The Moli-sani project: computerized ECG database in a population-based cohort study

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Abstract

Computerized electrocardiogram (ECG) acquisition and interpretation may be extremely useful in handling analysis of data from large cohort studies and exploit research on the use of ECG data as prognostic markers for cardiovascular disease.

The Moli-sani project (http://www.moli-sani.org) is a population-based cohort study aiming at evaluating the risk factors linked to chronic-degenerative disease with particular regard to cardiovascular disease and cancer and intermediate metabolic phenotypes such as hypertension, diabetes, dyslipidemia, obesity, and metabolic syndrome. Between March 2005 and April 2010, 24325 people aged 35 years or older, living in the Molise region (Italy), were randomly recruited. A follow-up based on linkage with hospital discharge records and mortality regional registry and reexamination of the cohort is ongoing and will be repeated at prefixed times.

Each subject was administered questionnaires on personal and medical history, food consumption, quality of life (FS36), and psychometry.

Plasma serum, cellular pellet, and urinary spots were stored in liquid nitrogen.

Subjects were measured blood pressure, weight, height, and waist and hip circumferences, and underwent spirometry to evaluate pulmonary diffusion capacity, gas diffusion, and pulmonary volumes. Standard 12-lead resting ECG was performed by a Cardiette ar2100-view electrocardiograph and tracings stored in digital standard communication protocol format for subsequent analysis.

The digital ECG database of the Moli-sani project is currently being used to assess the association between physiologic variables and pathophysiologic conditions and parameters derived from the ECG signal.

This computerized ECG database represents a unique opportunity to identify and assess prognostic factors associated with cardiovascular and metabolic diseases.

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Introduction

Resting electrocardiogram (ECG) carries important independent prognostic information for future cardiac events. 1,2 The presence of ECG-revealed ischemia, abnormal Q/qs patterns, or ST-T abnormalities is an index of doubled risk of cardiovascular disease (CVD) 3,4 and death 5-7 and has a prognostic value for CVD more powerful than that derived from established conventional risk factors. 8
However, performing ECG in population-based studies may lead to enormous numbers of tracings to be interpreted. Computerized ECG acquisition and interpretation may be extremely useful in handling analysis of data from large cohort studies and exploit research on the use of ECG data as prognostic markers for CVD.

The advent of computer technology with improved powers of signal processing has 2 major advantages compared with human readers: the intra- and interobserver variability in ECG interpretation and coding is reduced and it saves time spent by physicians, and related costs. Therefore new knowledge about computerized ECG recording variables that are of potential clinical value should produce an index of risk of fatal or nonfatal myocardial infarction that would be useful for primary or secondary prevention.9,10

Diagnostic ECG interpretation by a computer can be very helpful in population-based research, being potentially comparable to ECG interpretation by research physicians, but more efficient and therefore less expensive.11

The Moli-sani project (http://www.moli-sani.org) is a population-based cohort study aiming at evaluating the risk sequences on cardiovascular, cancer, and metabolic diseases is helpful in population-based research, being potentially comparable to ECG interpretation by research physicians, but more efficient and therefore less expensive.9,10

The Moli-sani project (http://www.moli-sani.org) is a population-based cohort study aiming at evaluating the risk factors linked to chronic-degenerative disease with particular regard to CVD and cancer and intermediate metabolic phenotypes such as hypertension, dyslipidemia, diabetes, obesity, and metabolic syndrome.12

A complex equilibrium does exist between genetics and lifestyle often setting the border between health and sickness. To better understand such an equilibrium and its consequences on cardiovascular, cancer, and metabolic diseases is a major aim of the Moli-sani project.

Studying either cardiovascular, cancer, or metabolic risk factors in the light of their relationships and mutual influence might offer a powerful tool for exploring the concept of “common soil” for these diseases,13,14 an approach that is attracting increasing attention. Moreover, the Moli-sani project will investigate easy-to-use and relatively low-cost tools that could be used in public health and in the clinical practice for an early identification of subjects at risk of developing chronic disease.

