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Editorial

Development of Genetics in the World and in Croatia – Forty Years of the Croatian Society of Human Genetics of the Croatian Medical Association

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A B S T R A C T

Resulting from several basic scientific disciplines, genetics has made impressive progress in the last century by discoveries of the heredity rules and genome structure, and by identification of the genes that determine the occurrence and characteristics of human diseases. In Croatia, the development of genetics began in the middle of the past century by the pioneering work of clinicians and basic scientists, which resulted in significant development of this scientific discipline that has quickly found its practical application in clinical genetics-cytogenetics, molecular genetics and prenatal diagnosis. The rapid advancement of technology and knowledge of genetics in recent decades has led to the development of genomics and related disciplines, entering the revolutionary new era of personalized medicine. Currently, much more data can be collected than interpreted. The data of electronic medical records, genomics, epigenetics, proteomics, metabolomics and microbiomics should be integrated and interpreted at the level of individual genome. Extensive use of new information will open a range of ethical issues that we must face timely. It is expected that in the forthcoming years, we will be able to learn more about genetics than what we have learned throughout the history of medicine. We must be prepared to welcome this new knowledge, reflecting on the positive and negative aspects of the latest achievements in the field of genetics. We hope that the experts dealing with human genetics in Croatia will successfully continue their work to enable practical application of the latest achievements in genetics, expanding our understanding of the concept of health and disease.

***Key words**: genetics, medical, counseling, cytogenetics, genomics, Croatia*

Introduction

Resulting from several basic scientific disciplines, genetics as the science of heredity and modifiable traits of living organisms was formally recognized only in the 19th century, based on the works of Gregor Mendel, Father of modern genetics, who published the results of his experiments with pea plants as early as 1865. Around 1900, results of these studies were confirmed by three scientists, H. de Vries, K. Correns and E. Tschermak, independently of each other. Soon after Mendel's observations, in 1883–1884, on cell staining during cell division E. van Beneden detected more intensively stained and visible structures in the cell nucleus, which he named chromosomes. The term genetics (Gr. *genesis*, origin, inception) was first used by W. Bateson in 1905, whereas in 1909 W. Johannsen named Mendel's factors as genes and introduced the terms genotype for the sum of all genes in an organism and phenotype for the outer expression of the gene determined features. In 1911, Th. H. Morgan demonstrated the genes to be linearly distributed on the chromosomes. In 1927, J. H. Muller verified that X-rays can experimentally induce gene changes, i.e. mutations, today known to be also inducible by many other external factors. During more than 150 years of its existence, Mendel's genetics has been enriched with novel, frequently quite revolutionary discoveries, while the advancements in complementary sciences, in particular biolo-

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gy, chemistry, physics and mathematics, have contributed to additional development of the genetic science and its practical application in various fields of biologic and medical disciplines.

Being deeply aware that life is a continuity that neither begins nor ends with us, we have been searching for some written word, some »Yesterday«, some trace of the initial scientific or professional interest in the role of heredity in our country. The idea of heredity as an essential element of human health has been present since ancient times in folk stories, records, beliefs, proverbs or sayings. In Croatia, genetic science was founded by Alois Tavčar, agronomist. He wrote the first book on agricultural genetics, entitled »Temelji nauke o nasljedstvu« (The Fundamentals of Heredity Science) in 1930, on the issues of hybridization in agriculture. The roots of the professional-scientific knowledge about the role of genetics in medicine date back to the late 1930s, in the first professional works of Boris Zarnik, Professor of Biology, and Ernest Mayerhofer, Professor of Pediatrics at the School of Medicine in Zagreb, tackling the then popular field of eugenics, and the works of Stjepan Urban, Professor of Biology from Rijeka. Several years later, Andrija Štampar and Ante Vuletić from the School of Public Health in Zagreb wrote the first papers on population genetics, and Đurđa Mušić-Severova published a case report of a child with Down syndrome as the first clinical description of a genetic disorder in 1934.

In Croatia as well as worldwide, a true revival and breakthrough in many sciences occurred after World War II, with the scientists' interest focused on the substance of heredity, human genome in the mid-1950s. A revolutionary novelty in the development of genetics was Watson and Crick's discovery of the deoxyribonucleic acid (DNA) composition and structure in 1953[1]. This interest was additionally enhanced by fear from the possible undesired mutations caused by atomic and other types of radiation from the bombs thrown on Hiroshima (uranium) and Nagasaki (plutonium). The tragedy of the destroyed Japanese towns urged and accelerated research and search for new technologies to be used in scientific work including human genetics. Advances and collaboration of medicine and complementary sciences have resulted in the advent of many novel methods such as cytogenetic methods that facilitate identification of the human hereditary basis and understanding of the pathologic conditions associated with impairments in the number and structure of chromosomes.

The beginnings of genetics as a scientific discipline related to human physiology and pathology in Croatia date from 1959, owing to the enthusiasm and vision of Ljiljana Zergollern, pediatrician. In collaboration with Danilo Tepavčević, endocrinologist, she issued the first cytogenetic finding in a human, thus confirming the revolutionary discoveries by Jerome Lejeune and Charles E. Ford, who several months earlier demonstrated the changes in the chromosome number and/or structure to be the cause of Down syndrome. In 1964, the first Centre for Human Genetics in former Yugoslavia, consisting of the ward for children suspected to carry some hereditary burden, genetic counselling unit and cytogenetic laboratory, was established at Department of Pediatrics, Zagreb University Hospital Centre and School of Medicine, University of Zagreb, with financial support from the Association for the Aid of Crippled Children from New York, USA. In 1984, the Centre grew into Department of Human Genetics, headed by Ljiljana Zergollern until 1991. In 1993, Department of Human Genetics merged with Department of Metabolic Diseases, Zagreb University Hospital Center to become Department of Genetics and Metabolic Diseases, successfully headed by Professor Davor Begović, Professor Ljiljana Zergollern's disciple. In 2009, this Department was divided into two specialist departments, Department of Medical Genetics and Department of Metabolic Diseases.

