1994, 43 (3/4): 355–361
PL ISSN 0023-4249

Polskie Towarzystwo Przyrodników im. Kopernika KOSMOS

GENETICS IN DEVELOPMENT OF MEDICAL SCIENCES. IMPORTANCE AND PERSPECTIVES

Genetic studies concerning humans were for several decades very limited because of the lack of appropriate experimental research methods. Within the last several years medical genetics has been developing rapidly due to introduction of entirely new research techniques. Human genetic information is contained in 46 chromosomes which occur in practically all the cells of our organisms. The whole human genome consists of a DNA composed of 3 milliard pairs of nucleotides. Only a small part of the genome, not exceeding a few percent, is engaged in coding of structural genes, the number of which is estimated at about 50 thousand. The major part of the genome consists of non-coding sequences: introns or repetivive sequences, the function of which has not been so far established.

The dynamic development of new techniques of molecular genetics has created qualitatively new possibilities for gaining knowledge of genetic diseases, as well as for diagnosis and therapy. Development of the genetic engineering techniques began by the discovery of restriction enzymes which are able to cut DNA at precisely defined sites. Studies on human genes became possible due to progress in various methods of DNA analysis, including cloning and sequencing. However, the discovery and bringing into practice the polymerase chain reaction, (PCR) constituted the real turning point. The author of the PCR technique, Kary Mullis, was awarded the Nobel Prize in 1993. The use of PCR in basic research and for diagnosis of genetic diseases gives the possibility of studying, by a relatively simple technique, of even a single human gene.

The exceptional value of the PCR technique consists in its making possible isolation and several-million-fold amplification of a single gene or its fragment. This task can be compared to the proverbial looking for a needle in a haystack, sensitivity of PCR permitting to find it with great precision and, moreover, to study it. Generally speaking, the range of possible practical applications of genetic engineering, not only in medicine but also in biology, argiculture and industry, is difficult to imagine and, may be, also difficult to accept. As concerns medical sciences, molecular genetics creates completely new possibilities of studying the ethiopathogenesis of several diseases, not only those caused by genetic defects.

Medical practice has taken advantage of the achievements of genetics very rapidly. As a particularly good example can serve prenatal diagnosis: Jacek Zaremba, *Prenatal diagnosis*. The main aim of this method is to make possible early diagnosis of diseases and developmental defects in the foetus. Initially, prenatal diagnosis was performed using the amniotic fluid and the cells of foetal origin present in this fluid, inter alia, the desquamated cells of the gastrointe-

stinal and urinary tracts. Within the last dozen years they became superseded by trophoblast cells. The test consists in withdrawing a small amount of chorion, sufficient for basic cytogenetic and biochemical studies, and the PCR method eliminates the necessity of tedious culturing of the cells *in vitro*. It is also possible to isolate, from the withdrawn material, the DNA for molecular testing for selected genetic defects. It is advisable to make prenatal diagnosis in various situations when there is a risk of a woman giving birth to a diseased child. In about 97% of the cases the results of prenatal diagnosis point to normal development of the foetus. Such a favorable result, especially in the case of a pregnant woman suffering from severe anxiety neurosis liberates her from the fear of giving birth to an abnormal child and contributes in this way to maintenance of the pregnancy. It is possible at present to diagnose several diseases and defects even before the birth of the child and in many cases this might permit early beginning of the treatment. Despite their advantages, the prenatal diagnosis tests are made in Poland only on a very small scale and but in a few medical centers. On the other hand, in the leading foreign laboratories the progress is so great that attempts have been made at preimplantation diagnosis which consists in diagnosing genetic effects on the basis of testing a single cell withdrawn from a developing embryo prior to its implantation into the uterus.