The aim of this article was to describe the Moli-sani database and biobank as a unique tool for electrocardiographic studies in the prevention of cardiovascular and metabolic disease.

Study population

The cohort of the Moli-sani Study was randomly recruited in the Molise region from city hall registries by a multistage sampling.15,16 First, townships were sampled in major areas by cluster sampling; then, within each township, participants aged 35 years or older were selected by simple random sampling. Recruitment was based on household to allow household and large pedigree construction for genetic analysis. Exclusion criteria were pregnancy at the time of recruitment, disturbances in understanding or willingness, current poly-traumas or coma, or refusal to sign the informed consent. Thirty percent of subjects refused to participate; these were generally older and had a higher prevalence of CVD and cancer.

Between March 2005 and April 2010, 24318 subjects were recruited in 2 different centers: the Catholic University at Campobasso (n=19,211; 79%) and San Timoteo Civil Hospital at Termoli (n=5107; 21%), by research personnel trained accurately. The recruitment strategies were carefully defined and standardized across the 2 recruiting centers. Structured digitized questionnaires were administered to collect personal and clinical information.

A follow-up based on linkage with hospital discharge records (SDO) and mortality regional registry was started in May 2010 after a mean period from the recruitment visit of 2.5 years. Such a follow-up will be updated every 6 months. In July 2010, a telephone follow-up on the whole cohort was also completed.

The Moli-sani study complies with the Declaration of Helsinki and was approved by the Catholic University ethics committee. All participants enrolled provided written informed consent.12

Electrocardiograms

Electrocardiograms were performed by trained research personnel, following standard procedures in order to minimize errors in ECG procedures, such as inadequate skin contact, wandering baseline, incorrect lead placement, reversed lead wires, and A-C interference. To ensure standardization, periodic evaluation visits were made by an expert cardiologist. For quality control, ECGs performed by an individual operator were periodically replicated and evaluated.

Standard 12-lead resting ECG was measured using a Cardiette ar2100-view electrocardiograph, a standard electrocardiographic acquisition unit which acquires synchronized 10-second, 12-lead ECG and can transmit the waveform data to a workstation in real time via a USB cable. Disposable electrodes were used.

Digital ECGs, stored in standard communication protocol-ECG format,17 were subsequently processed by a commercial computerized system for the analysis of resting ECG (CalECG version 3.2.0, AMPS-LLC, New York, NY, USA).18 CalECG has a built-in measuring algorithm for measuring/annotating ECGs; in addition, it embeds the “University of Glasgow” diagnostic algorithm.19 CalECG manages the link of all the patients’ demographic data from the standard communication protocol-Cardiette data structure to the embedded Glasgow module.

Electrocardiographs were also printed and evaluated by local cardiologists for participants’ records.

Recruitment procedures

Each subject was asked to answer 3 different types of questionnaires, namely, an anamnestic, a food frequency, and a quality-of-life (FS36) questionnaire, and underwent psychometric tests to evaluate any depressive status, stress response, and attitude to suicide. Table 1 shows the structure of the anamnestic questionnaire.

Dietary assessment

The European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ was used to determine daily nutritional...
Table 1
Anamnestic questionnaire structure.

- Personal data
- Social status presently and at childhood
- Physical activity
- Smoking habits
- Hormonal status in women
- Body weight history
- Inflammation history
- Influenza vaccine history
- History of hypertension, diabetes and dyslipidemia
- History of cardiovascular disease and relative treatment
- Rose’s questionnaire for angina and intermittent claudication
- History of cancer
- History of pulmonary diseases
- History of depressive or anxious status
- History of other chronic disease (hematologic, kidney, gastrointestinal, endocrinologic, neurologic)
- History of surgical interventions in the past 5 years
- Pharmacological enquiry on chronic or acute (last 15 days) medications

