**Supplementary Material**

Supplementary Methods

*Study design and data collection*

Baseline and follow-up data were collected from the population based multi-ethnic HELIUS (Healthy Life in an Urban Setting) study, consisting of inhabitants of the city of Amsterdam, the Netherlands. A detailed description of HELIUS has been previously published.(1, 2) The goal of HELIUS is to evaluate differences in the (causes of the) unequal burden of disease and clinical course across different ethnic groups, and to ultimately improve ethnicity specific health care. Initial inclusion at baseline (2011-2015) consisted of almost 25,000 participants, who were randomly sampled according to ethnic origin (i.e. Western European (Dutch), South Asian-South American (South Asian-Surinamese), Middle Eastern (Turkish), Northern African (Moroccan), African-South American (African-Surinamese), and Sub-Saharan Western African (Ghanaian) origin) through the municipality register of Amsterdam. The study was approved by the Medical Ethical Committee of the Amsterdam University Medical Centers and participants provided written informed consent. Between April 2019 and December 2022, the first follow-up visit took place., data collection of which was still ongoing during the current study. Therefore, data that were collected until December 31st 2020 could be included in this study. During both visits, data were obtained by questionnaire/interview and physical examinations were performed (including the collection of biological samples). A person was defined as of non-Dutch ethnic origin if he/she fulfilled one of two criteria: (1) he/she was born outside the Netherlands and has at least one parent born outside the Netherlands (first generation) or (2) he/she was born in the Netherlands but both parents were born outside the Netherlands (second generation). For the Dutch sample, we invited people who were born in the Netherlands and whose parents were born in the Netherlands. A limitation of the country of birth indicator for ethnicity is that people who are born in the same country might have a different ethnic background, which in the Dutch context is applicable to the Surinamese population. Therefore, after data collection, participants of Surinamese ethnic origin were further classified according to self-reported ethnic origin (obtained by questionnaire) into ‘African’, ‘South-Asian’, or ‘other’. A full overview of the geographic origin of each of the included ethnic groups, including a map with the migration patterns of each group has been published previously.(3)

*Participant inclusion and exclusion*

To establish normal values, we first identified ‘apparently healthy’ participants from the total HELIUS cohort. This process included an iterative process in which medical history data retrieved from the questionnaires was combined with the physical examination, blood test results and ECG diagnoses as published earlier.(3) In brief, 22,164 participants took part in the baseline physical examination and filled in a questionnaire. Participants were excluded as possibly not completely healthy, based on different elements from these investigations. As such, we defined categories of ‘arterial disease’, hypertension, diabetes mellitus, chronic kidney disease, the use of potential ECG-modifying drugs, and ECG-abnormalities. For the exclusion of arterial disease, self-reported stroke, self-reported transient ischemic attack / myocardial infarction, (coronary) bypass surgery, percutaneous intervention, the use of anti-thrombotic drugs, anticoagulants, or nitrates, were used. For the exclusion of hypertension, we used a reported history of hypertension, antihypertensive medication use, or measured hypertension defined as a systolic blood pressure ≥140mmHg or a diastolic blood pressure ≥90mmHg (World Health Organization criteria), each based on a mean of 2 measurements during the study visit. As anti-hypertensive agents can also be used for other conditions, participants who used these agents without self-report or measured hypertension were also excluded to avoid inappropriate inclusion of participants with (collaterally) treated hypertension. For the exclusion of diabetes mellitus, self-reported diabetes, a fasting glucose (≥7mmol/L), hemoglobin A1c (≥48mmol/mol), or the use of glucose-lowering medications, was used. Exclusion for chronic kidney disease was based on a Chronic Kidney Disease Epidemiology Collaboration stage ≥3 (estimated glomerular filtration rate <60mL/min per 1.73m2) or a Kidney Disease Improving Global Outcomes albumin-to-creatinine ratio ≥3mg/mmol. For possible ECG-modifying medications, we determined exclusion based on self-reported use of any antiarrhythmic Vaughan-Williams classification drugs plus digoxin or the daily use of a psychotropic drugs. In addition, we excluded participants with the following ECG abnormalities: (supra)ventricular arrhythmia, 2nd or 3rd degree atrioventricular block, pre-excitation, atrial or ventricular pacing or a QRS-duration of 120ms or more.

