Human Genetics

Presented by the Departments of Human Genetics, Medicine, Obstetrics and Gynecology, Pediatrics, Radiation Biology, Microbiology, and Radiology

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Ed. Note: The format of the MCV/Q has been changed slightly to accommodate the unusual number of papers for this special issue. We will return to the style of previous issues beginning with Volume Fourteen.
I. INTRODUCTION

The Relevance of Genetics to Medicine

This issue of the *MCV Quarterly* focuses on the explosion of knowledge in the field of human genetics. The activities of the new Department of Human Genetics at the Medical College of Virginia encompass the traditional medical school triad of teaching, patient care, and research, and an active graduate program has been developed with curricula leading to masters and doctorate degrees. The program is supported by a recently awarded National Institutes of Health predoctoral training grant as well as State and local funds from the A. D. Williams Foundation.

Clinical services are provided in a National Foundation-March of Dimes Genetic Counseling Clinic and through an interdepartmental Antenatal Diagnosis Program. At present, however, as shown by some of the case reports in this issue, research and clinical service are inextricably intertwined.

There has, perhaps, been no recent advance in medical technology that has greater potential for improving the human condition than the development of reliable methods for the prenatal diagnosis of a growing list of specific genetic diseases. The Antenatal Diagnosis Program at MCV has been developed with support from the State Department of Health and has at its disposal the most modern equipment available for these test procedures. Neonatal screening for genetic disease and carrier detection programs are two logical complements to prenatal diagnosis.

It is becoming increasingly clear that the major causes of morbidity and mortality in Western cultures are neither entirely environmental nor genetic in etiology but result, rather, from an interaction between the host and a pathogenic agent in a constantly changing environment. The concept of genetic risk factors may well emerge as the most significant and effective new health care strategy to appear during the last quarter of this century. Experience with the treatment of genetically determined metabolic diseases suggests that when a specific genetic liability can be diagnosed, patients and their parents are in general highly motivated to comply even with extremely elaborate treatment regimes. Wouldn't an individual who had seen a parent or close relative die of hypertension, heart disease, cancer, or pulmonary disease have a strong motivation to comply with a program of presymptomatic screening and treatment if he or she could be shown to carry a specific genetic risk factor? Improved public education will be essential for the acceptance of this approach to disease prevention. Since all normal individuals carry abnormal recessive genes, the diagnosis of a genetic disease or carrier state in a family should not be viewed as a stigma but rather as a readily identifiable risk factor that can be exploited by the prudent physician to plan a highly individualized health maintenance program for the patient. At present, relatively few alleles have been identified which can be detected in the heterozygous state that are associated with an increased disease risk; however, the number is growing, and it seems likely that this will continue to be a very active area of research in the future.

Genetic diseases are estimated to account for 15% of all pediatric hospital admissions. Recognizable chromosome abnormalities account for more than 50% of all early spontaneous first trimester abortions. A single genetic disease, Usher syndrome, accounts for more than 60% of all adults seeking rehabilitative services for the combined handicap of deafness and blindness. The lifetime cost of caring for the 3,000 to 4,000 new children who are born each year with Down syndrome is estimated to be in excess of $60,000,000 per year. More than 2,000 genetic diseases have now been described, most within the past two decades, and at least one genetic locus has now been mapped to each of the 23 pairs of chromosomes, most within the past two years. These facts presage the increasingly important role that genetics will play in medicine and human affairs in future years.

WALTER E. NANCE, M.D., PH.D.
Professor of Human Genetics, Pediatrics, and Medicine

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II. HISTORY

The Growth and Development of Genetics at MCV

J. IVES TOWNSEND, PH.D., Associate Professor of Human Genetics

Genetics was well established at the Medical College of Virginia by the time I joined the faculty in 1960. At least one course in genetics was required of students in the Schools of Medicine, Dentistry, Pharmacy, and Nursing. The Department of Biology and Genetics, which was in the School of Pharmacy and chaired by Dr. Roscoe D. Hughes, also had a modest graduate program in genetics and two genetics research programs, one on facial growth in twins, supported by a grant from the National Institutes of Health, and the other on the genetics of drosophila, supported by the National Science Foundation.

In 1938, Dean Wortley Fuller Rudd of the School of Pharmacy had hired Dr. Hughes to teach general biology and comparative anatomy to the undergraduate pharmacy students. Dr. Hughes, a graduate of the US Naval Academy at Annapolis, had just received his Ph. D. degree at Columbia University for cytogenetic studies on drosophila under the tutelage of the distinguished cytologist, Franz Schrader; this was the same department in which the Nobel Prize winner, Thomas Hunt Morgan, had begun his pioneer work three decades earlier. Dr. Hughes had the added advantages of direct association with Morgan and other great geneticists, including Leslie C. Dunn and Calvin B. Bridges.

It is hardly surprising then that after his arrival at MCV, Dr. Hughes wanted to introduce genetics into the various professional curricula; however, except for simple Mendelian principles taught in the general biology course to pharmacy and nursing students, no opportunities arose until sometime after World War II. By the late 1940s, the controversy over the dangers of fallout from atomic bomb tests had made the general public as well as clinicians aware that genes affect the health of everyone and undoubtedly helped to create the opportunities that Dr. Hughes had been looking for. Dr. Hughes reported, “Beginning in 1949, three or four lectures in genetics were given to second-year dental students. The number gradually increased to eleven. In 1954, the course was placed on a formal basis and from then on carried academic credit. Also, during this period occasional lectures were given on special topics to other classes.” This was, I believe, the first required genetics course in a dental curriculum anywhere.

Medical Genetics first appeared as a required eleven-hour course in the MCV Bulletin, Winter, 1951. While this was probably not the first required genetics course in a medical curriculum, it was certainly among the first. Genetics remains a required subject in the Schools of Medicine and Dentistry.

In 1953, Marion Waller, now Professor of Medicine at MCV, received the first graduate degree to be earned in the Department of Biology. As has been true of all research conducted in that department or in one of its successors, her research was in genetics.

The next year, Dr. Bertram L. Hanna, a mathematical geneticist, joined the faculty of the department, doubling the number of faculty above the rank of Instructor. Expansion of genetics courses in the various professional schools led to the department being renamed, Department of Biology and Genetics. (Although this name appeared in the MCV Bulletin for years beginning in April, 1956, Dr. Hughes once...
told me that he amended the name himself and soon everyone followed his lead without there ever having been any official approval.)

In 1963, the department was offering nine courses in its graduate program. By the fall of 1965, there were seven genetics graduate students in residence; this number had risen to nine by the fall of 1972.

After MCV and Richmond Professional Institute merged to form Virginia Commonwealth University in 1968, and Dr. Hughes retired in 1970, the responsibility for teaching anatomy to the pharmacy students was assigned to the Department of Anatomy; the Department of Biology and Genetics had become, in effect, a department of genetics. The newly appointed president of VCU, Dr. Warren W. Brandt, decided, however, that four faculty members were too few to constitute a "viable" department; so the department's name was changed to Program in Human Genetics, and the program was transferred to the School of Basic Sciences and in 1974, moved from McGuire Hall to the eleventh floor of Sanger Hall.

Although the genetics faculty has ceased having any role in the education of undergraduate pharmacy students, it has maintained responsibility for lectures on the genetics of special pathological conditions discussed in Nursing Health Science. Lectures on appropriate genetics topics are still given in the dietetic intern program and to students in blood banking.

Because a study made by the Virginia State Council of Higher Education in 1971 considered the number of graduate degrees earned in the Program in Human Genetics at MCV to be too few, the VCU administration approved a plan to expand the program at the earliest opportunity. Implementation began when Dr. Walter E. Nance was recruited as Chairman in September 1975. There shortly followed the third name change to succeed Department of Biology—Department of Human Genetics.

Since 1975, rapport with clinical departments has been strengthened, especially with the Department of Pediatrics, which had been closely involved with the Program in Human Genetics, and with the Department of Obstetrics and Gynecology, which had not. Faculty joint appointments have been made to several clinicians who have especial interest in genetics and are involved in teaching clinical genetics or in sponsoring graduate students in genetics.

In addition, both the space occupied by Human Genetics in Sanger Hall and the number of graduate students in the Department have doubled; the number of nonprofessional employees in the Department has grown more than sixfold; the amount of external grant support awarded to the Department's faculty has increased to more than $600,000 per year; and the Department has received a predoctoral training grant from the National Institutes of Health which will provide stipends for six students. During the coming year two additional faculty members will be recruited, increasing to six the number with primary appointments in Human Genetics. After the benign neglect of genetics, these developments promise to fulfill the dream of having an outstanding human genetics center at MCV, a dream long held by MCV's first geneticist, Dr. Hughes.

REFERENCES


Indications for Antenatal Genetic Diagnosis

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The diagnosis of chromosomal and metabolic abnormalities by mid-trimester amniocentesis is now an established part of antenatal care. Jacobson published the first use of this technique in 1967, followed in 1968 by Nadler. By 1974, over 3,000 pregnancies had been studied permitting the prenatal diagnosis of a wide variety of chromosomal anomalies and more than 60 different inborn errors of metabolism during the second trimester of pregnancy at a time when termination of the pregnancy can be safely performed if an abnormality is detected. During this period, the National Institute of Child Health and Human Development established a National Registry for amniocentesis in an attempt to document the safety and accuracy of this technique. Its report was published in 1975 and dealt with a comparison of 1,040 pregnancies studied by mid-trimester amniocentesis and 992 matched control pregnancies. The conclusions from the study were that mid-trimester amniocentesis was a safe procedure when performed by a qualified physician and that the accuracy rate of antenatal genetic diagnosis was 99.4%. Since 1975, the use of this procedure has become almost routine in pregnancies at risk for identifiable genetic abnormalities because of maternal age or family history. It should be emphasized that in the vast majority of cases prenatal genetic diagnosis is a life-saving procedure. Many women who are at high risk would not choose to continue their pregnancy unless they could be assured that there is no detectable fetal abnormality. In the series of 1,040 amniocentesis procedures mentioned above, only 34 abnormalities were detected; of these, 27 women elected to terminate their pregnancy. In addition, 11 male fetuses were identified among the 21 at risk for X-linked disorders and 8 of these 11 women elected to terminate their pregnancies.