In 1961, population geneticists Silvije Vuletić, Davor Ivanković and Josipa Kern from »Andrija Štampar« School of Public Health joined the small group of human geneticists, mostly Ljiljana Zergollern's coworkers. Over time, this heterogeneous group of experts and scientists engaged in the field of human genetics increased, being gradually joined by professionals of various educational backgrounds, i.e. physicians, biologists, biochemists, pharmacists, dental medicine doctors, etc. Their work covered the then few fields of human genetics such as population genetics, cytogenetics, clinical genetics, anthropology, and the beginnings of molecular and biochemical genetics. They all shared the enthusiasm for work in the field that by its nature demanded and stimulated multidisciplinary collaboration. Their partnership and associating started in 1972 at the Croatian Medical Association with foundation of the Section of Human Genetics. As early as 1974, members of the Section of Anthropology contributed substantially to the recognition of human genetics. In 1977, the Section grew to the Croatian Society of Anthropology, president Hubert Maver, now so enthusiastically led by Academician Pavao Rudan. The efforts invested by the Society members resulted in foundation of the Institute of Anthropology in Zagreb in 1992.

From 1975 onward, scientists from the »Rudjer Bošković« Institute (RBI) joined the Section of Human Genetics. At RBI, biomedical and genetic research started as early as 1955. From 1968, a group of scientists-theoreticians from RBI Department of Biology and Experimental Medicine, headed by Academician Željko Trgovčević and Vera Zgaga, PhD, Drago Petranović, PhD and Mirjana Petranović, PhD, contributed to better understanding of the human genome and the effect of UV rays on DNA synthesis, damage and repair by their experimental work on prokaryotic bacteria. Study of programmed cell death was a field of special interest of these scientists. At the turn of the 9th and 10th decade of the 20th century, fast development of molecular medicine and molecular genetics was supported by construction of the new centre at RBI, along with strong advancement in molecular biology; thus, the former department was divided into two organizational units, Department of Molecular Medicine and

Department of Molecular Genetics in 1995. Headed by now Academician Krešimir Pavelić, Department of Molecular Genetics turned to the leading national institution for basic and applied research as well as for their use in the field of molecular medicine. The projects of cancer research, cell biology, immunology, virology, neuropharmacology, endocrine pathology, etc., and promotion of molecular technologies and their use in clinical practice were valuable contribution of the Department to the development of clinical genetics in Croatia. In the 1970s, Department of Tissue Typing, Department of Urology, Zagreb University Hospital Centre, headed by Academician Andrija Kaštelan, transplantation immunologist, joined the work of the geneticists.

Department of Clinical Genetics with Laboratory of Cytogenetics, headed by Doctor Ivo Ligutić and his co-workers Professor Iskra Petković and Professor Lukrecija Brečević, was founded at the Zagreb University Children's Hospital in 1972.

At the beginning of 1983, Clinical Department of Laboratory Diagnosis, re-established and modernized by Professor Ana Stavljenić Rukavina, turned to an unavoidable collaborator in the work of the Department of Human Genetics, Clinical Department of Pediatrics, Zagreb University Hospital Center, first through the use of international achievements in the field of biochemistry, and later through the Croatian pioneer activities in molecular diagnosis of the rare monogenic disorders. The collaboration of now Professors Jadranka Sertić, Ksenija Fumić and Renata Zadro with human geneticists from Paris, mediated by Professor Zergollern, proved invaluable for the implementation of cystic fibrosis diagnosis in Croatia. Establishment of the Laboratory of Immunocytogenetics, led by Sanja Davidović, brought substantial improvement in the use of cytogenetics in chromosomal analysis of hemoblastosis, which in turn contributed to development of the expert team of Professor Boris Labar, who established Clinical Unit of Bone Marrow Transplantation as the first one in East Europe, in 1982.

Since 1991, the Laboratory of Calcified Tissues at the School of Medicine, University of Zagreb, established and led by Academician Slobodan Vukičević, has greatly contributed not only to Croatian but also to international osteology by their pioneer scientific as well as daring practical work on osteogenic tissue cloning.

In 1993, with establishment of the independent Republic of Croatia, the Section of Human Genetics, then with more than 100 very active members, grew into the Croatian Society of Human Genetics (CSHG) of the Croatian Medical Association, with Ljiljana Zergollern elected its first president. Because of the great number of members and their varied interests, the CSHG was divided into two sections, Section of Medical Genetics, led by Ivo Ligutić, pediatrician, and Section of Molecular Genetics, led by Academician Krešimir Pavelić. In 1997, Professor Ana Stavljenić Rukavina was elected CSHG president and Professor Ljiljana Zergollern honorary president for life. In the same year, the first CSHG branch office with 37 members was established in Split, led by Professor Vida Čulić. In 2013, the CSHG Section of Cytogenetics and Molecular Karyotyping, led by Leona Morožin Pohovski, PhD, was established for harmonization of its activities and exchange of experiences in this dynamic field of human genetics.

In Rijeka, a very active group of geneticists, led by Professor Miljenko Kapović, has assembled at the Department of Biology, School of Medicine, University of Rijeka, to mention only Professor Bojana Brajenović and Assistant Professor Saša Ostojić, for their outstanding work and enthusiasm. The work of Damir Letinić, pediatrician from Zadar, the research by the late Professor Igor Medica, pediatrician from Pula, and Dr Čalo's genetic counseling office from Osijek show that human genetics has expanded all over Croatia. In Split, the Department of Forensic Genetics has been established recently, led by Professor Dragan Primorac and Professor Šimun Anđelinović. In 2005, Professor Ivo Barić was elected CSHG president, and since 2009 CSHG president is Professor Ingeborg Barišić. Currently, there are around fifteen departments dealing with human genetics in Croatia, while CSHG members are active not only in Croatia but also in many international professional societies all over European Union (EU).

