0001) (Table 3).

**Table 3. Heritability of heart rate variations**

|                      | Heart rate |
|----------------------|------------|
| Fully adjusted Heritability $^a$ | 0.32       |
| P-value              | <.0001     |

$^a$ Includes adjustment for age, and gender, BMI, Smoking and alcohol.
In Table 4 are presented LOD scores greater than 1.0, nearest markers, chromosomal locations and candidate genes. In the adjusted model a maximum LOD score of 2.03 was seen on chromosome 18 at 77 cM. The second largest LOD score of 1.52 was seen on chromosome 5 at 216 cM.

Table 4. LOD scores, chromosomal locations, and nearest marker data for all LOD scores > 1.00

| Trait         | Marker at or Near Peak | Chromosome Location | deCODE (cM) | Maximum LOD Score | Candidate gene |
|---------------|------------------------|---------------------|-------------|-------------------|----------------|
| Heart rate    | D1S186                 | 1                   | 62          | 1.13              | ET2 ZC3H12A    |
|               | D3S1515                | 3                   | 21          | 1.32              | CAV 3          |
|               | D5S408                 | 5                   | 216         | 1.52              | NSD1 F12       |
|               | D6S1567                | 6                   | 39          | 1.35              | E2F3           |
|               | D18S474                | 18                  | 77          | 2.03              | LIPG SLC14A2   |

a Includes adjustment for age, gender, BMI, Smoking and alcohol.
Figure 1 shows the chromosomal regions linked to heart rate genome-wide linkage analysis.

**Figure 1.** Genome–wide linkage analysis of chromosomal regions linked to heart rate
As shown in figure 2, there is suggestive evidence of linkage (LOD score=2.03) of a quantitative trait locus (QTL) for heart rate on chromosome 18 at 77 cM.

**Figure 2.** Evidence of linkage (LOD score = 2.03) of a quantitative trait locus (QTL) for heart rate on chromosomes 18 at 77 cM
IV. DISCUSSION

It has long been known that heart rate is under the control of the parasympathetic and sympathetic nervous system, and that heightened sympathetic tone increases the heart rate.\textsuperscript{1, 10, 26, 27} In more recent studies, they pointed out that genetic components may play an essential role in the regulation of heart rate variability.\textsuperscript{34,39} The Framingham heart study also demonstrated that genetic factors are involved in heart rate variability.\textsuperscript{35}

In the GENDISCAN study, the degree of heritability of heart rate was 0.32. This value is somewhat higher than the figure (0.21) reported for Framingham Heart Study participants,\textsuperscript{34} but is lower than the figure (0.41) reported for participants of Netherlands Twin Register.\textsuperscript{17} All the three studies provide evidence for a strong genetic component in heart rate variability.

The GENDISCAN project is the first largest family studies in Asian population in Asia. The unique property of this study is that subjects are members of large extended families in isolated rural area.\textsuperscript{49}

We showed a peak with a maximum LOD of 2.03 on chromosome 18 at 77 cM. The \textit{SLC14A2} (solute carrier family 14 urea transporter) gene which lies near this loci, encodes UT-A protein expressed in the heart.\textsuperscript{8} The expression of UT-A protein in failing left
ventricle is 1.4-4.3 fold to that in normal nonfailing ventricle.\textsuperscript{8}

The \textit{LIPG} (endothelial lipase precursor) gene also lies on chromosome 18 at 77cM. This gene encodes the protein that process substantial phospholipase activity and plays an important role in lipid metabolism. More recently, it has been known that the significant association between 584C/T SNP of \textit{LIPG} gene and an acute myocardial infarction independent of HDL-C levels in a Japanese population.\textsuperscript{33}

It is also of interest that chromosome 5 yielded the second largest linkage peak which corresponds to its 216 cM region. An analysis of the database indicated that the chromosomal region 216 cM on chromosome 5 contains \textit{NSD1} (nuclear receptor SET domain containing gene1) gene. The protein encoded by \textit{NSD1} gene enhances transactivation of androgen receptor. It has been reported that the intragenic mutation of this gene is associated with the high frequency of congenital heart defects or heart conduction.\textsuperscript{3}

The \textit{F12} (coagulation factor XII) gene also located at 216 cM on chromosome 5. This gene encodes coagulation factor XII which circulates in blood as a zymogen. The \textit{C46T} polymorphism of \textit{F12} is associated with a reduction of plasma FXII levels and a development of myocardial infarction, particularly in hypercholesterolemic patients.\textsuperscript{30}
The homozygosity for the C46T polymorphism of the F12 gene is significantly associated with high risk of coronary artery disease in the Spanish population.\textsuperscript{31}

