

“10 001 Dalmatians:” Croatia Launches Its National Biobank

In 2006, the Croatian Ministry of Science, Education, and Sports has completed the review process of more than 3000 research proposals for the new 5-year funding cycle (2007-2011). The grant submission process was similar to the procedure used by the European Commission (Framework Programmes, FP). The new grant program encouraged the formation of national-level consortia, which linked several research projects from different institutions into greater and more efficient programs, and favored the projects with international collaboration. It also ensured greater attention to ethical aspects of the submitted proposals (1).

Aside from some persisting problems such as excessive length of the review process, which took 10 months, and high overall success rate, which was over 70% after the first call, the new process nevertheless represented a substantial improvement over previous practices in terms of transparency, supporting web-based technology, and the attention it paid to ethical aspects of the proposed research. The most interesting outcome of this process were a few rare and genuine attempts to synchronize and coordinate several institutions within the country into larger “research programs.” Such clusters of research groups aim to become competitive at the European level and join the successful consortia within the new FP7. There is hope that they could serve as examples that could increase the international impact of the Croatian scientific production, which was heavily affected during and after the war years (1991-1995).

One of the best examples is “The Croatian National Biobank,” a consortium now linking more than 20 research projects, either awarded or presently under review, from all four Medical Schools in Croatia, several teaching hospitals, and public health institutes. It is coordinated from the recently founded Croatian Centre for Global Health, based at the University of Split School of Medicine (2). In its infancy, the national Biobank will rely on 3 large resources that should ensure and maintain its international competitiveness: 1) “10 001 Dalmatians” study of Croatian island isolates; 2) a hospital-based DNA bank with thousands of cases with most common complex diseases; and 3) a large cohort from general population to serve as a control sample.

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The first resource is an internationally already recognized study of genetic and environmental determinants of health and disease in genetic-isolate island populations from Dalmatia, Croatia. This effort, aiming to recruit 10 001 examinees, has developed during the recent years, in collaboration mainly with the scientists from Scotland, but also from Sweden, The Netherlands, Italy, and Germany. In the period 2001-2007, it already received substantial competitive funding from The Croatian Government, European FP6, UK Medical Research Council, UK Royal Society, The Wellcome Trust, US National Institutes of Health, and The British Council. This “flagship” of the post-war Croatian science has had 3 publications in one of the leading journals in the field of genetics, *Nature Genetics* (3-5) and several more articles in other high-impact genetics journals (6-13). The project has also contributed to the characterization of the two indigenous Mendelian genetic disorders (14,15). In 2006, the *Croatian Medical Journal* devoted a theme issue, with 17 articles, to the results arising from this research program (16,17).

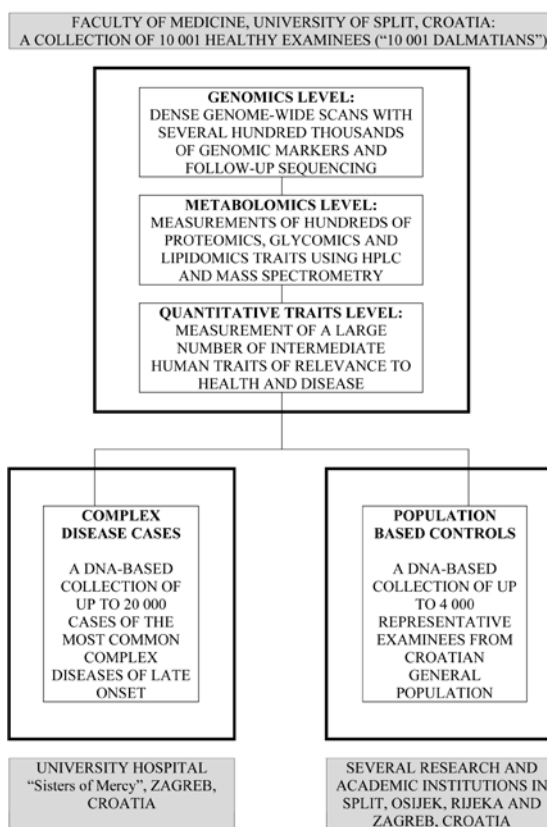
The second resource, planned as a large and important building block of the Croatian National Biobank, is a net-