Since their establishment to the present, the activities of the CSHG have been numerous and heterogeneous. Six symposia held in Zagreb and three international summer schools in human genetics held at the Interuniversity Centre Dubrovnik (interrupted by the war in Croatia) offered an opportunity for the geneticists from abroad to be introduced in our and for us to be informed on their achievements in the field of human genetics. The work of our geneticists was recognized by Ljiljana Zergollern elected vice-president and then president of the European Society of Human Genetics (1979–1982). In 1982, the Conference of European Society of Human Genetics was held in Dubrovnik, which proved very successful and well-attended. In 1989, 30th anniversary of the work on human genetics in Croatia was celebrated under the auspices of the Academy of Medical Sciences of Croatia, with a rich scientific program. In 1994, the First Croatian Congress of Human Genetics was held in Zagreb, with more than 150 participants from Croatia and abroad. The tradition of human genetics congresses has been successfully continued to the presence, i.e. to the last one, Fifth Congress of Human Genetics with International Participation, held in 2011 in Bol on the Island of Brač.

Besides national congresses, a number of international congresses were held in Croatia, e.g., 8th Balkan Congress of Human Genetics in Cavtat 2009, Conference on Cystic Fibrosis in South-East Europe in Dubrovnik 2010 and 11th International Conference on Osteogenesis Imperfecta, held in Dubrovnik in 2011 owing to great efforts invested by Professor Darko Antičević. CSHG has also sponsored the conferences of the International Society for Applied Biological Sciences (ISABS), organized for years by Professor Dragan Primorac. In 2013, CSHG acted as co-organizer of the 12th European Symposium

on Congenital Anomalies, held in Zagreb in the organization of the European Concerted Action on Congenital Anomalies and Twins (EUROCAT), one of its founders being Professor Ivo Švel, whose work was continued by Doctor Ivo Ligutić and Professor Ingeborg Barišić who is current president of EUROCAT. The last important CSHG meeting were the 1st Days of Human Genetics, dedicated to Professor Ljiljana Zergollern-Čupak. A class I postgraduate study of the School of Medicine on the State-of-the-Art in Medical Genetics was organized in the frame of this meeting. The lectures held presented new achievements in Croatia and in the world, with the aim of expert scientific education of young human geneticists.

The publication activity of the Croatian human geneticists shows many valuable papers, monographs and books by a number of authors, articles in Croatian and international journals, etc. The first university textbook in human and medical genetics was written by Professor Ljiljana Zergollern as early as 1977, run through four printings, entitled »Uvod u humanu genetiku i kliničku citogenetiku« (Introduction in Human Genetics and Clinical Cytogenetics), issued by »Sveučilišna naklada Liber«[2]. It was followed by other books and textbooks penned by Professor Zergollern in collaboration with many CSHG members[3–8].

Formal education in human genetics was initiated as a subject „Fundamentals of Human Genetics" at the Education-Rehabilitation School, University of Zagreb, as early as 1969, offering young scientists an opportunity to acquire MS and PhD degree in the field. This form of education has continued to the present, led by Professor Ingeborg Barišić. In 1972, Professor Ljiljana Zergollern introduced the four-semester Postgraduate Study in Medical Genetics at the School of Medicine in Zagreb, as the first in the region. During the 20-year period (1973–1993), nine generations of students attended the study covering theoretical, medical, ethical and juridical genetics, with lecturers from Croatia and abroad. These activities were crowned by about 40 MS theses and a dozen of doctoral dissertations in the field of human genetics, defended at the University of Zagreb. The subject of orofacial genetics was introduced as early as 1981, thanks to the then young dental doctor, now Professor Ilija Škrinjarić, first in postgraduate education and since 1983 in undergraduate education at the School of Dental Medicine in Zagreb, and later also in Rijeka. This greatly improved education and knowledge of the role of human genetics in modern dental medicine. Organization of the study in forensics at the University of Split as the first one in the region should be noted. Lectures on the subject of Fundamentals of Human and Population Genetics, led by Professor Ingeborg Barišić at the School of Pharmacy and Biochemistry in Zagreb started in 2007, and since 2010 Medical Genetics is a regular study subject at all medical schools in Croatia. In Zagreb, the subject is led by Professors Florijana Bulić Jakuš and Ingeborg Barišić.

Development of Cytogenetics in Croatia

Cytogenetics is the first human genetics subspecialty developed from cytology and genetics as a branch of medicine investigating chromosome number, structure and dynamics during the cell cycle and division. The discovery that man has 46 chromosomes, made by Tjio and Levan in 1956, was the turning point at which the study of human chromosome began. In 1960, a group of cytogeneticists from Denver, USA, issued basic guidelines on chromosome terminology and arrangement. A breakthrough occurred at the beginning of the 1970s with the introduction of banding techniques for chromosome identification by their unique band arrangement, so that each chromosome is allocated its place and particular number in the karyotype. Bands have enabled detection of numerous structural changes on chromosomes (insertions, inversions, duplication, deletions and translocations) that could not be observed before.

Always trying to keeping up with international achievements, we made the first karyotype in a child with Down syndrome, then also in a child with Turner syndrome using *camera obscura* instead of a photomicroscope, borrowed centrifuge and improvised thermostat in Zagreb in October 1959, only several months after Lejeune (Montreal–Paris, 1958) and Ford (Oxford, 1959). Since then, cytogenetics has been progressing rapidly in our setting, pointing to the association of chromosome aberrations not only with various syndromes in human pathology, but also with carcinogenesis, monogenic disorders, alterations caused by viral diseases, and lesions induced by x-ray and other harmful factors. Besides Department of Human Genetics, Department of Pediatrics, Zagreb University Hospital Centre, organized work in the field of cytogenetics has also been introduced in other institutions in Zagreb, and then all over Croatia. At Department of Gynaecology and Obstetrics, »Merkur« University Hospital in Zagreb, the work in cytogenetics was started by Professor Zvonimir Singer and biologist Mirko Beck in 1963; in Zagreb University Children's Hospital by Head Doctor Ivo Ligutić and Professor Iskra Petković in 1971; and in »Sestre milosrdnice« University Hospital Center by paediatrician Ljerka Schmutzer and dental medicine doctor Goran Horgas in 1972. In 1992, at the request by Professor Asim Kurjak, gynecologist, Professor Ljiljana Zergollern with Feodora Stipoljev, biologist, established Cytogenetic Laboratory with Genetic Counselling Unit at »Sveti Duh« University Hospital. At Department of Biology, University of Rijeka, biologists Vera Tomašić, Milica Kružić and Stjepan Urban, with the help of cytogeneticists from Zagreb started pioneer work in the fields of cytogenetics and population genetics in the Primorje area. Later on, cytogenetic laboratories were also organized in Split and Osijek.