The ZC3H12A (zinc finger CCCH type containing 12A) gene was located at 62cM on chromosome 1, which is a monocyte chemotactic protein-1 (MCP1) induced protein. MCP1 mediated inflammation plays a critical role in the development of cardiovascular disease.\textsuperscript{1,4,41} In a recent study, it has been reported that MCP1 causes cell death of cardiomyocytes and plays an important role in the development of ischemic heart disease.\textsuperscript{41}

The ET2 (endothelin 2) gene at 62 cM on chromosome 1 was founded. High circulating plasma levels of ET have been reported in essential hypertension.\textsuperscript{32} The polymorphism in the ET2 gene influences the hypertension when blood pressure is assessed as a quantitative trait.\textsuperscript{32} In addition, ET2 messenger transcript is known to be present in varying quantities in human heart and kidney, two of the main target organs of hypertensive complications in severe hypertensives. Thus, the variability of ET2 tissue expression and, in particular, the ET2 linkage data makes it an ideal candidate gene for human essential hypertension.\textsuperscript{32}

The E2F3 gene at 39 cM on chromosome 6 encodes a
transcription factor of the \textit{E2F} family. This family protein has an important role of controlling tumor suppressor proteins and cell cycle.

The region at 21 cM on chromosome 3 has includes \textit{CAV3} gene, which encodes caveolin-3 muscle-specific protein. This finding is interesting in the background of evidence that \textit{CAV3} null mice show perivascular fibrosis, cellular infiltration in cardiac tissue and cardiac myocyte hypertrophy, which exhibits inter- and intrafamilial variations ranging from benign to malignant forms with high risk of cardiac failure and sudden cardiac death.\textsuperscript{4, 6, 40}
V. CONCLUSION

We identified susceptible loci for heart rate variability in an Asian population. This study strongly indicates that heart rate is controlled by genes mapped to several loci.

We believe characterization of genes that affects heart rate variability can lead to unraveling of the pathogenetic mechanisms underlying heart rate variability and its association with cardiovascular disease and to rational therapeutic interventions.
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국문요약

몽골 고립 부족에서 심박수의 유전율과 유전자 자리에 관한 연구

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심혈관계 질환의 독립적인 위험 인자로서 그 동안 높은 심박수(elevated heart rate)에 대한 의학적인 관심이 있었지만, 그 정확한 상관관계는 알려지지 않았다. 본 연구에서는, 1,002명의 사람(평균 연령 30.6세, 54.5%가 여성)이 포함된 95개의 몽골 대가족에 대한 전유전체 연관 분석(genome-wide linkage scan)을 시행하여, 심박수에 영향을 미치는 연속 형질 유전자 자리(QTL: quantitative trait locus)를 찾고자 하였다. 연구 집단의 DNA는 deCODE사(社)에서 제공하는 1000개의 단순 반복 염기 서열 표지자(microsatellite marker)를 이용해 전 유전체를 3cM의 해상도로 분석하였다. 심박수와 관계된 공변량(covariate)을 알아내어 최종 분석에서 제거하기 위하여
상관 분석을 시행하였다. 심박수의 성별, 흡연 여부, 음주 여부에 대한 t-test 역시 시행하였다. 위와 같은 분석을 통해 심박수에 영향을 미치는 인자들에 대한 모델을 구축하였고, 본 모델을 분산 성분(variance component)를 이용한 비모수적 전 유전자 연관 분석(nonparametric genome-wide linkage analysis)에 적용하였다. 본 방법을 통해 각 유전자 자리의 다지점 LOD (multipoint logarithm of odds) 점수와 그에 해당하는 p 값이 결정되었다. 심박수에 대한 유전율(heritability)은 0.32 (p<0.0001)로 추정되었으며, 18번 염색체의 77cM 위치에서 가장 높은 LOD 점수인 2.03이 관찰되었다. 두 번째로 높은 LOD 점수는 1.52로써, 5번 염색체의 216cM 위치에서 관찰되었다. 본 연구를 통해 매우 방대한 유전자 상에서 심박수에 영향을 미치는 유전자좌를 선별할 수 있었으며, 본 유전자 자리에 위치한 후보 유전자들을 분석할 수 있었다.

핵심 단어: 전유전자 연관 분석, 심박수, 유전율