Because the number of qualified laboratories is limited, the cases which can be studied must be restricted to certain high-risk categories. The largest group of pregnant women at increased risk of producing an abnormal child are patients over 35 years of age. It is well established that as the mother ages, the incidence of nondisjunction in her gametes increases. Table 1 shows the relation between the incidence of trisomy 21 and maternal age. Another indicator of the effect of maternal age is that while only 13% of pregnancies occur in mothers over 35 years old, these pregnancies produce 51% of all cases of Down syndrome. Recent studies employing normal chromosomal markers have clearly shown that the extra chromosome in this syndrome is not invariably maternal in origin. If only those cases in which the nondisjunction occurred in the mother were considered, it seems likely that a more striking relationship to the maternal age would be observed. Age 35 is considered by many to be the maternal age at which the risk is sufficiently great to recommend amniocentesis, but this figure is obviously somewhat arbitrary.

The second largest group of patients for whom antenatal diagnosis is recommended are women who have already had a child with trisomy 21. The risk of producing another mongoloid child in this group is 1% to 2%. Needless to say, these mothers are also extremely anxious about a recurrence, and in most cases the results of the cytogenetic analysis serve to allay their fears.
A small group of patients at considerable risk for chromosomally abnormal children are families in which one of the parents carries a chromosomal translocation. In these cases, the risk varies depending on the translocation. For example, in the case of a t(21;21) carrier, the risk of bearing a mongoloid child is 100%; while in the case of the more common D/G translocation t(15;21), the risk of producing a child with translocation Down syndrome varies from about 10% to 30% depending upon whether the father or the mother is the translocation carrier. This translocation group is responsible for "familial mongolism" and is fortunately quite rare. Of all mongoloid children, 2% to 3% are of the translocation type; of these, only about half are familial and the remainder are de novo translocations.

Approximately 150 genetic diseases have been recognized which show an X-linked pattern of transmission, and many such as the X-linked hemophilies, Duchenne-type muscular dystrophy, and agammaglobulinemia are associated with serious or even lethal disease. X-linked disorders only affect male offspring, although carrier females may occasionally show mild symptoms. Specific intrauterine diagnosis is not yet possible for most X-linked traits; however, since it is possible to diagnose the sex of the child in utero, carrier females can avoid giving birth to additional affected males if they are willing to carry only female infants to term. Some couples find this an acceptable method of completing their families without the fear of a recurrent abnormality; others do not. In any case the decision about whether or not to use this approach is made by the parents themselves. The dilemma of terminating potentially normal pregnancies would be solved if specific tests were available to diagnose each X-linked disease. Currently, this is possible only for a relatively few X-linked diseases in which the specific enzymatic defect has been identified. These conditions include Hunter syndrome, the Lesch-Nyhan syndrome, and Fabry disease. In addition, approximately 40 rare autosomal recessive traits are known in which the enzyme defect can be detected with varying reliabilities in fibroblasts.

The last category of patients at risk for specific fetal abnormalities are those who have had a previous child with a neural tube defect (anencephaly, encephalocele, meningomyelocele, and spina bifida). As a group, the recurrence rate for any of these disorders is about 5%. These abnormalities are not chromosomal defects, therefore, the fetal karyotype is not useful. However, it has been well proven that the level of alpha-fetoprotein in the amniotic fluid at 16 to 18 weeks gestation correlates with these defects; a high level of alpha-fetoprotein (greater than 5 standard deviations above normal) is diagnostic of an open neural tube defect. Amniography, fetoscopy, and ultrasound can also be utilized to document these abnormalities.

In summary, the general indications for amniocentesis for prenatal diagnosis are as follows:

1. Maternal age greater than 35 years at time of conception.
2. History of a previous child with Down syndrome or any other trisomy.
3. Documented chromosomal translocation in either parent.
4. Previous child with a serious inherited X-linked disease or an autosomal genetic disease that can be detected prenatally.
5. Previous child with a midline neurologic defect.

Table 2 is a summary of the indications for amniocentesis performed at MCV since 1973.

Patients who have been counseled and have a definite indication for prenatal genetic diagnosis, and who have given their informed consent, are first scheduled for an ultrasound examination. This non-invasive scanning procedure can locate the placenta, identify the best area for the amniocentesis, and rule out twin gestations. It also accurately measures the
TABLE 2
Indications for Prenatal Diagnosis at MCV (9-1-73 to 7-1-77)

<table>
<thead>
<tr>
<th>Indications</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous trisomy 21</td>
<td>28</td>
</tr>
<tr>
<td>Maternal age 40 yr or greater</td>
<td>77</td>
</tr>
<tr>
<td>Maternal age 35 yr to 39 yr</td>
<td>65</td>
</tr>
<tr>
<td>Close relative with trisomy 21</td>
<td>8</td>
</tr>
<tr>
<td>Previous child with hemophilia A</td>
<td>1</td>
</tr>
<tr>
<td>Previous spina bifida + cleft palate</td>
<td>1</td>
</tr>
<tr>
<td>Previous spina bifida or hydrocephalus or meningocele</td>
<td>10</td>
</tr>
<tr>
<td>Previous anencephalic</td>
<td>22</td>
</tr>
<tr>
<td>Previous Potter syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Anophthalmia</td>
<td>2</td>
</tr>
<tr>
<td>Previous G/G translocation carrier</td>
<td>1</td>
</tr>
<tr>
<td>Previous D/G translocation carrier</td>
<td>1</td>
</tr>
<tr>
<td>Elevated maternal creatine phosphokinase</td>
<td>1</td>
</tr>
<tr>
<td>Previous child with Sandhoff disease</td>
<td>1</td>
</tr>
<tr>
<td>Four previous spontaneous abortions</td>
<td>1</td>
</tr>
<tr>
<td>Previous trisomy 18</td>
<td>1</td>
</tr>
<tr>
<td>Previous trisomy 13</td>
<td>1</td>
</tr>
<tr>
<td>Previous XX/XO mosaics</td>
<td>1</td>
</tr>
<tr>
<td>Three previous “retarded” children</td>
<td>1</td>
</tr>
<tr>
<td>Previous child with “multiple defects”</td>
<td>2</td>
</tr>
</tbody>
</table>

Fetal head to document fetal age. After the ultrasound procedure, the patient's abdomen is prepared and under sterile conditions the amniocentesis is performed by an experienced obstetrician. The amniocentesis procedure involves the introduction of a small bore needle through the mother's abdominal wall into the uterus and amniotic cavity under local anesthesia. The ultrasound examination aids in selecting the proper placement of the needle to avoid the baby and to locate the amniotic fluid. Twenty milliliters of fluid are withdrawn and the needle removed. Most patients have no ill effects from the procedure and can go home shortly after it is performed. The optimum time to obtain the amniotic fluid is from 15 to 16 weeks gestation.

Preliminary results are usually available within three weeks of the procedure and the final report is issued one to two weeks later.

Since most of the patients have a rate of below 5% of bearing an affected child, the results are usually that the fetus has a normal chromosome complement. This greatly relieves the parents' anxiety and allows them to have a happy prenatal course and enjoy a normal baby. If, however, a mongoloid fetus or a fetus with anencephaly is diagnosed, the parents are counseled and pregnancy termination suggested. This decision is left to the parents once the facts are presented to them, and even for those parents who felt before the study that there would be no question of terminating the pregnancy if the fetus were abnormal, the decision is a difficult one.

As of July 1, 1977, at MCV, three patients, of 216 studied, have been diagnosed with trisomy 21 in utero. All three patients underwent therapeutic abortion. In addition, the diagnosis of a fetus with Turner syndrome (45 XO) was made and the parents elected to continue the pregnancy since the abnormality detected was not life-threatening. Table 3 summarizes the results of the antenatal testing at MCV.

The future of antenatal genetic diagnosis is an exciting one. Recent advances include the application of chromosomal banding techniques to identify subtle abnormalities and rearrangements; ultrasonography, using high resolution gray scale equipment to permit delineation of fetal soft tissue and skeletal anomalies; and fetoscopy, using a small fiberoptic instrument to view the fetus directly and observe its development. With these advances in technology, it should be possible to enable even more women at high risk for genetically diseased offspring to bear healthy children.

Table 1 is adapted from Science (169:495–497, 1970).

REFERENCES


Recent Advances in Cytogenetic Technology for Antenatal Genetic Testing

JUDITH A. BROWN, PH.D., ANNA CARR, ELIZABETH S. COOPER, AND DEBORAH W. HERITAGE, Cytogenetic Laboratory, Department of Human Genetics

The examination of human chromosomes has been a part of the physician's laboratory armamentarium since the correct diploid number of human chromosomes was established and a method was developed for the in vitro growth of peripheral blood leukocytes to yield metaphase chromosomes. The discovery that on ultraviolet microscopy (UV), metaphase chromosomes stained with fluorochrome dyes displayed a characteristic pattern of bright and dull bands unique for a given pair of homologous chromosomes, was a major technological breakthrough in human cytogenetics; for the first time, every chromosome in the karyotype could be unequivocally identified. Although the short storage life of fluorochrome-stained chromosomes and the costs of UV microscopy have limited the usability of fluorescence banding, the introduction of one discriminating procedure quickly led to the development of an array of similar banding techniques for conventional microscopy that yield comparable information. Some of these technical procedures depend on enzyme and/or heat denaturation of the chromosomes, resulting in the characteristic banding patterns seen by the trypsin-Giemsa method, the 5M urea method, and the acid-saline-Giemsa technique. A typical human karyotype prepared from metaphase chromosomes treated with trypsin, stained with Giemsa, and photographed with brightfield photomicrographic techniques is shown in Figure 1. Careful examination of this karyotype reveals that each chromosome in the homologous pair has an array of dark and light bands identical with those of its homolog and that each homologous pair, autosomes number 1 to number 22, has a characteristic, easily identifiable banding pattern.