intakes consumed in the past year. This questionnaire was designed and previously validated in a multicenter study performed in 10 European countries to evaluate the relation between diet and cancer. The questionnaire, computerized with a custom-made software, allowed researchers to interview participants in a fully interactive way, including illustrations of sample dishes of defined sizes or by reference to standard portion sizes. All the answers provided by the participants were recorded in a database in real time. The NAF software (Nutritional Analysis of Food Frequency Questionnaires, National Cancer Institute, Milan, Italy) was used to transform information about food composition into daily intake of food items (grams per day), energy (kilocalories or kilojoules per day), and macro- and micronutrients (grams or milligrams per day). Nutrient data for specific foods were obtained from the food composition database for epidemiologic studies in Italy.

On the basis of the recorded data, each subject was provided with his/her own cardiovascular risk according to the algorithm in the cuore.exe software elaborated in the Cuore Project.

Biochemical measurements
Venous blood samples were obtained by clean venipuncture between 7:00 am and 09:00 am from participants who had fasted overnight and had refrained from smoking for at least 6 hours. Glycaemia, cholesterol, high-density lipoprotein, low-density lipoprotein, C-reactive protein, D-Dimers, and blood cell count were measured within 3 hours from blood sampling of fresh samples in the centralized Moli-sani clinical chemistry laboratory set up in both recruiting centers.

High-sensitivity C-reactive protein was measured in fresh serum, by a latex particle-enhanced immunoturbidimetric assay (IL Coagulation Systems ACL9000, Milano, Italy). Inter- and intraday coefficient of variation were 5.4% and 4.2%, respectively. D-Dimers were measured in fresh citrate plasma, using HemosIL, an automated latex immunoassay on the IL Coagulation System ACL9000. Inter- and intraday coefficient of variation were 5.4% and 7.6%, respectively.

Serum lipids and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab 350, Milano, Italy). Low-density lipoprotein cholesterol level was calculated according to Friedewald.

Blood cell counts including platelets, white and red blood cells, mean platelet volume, mean corpuscular volume, and hemoglobin were performed by the Coulter LH Hematology analyzer (Becker-Coulter, Brea, CA, USA).

Citrated plasma, EDTA plasma, serum, cellular pellet, and urinary spots were stored in liquid nitrogen in a dedicated biobank (Moli-bank, http://biobank.molisani.org/).

Anthropometric and blood pressure measurements
Body weight and height were measured on a standard beam balance scale with an attached ruler with subjects wearing no shoes and only light indoor clothing. Body mass index was calculated as weight in kilograms divided by height in meters squared. Waist circumferences were measured according to the NIH, Heart, Lung, and Blood guidelines.

Blood pressure (BP) was measured by an automatic device (OMRON-HEM-705CP: Omron Electronics SpA, Milano, Italy) 3 times on the nondominant arm, and the average of the last 2 values was taken as the BP.

Definition of risk factors
Hypertension was defined by values of systolic BP of 160 mm Hg or higher and/or diastolic BP of 95 mm Hg or higher, or current pharmacological treatment. Hypercholesterolemia was defined by levels of cholesterol 240 mg/dL or higher, or current pharmacological treatment. Diabetes was defined by fasting glucose level of 126 mg/dL or higher, or current treatment with antidiabetic drugs.

Subjects were classified as nonsmokers if they had never smoked cigarettes or had smoked less than 100 cigarettes in their lifetime, as ex-smokers if they had smoked more than 100 cigarettes in the past but had stopped smoking for at least 1 year, and as current smokers if they have smoked more than 100 cigarettes in their lifetime and either still smoke or had quit smoking for less than 1 year.

Socioeconomic status was defined by a score based on 7 variables (income, education, job, housing, ratio between the number of live-in partners and the number of rooms—both current and during childhood—and availability of hot water at home during childhood); the higher the score, the higher the level of socioeconomic status. Physical activity was assessed by a structured questionnaire and expressed as daily energy expenditure in metabolic equivalent task-hours.