After these exclusion criteria were applied, the apparently healthy participants were included for the current analysis. From a subset of these participants, follow-up data was available. This enabled establishment of a cohort which remained free of arterial disease, diabetes mellitus or hypertension during follow-up: all participants who developed these comorbidities during follow-up were additionally excluded for the apparently healthy follow-up cohort. This follow-up cohort allowed validation and sensitivity analyses in the baseline cohort to determine whether results during the baseline evaluation might be affected by subclinical disease or comorbidities that only emerged several years later.

*ECG analyses*

We collected a standard 12-lead supine resting ECG, recorded met GE MAC5500 (500 samples/sec). As previously described,(3) these ECGs were thoroughly evaluated including both visual assessment by a cardiologist (assessing also the technical quality) as well as manually verified automated analyses. ECG data was processed using the Modular ECG Analysis System (MEANS) program.(4) MEANS determines from a single averaged representative beat common P-wave, QRS, and T-wave onsets and offsets for all 12 ECG leads together. Subsequently, all on- and offsets of all ECGs were then manually verified and adjusted where necessary. Using MEANS, predefined ECG parameters were subsequently extracted consisting of heart rate, P-wave duration, PR-interval, QRS-duration, QT-interval, QTc (heart rate correction performed with Bazett’s formula), frontal P-wave axis, frontal QRS-complex axis, frontal T-wave axis and amplitudes of QRS and T-wave for all leads. (5) In addition, to evaluate the interaction between depolarization and repolarization, the spatial QRS-T angle and the (activation sequence independent) ventricular gradient of the spatial QRS-T integral and sum absolute QRS-T integrale were determined after XYZ-reconstruction from the standard ECG leads.(5, 6)

A three-way approach was subsequently used to assess ECG abnormalities to allow exclusion (see above). MEANS derived Minnesota coding was combined with the GE Marquette 12SL report and visual assessment by a cardiologist for each ECG.(7) In the event of discrepancies among the 3 methods, recommendations published by international expert groups and consensus among the ECG team were used to reach a final diagnosis.(8, 9)

For defining high QRS voltage, initially the European Society of Cardiology hypertension guideline for electrocardiographic criteria of left ventricular hypertrophy (LVH) (SV1 + RV5 >35mm) were utilized.(10) However, as these criteria resulted in a very high prevalence of ECG-LVH in our normotensive participants without known previous hypertension nor the use of antihypertensive drugs (indicative of low sensitivity), we additionally determined composite high QRS voltage criteria (combining SV1 + RV5 > 3.5 mV, RaVL + S V3 > 2.8 mV (men), 2.0 mV (women) and R aVL > 1.1mV).(3, 11) For low QRS voltage, peak-to-peak QRS amplitudes of <0.5mV in all limb-leads or <1.0mV in all precordial leads was used. Abnormal Q-waves were defined as Q-waves (or high R-waves in V1/V2) not fitting the defined normal limits set up in the Minnesota coding. (12)

In addition, ECGs were assessed for the presence of early repolarization pattern (ERP) with fully automated assessment by the University of Glasgow ECG core laboratory. ERP was determined by end-QRS notching or slurring (irrespective of ST-segment elevation) in at least 2 contiguous leads (lateral ERP [aVL, I], inferior ERP [II, aVF, III], anterolateral ERP [V4-V6]) with J-peak or end-QRS slur onset ≥0.1mV.(13)

An overview of the exclusion process resulting in both the apparently healthy baseline and follow-up cohort can be found in Figure 1.