In order to establish a standardized nomenclature to describe the chromosomes and chromosome regions, as revealed by the banding techniques, a committee of international experts in human cytogenetics met in Paris, France, in 1971. The committee retained the previously established designation of the short arm of the chromosome as “p” and the long arm as “q” and agreed to divide the chromosome arms into a number of regions according to the
position of the centromere and the banding characteristics of the arm. For example, the long arm of chromosome 7 (7q) (Fig 1) consists of three regions (1 to 3) which are more or less delimited by the two major dark bands and the lighter staining terminal band. If a patient has a deletion in the long arm of this chromosome beginning with the second dark band from the centromere and including all the rest of the long arm to its terminus (ter), the designation would be del (7) (pter → q21:). Such a detailed description of the karyotype abnormality makes it possible to associate the physical abnormalities of a given patient with a given chromosome abnormality and thus establish the phenotype-karyotype correlations which are extremely important in the diagnosis of the so-called chromosome syndromes.

Major indications for a karyotype examination are the evaluation of a newborn with multiple congenital abnormalities, psychomotor retardation of unknown etiology, or ambiguous genitalia; of an adolescent with short stature and/or delayed puberty; of selected cancers such as chronic myelogenous leukemia, retinoblastoma, and cancer of the bladder; of patients with a history of multiple spontaneous abortions or infertility; and of high-risk pregnancies by antenatal genetic testing. Whenever one of the above conditions occurs, aberrations in the chromosomes are a frequent finding. Recently completed newborn surveys show that approximately 1% of all live-born children have a significant chromosome abnormality which leads to severe physical and mental handicap. Chromosome studies of fetal tissues obtained from early spontaneous abortions clearly indicate that karyotype abnormalities are a contributing factor in more than 50% of cases.

The utilization of chromosome banding procedures in antenatal genetic testing has resulted in a more accurate description of the fetal karyotype and increased diagnostic capabilities. Heretofore, it was possible only to document the existence of an abnormal number of chromosomes—a condition known as aneuploidy—as found in Down syndrome, D-trisomy, or E-trisomy, while major or minor structural aberrations went undetected. At the same time, greater expertise in the interpretation of the karyotype result is demanded, and frequently chromosome studies of the parents are essential for a correct interpretation of the cytogenetic findings in the fetus (Figs 2 through 4). The fetal karyotype in Figure 2 shows two chromosomes, chromosome 15 and chromosome 21, with a considerable amount of extra chromatin in the short arm (indicated by arrows), which can easily be construed as an abnormality.

Fig 1—Normal female karyotype. Chromosomes were treated with trypsin and stained with Giemsa. (Chromosome magnification, X4000.)

Fig 2—Karyotype of a male fetus. Extra chromatin on the short arm of chromosome 15 and of chromosome 21 is indicated by arrows. (Chromosome magnification, X4000.)
Examination of the parental karyotypes shows that the mother (Fig 3) possesses the variant chromosome 21 and the father (Fig 4) the variant chromosome 15. These chromosomal differences, referred to as normal or polymorphic variants, are often seen in humans with a frequency that depends on the particular chromosome; they are compatible with clinically normal phenotypes. The fetal karyotype in Figure 2, therefore, is normal. Another kind of polymorphism which can confuse the correct interpretation of a fetal karyotype is centromeric heterochromatin (Fig 5). In this case, one of the homologs of chromosome pairs 1, 9, and 16 is larger than its normal homolog—that is, heteromorphic—and the size difference can be attributed to a larger amount of inactive chromatin-heterochromatin as revealed by an alkali treatment of the chromosomes, developed by Arrighi and Hsu. These so-called C-band polymorphisms are encountered in the normal population with a frequency that, depending on the chromosome, shows characteristic racial distributions.

The in vitro culture of amniotic fluid cells for antenatal genetic testing can be complicated in approximately 1% of cases by contamination of the culture with cells of maternal origin. Here, whereas fetal cells can be readily discriminated if the fetus is...
male, a knowledge and use of chromosome polymorphisms is used when the fetus is female to rule out the presence of maternal cells and assure that the karyotype is fetal. This technical difficulty can further be circumvented by establishing two or more cultures from the amniotic fluid sample and subculturing the cells at least once prior to karyotype evaluation. Assaying duplicate cultures also increases the accuracy of the test where chromosome mosaicism may result in an abnormal offspring.

The application of chromosome banding techniques has vastly increased the precision with which structural rearrangements can be characterized. This in turn has contributed to the mapping of specific genes to specific regions and bands of the chromosome and has led to the recognition of a growing number of syndromes resulting from small duplications, deficiencies, or rearrangements of the chromosome material. Even the smallest duplication or deletion recognizable by current technology can result in major phenotypic abnormalities. For example, Figure 6 shows an array of pairs of chromosome 4 ascertained in a child referred to MCV because of severe multiple congenital anomalies noted at birth. Briefly, this infant female had microcephaly with a midline occipital scalp defect, mild hypotelorism, low-set, simple ears with preauricular pits, bilateral cleft lip and palate, and a bulbous nasal root. She also had mild flexion deformities of both thumbs and hypoplastic nails, as well as congenital heart disease and a shallow pilonidal dimple. Cytogenetic evaluation revealed a modal number of 46 chromosomes, but as this figure demonstrates, one of the chromosomes 4 consistently showed a lesser amount of short arm material than the homolog, and banding analysis revealed a terminal deletion in the short arm: 46,XXdel(4)(p15 or p16).

Unfortunately, in the above case, the cytogenetic diagnosis was made postnatally. Further refinements of our current technology will soon permit such minor chromosome anomalies to be detected prenatally, and as more patients elect antenatal genetic testing and as banding and optical technology are improved, karyotype analysis of the fetus will become an even more precise diagnostic tool.

REFERENCES

IV. CLINICAL PROGRAM

The Genetic Counseling Program at MCV

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The Genetic Counseling Clinic at the Medical College of Virginia, established by Drs. Peter Mamunes and R. B. Young in 1973, has been supported since its inception by a clinical service grant from the National Foundation—March of Dimes; it is one of 83 genetic counseling programs in the United States and one of three in Virginia that receive support from the Foundation. The Clinic provides counseling and diagnostic services for a variety of genetic diseases and is the focus of clinical teaching and research activities of the Department of Human Genetics. The Clinic is staffed by members of the Departments of Human Genetics, Obstetrics, and Pediatrics, as well as consultants from many other clinical disciplines.

Following referrals, patients are sent a questionnaire to initiate the collection of relevant medical and genetic data; the patient is then scheduled for a Clinic appointment. At the time of the Clinic visit, the graduate student interviews the patient and documents sufficient family history information to permit the construction of a pedigree. Following review of the collected data and physical examination of the patient by the staff physician, the patient and family are invited to continue the counseling session in a room equipped with a microphone and a two-way mirror. In this setting, students, house officers, and staff members can participate in the counseling session in an unobtrusive manner, and discuss it later. About 90% of the patients and families are willing to participate in this educational program; those who object are counseled privately.

After a year's experience in the Genetic Counseling Clinic, graduate assistants are assigned to specialty areas such as hemophilia, cystic fibrosis, and endocrine clinics, where they collect family history data in selected cases, under the supervision of the Clinic director and augment the genetic counseling the families have previously received. Although virtually all patients seen by the graduate assistant in these clinics have a clearly defined Mendelian disease, many may not have received adequate genetic counseling in the past.

Since its beginning in 1973, the Clinical Genetics Program has evaluated or counseled 698 individuals (Table); of these, 33.7% were diagnosed as having a simple inherited Mendelian trait and 45.6% a recognizable chromosomal disorder. Twenty-one dominantly inherited traits were diagnosed including achondroplasia, neurofibromatosis, Huntington chorea, Marfan syndrome, retinitis pigmentosa, hypochondroplasia, Noonan syndrome, aniridia, limb-girdle dystrophy, Treacher-Collins syndrome, and tuberous sclerosis; 28 recessive traits were diagnosed including cystic fibrosis, alpha-1-antitrypsin deficiency, recessive deafness, Tay-Sachs disease, Werdnig-Hoffman disease, galactosemia, phenylketonuria, Gaucher disease, Usher syndrome, and maple syrup urine disease. X-linked traits seen in the clinic included Duchenne muscular dystrophy, hemophilia, Norrie disease, anophthalmia and Charcot-Marie-Tooth disease. Most of the cases seen with chromosome anomalies had Down syndrome, but the
population included patients with D and E trisomy, the cri du chat syndrome, Turner syndrome, Klinefelter syndrome, the 4p-syndrome, and a variety of structural rearrangements.

Patients classified as having multifactorial diseases included individuals with midline neurologic defects, diabetes, uncomplicated cleft lips and/or palate, clubfeet, seizures, and certain patients with familial mental retardation that could not be otherwise classified. Patients falling into the other categories included individuals with multiple malformations or repeated abortions with normal chromosomes and no other definite causes, or patients with psychomotor retardation of unknown or environmental etiology.

During the year 1976 (Figure), patients came from all parts of the state either to the Genetic Counseling and Amniocentesis Clinics or for diagnostic karyotypic analysis. This pattern of referrals is largely a result of the central location of the Clinic in a large urban area.