The advent of the methods of molecular cytogenetics such as fluorescent in situ hybridization (FISH) in the 1980s made a new breakthrough, followed by the methods of Multiplex Ligation-dependent Probe Amplification (MLPA) and chromosomal microarray analysis (CMA) at the beginning of the 21st century. After professional

training in The Netherlands, biologist Ružica Lasan-Trčić was the first to introduce FISH method in routine practice at the Zagreb University Hospital Center in 1996[9]. At Department of Clinical Genetics, Zagreb University Children's Hospital, Professor Iskra Petković was the first to use FISH technique in addition to classic FISH diagnosis in screening for chromosome subtelomeric regions in 2001[10]. Then, FISH diagnosis was soon introduced in other institutions in Croatia. At Zagreb University Children's Hospital, Leona Morožin Pohovski, PhD, and Ivona Sansović, PhD, introduced MLPA in the diagnosis of microdeletions/duplications, subtelomeric aberrations, frequent mutations and major deletions in more common monogenic disorders[11,12].

As novel methods of molecular karyotyping, such as genome microarray and next generation sequencing have been introduced in clinical practice, they are expected to be included in the clinical routine also in Croatia in the near future.

Development of Prenatal Diagnosis in Croatia

Following the respective events worldwide, prenatal diagnosis was very early introduced in Croatia as well. Professors Milan Bulić and Jovan Vujić from »Merkur« University Hospital were the first to employ ultrasound in morphological fetus analysis in 1969. The team consisting of Bulić and Vujić (amniocentesis), Singer and Beck (amniotic fluid cell culture and fetal karyotype analysis) and Zergollern (genetic counselling) started working in spring 1969, along with the pioneer work on the prenatal diagnosis of chromosomal disorders by culture and cytogenetic testing of amniotic fluid cells. The first amniocentesis performed in the 16th week of gestation, was just the first step of this team's 'pilot study', approved as a routine method only in 1971. In Croatia, the first child undergoing amniocentesis and cytogenetic chromosome analysis was born in February 1972 as the first such child in the former Yugoslavia, where Zagreb was the only center developing »aggressive prenatal diagnosis«. Professor Vujić was brave enough to perform a number of fetoscopy procedures; however, he soon abandoned this diagnostic technology due to the high risk for the fetus. In 1974, Professor Zvonimir Singer started performing placentocentesis, later joined by Marjan Podobnik, using the tissue obtained for both cytogenetic and molecular analysis, and for determination of the placental condition and maturity. In the same year, the team from the Department of Gynecology and Obstetrics, Zagreb University Hospital Centre (V. Latin, A. Kurjak and B. Rajhvajn Jr.) introduced amniocentesis in their routine and established long-term collaboration with Department of Human Genetics, Zagreb University Hospital Centre, where cell culture and karyotype analysis was taken over by Dubravka Mužinić, competent disciple and collaborator of Professor Lj. Zergollern, with a group of laboratory technicians. Other centers have also been established elsewhere. In 1986, Department of Biology, Rijeka School of Medicine, introduced amniotic fluid cell culture. A team of cytogeneticists led by Professor Bojana Brajenović-Milić were the first in Croatia to introduce prenatal screening for Down syndrome in maternal blood in 1996[13]. In 1992, cytogenetic laboratory was established at »Sveti Duh« University Hospital, with great help offered by Professor Zergollern, where all techniques of prenatal diagnosis have been introduced to date. Assist. Professor Feodora Stipoljev with the help of her laboratory staff introduced analysis of chorionic villus biopsy, currently available only at their institution in Croatia[14]. The cytogenetic laboratory led by Professor Vida Čulić started performing amniotic fluid culture and analysis in 2007. In 2001, Kristina Crikvenac-Gornik, biologist, developed a method of microsatellite loci for the diagnosis of most common aneuploidies and analysis of STR loci for the diagnosis of uniparental disomy[15]. Jasenka Wagner from Osijek introduced fetal sex determination from maternal blood in 2008[16]. Development of biochemical methods and molecular diagnosis techniques enabled introduction of the prenatal diagnosis of monogenic disorders such as cystic fibrosis, fragile X syndrome or spinal muscular atrophy in Croatia; however, assistance of the respective laboratories from abroad is still required in case of rare hereditary disorders.

Development of Molecular Diagnosis in Croatia

In Croatia, the use of molecular diagnosis in clinical practice dates back to the 1990s with the molecular diagnosis of cystic fibrosis[17,18]. Since then, genetic assays for many monogenic and multifactorial disorders have been developed at Clinical Laboratory of Molecular Diagnosis, Zagreb University Hospital Center. The diagnosis of metabolic disorders has been developing at the Clinical Laboratory of Hereditary Metabolic Diseases, headed by Professor Ksenija Fumić. Department of Functional Genomics was established in 2003, supported by the Ministry of Science, Education and Sports, as a joint project of the School of Medicine, University of Zagreb and Zagreb University Hospital Centre. Until 2005, Department was headed by Professor Dmitrij Krainc. Later, Department grew into Department of Translational and Clinical Research, headed by Professor Fran Borovečki. In the 1990s, diagnosis of genetic disorders was also introduced at the RBI Department of Molecular Biology headed by Academician Krešimir Pavelić, then by Professor Jasminka Pavelić[19–22]. Currently, a number of projects have been carried out at the Department, led by Oliver Vugrek, including development of molecular target cancer and viral disease therapy, development of new diagnostic tools and studies aiming at decoding the molecular basis of diseases, and at development of tools based on omics approach. During the war in Croatia, Professor Dragan Primorac in collaboration with a group of scientists from Split led by Professor Šimun Anđelinović worked on the identification of Patriotic War victims and set fundamentals for the development of modern forensics in Croa-

tia[23]. Molecular diagnosis of monogenic and multifactorial disorders has been developed at Zagreb University Children's Hospital[24,25], and of tumors in Split (diagnosis of leukemias and lymphomas).