*Normal limits- GAMLSS*

Ethnicity specific normal limit curves for each of the above-mentioned ECG measurements were developed using the 2.5th and 97.5th centile curves derived from univariate distributional regression models (Generalized additive models for location, scale and shape; GAMLSS), where the ECG measurements are modelled against age using a distributional regression approach. Data were stratified by ethnicity and sex. GAMLSS are often used to define normal limits because these models are highly adjustable for many different distributions. These models can be used to determine percentile curves based on centile estimation by the model that is fitted to a dataset.(14) Because all ECG parameters are quantitative parameters, we chose the Box-Cox family of t-distributions to model the distribution of ECG parameters given age. This means that for any age, (i) the mean of the ECG parameter (the location parameter, mu) is estimated, as well as (ii) the standard deviation of the parameter (the scale parameter, sigma), (iii) the optimal Box-Cox power parameter to achieve distributional symmetry and reduce skewness and kurtosis (the nu parameter) and (iv) the degrees of freedom of the t-distribution (the tau parameter) of the ECG parameter. To achieve smoother, but not necessarily linear, percentile curves over the age-domain, we modelled the means and standard deviations using spline functions of age. The Box-Cox power parameter and the degrees of freedom of the t-distribution were assumed to be constant over the age domain.

To assess differences between normal limits curves of groups with a migration background compared to those with Western-European origin, Wald-tests were used to evaluate significance for all the parameters of the ethnicity-specific GAMLSS models. The data were analyzed using Rstudio (v3.6.1). and GAMLSS models were created using the gamlss() function from the R-package ‘gamlss’.(15)

*Normal limits – Percentiles*

To determine ethnicity-, age- and sex-specific normal limits for the ECG, the apparently healthy dataset was split into four groups, based on sex (male or female) and age (below 40 years or 40 years and older), in correspondence to the GAMLSS curves and previous research on normal limits for the ECG by this group.(3) For each of these groups, normal limits were determined by calculating the 2.5th and 97.5th percentile, in addition to mean and standard deviation for the above-mentioned (Box-Cox transformed) ECG measurements.

*Comparison between baseline and follow-up data*

To assess the validity of the normal limits derived from the apparently healthy (baseline) cohort, we compared those results and GAMLSS percentile curves with the follow-up cohort using the same methods.

1. Stronks K, Snijder MB, Peters RJ, Prins M, Schene AH, Zwinderman AH. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. BMC Public Health. 2013;13:402.

2. Snijder MB, Galenkamp H, Prins M, Derks EM, Peters RJG, Zwinderman AH, Stronks K. Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. BMJ Open. 2017;7(12):e017873.

3. Ter Haar CC, Kors JA, Peters RJG, Tanck MWT, Snijder MB, Maan AC, et al. Prevalence of ECGs Exceeding Thresholds for ST-Segment-Elevation Myocardial Infarction in Apparently Healthy Individuals: The Role of Ethnicity. J Am Heart Assoc. 2020;9(13):e015477.

4. van Bemmel JH, Kors JA, van Herpen G. Methodology of the modular ECG analysis system MEANS. Methods Inf Med. 1990;29(4):346-53.

5. Kors JA, van Herpen G, Sittig AC, van Bemmel JH. Reconstruction of the Frank vectorcardiogram from standard electrocardiographic leads: diagnostic comparison of different methods. Eur Heart J. 1990;11(12):1083-92.

6. Kardys I, Kors JA, van der Meer IM, Hofman A, van der Kuip DA, Witteman JC. Spatial QRS-T angle predicts cardiac death in a general population. Eur Heart J. 2003;24(14):1357-64.

7. Kors JA, van Herpen G, Wu J, Zhang Z, Prineas RJ, van Bemmel JH. Validation of a new computer program for Minnesota coding. J Electrocardiol. 1996;29 Suppl:83-8.

8. Rautaharju PM, Surawicz B, Gettes LS, Bailey JJ, Childers R, Deal BJ, et al. AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram: part IV: the ST segment, T and U waves, and the QT interval: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society: endorsed by the International Society for Computerized Electrocardiology. Circulation. 2009;119(10):e241-50.

9. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth Universal Definition of Myocardial Infarction (2018). Circulation. 2018;138(20):e618-e51.

10. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34(28):2159-219.

11. Bacharova L, Chen H, Estes EH, Mateasik A, Bluemke DA, Lima JA, et al. Determinants of discrepancies in detection and comparison of the prognostic significance of left ventricular hypertrophy by electrocardiogram and cardiac magnetic resonance imaging. Am J Cardiol. 2015;115(4):515-22.

12. Blackburn H, Keys A, Simonson E, Rautaharju P, Punsar S. The electrocardiogram in population studies. A classification system. Circulation. 1960;21:1160-75.