**Figure**—Pattern of referrals to Genetic Counseling Program from Virginia's health care district, 1976.

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**Population Screening for Genetic Disease**

**PETER MAMUNES, M.D., Professor of Pediatrics and of Human Genetics**

Recent advances in genetics and laboratory techniques have raised difficult issues for both the medical and lay communities. The desirability of initiating population screening programs is an example of one such issue that has engendered considerable confusion concerning its intent—so much so that the National Academy of Sciences recently reviewed this subject and in 1975 published a book entitled, *Genetic Screening—Programs, Principles and Research.*

This is paper #38 from the Department of Human Genetics of the Medical College of Virginia.

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This presentation will describe the four major forms of population screening for genetic disease and, from the Virginia experience with some of them, demonstrate their strengths and shortcomings.

Most readers are already aware of programs to screen for disorders such as hypertension, diabetes, cervical cancer, glaucoma, and other diseases. These programs were established with the idea that they are inexpensive, can be detected by a simple test, and that effective treatment is available. The same principles apply to the four major types of population screening programs for genetic disorders.

1. Carrier detection. Almost all persons are believed to carry approximately four lethal or deleterious recessive genes; however, their existence is usually never known unless the spouse has the same gene, in which case there is a one-in-four chance that each child will inherit a "double dose" of the abnormal gene and develop the disorder. Fortunately, most serious recessive diseases are rare, but there are two—sickle cell disease and Tay-Sachs disease—which are especially prevalent in specific subpopulations and for which simple, reliable, and relatively inexpensive carrier tests are available. Screening programs for detection of heterozygotes (carriers) in both disorders have existed in Virginia for at least five years. Elsewhere in this issue experience with the Medical College of Virginia Sickle Cell Program is described.

Tay-Sachs disease affects approximately 1:4,000 Ashkenazi Jewish births and is invariably lethal by the fourth year because of the abnormal accumulation of a sphingolipid, ganglioside GM_{2}, in the central nervous system. Hexosaminidase A, the enzyme which is necessary for the normal degradation of this material, is inactive in these patients and is present in only approximately 50% of normal activity in the serum, white blood cells, and other tissues of carriers. Women who are at risk to give birth to such children can therefore be identified before pregnancy. If both parents are carriers, an amniocentesis performed in the second trimester can determine the enzyme status of the fetus. If Tay-Sachs disease is found, the parents then have the option to terminate the pregnancy to avert the birth of an affected child.

Measurement of the serum enzyme level is not in itself technically difficult, but so many factors affect its activity (for example, pregnancy, cleanliness of test tubes in which blood is collected, the time during which the serum remains at room temperature before freezing, storage temperature prior to testing, medications taken by patient) that it is not practical for the local physician to forward blood to the testing laboratory from patients wishing their carrier status determined. Although with special precautions and arrangements, testing of remote patients can be done, a more desirable procedure is to schedule periodic mass community testing at various sites. These mobile screening clinics not only obviate the above-mentioned difficulties but they offer a better opportunity to ensure that tested individuals have been properly informed regarding the purpose and possible consequence of the testing. Because this concept of carrier testing is a new one, the most difficult aspect of such screening is to educate the public in the importance of voluntary participation. Most people will not have heard of the disease, and what is more significant they find it difficult to believe that they have a 1:30 chance of being carriers. A second obstacle to successful screening is the lack of funding for such programs. After initial equipment cost outlay, the cost for the Tay-Sachs test averages $7.00 ± $2.00, depending on the volume of tests, per individual. The MCV Tay-Sachs Screening Program, operative since 1972, was fortunate to obtain initial support from the Virginia State Department of Health and local Jewish community organizations so that it has been able to offer the test without a fixed charge to tested persons.

As a result of Virginia screening, over 3,700 Jewish persons have been tested at sites in their own cities of Richmond, Norfolk, Newport News, Roanoke, and Fredericksburg; the metropolitan northern Virginia cities have used testing sites in southern Maryland and Washington, D.C., provided by Johns Hopkins Hospital. Over 100 carriers have been identified and counseled, but surprisingly no carrier couple has thus far been found. A full 25% of all adult Jewish people in these cities have been tested. In almost all of the more populous United States cities where testing has been offered for several years, no more than 5% to 10% have appeared for this voluntary test. Clearly, a significant educational effort is needed on two fronts: to convince governmental agencies of the need for appropriate funding for these programs and to inform the public regarding the concept of disease prevention by carrier detection. Support of these efforts is needed soon, for in the very near future carrier detection tests for other more common recessive diseases, such as cystic fibrosis, promise to be available.

Phenylketonuria (PKU) carrier detection is a second test that we currently provide, using a dis-
criminant function, based on serum amino acid levels, that we have recently developed in our laboratory. In this case, since there is no ethnic group in which the gene has a high incidence, we offer the test to the collateral relatives of all new cases of PKU that come to our attention.

2. Antenatal screening. There is a small but clearly definable group of pregnant women for whom screening is indicated because of their substantially increased risk of bearing a child with a genetic disorder. The Antenatal Testing Program, jointly administered by the Departments of Obstetrics and Gynecology, Human Genetics, and Pediatrics, is discussed elsewhere in this issue.

3. Mass neonatal screening. The prototype in this level of screening is the Guthrie test to detect PKU, an assay performed on a dried blood spot obtained in the first week of life. Virginia State law established the program in 1966 and made it the joint responsibility of the parent and/or the physician to provide that the neonate be screened for this condition. Since that time, four to five children with PKU per year (approximately 1:16,000 births) have been discovered and mental retardation averted by maintaining them on a phenylalanine-restricted diet for the first six to seven years of life. The cost for early screening and treatment is approximately one tenth the cost of special education or institutionalization of late-diagnosed, retarded PKU children.

With the demonstrated success of the PKU screening program, many states and foreign countries have established screening programs for other disorders using the same blood spots. The grafting of these additional programs onto an already-existing one and the availability of a machine to handle the blood-spotted filter paper have minimized their cost. The decision of which additional disorders to screen should relate to the following major factors:

a) frequency of the disorder,
b) severity of the untreated disorder,
c) simplicity and sensitivity of the test, and
d) availability of treatment to ameliorate or prevent the disorder.

Under the above considerations, hypothyroidism (by radioimmunoassay of thyroxine), galactosemia (by bacterial inhibition assay), and several aminoacidopathies including homocystinuria, maple syrup urine disease (MSUD), and histidinemia (all also by bacterial inhibition assay) qualify. Because of its frequency (1:6,000) and ease of treatment, and the severity of the mental retardation that ensues due to delayed diagnosis, hypothyroidism warrants immediate development of screening programs.4,5 Therefore, a neonatal hypothyroid screening program in Virginia is planned for 1978. It is hoped that additional programs for galactosemia and other aminoacidopathies will also be initiated soon.

The spectrum of metabolic disorders screened can be considerably expanded if the urine of two- to three-week old infants is also tested by various simple chromatographic and high-voltage electrophoretic techniques. Such a program, using specimens collected on filter paper at home by the parent and mailed to the central laboratory performing the blood tests, has already been in effect for several years in Massachusetts6 and several other states. This procedure also serves as a follow-up test to detect neonates with false-negative blood results for the previously mentioned aminoacidopathies, including PKU.

Except for the PKU program, testing for other disorders in Virginia and most other states is and will be voluntary. An advisory committee of consumers, experts in metabolic disease, geneticists, laboratory personnel, clergy, and ethicists is clearly needed to serve as an advisory body to determine the propriety and mode of administration of these proliferating programs.

4. Urine metabolic screening. While the total incidence of inborn errors of metabolism is probably no greater than 1:5,000, these disorders are more often seen in children with mental retardation, seizures, hepatosplenomegaly, recurrent or persistent acidosis, failure to thrive, or unusual odor to urine or sweat. Where the etiology of any of these conditions is unclear, one of the inborn errors of metabolism must be considered; therefore, a variety of simple tests on a random urine specimen is recommended to screen for over 30 such disorders of amino acid, mucopolysaccharide, and carbohydrate metabolism.7 At the pediatric metabolic laboratory the following tests are routinely performed weekly on a 20 ml acidified random urine specimen received by mail: a) ferric chloride and dinitrophenylhydrazine tests to detect the alpha-keto acid excesses found in PKU, tyrosinemia, MSUD, and histidinemia; b) silver nitroprusside test for homocystinuria; c) Benedict's tablet for reducing substances; d) gross turbidity test for mucopolysaccharides; e) isatin spot test for the iminoaciduria of proline or hydroxyproline excess; f) nitrosonaphthol test for tyrosinosis; and g) high-volt-
age separation of all urinary amino acids to detect any excesses. Although the yield of newly diagnosed inborn errors by this screening program is small, those few cases detected are important because they may lead to a specific diagnosis that permits a prognosis, a recurrence risk for other family members, and counseling about the possibility of treatment or prenatal diagnosis for future pregnancies.

Clearly, genetic screening programs are an important component of the primary care physician's efforts in preventive medicine. Participation in and support of these programs is therefore strongly urged.

REFERENCES


The Virginia Sickle Cell Anemia Awareness Program (VaSCAP): Education, Screening, and Counseling

FLORENCE N. COOPER AND ROBERT B. SCOTT, M.D., Department of Medicine

In 1968, a program of screening for sickle trait carriers was begun as part of the work of the Hematology Division, Department of Medicine, at the Medical College of Virginia. It was felt that sickle cell anemia was more of a public health problem than was generally recognized, and in addition to instituting screening and education programs, data were collected to document the relative neglect of the problem.