For a long time human genetics has been based to a large extent on classical cytogenetic studies, at present, however, molecular studies begin to predominate. Both for cytogenetics and molecular genetics sex chromosomes continue to present a challenging problem of great practical significance. The paper by BOGDAN KAŁUŻEWSKI entitled *Do we need sex chromosomes* provokes many reflections. Although the feeling of one's sexual identity is usually quite obvious to the person concerned, the problem of sexual differentiation and identification is neither simple nor unequivocally formulated. Sex can be defined as: chromosomal, related to the occurrence in cell nuclei of sex chromatin X in a woman, and of sex chromatin Y in a man; gonadal sex defined by the presence of a primary gonad; endocrinological sex determined by the activity of particular sex hormones; phenotypic sex defined according to the internal and external sex organs present; and psychological sex comprising the psycho-sexual and psycho-social spheres. The author describes the most spectacular race for priority in identifying the genes which determine development of the primary gonad. So far, we have gained better understanding of the sex determination mechanism, however, one should consider simultaneously possible involvement of still other genes in normal sex differentiation. To see how much remains to be done, take for example antigen H-Y for which, after so many years of research, the structural gene has not been located. To the question posed in the title of BOGDAN KAŁUŻEWSKI'S paper the answer should be in the affirmative: a normal karyotype 46,XY or 46,XX is indispensable for normal development of a man or a woman, respectively.

It would not be possible to diagnose several genetic diseases without taking advantage of molecular studies. The classical cytogenetic analysis of chromosomes permits to detect only the genetic disturbances due to those genetic defects which are related to chromosome aberrations. The majority of the five thousand

genetic diseases are undetectable by the simple analysis of the karyotype. Duchenne muscular dystrophy can serve as a good example of usefullness of molecular studies for precise determination of the genetic defect. Duchenne dystrophy is caused by mutations in the largest human gene so far known, the *DMD* (Duchenne muscular dystrophy) gene. It is 2.5 million nucleotides in length, is composed of 78 exons interspersed with introns, on average of 25 kb, and is located on chromosome X. cDNA deprived of introns is of a rather small size being composed of over 13 thousand nucleotides, of which about 11 thousand code for a protein called dystrophin. DMD is lethal disease encountered at a frequency of 1 per 3500 male newborns. In about 70% of the cases it is caused by deletions in the *DMD* gene, which can be detected by multiplex PCR technique using 21 pairs of primers. JOLANTA KWIATKOWSKA , DOBRAWA LISIECKA, RYSZARD SŁOMSKI, BOŻENA SIEMIENIAKO, JADWIGA SOWIŃSKA, BOŻENA GALAS-ZGORZALEWICZ, EWA EMICH-WIDERA, ELŻBIETA MARSZAŁ i TADEUSZ CIESIELSKI — *Molecular diagnostics of Duchenne/Becker muscular dystrophy* — discussed not only various aspects of molecular diagnosis of Duchenne/Becker muscular dystrophy but also the possibility of carrier screening by testing for polymorphism of the PCR product restriction fragments and determination of microsatellite sequences within the *DMD* gene. It seems that in the near future it will be possible to identify precisely the DMD carriers by studying microsatellite sequences related to specific mutations in the *DMD* gene.

Selected aspects of the dependence in mucoviscidosis, of phenotype on genotype have been discussed by MICHAŁ WITT — *Selected aspects of genotype – fenotype relationship in cystic fibrosis*. Mucoviscidosis (cystic fibrosis, CF) is an autosomal recessive disease encountered once per 2500 births. The defective gene responsible for mucoviscidosis is located on chromosome 7, and has been cloned and sequenced. It is most astonishing that this grave genetic disease is caused by over 400 different mutations which determine to some extent its clinical pattern. Unfortunately, there is no simple genotype/phenotype correlation, and the disease itself appears in very diverse forms. Attempts at a correlation of, e.g., disturbed functioning of the respiratory system or susceptibility to bacterial infections to specific mutations gave no unequivocal results. The diversity of phenotypes related to gene *CFTR* mutations points to the possible occurrence of the disease in very varied forms.