Metabolic syndrome was defined according to the Adult Treatment Panel III criteria. Global individual CVD risk was calculated by applying the risk equations of the CUORE project. Subjects older than 69 years or with a history of cardiovascular events were excluded from this analysis.

Pulmonary function tests
Pulmonary function maneuvers were performed by trained operators, according to the American Thoracic Society/European Respiratory Society recommendations,
with 3 V-Max Encore 22D equipped with plethysmography V623 Autobox and 2 V-Max Encore 20, all with the same Mass Flow Sensor model (Sensormedics Viasys, Sensormedics-Titalia, Milano, Italy).

Daily volume calibration was performed with a 3-L syringe. Volume variability higher then 0.5% from the real value (3 L) was discarded and calibration was repeated. At the end of each test session, technicians evaluated the acceptability (including start, duration, and end of test) and the reproducibility of the maneuvers. Subjects with HQ spirometry randomly performed plethysmography and a diffusion test. All tests were performed in the morning after technical explanation, with subjects in a sitting position and with the use of a nose clip.

Results

After exclusion of subjects with incomplete questionnaires and without ECG data, 24090 subjects were analyzed. General characteristics of men and women included in the study are listed in Table 2.

The mean age of the population was 55±12 years in women and 56±12 years in men. Sixty-six percent of the women never smoked, whereas 20% currently smoked; in men, these percentages were 32% and 26%, respectively. The prevalence of obesity, hypertension, hypercholesterolemia, and diabetes was 31%, 29%, 7%, and 4%, and 29%, 29%, 9%, and 6% in women and men, respectively. The metabolic syndrome was present in 26% and 29% of female and male subjects, respectively. In women and men, history of coronary artery disease was present in 2.0% and 4.8%, of cardiovascular disease in 3.8% and 8.0%, and of cancer in 3.8% and 2.4%, respectively.

Electrocardiogram variables are shown in Table 3. They are similar to those presented in an earlier report.

Differences in numerical ECG values were observed between sexes. Table 3 also shows the distribution of principal cardiac disorders by ECG digital diagnosis. Thirty-nine percent of ECG results in women and 42% in men were normal. Complete (right or left) bundle branch blocks were observed in 1.9% of women and in 5.0% of men. As expected, the prevalence of myocardial infarction was higher in men than in women. T-wave axis deviation was categorized as normal (15° to 75°), borderline (−15° to 15°), or abnormal (−180° to −15° or 75° to 180°). The last category was found in 3% of both genders. Left ventricular hypertrophy defined by Cornell voltage-duration product and Sokolow-Lyon voltage criteria was found in 10 women and in 18 men.

Discussion

The population of the Moli-sani study, although recruited in the central-southern region of Italy, shows characteristics similar to those of the general Italian population described within the frame of the Italian Cardiovascular Observatory, which recruited a random sample of male and female subjects, aged 35 to 79 years, all over Italy. Of interest, the higher prevalence of diabetes in men as compared to women, also reported by the observatory, confirms the observed major sex shift, with the increase in male/female ratio, in the course of the 20th century. Possible explanation for the changing sex ratio is the more sedentary lifestyle leading to increased obesity in men.

The prevalence of reported ischemic cardiovascular disease, as expected, was higher in men than in women, as