Genomics, Pharmacogenomics and Personalized Medicine

New technologies for genome analysis have led to the development of genomics, a scientific discipline investigating the structure, organization and functioning of the genome. The field of genomics also includes the study of intragenomic phenomena such as epistasis, pleiotropy and other interactions between gene loci, and in broader sense the research not only at the DNA level (genotype) but also at the level of mRNA (transcriptomics) and proteins (proteomics).

Whole genome studies, and exome and RNA sequencing have dramatically increased the number of known somatic alterations, i.e. mutations, amplifications, deletions and translocations associated with tumors development. Molecular information can be used for developing target therapy for particular patient subgroups, as well as for monitoring therapeutic efficacy and prognosis of disease aggressiveness, thus avoiding unnecessary and expensive procedures in patients not expected to respond to therapy[26]. Progress in this field of genetics is expected to be achieved at the RBI Department of Molecular Medicine by use of the new methodologies through multidisciplinary collaboration of molecular biologists, chemists, biomedical experts and bioinformatics professionals, which may hopefully result in the development of novel anti-tumor agents.

Some ten thousand monogenic disorders have been identified to date, most of them very rare, however, yet involving millions of people all over the world. These new technologies enable us to identify a hundreds of genes responsible for the occurrence of etiologically heterogeneous entities such as epilepsy, autism or intellectual disabilities, as well as gene mutations responsible for the occurrence of rare and poorly known diseases[27]. In Croatia, groups of scientists are also engaged in a number of multicenter studies investigating the complex genetic basis of multifactorial disorders, to mention only Professor Gordan Lauc and his co-workers in Genos, scientists from the Institute of Anthropology and Croatian Centre for Global Health, until recently led by Professor Igor Rudan[28,29].

Clinically relevant examples of the impact of genetics on drug metabolism have already been known for decades and belong to the field of pharmacogenetics. In Croatia, this field has been very successfully dealt with by Professor Nada Božina, head of the Clinical Laboratory of Pharmacogenomics and Individualized Therapy, Department of Laboratory Diagnosis, Zagreb University Hospital Center[30]. This discipline has recently evolved to pharmacogenomics, a discipline studying the role of hereditary and acquired genome variations in the patient response to the drug administered. Genome sequencing can identify variations influencing individual metabolism, thus also drug response; based on this information, drug type, dosage and therapy duration can be chosen, avoiding the possible side effects. In the future, it is expected that we will choose the type and dosage of drugs individually for each patient rather than matching it to the average obtained by clinical trials. In addition, sophisticated instruments can rapidly identify and analyse genetic structure of microorganisms, i.e. viruses and bacteria (microbiomics), which will facilitate the search for new drugs against the pathogens undergoing mutation and developing resistance. Quite intriguing is also the new field of metagenomics that investigates the interaction between the host genome and the microorganisms, aiming to facilitate understanding of complex diseases such as inflammatory ones.

The concept of personalized medicine, i.e. the use of the individual's genetic composition to predict the risk of disease development and its course, and to adjust the treatment protocol according to the unique patient requirements, has been known for more than hundred years now. However, it was the introduction of high-resolution technology in genome analysis that brought revolutionary progress in the field of personalized medicine. Along with progress in computer biotechnology, these technologies enabled simultaneous analysis of thousands of molecules in the genome and interpretation of large groups of data obtained in these experiments. The introduction of target therapy has increased the need of detailed molecular characterization of the disease and identification of biomarkers for the prognosis and prediction of therapeutic success and disease outcome[31]. At the RBI, this topic is especially tackled at the Laboratory of Personalized Medicine, led by Professor Sanja Kapitanović. The scientific activities of this laboratory are focused on the study of the molecular-genetic basis of the occurrence and progression of sporadic tumors of the colon and on the pharmacogenetic studies of the efficacy or toxicity of anti-tumor therapy.

In the past decade, the interest in the field of epigenetics has increased considerably. Epigenetics is a discipline investigating hereditary alterations in gene expression that cannot be ascribed to DNA sequence changes. The main epigenetic mechanisms include DNA methylation, modification of chromatin structure, loss of imprinting, and noncoding RNA. An important characteristic of these mechanisms is that they can be inherited *via* meiotic and mitotic division, while being capable of being deleted later during cell differentiation or upon exposure to extracellular and environmental factors. Epigenomics is a science investigating the genome epigenetic marks and their effect on the global gene expression and trying to explain why the cells and organisms with identical DNA can have so dramatically different phenotypes. Just as variations in DNA sequence (previously called mutations) can contribute to development of disease, epigenetic changes such as impaired DNA methylation or histone modification can favor development of multifactorial diseases (e.g., cardiovascular disease, hypertension, dia-

betes mellitus, some infectious diseases) and neoplasms (e.g., leukemia, lymphomas, sarcoma, and carcinoma of the breast, lung, prostate, liver and colon). Recently, a great body of data has been acquired on the epigenetic mechanisms and involvement of epigenetic regulation of gene expression as the underlying pathogenic mechanism in the development of other phenotypes and diseases such as mental disorders, autism and epilepsy. Dietary habits and other external factors can alter epigenetic regulation, which explains the association between the lifestyle and the risk of disease development observed by the scientists. Epigenetic marks (DNA methylation, mRNA and microRNA expression, etc.) can serve as biomarkers for risk stratification, early detection and classification of diseases, and also as the targets for treatment and chemoprevention, thus promising development of novel therapeutic strategies.