work of hospital-based registries of patients with specific diseases. The project, which received ethical approval of the ethics committee of the Sisters of Mercy University Hospital in Zagreb, aims to create a large DNA bank of up to 20 000 cases of complex chronic diseases by 2011. To achieve this, participating hospitals and public health institutes will form several national registries of diseases of unknown etiology and without preventable risk factors, such as amyotrophic lateral sclerosis, multiple sclerosis, diabetes type 1, specific early-onset cancers, and a spectrum of childhood illnesses. In addition, cases of the most common complex diseases, which form the greatest share of the overall disease burden in the Croatian population, will be collected: myocardial infarction; cerebral stroke; breast, lung, and colorectal cancer; type 2 diabetes; depression; schizophrenia; renal stones and gallstones; gout; eye diseases; Parkinson and Alzheimer diseases; osteoporosis; rheumatoid arthritis; and others.

Finally, a representative sample of several thousands of individuals from general Croatian population will be collected from many of the participating institutions as a control population for the two resources described above. This will be a "targeted sample," which will correspond closely to the demographic characteristics of the Croatian population. It will also be DNA-based. In this sample, information on the lifestyle, habits, exposure to health risks, health attitudes, and medical history will be documented using standard and internationally validated questionnaires.

The main aim of the research within the Croatian National Biobank will be to discover and illuminate the nature of the relationships between: 1) genomic sequence and sophisticated circulating metabolites; 2) circulating metabolites and quantitative biological traits of relevance to human health and disease; and 3) quantitative biological traits and human diseases of complex etiology. The Croatian National Biobank will take into consideration 4 general levels of complexity involved in the development of human diseases: 1) genomics level; 2) "metabolomics" level (including proteomics, glycomics, and lipidomics); 3) level of intermediate quantitative traits (eg, blood pressure, forced expiratory capacity, cholesterol levels, etc.); and 4) endpoint that results in a complex disease phenotype. It will probably be easier to demonstrate and explore the associations between "neighboring" levels of complexity, while the associations across the levels will be more likely to be weak and of relatively small effect size. At each of these levels, modifiers such as environmental, cultural, socio-economic, and psychological influences can also play an important role.

Figure 1.



Schematic representation of the resources that will contribute to The Croatian Biobank in the future. HPLC – high-performance liquid chromatography.

The interactions between those "outside" influences and biological factors will also be studied.

Figure 1 shows the structure of the Croatian National Biobank, presenting all contributing resources. The central resource will be a databank of 10 001 examinees collected at the Croatian Centre for Global Health in Split, Croatia. In these healthy examinees, genome scanning will be performed (using dense genome-wide scans with hundreds of thousands of genomic markers), followed by sequencing. In addition, hundreds of metabolites will be measured in plasma of all individuals, based on high-throughput technologies such as mass-spectrometry and high-performance liquid chromatography. This will involve proteomics, glycomics, and lipidomics measurements ("metabolomics"). Genetic sequence variants and structural genetic variants will then be associated with chang-

es in the levels of individual metabolites and the effects of metabolite changes on complex quantitative traits will also be assessed. Eventually, public health relevance of identified genetic variants that could be used in genetic testing, metabolites that could potentially represent disease markers, and quantitative traits that could represent significant health risks will then be assessed in large collections of diseased individuals and controls from the general population. The diseased individuals will be approached in participating hospitals, while the examinees for the control sample will be recruited through a network of collaborating institutions (such as the National Institute for Public Health and 4 University Medical Schools in all parts of Croatia) (Figure 1).

More than 150 Croatian researchers from a variety of scientific backgrounds and disciplines will also be involved in this project and will continuously seek and expand their international collaborations. This should ensure sustainable growth of the program and its lasting international competitiveness. We hope that the Croatian National Biobank could substantially increase international visibility and productivity of Croatian biomedical research in the 21st century.

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