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**Table 2**

Characteristics of women and men of the Moli-sani study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Women (n = 12,529, 52%)</th>
<th>Men (n = 11,561, 48%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs (SD)</td>
<td>55 (12)</td>
<td>56 (12)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>8231 (66)</td>
<td>3690 (32)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>2539 (20)</td>
<td>3007 (26)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>1742 (14)</td>
<td>4853 (42)</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status score (SD)</td>
<td>3.47 (1.38)</td>
<td>3.48 (1.40)</td>
<td>.016</td>
</tr>
<tr>
<td>Physical activity, MET-h/d (SD)</td>
<td>42.6 (7.4)</td>
<td>43.7 (9.6)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI, kg/m² (SD)</td>
<td>27.9 (5.4)</td>
<td>28.2 (4.1)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI 25-30kg/m², n (%)</td>
<td>4478 (36)</td>
<td>5793 (32)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI≥30kg/m², n (%)</td>
<td>3837 (31)</td>
<td>3366 (29)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>4601 (37)</td>
<td>4904 (43)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>788 (6.4)</td>
<td>1436 (12.6)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>4035 (33)</td>
<td>3443 (30)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Metabolic syndrome, n (%)</td>
<td>3198 (26)</td>
<td>3274 (29)</td>
<td>.0002</td>
</tr>
<tr>
<td>C-Reactive protein (mg/dL)</td>
<td>2.7 (3.3)</td>
<td>2.5 (3.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>246 (2.0)</td>
<td>670 (5.8)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>469 (3.8)</td>
<td>915 (8.0)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Malignancies, n (%)</td>
<td>472 (3.8)</td>
<td>275 (2.4)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

**Table 3**

Digital ECG variables and diagnosis in women and men of the Moli-sani study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Women (n = 12,529, 52%)</th>
<th>Men (n = 11,561, 48%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>69 (10)</td>
<td>65 (10)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>T-wave axis deviation, n (%)</td>
<td>9795 (79)</td>
<td>8223 (72)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Normal</td>
<td>2284 (18)</td>
<td>2848 (25)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Borderline</td>
<td>347 (3)</td>
<td>401 (3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Abnormal</td>
<td>430 (18)</td>
<td>423 (19)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>QT Interval (ms)</td>
<td>417 (28)</td>
<td>414 (30)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>50 (24)</td>
<td>47 (24)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>QT Dispersion (ms)</td>
<td>146 (25)</td>
<td>153 (27)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ST Duration (ms)</td>
<td>124 (24)</td>
<td>105 (26)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Normal ECG, n (%)</td>
<td>4926 (39.3)</td>
<td>4880 (42.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Left bundle branch block, n (%)</td>
<td>112 (0.9)</td>
<td>69 (0.6)</td>
<td>.0017</td>
</tr>
<tr>
<td>Right bundle branch block, n (%)</td>
<td>126 (1.0)</td>
<td>389 (3.4)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Right bundle branch block incomplete, n (%)</td>
<td>216 (1.7)</td>
<td>463 (4.0)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Anterior myocardial infarction, n (%)</td>
<td>9 (0.07)</td>
<td>28 (0.24)</td>
<td>.0010</td>
</tr>
<tr>
<td>Inferior myocardial infarction possible, n (%)</td>
<td>408 (3.3)</td>
<td>150 (1.3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Inferior myocardial infarction, n (%)</td>
<td>351 (2.8)</td>
<td>664 (5.7)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Left ventricular hypertrophy—Sokolow and Cornell product, n (%)</td>
<td>10 (0.08)</td>
<td>18 (0.16)</td>
<td>.11</td>
</tr>
</tbody>
</table>
well as the prevalence of anterior and inferior myocardial infarction at the ECG. However, the frequency of possible anterior myocardial infarction was higher in women, probably because of the less uniform placement of precordial electrodes in women. To avoid misclassification, ECG digital interpretation, reporting “possible” alterations, requires further validation by a cardiologist and from clinical records, or exclusion from the analysis.

The collection of ECG data in the Moli-sani study will allow longitudinal analyses to examine the relationship of specific ECG abnormalities to subsequent events of cardiovascular disease and will provide a unique opportunity to understand ECG abnormalities in high-risk groups which could enhance future cardiovascular disease detection and prevention efforts. Moreover, it will provide a description of the age- and sex-specific rates of ECG abnormalities in a general population.

The computerized ECG database of the Moli-sani project represents a unique opportunity to identify and assess prognostic factors associated with cardiovascular and metabolic diseases.

Appendix. Moli-sani project investigators

Chairperson: Licia Iacoviello (Campobasso, Italy).

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References