In spite of all difficulties, we certainly enter a new revolutionary era of personalized medicine. Currently, we can collect considerably more data than we can interpret. The data of electronic medical records, genomics, epigenetics, transcriptomics, proteomics, metabolomics and microbiomics should be integrated and interpreted. Therefore, when speaking about the impact of genomics on the health and disease, sequencing of individual genomes of healthy persons (integrative personal omics profiling, iPOP) integrating data on all »omics«, i.e. transcriptome, metabolome and proteome into the personal omics profile to be observed in health and disease is of utmost importance[32]. Although quite promising, it may be perceived as a favorable circumstance that these methods are still too expensive for routine use in medical practice. Their wide use will certainly open an array of ethical questions we have to be ready to face on time. These include questions of data availability, in particular to family members that might carry the same risk as the proband, but also related to wider community (employer, insurance companies, healthcare system and legal system). Accidental findings pose a special practical challenge. Is it necessary to inform the patient only on the results related to the disease for which he/she has undergone testing or on all his/her genome findings? Should the patient be informed on the variants associated with the risk of developing a disease for which there is no cure or preventive methods? How to ensure confidentiality of such a huge amount of data? How to regulate genetic testing which is commercially available on the market without appropriate genetic information? In the years to come, we can expect to learn more about genetics than we have learned throughout the history of medicine, and we should be properly prepared for it, considering seriously all the favorable as well as unfavorable aspects of modern achievements in the field of genetics[33].

Genetic Counselling

In 1975, the American Society of Human Genetics adopted the probably best known and most widely accepted definition of genetic counselling to date, stating that it is a communication process that deals with the human problems related to the occurrence or risk of occurrence of a genetic disorder in a family. It includes one or more competent professionals trying to help an individual or a family to (a) comprehend medical facts including diagnosis, probable course of disease and treatment options; (b) understand the way in which heredity influences disease development and risk of disease recurrence in a family; (c) comprehend all the options available considering the risk involved; (d) use genetic information in a reasonable way in order to promote health, reduce psychological stress and upgrade personal control; (e) choose the mode of action he/she finds appropriate according to the risk involved, family planning and ethical issues, and to act in line with his/her own decision; and (f) achieve the best possible adjustment to the disease affecting the family and to the implied risk of disease recurrence[34]. The first genetic counselling unit in Croatia was established by Professor Zergollern in 1964 at Department of Human Genetics, Department of Pediatrics, Zagreb University Hospital Centre, which was followed by the genetic counselling units in Zagreb University Children's Hospital in 1972, and then also in Split and Osijek. Genetic counselling should be as a rule administered by a properly trained professionals. In Croatia, respective education is acquired through residency in pediatrics, sub-residency in medical genetics. In special cases, other specialists can be trained in particular issues (for example, gynecologist for prenatal screening of older pregnant women or oncologist for particular tumor types), which requires specific education and accreditation issued by the Croatian Society of Human Genetics, Croatian Medical Association[35]. Fast development of the knowledge and understanding the molecular basis of genetic disorders has opened new diagnostic options such as prenatal, pre-symptomatic and predictive genetic testing, determination of carrier status for genetic disorders, and even detection of hereditary disorders through various screening programs for risk groups or the population at large. These complex procedures require thorough information on all medical facts. Therefore, genetic counselling should be an integral part of genetic testing, but must not be coercive. Based on quality information, each person makes his/her own decision independently, which he/she considers best for him/her and his/her family. Maximum privacy and confidentiality should be ensured for both the information communicated by the person and the information obtained by genetic testing. On genetic counselling, the whole family should be provided with any psychological and moral support required in the context of their family values and beliefs, family relationships, and in particular their cultural and social environment[36,37].

The Future of Human Genetics in Croatia

Until now, we have been able to follow the developments of human genetics in the world with our technical and other economic resources; however, this develop-

ment is so fast that we frequently have to give up, wondering whether we can ensure a satisfactory development of clinical genetics disciplines in the forthcoming years. Considering the progress of our science that has succeeded in keeping pace with the globalization trends to date, we do hope that our society members working at various institutes, departments and laboratories will continue working in the field of human genetics according to the bioethical principles, applying their results in the diagnosis and therapy of neoplastic, chromosomal, gene and other diseases associated with genome changes.

Although ethics and jurisdiction with legislation are outrun by the too fast technologic and genetic achievements, they have been integrated in the CSHG activities, so that jurists, philosophers and theologians took active part as invited lecturers at CSHG conventions and symposia since as early as 1974. In 1995, the newly founded Croatian Society of Bioethicists with Van Potter and Professor Šegota as active founders supported the geneticists' efforts to halt the general drop in morality and ethics, visible in many aspects of the modern society, even in biomedical and biologic sciences, which can have unpredictable consequences for the humanity. In this tumultuous time of the rule of biotechnology, *homo sapiens* should stay *homo sapiens* and find the right way to preserve life and health of all living creatures, primarily man, while not arresting the progress of biomedical and technologic sciences. Not everything that is possible in science is acceptable or ethical. According to Bishop Valentin Pozaić, technology has no conscience, but a scientist should have it. Just think how good intended were the scientists that discovered powder, atomic and nuclear energy, antibiotics, etc. Instead of proper use, the wrong and conscience uncontrolled employment of these discoveries led and is still leading to its mere contrast causing numerous tragedies. Apart from the positive effects of such discoveries, favorable for the humankind, today's conscientious scientists, philosophers and writers such as Hawking, Fukuyama or Rifkin are particularly worried by the biotechnology with genetic engineering. Rotblat, atomic physicist and 1995 Nobel Prize Winner, is afraid of mankind destruction by genetic engineering rather than atomic energy. Is it possible that the expensive biotechnology, modifying bioevolution into biorevolution by its over-dynamic behavior, would eventually lead to chaos through which nature will not be maintained but destroyed?