Paper by JERZY BAL and DOROTA MACIEJKO *Muscoviscidosis — from gene to therapy* deals with mode of inherity, epidemiology of cystic fibrosis, structure and function of *CFTR* gene as well as molecular diagnosis. Authors discuss the matter of verification of clinical diagnosis by the study at the molecular level. They present the principle of detection of *CFTR* gene mutation carriers and prenatal diagnosis of muscoviscidosis. Finally they discuss clinical trials of gene therapy for cystic fibrosis.

Phenylketonuria is a rather common genetic disease. It can serve as an example of a genetic disease that can be effectively treated by application of a diet poor in phenylalanine. The therapy can be started quite early due to the commonly applied screening of newborns by Guthri test. Early implementation of the phenylalanine-poor diet permits to eliminate the most important clinical symptom of phenylketonuria, i.e. deep mental impairment. Despite the treat-

ment the affected children usually learn with difficulty, and in some cases psychical disturbances are observed. JADWIGA JARUZELSKA — *Phenylketonuria — from genotype to the clinic* — discusses the mutations responsible for phenylketonuria, its diversified clinical manifestations, problems of treatment and management of phenylketonuria children, and search for new forms of therapy.

The molecular basis of Hunter syndrome, a rather rare genetic disease, has been presented by EWA POPOWSKA — *Molecular basis of Hunter syndrome.* This syndrome, in its grave form, characterized by deep mental retardation, marked changes in circulation and the bone-joint system, leads usually to death before the patient has reached the 15th year of life. In its mild form the syndrome does not lead to disturbances in mental development, and changes in the circulatory and the skeletal bone-joint systems are much less apparent. To put it in a simplified form, deletions of the whole gene or its fragment, insertions altering the reading frame, point mutations in conserved or unique regions, and mutations introducing the termination codon, especially into the initial region of the transcript, seem to be responsible for the grave form of the disease. On the other hand, mutations altering the functionally less important protein regions lead to the mild form of the disease. The possibilities of the Hunter syndrome therapy are limited to application of motor rehabilitation, mental training and temporary pharmacological treatment.

Defects in the alfa-1-antitrypsin coding gene can lead to liver cirrhosis and pulmonary emphysema. ANNA KOWALSKA and BOŻENA PIŁACIK present in their paper *Deficiency of alpha-1-antitripsin* the molecular basis of alfa-1-antitrypsin deficiency and the role of this enzyme in pathogenesis of liver and lung diseases. They point also to the significance of alfa-1-antitrypsin deficiency in rheumatoid arthritis and in pathogenesis of some skin diseases which so far has not been fully elucidated. Lowering of the level of alfa-1-antitrypsin, the enzyme inhibiting several proteases, raises the risk of pulmonary diseases not only in homozygotes but also in heterozygotes, especially after exposure to tobacco smoke and other noxious, alfa-1-antitrypsin inactivating factors. The treatment applied consists in administration of alfa-1-antitrypsin preparation. It seems that it might be possible, in the near future, to apply gene therapy in treatment of this disease.

Diabetes mellitus of type I, i.e. insulin dependent, is an example of a disease dependent on multiple factors, many genes playing an essential role in its development. MAŁGORZATA JUNGERMAN presents a review of studies on genetic conditioning of insulin-dependent diabetes — *Insulin dependent diabetes mellitus — a complicated example of multifactorial disorder.* She draws attention to the complexity of factors leading to development of diabetes, and to the practical importance of research aiming at early detection of persons who are prone to become diabetic. In the future, identification of both genetic and environmental factors involved could permit early prevention of the insulin-dependent diabetes.