A survey of the City of Richmond was conducted to ascertain the level of awareness among
black adults of sickle cell anemia, a severe chronic disease affecting one of every 500 black babies. The results showed that only 3 of every 10 black adults had ever heard of this disease, and of those who recognized it, few understood the nature of the illness. Additional background information was collected which further showed the lack of professional and public understanding of the importance of sickle cell anemia as a public health problem. These data, which attracted widespread attention, showed that sickle cell anemia research received far less support than other much less common chronic illnesses.

Public attention was also focused on sickle cell anemia by publicity concerning a new "test tube" test for sickling, and claims for therapeutic benefit from intravenous urea solutions. The heightened interest in sickle cell disease finally resulted in the Sickle Cell Anemia Prevention Act of 1972, which included a series of sickle cell research and treatment centers plus screening, education, and counseling clinics throughout the nation. At that time VaSCAP competed successfully for a contract under the national program, and expanded from a small screening effort to a larger, well-staffed clinic which could extend its effects state-wide.

Although the ability to detect healthy carriers of the recessive sickle trait had existed for years, no widespread screening and genetic counseling had taken place prior to these new programs. Another factor which made mass screening economically feasible was the development of rapid, simple instruments for performing hemoglobin electrophoresis. Electrophoresis has distinct advantages over the classical sickle cell "preptrip" or the newer test tube tests since it can distinguish sickle trait from the homozygous state, sickle cell anemia, and can also detect numerous other hemoglobin variants.

The purpose of screening for abnormal hemoglobins is to give young people an opportunity, not previously available, to know in advance of childbearing whether they run the risk of having children with sickle cell anemia. The frequency of sickle trait is about 8% of the black population, or 1 in every 12 blacks. The chance, then, that both potential parents in a couple are trait carriers is 1 in 144; thus slightly less than 1% of black couples are composed of two trait carriers and are at risk for bearing children with sickle cell anemia. The chance is 1 in 4 with each pregnancy that a child will be born with this incurable illness.

Screening should not be performed without adequate prior education. To implement this principle, VaSCAP has developed a number of audiovisual programs and written materials suitable for a wide variety of audiences. Included in these are the Sickle Cell Anemia Fact Book, a booklet for teachers, nurses, and other professionals, and Our Sickle Cell Story Fact Book, for elementary and middle school students. These, as well as brochures, posters, and fact sheets are distributed through the Virginia State Department of Health, to many areas of Virginia and throughout the nation. Since its inception, VaSCAP has provided educational sessions to over 200,000 individuals in groups of various sizes. It has also reached many more through programs, spot announcements, and structured educational models for specific target groups with the result that screening for hemoglobin genes has become an acceptable procedure among the general population. At present over 42,000 persons have been screened by hemoglobin electrophoresis and 3,951 have been found to have hemoglobin variants.

The ultimate result of education and screening is counseling those found to have sickle or other mutant hemoglobin genes. Counseling is aimed at educating the individual so that he or she can know the nature of the sickle gene, understand what sickle cell anemia is, understand the probabilities of transmitting the genes, and then be able to make informed decisions about childbearing. There is a set of slides available which has been developed by the VaSCAP staff. These explain the probabilities of inheritance for each specific hemoglobin variant. Individuals newly diagnosed with sickle cell anemia or one of its variants have been referred to a family physician or to the Hematology Clinic at MCV.

Approximately 8% of Virginia blacks have been found to have sickle cell trait, and 2% carry hemoglobin C trait. About 0.3% have the gene for hereditary persistence of fetal hemoglobin (HPFH), which is a gene mutation not associated with any disease. In addition, a large number of less common mutant hemoglobin genes have been recognized.

Though the alkaline electrophoresis test is the primary method used for the detection of hemoglobin genes, there are other methods which may be required in order to provide more conclusive results. The laboratory of the VaSCAP clinic provides both alkaline and acid or citrate agar electrophoresis. A mini-columns for hemoglobin A and citrate agar electrophoresis. A mini-columns for hemoglobin A and citrate agar electrophoresis. A2 quantitations are very useful in detecting the Beta thalassemias; fetal hemoglobin staining aids in determining the presence
of HPFH. Family studies are necessary in certain cases and the clinic has screened as many as four generations in some families.

All of the services have been provided without charge to the public or physicians for whom diagnostic testing has been performed. The emphasis has been on testing persons in or approaching the child-bearing age.

Education programs have been carried out in most high schools and colleges in central Virginia and the Tidewater area. Church groups and community organizations regularly request programs, many of which are provided in evenings or on weekends. An important part of the educational effort has been to provide workshops to train public health nurses in genetic counseling relating to hemoglobinopathies. State legislation has provided funds for nurses to be designated as genetic counselors in all of the Health Department clinics in the State. VaSCAP has also worked closely with local health departments in providing consultations concerning diagnosis and counseling of individuals with unusual hemoglobin variants.

The number of individuals served by the VaSCAP clinic is a tangible measure of its performance, but it does not reflect all of the benefits to the public and health professionals. The tragic lack of public awareness of the importance of sickle cell anemia that characterized the beginnings of this program has disappeared. For health professionals, especially at MCV, the subject of hemoglobinopathies has been emphasized repeatedly, and the level of interest and understanding of the disease and its variants have risen considerably. Of particular significance are the improved diagnostic facilities provided by VaSCAP clinics which have made it possible to stress the more rigorous modern standard of diagnosis for hemoglobin disorders. Perhaps most important to the field of genetics generally has been the extensive practical experience gained by the first mass screening efforts for a heritable disease. The ease with which recessive hemoglobin genes are detected made it the first such program to be implemented; as additional gene tests are developed, other genetic diseases will be the targets of similar programs.

REFERENCES
5. COOPER FN, SCOTT RB: Our Sickle Cell Story Fact Book. Virginia Sickle Cell Anemia Awareness Program, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University, 1975.
The existence of a relationship between cancer and heredity has been recognized for years, but only recently has emerging knowledge in this area enabled physicians to detect and treat cancer earlier in affected individuals and to prevent its occurrence in others.

The genetics of human cancer can be divided into three categories: first, cancers inherited by direct gene transmission; second, familial disorders not characterized by malignant disease themselves but which predispose to an increased likelihood of developing cancer; third, cancers of one or more types occurring with an increased incidence within a family but with a mode of genetic transmission that may not be clear.

A relatively small proportion of cancers are inherited as a result of direct single gene transmission. One example is retinoblastoma, a highly malignant tumor of the eye which presents in early childhood, and which occurs in two different forms. The less common familial form of the disease is usually transmitted as a highly penetrant dominant trait, with affected individuals often having bilateral involvement and a somewhat earlier age of onset. The more common form is characteristically sporadic, with affected individuals having unilateral disease and a later age of onset. As more affected persons will now reach adulthood and reproduce because of improved treatment, clarification of the mode of inheritance is mandatory to allow proper genetic counseling.

Multiple endocrine adenomatosis type II or Sipple syndrome consists of the association of medullary carcinoma of the thyroid, pheochromocytoma, and parathyroid hyperplasia. This disorder appears to be inherited as an autosomal dominant but with incomplete penetrance. As with many inherited cancers, there is an increased incidence of bilateral organ involvement. Clinically, the patient may present with evidence of carcinoma in only one system; for example, medullary carcinoma of the thyroid may be present without a palpable thyroid nodule. However, appropriate testing may show occult disease in the other organs. An elevated serum calcitonin level may suggest an occult cancer in predisposed individuals; therefore, diagnosis of any of the features of this unusual syndrome should lead to careful exclusion of the others, as well as to a search among close relatives for additional, possibly presymptomatic, cases.

Familial polyposis coli is an autosomal dominant disorder in which adenomatous polyps may be so numerous that they actually carpet the entire colon and rectum; Gardner syndrome is a distinct variant that includes associated sebaceous cysts and osteomas of bone. Early recognition of these disorders is critical since virtually 100% of affected persons will develop carcinoma of the colon by age 50 unless a prophylactic colectomy is performed. A number of oncologists now recommend total colectomy as soon as the disorder is discovered in childhood unless it is possible to follow the affected child with regular sigmoidoscopies and barium enemas. The management of these families can be extremely difficult since it is essential both to stress the importance of regular examination and to avoid contributing to an obsessive cancer phobia.

Neurofibromatosis is an autosomal dominant disorder characterized by café au lait spots and multiple neurofibromas. The neurofibromas show a sarcomatous degeneration in about 10% of patients, and there is an increased risk of gliomas of the brain or optic nerve, meningiomas, acoustic neuromas, and pheochromocytomas.

Xeroderma pigmentosa is a rare disorder manifested by sun sensitivity and the development of freckles on sun-exposed areas which then become dry and scaly. Basal cell and squamous cell carcinomas of
the skin later develop, and even if they are arrested, affected individuals have an increased risk of melanoma.\textsuperscript{1} Xeroderma pigmentosa is transmitted as an autosomal recessive trait. The defect has been shown to be a specific deficiency in one of the several enzymes normally required to repair ultraviolet radiation damage to the DNA of epidermal cells.\textsuperscript{4} When the precipitating causes of the problem are appreciated, the physician can teach affected individuals to protect themselves from the carcinogenic effects of ultraviolet radiation without undue disruption of a normal lifestyle.\textsuperscript{5}

Immune deficiency disorders are associated with an increased incidence of cancer, particularly lymphoma. The prime examples are the recessively inherited syndromes such as X-linked agammaglobulinemia, ataxia telangiectasia, and Wiskott-Aldrich syndrome. Individually, each of these recessive syndromes is rare, and in the aggregate, affected homozygotes (or hemizygotes if the trait is X-linked) constitute only a small proportion of all patients who develop cancer. However, there is increasing evidence that heterozygous carriers of at least some of these recessive genes may also be predisposed to cancer. Carriers of rare recessive genes are, of course, much more numerous in the general population than are affected homozygotes; in fact, the rarer the recessive trait, the greater the relative frequency of heterozygous carriers in the population in comparison with affected homozygotes. Therefore, a small effect of cancer risk in heterozygotes could have much greater public health significance than would a major effect in homozygotes. Swift\textsuperscript{6} has provided convincing evidence that heterozygous carriers of the genes for ataxia telangiectasia and Fanconi anemia have a three- to fourfold increased risk of developing cancer; he has estimated that at least 3\% of all cancers arise in individuals who are heterozygous for one or the other of these two genes alone. At present, no laboratory test is available to identify carriers.