Genetic engineering has contributed and continues contributing to human welfare by the results of the work of many scientists including human geneticists. Drugs, multivalent oral vaccines and vaccines against certain malignancies, cloning of tissue cells and organs for experimental or therapeutic use in homo-, hetero- and xenotransplantation, transgenic animals, in vitro fertilization, preimplantation and presymptomatic diagnosis of some diseases, gene therapy, stem cell experiments and therapy, achievements in genomics, proteomics and oncogenomics, and the supreme success in the cloning of mammals, even primates, and perhaps man somewhere in secret, make the future of human genetics. Technically, we are not far from the possibility, already practiced in the USA, to create children with particular characteristics, and to substitute the hateful racial discrimination by the genetic one. All this is currently part of the work of the Croatian geneticists as well. As according to Pope John Paul II, the omnipotent, rich industry has, like merchants, entered the temple of science, we should be worried whether, in the times of deep economic and even deeper moral crisis, there are some among us who are ready to put their personal profit ahead of moral postulates. However, although Pandora's box has long been wide open, there is still hope that the development of top-line genetics, and thus of biomedical science will prevent it before entering the programmed computer intelligence (Hawking) and prior to our post-human future (Fukuyama), when the new, through eugenetic game affluent genetically improved people will threaten those unimproved, poor, occasionally born with congenital anomalies. Genetic lottery with nature can be considered unjust individually, but is fully egalitarian because it does not endanger human nature, human dignity and rights and because it may involve anybody irrespective of the class and nationality. Although we would like to be optimistic, we do share the worries of some scientists-medical technologists, who do not see only good results in the progress of science, but look at it with skepticism, in fear from the far-reaching consequences of these achievements for human race because they feel that some scientists of today substitute revolution for evolution in nature, playing God[38] and claiming right to modify nature in a fast and revolutionary manner. The capacity of human imagination is immeasurable and more valuable than the knowledge acquired. Science fiction has the feature of coming true, while the works once perceived as fanciful not infrequently are realized as visionary achievements. Until recently, organ transplantation seemed impossible, inhuman and impermissible, while nowadays we speak of organ and organism cloning, gene and organ chips, genetic identity cards, etc.

What is the »tomorrow« of human genetics in Croatia? We believe that the community of experts dealing with human genetics in Croatia will grow further and that they will, in spite of the current financial restrictions, have an opportunity to develop both practical use in the diagnosis, treatment and prevention of hereditary disorders and the basic genetic research that will expand our horizon and understanding of the concept of health and disease. We do hope that it will be done within the modern model of integrating bioethics which takes in consideration the standpoints of all relevant scientific disciplines, as well as the broader cultural perspective, and discuss more broadly the role of genetic science in modern civilization.

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REFERENCES

1. WATSON JD, CRICK FHC, Nature, 171 (1953) 737. — 2. ZERGOLLERN-ČUPAK LJ, Uvod u medicinsku genetiku s kliničkom citogenetikom (Sveučilišna naklada Liber, Zagreb, 1977). — 3. ZERGOLLERN-ČUPAK LJ, Uvod u medicinsku genetiku s kliničkom genetikom (Sveučilišna naklada Liber, Zagreb, 1980). — 4. ZERGOLLERN LJ, KURJAK A, Prenatalna dijagnostika (JUMENA, Zagreb, 1984). — 5. ZERGOLLERN LJ, Medicinska genetika 1, 2nd ed. (Školska knjiga Zagreb, 1991). — 6. ZERGOLLERN LJ, Medicinska genetika 2 (Školska knjiga Zagreb, 1994). — 7. ZERGOLLERN LJ, Humana genetika, 3rd ed. (Medicinska naklada, Zagreb, 1994). — 8. ZERGOLLERN LJ, Pedijatrija 1 i 2 (Naprijed, Zagreb, 1994). — 9. LASAN-TRCIĆ R, HITREC V, LETICA LJ, ĆUK M, BEGOVIĆ D, Croatian Medical Journal, 44 (2003) 477. — 10. PETKOVIĆ I, BARIšIĆ I, Paediatr Croat, 47 (2003) 1. — 11. MOROZIN POHOVSKI L, DUMIĆ K, ODAK LJ, BARIŠIĆ I, Mol Cytogenet, 6 (2013) 7. DOI: 10.1186/1755-8166-6-7. — 12. SANSOVIĆ I, BARIŠIĆ I, DUMIĆ K, Biochem Genet, 51 (2013) 189. DOI: 10.1007/ s10528-012-9554-9. — 13. BRAJENOVIĆ-MILIĆ B, TISLARIĆ D, BACIĆ J, PARAVIĆ J, SLIVAR AE, KAPOVIĆ M, KOŠEĆ V, RISTIĆ S, RAJHVAJN B, Fetal Diagn Ther, 13 (1998) 367. — 14. VIČIĆ A, STIPOLJEV F, Paediatr Croat, 57 (2013) 392. DOI: 10.13112/PC.2013.12. — 15. CRKVENAC-GORNIK K, GRUBIĆ Z, STINGL K, MUŽINIĆ D, BRKLJAČIĆ-KERHIN V, BEGOVIĆ D, Coll Antropol, 31 (2007) 859. — 16. WAGNER J, DZIJAN S, PAVAN-JUKIĆ D, WAGNER J, LAUC G, Prenat Diagn, 28 (2008) 412. DOI: 10.1002/pd.1986. — 17. ZERGOLLERN LJ, STAVLJENIĆ-RUKAVINA A, BARIŠIĆ I, SERTIĆ J, Acta Med Croat, 46 (1992) 181. — 18. ZERGOLLERN LJ, STAVLJENIĆ-RUKAVINA A, BARIŠIĆ I, SERTIĆ J, Period Biol, 95 (1993) 359. — 19. HEĆIMOVIĆ S, BARIŠIĆ I, PETKOVIĆ I, BARIĆ I, LIGUTIĆ I, PAVELIĆ K, Clin Genet, 52 (1997) 147. — 20. TANACKOVIĆ G, BARIŠIĆ I, GJERGJA-MATEJIĆ R, HEĆIMOVIĆ S, PAVELIĆ J, Clin Genet, 58 (2000) 333. — 21. PAVELIĆ J, KRIZANAC S, KAPITANOVIĆ S, PAVELIĆ L, SAMARZIJA M, PAVICIĆ F, SPAVENTI S, JAKOPOVIĆ M, HERCEG-IVANOVI Z, PAVELIĆ K, Am J Respir Cell Mol Biol, 32 (2005) 65. — 22. MATIJEVIĆ T, KNEZEVIĆ J, BARIŠIĆ I, RESIĆ B, CULIĆ V, PAVELIĆ J, Ann N Y Acad Sci, 1091 (2006) 225. — 23. MARJANOVIĆ D, KONJHODZIĆ R, BUTORAC SS, DROBNIC K, MERKAS S, LAUC G, PRIMORAC D, ANDJELINOVIĆ S, MILOSAVLJEVIĆ M, KARAN Z, VIDOVIĆ S, STOJKOVIĆ O, PANIĆ B, VUCETIĆ DRAGOVIĆ A, KOVAČEVIĆ S, JAKOVSKI Z, ASPLEN C, PRIMORAC D, Croat Med J, 52 (2011) 235. — 24. SANSOVIĆ I, KNEŽEVIĆ J, MUSANI V, SEEMAN P, BARIŠIĆ I, PAVELIĆ J, Genet Test Mol Biomarkers, 13 (2009) 693. DOI: 10.1089/gtmb.2009.0073. — 25. SANSOVIĆ I, DUMIĆ KUBAT K, BARIŠIĆ I, Paediatr Croat, 57 (2013) 408. DOI: 10.13112/PC.2013.14. — 26. ULAHANNAN D, KOVAC MB, MULHOLLAND PJ, CAZIER JB, TOMLINSON I, Chem Biol, 20 (2013) 660. DOI: 10.1016/j.chembiol.2013.05.001 — 27. SOON WW, HARIHARAN M, SNYDER MP, Mol Syst Biol, 9 (2013) 640. DOI: 10.1038/msb.2012.61. — 28. KRISTIC J, VUCKOVIC F, MENNI C, KLARIC L, KESER T, BECEHELI I, PUCIC-BAKOVIC M, NOVOKMET M, MANGINO M, THAQI K, RUDAN P, NOVOKMET N, SARAC J, MISSONI S, KOLCIC I, POLASEK O, RUDAN I, CAMPBELL H, HAYWARD C, AULCHENKO Y, VALDES A, WILSON JF, GORNIK O, PRIMORAC D, ZOLDOS V, SPECTOR T, LAUC G, J Gerontol A Biol Sci Med Sci, 69 (2014) 779. DOI: 10. 1093/gerona/glt190. — 29. LAUC G, HUFFMAN JE, PUČIĆ M, ZGAGA L, ADAMCZYK B, MUŽINIĆ A, NOVOKMET M, POLAŠEK O, GORNIK O, KRIŠTIĆ J, KESER T, VITART V, SCHEIJEN B, UH HW, MOLOKHIA M, PATRICK AL, MCKEIGUE P, KOLČIĆ I, LUKIĆ IK, SWANN O, VAN LEEUWEN FN, RUHAAK LR, HOUWING-DUISTERMAAT JJ, SLAGBOOM PE, BEEKMAN M, DE CRAEN AJ, DEELDER AM, ZENG Q, WANG W, HASTIE ND, GYLLENSTEN U, WILSON JF, WUHRER M, WRIGHT AF, RUDD PM, HAYWARD C, AULCHENKO Y, CAMPBELL H, RUDAN I, PLoS Genet, 9 (2013) e1003225. DOI: 10.1371/journal.pgen.1003225. — 30. BOŽINA N, PEJNOVIĆ L, Paed Croat, 57 (2013) 336. DOI: 10.13112/PC.2013.4. — 31. HUDSON TJ, J Intern Med, 274 (2013) 440. DOI: 10.1111/joim.12097. — 32. LI-POOK-THAN J, SNYDER M, Chem Biol, 20 (2013) 660. doi: 10.1016/j.chembiol.2013.05.001. — 33. YIM SH, CHUNG YJ, 11 (2013) 218. DOI:10.5808/GI.2013.11.4.218. — 34. FRAZER FC, Am J Hum Genet 26 (1974) 636. — 35. BARIŠIĆ I, Paediatr Croat, 54 (2010) 213. — 36. MCCONKIE-ROSELL A, SPIRIDIGLIOZZI GA, J Genet Couns, 13 (2004) 9. — 37. BARIŠIĆ I, Etički aspekti genetičkog testiranja u djece In: ČOVIĆ A, RADONIĆ M (Eds) Bioetika i dijete (Pergamena, Zagreb, 2011). — 38. MATULIĆ T, Nevjera i vjera – u četiri oka, (Glas Koncila, Zagreb 2012).