13. Macfarlane PW, Antzelevitch C, Haissaguerre M, Huikuri HV, Potse M, Rosso R, et al. The Early Repolarization Pattern: A Consensus Paper. J Am Coll Cardiol. 2015;66(4):470-7.

14. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape. J R Stat Soc C-Appl. 2005;54:507-44.

15. Stasinopoulos DM, Rigby RA. Generalized additive models for location scale and shape (GAMLSS) in R. J Stat Softw. 2007;23(7).

*Table S1. Normal limits based on the AHA/ACCF/HRS guidelines.*

|  |  |
| --- | --- |
| ECG measurement | Current reference value for normal |
| Heart rate | 60-100 beats per minute |
| P-wave duration | <120 ms |
| PR interval | 120-200 ms |
| QRS width | 70-110 ms |
| QT interval | N.A. |
| QTc interval (Bazett) | 450 ms in males and 460 ms in females |
| P-wave axis | 0 – +75 ° |
| QRS- complex axis | -30 – +90 ° |
| T-wave axis | +25 – +75 ° |
| LVH Sokolow Lyon criteria | R in V5 or V6 + S in V1 > 35 mm. |
| LVH Cornell criteria | R in aVL + S in V3 >28 mm in males and >20mm in females |
| Spatial QRS-T angle | 105–135° is borderline abnormal and >135° is abnormal |

*Table S2. Comparison of GAMLSS curves for each ECG interval or axis measurement, stratified by ethnicity and sex.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Apparently healthy versus Healthy FU | | | | | | | | | | | |
| Dutch | | South Asian Surinamese | | African Surinamese | | Ghanaian | | Turkish | | Moroccan | |
| ECG parameter | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| Heart Rate | 0.626 | 0.949 | 0.941 | 0.469 | 0.823 | 0.257 | 0.602 | 0.588 | 0.298 | 0.097 | 0.654 | 0.948 |
| P-wave duration | 0.729 | 0.918 | 0.545 | 0.741 | 0.812 | 0.662 | 0.603 | 0.432 | 0.737 | 0.777 | 0.663 | 0.819 |
| PR interval | 0.952 | 0.880 | 0.509 | 0.447 | 0.138 | 0.380 | 0.796 | 0.691 | 0.547 | 0.061 | 0.353 | 0.355 |
| QRS duration | 0.825 | 0.967 | 0.858 | 0.593 | 0.933 | 0.366 | 0.170 | 0.628 | 0.819 | 0.787 | 0.869 | 0.891 |
| QT interval | 0.736 | 0.630 | 1.000 | 0.732 | 0.990 | 0.358 | 0.574 | 0.751 | 0.680 | 0.392 | 0.854 | 0.188 |
| QTc interval | 0.175 | 0.548 | 0.876 | 0.422 | 0.742 | 0.414 | 0.503 | 0.549 | 0.471 | 0.108 | 0.351 | 0.125 |
| P-wave axis | **<0.001** | **0.002** | 0.760 | 0.427 | 0.725 | 0.770 | 0.594 | 0.168 | 0.541 | 0.783 | 0.298 | 0.581 |
| QRS axis | 0.380 | 0.546 | 0.869 | 0.945 | 0.265 | 0.300 | 0.188 | 0.740 | 0.956 | 0.831 | 0.186 | 0.917 |
| T-wave axis | 0.116 | 0.564 | 0.764 | 0.500 | 0.218 | 0.576 | 0.190 | 0.414 | 0.723 | 0.797 | 0.140 | 0.696 |
| Ventricular gradient | 0.649 | 0.632 | 0.675 | 0.222 | 0.084 | 0.913 | 0.646 | 0.540 | 0.604 | 0.990 | 0.438 | 0.914 |
| QRS-T angle | 0.877 | 0.864 | 0.788 | 0.155 | 0.202 | 0.557 | 0.636 | 0.982 | 0.951 | 0.320 | 0.074 | 0.776 |
| SAI QRST-T | 0.590 | 0.633 | 0.726 | 0.223 | 0.100 | 0.982 | 0.594 | 0.502 | 0.585 | 0.947 | 0.454 | 0.968 |

*Figure S1 Population pyramid. Age distribution separated by sex*