Detecting an increased familial risk of cancer and following the involved family members for evidence of developing neoplasia is an important activity for practicing physicians. The cancers may be always of the same organ, that is, a site-specific tumor may occur in familial aggregations, or they may be of multiple varied organs. The Table lists the site-specific malignant neoplasms with possible familial occurrence.\textsuperscript{7}

There is considerable heterogeneity in the genetics of certain cancers, especially breast cancer. Studies\textsuperscript{8–10} have helped elucidate some of the risk factors. It is important to know whether the disease has occurred in two or more female first-degree relatives, whether the disease was pre- or post-menopausal in onset, and whether it was bilateral. In the premenopausal age group, the relative risk of breast cancer among family members of an affected individual is approximately three times that of controls, whereas in the postmenopausal age group, the risk is only slightly greater than that of controls. Relatives of women with bilateral disease have a fivefold risk compared to controls. The relatives of women with premenopausal and bilateral disease have a relative risk of developing breast cancer that is almost nine times greater than that of controls, while the relatives of patients with postmenopausal bilateral disease have a risk that is four times greater. The risk to other family members also depends upon the relationship of prior affected persons to the proband. For example, if prior disease involved a proband's mother, the proband's sisters will have a 30\% incidence of cancer in the 20-to 29-year age group, a risk 47 times greater than that of the general population. Clearly, these facts imply a genetic factor in breast cancer which appears most consistent with polygenic inheritance of a predisposition. Physician recognition of and response to this inherited predisposition is important particularly in the high-risk situation. In one such family, for example, bilateral reduction mammoplasty with implantation of a subcutaneous prosthesis was recommended for a 19-year-old girl whose two sisters, mother, and maternal grandmother had had early onset of breast cancer.\textsuperscript{11}

The concept of “cancer families” has been broadened to include kindreds displaying familial occurrence of diverse neoplasms even of dissimilar cell types.\textsuperscript{12} The cancer family has the following characteristics: 1) an increased occurrence of adenocarcinoma primarily of colon and endometrium; 2) in-

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creased frequency of multiple primary malignant neoplasms; 3) early age of onset of cancer relative to the usual age of onset of that cancer in the general population; 4) vertical transmission of cancer consistent with an autosomal dominant inheritance pattern. One such family has been described in Virginia. 13

Environmental factors may also influence expression of the disease when there is a familial tendency to cancer. Studies by Tokuhata have confirmed an increased incidence of cancer of the lung in relatives of patients with lung cancer, and his data suggest that the combined risk of cigarette smoking and the familial host factor increase the liability of developing lung cancer in a synergistic rather than additive manner. 14

The interrelationships between heredity and environment in the occurrence of cancer are complex and in many cases not completely characterized, but certain facts are clear. Specific cancers can be inherited directly. Certain diseases that predispose to cancer are inherited, and specific cancers or specific tendencies to develop a variety of cancers are inherited in families. Examples of each of these have been discussed. The long-range challenge to physicians is to recognize these situations and to study them thoroughly to further our understanding of the causes of cancer. More immediately, however, the alert physician who takes a detailed family history can recognize the increased risk of a particular malignancy and, by careful examination, testing, advice, and follow-up, can provide early treatment or even prevent the development of a potentially fatal malignancy. 11

REFERENCES


The Genetic and Somatic Effects of Radiation: A Balance Between Benefits and Risks

TIMOTHY MERZ, PH.D., Professor and Chairman, Division of Radiation Biology, and Professor, Department of Human Genetics

The present clinical interest in mutagenic agents in general and radiation in particular can be seen in the current proliferation of popular articles and reports on the effect of mutagens and radiation on biological systems. In spite of the continuing controversy over the effects of radiation from environmental sources, the most significant problem today is the increasing x-ray exposure of individuals due to increasing radiological diagnostic capability and the expanding importance of radiographic procedures in medical diagnosis.

Although man has survived radiation from natural sources during the course of his evolution, there is a point at which the risk of exposure is greater than any benefit that might be derived. The exposure dose at which that risk occurs and how it is influenced by the rate of exposure are being studied here at the Medical College of Virginia and many other institutions. The human system is extraordinarily well adapted to respond to the effects of radiation, chemical mutagens, and clastogens (compounds that break DNA strands and chromosomes) through a repair mechanism which more often than not returns things to normal, but there is a limit to its capacity.

The genetic risk of radiation has been recognized since 1927 when Muller reported that radiation induced mutations in fruit flies. Auerbach, using nitrogen mustard during World War II, demonstrated that chemicals could produce the same kinds of effects as radiation. We are now becoming aware of the magnitude of the threat that chemical mutagens pose for us in our environment. Since most chemical mutagens and clastogens produce effects similar to those of radiation, have the same targets and a similar dependence on dose and rate of exposure, and since the consequences are much the same although the mechanisms are quite different, I will concentrate almost entirely on the effects of radiation.

Radiation does not cause new kinds of genetic alterations but an increase in the frequency of alterations above the spontaneous level. Also there is no obvious threshold dose of radiation; that is, there is no dose below which there is no effect. Although no threshold dose of radiation exposure has ever been established, it is probably true that very low doses impose a very low order of risk.

Human damage by radiation is divided into two groups—germ cell (or presumptive germ cell) damage and somatic cell damage. The primary events of alteration, be it the genome, induction of cancer, or death, are always intracellular, the target being the genetic material found in the nucleus and organized as chromosomes. Thus, whatever the nature and extent of damage done to humans, it is always first expressed by cells, then by tissues or organs, and finally by the individual. The repair of altered DNA is accomplished by a group of enzymes that work with great speed and accuracy. Most of the damage produced by a dose of radiation is repaired by the cell in a very short time, but repair systems do not repair all of the damage nor is all repair perfect. Many cells will, for several reasons, survive and retain their function in the face of sustained genetic alterations. First, cells maintain their synthetic function after exposure...
to doses of radiation that would kill human beings. Enzyme systems and systems that synthesize protein in general are not sensitive to large doses of radiation. Second, the loss of genetic material is not necessarily significant to cells that have differentiated and use only that information from the DNA that is pertinent to their particular task. Third, the loss of genetic material is most often the loss of one half of that which is supplied to the cells since it gets informational content from both parents. Fourth, some of the genetic material is redundant and the loss of part of it, while it may result in reduced capability, will not necessarily result in death. Although cells can function after exposure to radiation, a large number of them will lose their ability to divide and replace old cells. After doses of radiation of approximately 200 rads, many cells lose their reproductive integrity. That loss is defined as reproductive death. Death, reproductive or actual, is only one significant result of radiation. Changes in DNA base pairs, deletions of materials, or chromosomal alterations often result in mutations that are usually recessive and almost always deleterious. When expressed, they result in the loss of enzyme function and altered proteins. There is a growing list of known enzyme-deficient disorders and of structural protein abnormalities, such as abnormal hemoglobin variance, in man. Recessive mutations are not observed until they are present in both maternal and paternal chromosomes and therefore take at least two generations of breeding to be expressed. In somatic cells recessive mutations will often go unnoticed; large losses or rearrangement of material seen as chromosomal changes, chromosome and chromatid aberrations, exchanges of material between chromosomes, and large losses of pieces of chromosomes usually cause more trouble and often result in the death of the cell. In addition to causing changes in the structural integrity of chromosomes, radiation often results in changes of chromosome number either by the addition or deletion of whole chromosomes. Radiation-induced genetic damage can be expressed in many ways, the simplest of which go unnoticed. While death may be the most severe result of a mutation, cell death is a minimal problem for an organism whose tissues renew cell populations continually; however, the death of many cells in critical stem cell populations of renewal tissues such as the gut or the marrow will cause the death of the individual. But these events do not occur as the result of incidental exposure to radiation.

The effects of low doses of radiation, chemical mutagens or clastogenic agents can be listed for both germ cells and somatic cells. The expressions of damage to germ cells are abortions, stillbirths, and congenital defects including all the known trisomies such as Down syndrome. The fact that radiation can induce trisomic developmental anomalies in early cleavage cells and germ cells is based both on theoretical considerations and on observations from published studies. The failure of chromosomes to disjoin at mitosis or meiosis is a well known result of exposure to radiation and an event preliminary to the production of trisomics. Germ cells or early cleavage stage cells (embryogenesis) that are missing chromosomes due to nondisjunction or have extra chromosomes for the same reason usually fare poorly and often result in fetal death. The best studies of trisomic or polysomic conditions in man are those involving the sex chromosomes. Extra sex chromosomes and missing sex chromosomes often produce less deleterious consequences in fetal development than changes in number of other chromosomes, but they are never without later developmental consequences. The same developmental problems can be produced by the insertion of pieces of chromosomes in other chromosomes or the loss of pieces of chromosomes.