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RAZVOJ GENETIKE U SVIJETU I U HRVATSKOJ – POVODOM 40 GODINA HRVATSKOGA DRUŠTVA ZA HUMANU GENETIKU HRVATSKOGA LIJEČNIČKOG ZBORA

SAŽETAK

Nastala iz više bazičnih znanstvenih disciplina, genetika je doživjela izvanredan napredak u prošlom stoljeću otkrićima pravila nasljeđivanja, osnove građe genoma i identifikacijom gena koji određuju nastanak osobina i bolesti čovjeka. U Hrvatskoj je razvoj genetike započeo sredinom prošloga stoljeća pionirskim radom kliničara i bazičnih znanstvenika, što je rezultiralo značajnim razvojem ove znanstvene discipline koja je brzo našla svoju praktičnu primjenu u kliničkoj genetici-citogenetici, molekularnoj genetici i prenatalnoj dijagnostici. Brz napredak tehnologije i znanja o genetici u posljednjim desetljećima doveo je do razvoja genomike i srodnih disciplina pa se može reći da smo ušli u novu revolucinarnu eru personalizirane medicine. Danas možemo prikupiti puno više podataka nego što ih možemo protumačiti. Podatke elektroničkih medicinskih zapisa, genomike, epigenetike, transkriptomike, proteomike, metabolomike, mikrobiomike treba integrirati i interpretirati na razini genoma pojedinca.Široka uporaba novih informacija otvorit će niz etičkih pitanja s kojima se moramo na vrijeme suočiti. Očekuje se da ćemo u idućim godinama moći naučiti o gene-

tici više no što smo naučili u cijeloj povijesti medicine, a to moramo dočekati spremni, promišljajući sve pozitivne, ali i negativne aspekte suvremenih dostignuća u području genetike. Nadamo se da će stručnjaci koji se bave humanom genetikom u Hrvatskoj nastaviti uspješno sa svojim radom koji će omogućiti praktičnu primjenu suvremenih dostignuća u genetici te proširiti naše razumijevanje koncepta bolesti i zdravlja.