Studies on DNA are essential not only for gaining knowledge of molecular defects leading to the occurrence of genetic diseases but simultaneously play a steadily increasing role in molecular diagnostics. The latter finds broad application not only in various branches of medicine but also in biology. An excellent example of the progress in molecular genetics and its practical usefulness is

paternity testing. The necessity to ascertain contestable paternity occurs not only in lawsuits but is also indispensable in some cases concerning genetic counselling, prenatal diagnostics and transplantology. The standard method for individual identification introduced by Jeffreys rapidly found application in practice and, under the name of DNA fingerprinting, is at present widely used in molecular diagnostics. The currently applied DNA fingerprinting tests can be divided into two main groups: analysis of a single locus or of multiple loci. The conclusions concerning kinship of the persons examined in the cases of contestable paternity are formulated on the basis of comparison of the DNA fragments found in those persons. RYSZARD SŁOMSKI, JOLANTA KWIATKOWSKA, HANNA CHLEBOWSKA, BARBARA SIEMIENIAKO i MAGDALENA SŁOMSKA — *Polymorphic DNA sequences and their application in paternity* — present a critical review of the paternity testing techniques, drawing attention to some limitations of the methods applied and difficulties in interpretation of the results.

HENRYK HÜBNER and ANNA MORDALSKA discuss the very interesting problem of genomic imprinting, for which they propose a new Polish name — *Genomic imprinting in man*. This inborn imprint affects the expression of homologous alleles or regions of chromosomes inherited from either of the parents. There is ample evidence that chromosomes or genes can "remember" their parental origin. In some organisms genomic imprinting results in a reversible loss of activity of one of the two homologous DNA sequences. From the genetic point of view the effect of imprinting is equivalent to homozygosity at a defined locus or in a group of loci. The concept of genomic imprinting is not incompatible with the Mendelian law but explains the observed deviations from this law. Autosomal genes of maternal and paternal origin undergo, during development of the embryo, differentiated expression indispensable for proper development of the organism. Under pathological conditions, an embryonal neoplasma, hydatid mole, developing from chorion, contains 46 normal chromosomes, all of them of paternal origin. In the genetically opposite situation, when all the chromosomes are of maternal origin, there appears a neoplasma called teratoma. These neoplasmas result from diploidal, partenogenetic growth of either paternal or maternal cells. As cytogenetic evidence for genomic imprinting can serve the occurrence of uniparental disomy. It happens sometimes that, along with a completely normal karyotype, a sex-linked trait is transferred from father to son and not to the daughter. This phenomenon can be explained by assuming that chromosomes X and Y of the zygote were of paternal origin, and the ovum had no sex chromosomes. The significance of uniparental disomy in causing genetic disturbances leading to developmental defects finds its confirmation in the case of the Angelman syndrome, which results from a deletion of a part of maternal chromosome 15, and in the phenotypically different Prader-Willi syndrome, evoked by the deletion of paternal chromosome 15. The postulated molecular mechanisms of genomic imprinting are related to the process of DNA methylation occurring during mitotic divisions preceding meiosis, or during meiosis itself. Methylation affects chromatin conformation and, once introduced during gametogenesis, could be reproduced until activation of the gene.

At present, an increasing body of evidence points to a significant involvement of genetic disturbances in neoplasma development. DANUTA ROŻYNKOWA —

Perspectivess in cancer genetic research — discoveries and visions — discusses selected results of cytogenetic studies on leukemias and lymphomas, the studies which formed the first step in elucidation of the molecular mechanisms responsible for the phenomena of neoplastic transformation. She draws special attention to disturbances in functioning of cellular oncogenes and antioncogenes. According to her views, in diagnosis of neoplasma, cytogenetic and molecular tests supplement but can not replace pathomorphology, as the main role of molecular genetics consists in detecting the previously unknown mechanisms responsible for deregulation of transcription control, and for autonomy of cell growth.