The expression of somatic cell damage depends on when the damage is done. Damage done to fetal cells will often result in congenital defects and/or death. A minimal number of cells altered at the time when they are differentiating into a tissue or organ system can produce a partially or totally defective system. The earlier in development that exposure to radiation occurs, the greater is the risk of congenital defects. The risk of a particular system being defective is dependent on the stage of differentiation of that system at the time of radiation exposure. Differentiating cells are by far the most sensitive cells in the human system and they are apparently sensitive because their repair systems are not operating during the time they are differentiating. Differentiating cells are sixtyfold as sensitive as nondifferentiating cells. It is also clear that cells undergoing the complex process associated with organogenesis are particularly susceptible to genetic loss. Following organogenesis, the systems become considerably more resistant to radiation exposure or exposure to chemical mutagens. The first two trimesters of fetal development have been considered by radiologists to be so sensitive that most try to avoid extensive radiological investigations to women who may be pregnant. The last trimester has been considered to be a safe period
to the fetus, but current investigators have raised some questions about the extent of fetal resistance to radiation-induced damage even at that period of fetal development. Probably the most extensive investigation of the effect of radiation on developing fetuses is the Oxford Study directed by Stewart et al., which for all its excellence remains one of the most informative but controversial studies on this subject. Although it is a continuing investigation, the Oxford Study has already provided data that indicates that obstetric radiology as practiced some years ago in England increased the prevalence of childhood cancers in general and more specifically the risk of leukemia. That risk appears to be a function both of the number of x-ray films taken while the fetus is in utero and the stage of fetal development. Four other studies involving the effects of radiation on developing fetuses also indicate that obstetric radiology even to third trimester fetuses has probable deleterious effects. One study by Meyer, Tonascia, and Merz indicates that there is a 15% increase in fertility in young females exposed in utero also a suggestion of differences in growth, development, and behavior between exposed and control populations. Exposed women (in utero) have completed fewer grades of school, have poorer general health, more menstrual problems, more of certain diseases and accidents, and are heavier for their height than the control population. Mullineux and her colleagues working with rat fetuses irradiated in utero have observed significant changes in the behavioral activities of the rats that are exposed as compared with controls.

The major effect of very low doses of radiation on adult somatic cells is the induction of tumors. One of the difficulties of estimating the relationship between exposure to radiation and the induction of tumors is the long latent period between the induction and the growth of the tumors. There is little doubt, however, that tumors are induced by radiation at low-dose levels. The most common cancer induced appears to be leukemia possibly because no matter what area of the body is irradiated, circulating peripheral cells are always exposed and exposed frequently. To understand the risks involved in radiation exposure, one must remember that the dose of radiation calculated to double the natural incidence of diseases as a consequence of radiation exposure (the doubling dose) is somewhere between 20 rem and 200 rem. A rem is the absorbed dose in rads multiplied by modifying factors such as tissue sensitivity and the penetrating or ionizing qualities of the radiation in question, with the test or biomedical endpoint being measured. For most purposes one can substitute the radiation absorbed dose (rad) for rem: One rad equals an absorbed deposition of energy equivalent to 100 erg/gm of tissue. The maximum permissible exposure to non-radiation workers is 0.5 rads per year. Radiation exposures associated with typical diagnostic procedures such as chest films, gastrointestinal series or an intravenous pyelogram (IVP) are usually well under the permissible dose, the exception being the doses associated with the diagnosis and treatment of serious diseases. Most people never are exposed to as much as 20 rads of ionizing radiation in a lifetime. Even then the risk would be low but not insignificant since a 5 rem gonadal dose per generation to a population would increase disease due to mutation from 0.5% to 5.0% at equilibrium in the population.

The questions regarding the advisability of exposure to radiation are almost always those of risk vs benefit. Reasonable answers can be made only by using care, judgment and flexibility. Mutagenic agents are ubiquitous in the environment; they are always dangerous, but in many instances the benefits outweigh the risks associated with exposure. If a normal incidence of a disease or condition is 1/100,000 individuals, doubling the risk only increases it to 2/100,000. That risk is not meaningless, but in many instances it is low enough to negate concern when reasonable benefit is to be derived from exposure.

REFERENCES
Juvenile Hyperlipemia

WILLIAM W. MILLER, M.D., Professor of Pediatrics

Asymptomatic hyperlipemia occurs in a significant number of American children. It is important to recognize the condition during childhood because of its role as a risk factor in the early onset of coronary artery disease and because early dietary modification may prevent changes leading to premature heart disease.

A predisposition to heart attack exists within families, and now there is significant documentation of familial aggregations of coronary atherosclerosis as a concomitant of genetically determined risk factors such as hyperlipemia, hypertension, and diabetes mellitus.1–3 Of these three conditions, hyperlipemia, which often displays a clearly genetic component, is related most directly to the pathogenesis of atherosclerosis.

The following review will present the characteristics of juvenile hyperlipemia, note the known genetic influences, document its prevalence in children in the United States, and present recommendations for diagnosis and management.

Hyperlipemia is the manifestation of a variety of biochemical abnormalities producing hypercholesterolemia and/or hypertriglyceridemia, both of which are associated with an increased risk of coronary artery disease in adults. Because hyperlipemia occurs in early childhood and may lead to coronary atherosclerosis, the identification and treatment of children with hyperlipemia should receive more attention than it does at present. The definition of hyperlipemia is indistinct; normal blood levels of cholesterol and triglycerides are defined statistically by measurements in apparently healthy children. Although the levels can vary geographically and may be influenced by diet and other factors, “normal limits” have generally been based on 90th percentile fiducial limits calculated from relatively small samples (Table 1).4 A diagnosis of hyperlipemia is justified if lipid or lipoprotein concentrations, measured after a 12-hour fast, are above these “normal limits” on three separate occasions at least two weeks apart. Both cholesterol and triglyceride serum concentrations increase gradually throughout childhood and adolescence. Although the studies are limited, the normal range of cholesterol levels appears to be relatively narrow except during the first year of life when serum cholesterol is especially affected by the type of fat in the diet; consequently, unless the parents are hyperlipemic, diagnostic and therapeutic decisions are best delayed until after six months or one year of age, when a more mixed source of dietary fat is usual and there is a diminished variation of serum lipid concentrations. The changes in serum triglyceride concentrations during childhood are less well defined than are those for cholesterol, although a gradual increase is commonly observed. In children with persistent hyperlipemia, serum lipoprotein should be estimated by electrophoresis, and a survey of other family members is indicated. These additional studies can greatly facilitate a precise diagnosis when it is possible to demonstrate similar findings in a parent or sibling.

The most widely used classification of hyperlipemic diseases is based upon the serum lipoprotein pattern since blood lipids are bound to lipoproteins. The term “hyperlipoproteinemia” is therefore often preferred over “hyperlipemia” or “hyperlipidemia.” Six types of primary hyperlipoproteinemia are seen in adults; all except type III have been detected in childhood or adolescence. A useful classification of juvenile hyperlipoproteinemia is illustrated in the Figure.

Hyperlipoproteinemia I, also known as familial hyperchylomicronemia or lipoprotein lipase deficiency, is a rare autosomal recessive trait. The biochemical defect is a deficiency of the enzyme, lipoprotein lipase. In homozygous children the disease is usually characterized by elevated cholesterol and tri-
TABLE 1
Upper Limits of "Normal" of Plasma Lipid and Lipoprotein Cholesterol Concentrations (mg/100 ml) in Infants, Children and Adolescents

<table>
<thead>
<tr>
<th>AGE</th>
<th>TOTAL CHOLESTEROL</th>
<th>TRIGLYCERIDE</th>
<th>LDL CHOLESTEROL</th>
<th>HDL CHOLESTEROL</th>
</tr>
</thead>
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<tr>
<td>CORD BLOOD</td>
<td>95</td>
<td>65</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>1-19 yr</td>
<td>230</td>
<td>140</td>
<td>170</td>
<td>65</td>
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</tbody>
</table>

Glyceride levels, a deficiency of lipoprotein lipase activity in post-heparin plasma or adipose tissue, chylomicron uptake in the skin and reticuloendothelial tissue, abdominal pain, and pancreatitis which may be fatal. In children with xanthoma or pain the diagnosis is clinically obvious. However, in many children who are asymptomatic the diagnosis may be made accidentally by a finding of milky plasma, hepatosplenomegaly, or lipemia retinalis. Premature atherosclerosis has not been documented in these patients; however, the other symptoms and debilitating nature of the disease make treatment mandatory.

Hyperlipoproteinemia II, also known as familial hypercholesterolemia or familial hyperbetalipoproteinemia, is characterized by a deficiency of lipoprotein lipase activity, which results in the accumulation of chylomicrons and lipoprotein remnants in the bloodstream. This leads to the development of xanthomas (yellow, fatty deposits on the skin) and tendinous xanthomas (hard, painless nodules under the skin) in affected individuals. The diagnosis is typically made by detecting the characteristic phenotypic pattern, which includes a high level of triglycerides and low levels of high-density lipoprotein (HDL) cholesterol.

The phenotypic classification of hyperlipoproteinemia includes five major types (I, II, III, IV, and V) based on the results of electrophoretic analysis. Each type is further divided into subgroups (IA, IB, IIA, IIB, IIIA, IIIB, VAI, and VBB) to account for variations in lipid and lipoprotein profiles. For example, type I hyperlipoproteinemia is characterized by the absence of chylomicrons and very low density lipoproteins (VLDL), while type II hyperlipoproteinemia is characterized by the presence of chylomicrons and VLDL.