A significant discussion of the problems of oncogenes and antioncogenes has been presented by ANTONI HORST — *Products of the tumor suppressor genes.* So far, over 100 oncogenes and about 50 antioncogenes have been described. It is at present accepted that development of neoplasma is dependent on the one hand on the presence of an excess of growth promoting factors, i.e. oncogenes, both of endogenous and exogenous origin, and on the other hand on a deficiency or loss of the products of antioncogenes. Under normal conditions the cell cycle is strictly controlled by regulatory mechanisms. The products of oncogenes and antioncogenes plag the key role at successive phases of the cell cycle. The understanding of the complex mechanisms which regulate the cell cycle is of great importance for elaboration of new solutions in the therapy of neoplasmas.

Neoplastic transformation and development of neoplasma are also dependent on the metabolism of chemical carcinogens and the process of DNA repair, which both differ from individual to individual. ANDRZEJ L. PAWLAK — *Inherited susceptibility to cancer and other factors influencing cancer occurrence* and KRZYSZTOF SZYFLER — *Physiological and pathological variability of DNA repair in human* — write about the problems of hereditary proneness to neoplasma, and the factors affecting the risk of its development. In view of the possibility of early implementation in some cases of prophylactic measures, the practical significance of solving this problem is immense, especially for the neoplasma-prone persons. The factors underlying proneness to neoplasma are of a subtle character and are often difficult to define. The genetic sensitivity to environmental carcinogens has not been fully recognized yet. It is known that genetic polymorphism can exert its action at the stage of formation of DNA defects, of DNA repair, and control of proliferation of the transformed cells. In turn, disturbances in DNA repair have been described so far to be the main factor underlying proneness to neoplasma in the Fanconi syndrome, *xeroderma pigmentosum* and in *ataxia telangiectasia.* Due to the relationship existing the defect in DNA rapair and sensitivity to particular genotoxic agents, also the symptom-free carriers of the genes of the above-mentioned syndromes can show increased neoplasma proneness on exposition to specific genotoxic factors. Diversity in the course of DNA repair processes in the human population concerns both the level of the whole genome, DNA fragments, or even a single gene. The intensity of DNA repair processes in different parts of the genome is unequal. The efficiency of DNA repair becomes lowered with age, in correlation with increased frequency of occurrence of neoplastic dieases in more advanced age. In practice, it is possible to diagnose rather early the proneness to the large intestine cancer in persons

burdened by the so-called familial adenomatous polyposis coli. The diagnosis can be made even prior to the appearance of polyps in the intestinal mucosa. This opens new possibilities for prophylaxis of neoplastic transformation with the use of anti-inflammatory drugs. The problem of neoplasma proneness is related to the problem of genetic diversity of the population in the aspect of admissible exposition to genotoxic agents.

MARIA SĄSIADEK and JÓZEF JAGIELSKI — *Biological effects of mutagenic agents* discuss the biological impact of mutagenic agents during pregnancy, wich is, dependent to a large extent on the embryo development stage. In the first 10–14 days following fertilization, that is in the preimplantation period, the fertilized ovum cell has rather low sensitivity to the action of external agents. However, if damaged at that time, it usually undergoes atrophy. In the period of organogenesis the embryo is most sensitive to teratogenic agents. Depending on the time of their action, the injury can concern various organs. After the 8th week of pregnancy, mutagenic compounds can cause neoplastic transformation of somatic cells. The authors discuss also the necessity of gaining thorough knowledge of the mutagenic and carcinogenic properties of the chemical substances introduced into the environmental as a result of industrial development, as well as the importance of the detection, within the framework of the so-called biological monitoring, of early biological effects of these compounds on man.

Due to rapid development of the gene transfer technology — JERZY NOWAK — *Progress in gene therapy of hereditary diseases* — we can at present speak about the first positive results of gene therapy for hereditary diseases. It seems that, in the future, gene therapy will be applied for causal treatment of inherited diseases, mainly those caused by a defect in a single gene. One should, however, realize that gene therapy is only beginning to develop. At present we can not yet offer hope to the seriously ill that this new form of therapy could successfully cure them. However, one can be sure that, in the future, gene therapy will become an increasingly common and effective form of treatment and prophylaxis for various types of diseases.