Accurate diagnosis and management are crucial to prevent complications such as coronary artery disease, peripheral artery disease, and cerebrovascular events. Treatment options include lifestyle modifications, such as diet and exercise, and pharmacotherapy with statins and other lipid-lowering agents. Early intervention can significantly reduce the risk of cardiovascular disease in affected individuals.
teinemia, is inherited as an autosomal dominant trait. It is the most common form of juvenile hyperlipemia and is associated with a high risk of premature coronary artery disease. Hyperlipoproteinemia II accounts for many adults with myocardial infarction. Biochemical abnormalities are now believed to be the result of an absence or deficiency of a low-density lipoprotein receptor on cell membranes.5,6

The relatively common heterozygous form, hyperlipoproteinemia II A, has an estimated prevalence of four per thousand; in a Seattle study approximately one out of every twenty adult survivors of myocardial infarction was found to have this condition; in a similar study 38 of 64 survivors had abnormal lipoprotein patterns.9 These hyperlipemic fathers, all under 41 years of age, had 85 children of whom 30 also had hyperlipoproteinemia II. Heterozygous infants have plasma cholesterol levels that are two to three times normal by the end of the first year of life. In matings between a heterozygous and a normal parent, the affected children can be identified at birth by cord-blood elevations of low-density lipoprotein cholesterol to values greater than 41 mg/100 ml.10 Tendinous xanthoma, corneal arcus, and coronary artery disease develop in young adults, usually from 20 to 30 years of age. In about 10% of children with type II disease, there are mildly elevated levels of very low-density lipoproteins and triglycerides along with elevations in low-density lipoprotein cholesterol.11 This less common variation is classified as familial hyperlipoproteinemia II B.12

Homozygous children, who inherit a double dose of the mutant gene, are much more severely affected; the disease is often suspected initially because of the strong family history of heart disease. Cholesterol levels, which may range from 500 to 1000 mg/100 ml, are frequently markedly elevated even at birth, and corneal arcus and xanthoma appear before adolescence. Coronary artery disease is clinically apparent before the age of 20 and survival beyond 30 is rare.

In every family with a member who has experienced a heart attack before the age of 50, serum cholesterol and triglyceride levels should be measured in all first-degree relatives. Those with normal lipid values can be reassured about lipid risk factors and a significant number of those with hyperlipemia can be identified and treated early.

Hyperlipoproteinemia III, also known as "broad Beta disease," "floating Beta disease," or dysbeta-lipoproteinemia, has not been detected in children, as noted previously. Inheritance is probably either an autosomal recessive or an autosomal dominant with incomplete penetrance.13 The condition results in elevated levels of cholesterol and triglycerides with an abnormal cholesterol to triglyceride ratio in the very low-density lipoprotein.14

Hyperlipoproteinemia IV, is relatively rare in children and is also referred to as familial hypertriglyceridemia or endogenous hypertriglyceridemia. Triglyceride levels are always elevated, ranging from 150 to 1000 mg/100 ml, and in about 20% of patients the very low-density lipoprotein levels may result in a hypercholesterolemia, with levels up to 350 mg/100 ml.15 Clinical findings commonly include non-ketotic diabetes and, less commonly, subcutaneous xanthoma. Obesity and hyperuricemia are frequent. A significant proportion of adults under the age of 50 with angina or myocardial infarction have primary type IV disease.15 The condition appears to be heterogeneous. The biochemical abnormality is not known, but studies suggest a defect in very low-density lipoprotein catabolism.16,17 It may occur in relatives of patients with type III or type V lipoprotein abnormalities and in some families genetic transmission follows an autosomal dominant pattern with delayed expression.

Hyperlipoproteinemia V is rare in childhood. Triglyceride levels range from 500 to 6,500 mg/100 ml and cholesterol varies from 150 to 1,500 mg/100 ml. Clinical findings are similar to those with type I disease. Type V disease does not lead to premature atherosclerosis; it is frequently associated with obesity, hyperuricemia, and abnormal glucose tolerance. The inheritance of the primary disease is not known; a family history of diabetes is common and there is a high prevalence of type IV disease in first-degree relatives.

Many chronic childhood diseases are associated with secondary hyperlipemia (Figure). Although infants with hypothyroidism usually have normal lipids and lipoproteins, in older children hypothyroidism is often associated with hypercholesterolemia which can be reduced by adequate thyroid treatment. Most children with the nephrotic syndrome have hypercholesterolemia, with close inverse correlations between cholesterol and albumin levels. In children treated by renal dialysis, the practice of feeding high fat diets to provide sufficient calories for growth may result in secondary hyperlipoproteinemia and acceleration of atherosclerosis. Children with hepatic glycogenosis III have hyperlipoproteinemia, usually
with increases in very low-density lipoproteins. Since many of these children survive to adulthood, early modification of the hyperlipemia by diet is indicated. Children with obstructive liver disease often have hyperlipemia unless the disease is at an advanced stage. Diet and drug management of the hyperlipemia result in diminution in the symptoms and lesions of xanthomatosis. In adolescents and young adults other rare causes of hyperlipemia are pregnancy, alcoholism, adrenal disease, and the chronic administration of adrenal corticosteroid drugs.

Dietary restriction and selection of fats is indicated in all children with primary hyperlipemias (Table 2). Because skin xanthoma are diminished or eliminated in children undergoing treatment, it is reasonable to conjecture that regression of vascular lipid deposits, with a concomitantly decreased risk of coronary artery disease, also occurs. In type I disease a low-fat diet normalizes serum lipids and reduces or prevents abdominal pain and pancreatitis. In type II disease a low-fat, low-cholesterol diet with a high ratio of polyunsaturated to saturated fats normalizes lipids in type II A patients, reduces lipid levels significantly in type II B children, and diminishes xanthoma in both. In type IV children a low-calorie, weight-reduction diet is sufficient to normalize serum lipid. In children with type V disease a low fat, moderate cholesterol diet normalizes serum lipids and prevents abdominal pain.

A low-cholesterol diet, 100 to 150 mg/100 ml, is achieved by eliminating egg yolk, organ and fatty meats, and shellfish, substituting skim milk and low-cholesterol margarine for whole milk and butter, using only cottage cheese among the cheeses and prohibiting products with coconut oil in them. Diet management alone is inadequate in 85% of type II children, and it is often necessary to use cholestyramine, a nonabsorbable anionic resin and bile acid sequestrant, which produces an exchange of chloride ions for bile acids in the intestine. The acids are excreted in the feces, and the reduction in enzyme product inhibition increases the endogenous metabolism of cholesterol, resulting in diminished serum levels of very low-density and low-density lipoprotein cholesterol. In doses of 250 to 800 mg/kg/day, cholestyramine does not cause malabsorption, although constipation, epigastric discomfort, and bloating are common. Because the drug absorbs fat, it can also produce mild steatorrhea and poor absorption of fat soluble vitamins. Its disagreeable taste is masked by using fruit juice or incorporating it into cookies. The surgical treatment of children with type II disease by partial ileal bypass is not recommended at this time.

In homozygous type II B children, the prognosis for effective reduction in serum lipids is poor. Low-fat diets and large doses of cholestyramine, together with the administration of nicotinic acid may be effective. Nicotinic acid, however, is often poorly tol-

### Table 2

<table>
<thead>
<tr>
<th>TYPE</th>
<th>DIET</th>
<th>DRUG</th>
<th>EFFECT</th>
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<tr>
<td>I</td>
<td>Low fat (10–15 g/day)</td>
<td></td>
<td>Normalizes lipids</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prevents pain and pancreatitis</td>
</tr>
<tr>
<td>II A HETEROZYGOTE</td>
<td>Low cholesterol (100–150 mg/day)</td>
<td>Cholestyramine (250–800 mg/kg/day)</td>
<td>Normalizes lipids. Reduction of cardiovascular risk possible</td>
</tr>
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<td></td>
<td>High polyunsaturated/saturated ratio (2/1)</td>
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</tr>
<tr>
<td>II B HOMOZYGOTE</td>
<td>Low cholesterol (100–150 mg/day)</td>
<td>Cholestyramine 500–1500 mg/kg/day</td>
<td>Lowers lipids, Xanthoma diminish. Reduction of cardiovascular risk possible</td>
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<tr>
<td></td>
<td>High polyunsaturated/saturated ratio (2/1)</td>
<td>Nicotinic acid 25–75 mg/kg/day</td>
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<tr>
<td>IV</td>
<td>Low calorie weight control</td>
<td></td>
<td>Normalizes lipids. Reduction in cardiovascular risk possible</td>
</tr>
<tr>
<td>V</td>
<td>Low fat (20–30 g/day)</td>
<td></td>
<td>Normalizes lipids prevents pain</td>
</tr>
<tr>
<td></td>
<td>Moderate cholesterol (300–400 mg/kg/day)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Medium chain triglyceride</td>
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erated because of such side-effects as flushing, gastro-intestinal distress, temporary hyperuricemia, glucose intolerance, or hepatic dysfunction.

Nationwide reductions in dietary fat and cholesterol intake have been promulgated by various health organizations. Most advisors recommend a reduction of individual fat intake from 40% to 30% of the total calories and a decrease in cholesterol and saturated fats.

Juvenile hyperlipemia is relatively common in the United States, and most affected children are asymptomatic. Measurements of serum cholesterol and triglycerides are indicated in all infants and children in families with one or more members having a heart attack before the age of 50, because a significant proportion of these children will have hyperlipemia and will require dietary restrictions, and some will need drug treatment. Early recognition and management of juvenile hyperlipemia may diminish the incidence or severity of coronary artery disease decades later.

REFERENCES


V. RESEARCH

New Approaches to the Use of Twins in Biomedical Research

WALTER E. NANCE, M.D., PH.D., Professor of Human Genetics, Pediatrics, and Medicine

Human geneticists are often accused of being preoccupied with exotic syndromes that are of marginal relevance to the general population. Brilliant success has been achieved during the past two decades in defining the nature and function of the genetic material, the molecular pathology of a large number of metabolic diseases, the phenotype of more than 2,000 Mendelian traits, and more recently the chromosomal location of a rapidly expanding number of human gene pairs. In contrast, relatively little progress has been made in the genetic analysis of quantitative traits such as blood pressure, serum cholesterol, intelligence quotient (IQ), skin color, height, birth weight, or glucose tolerance. Traits of this type are not only of interest to society, but may also relate significantly to a variety of common diseases. With almost every continuously distributed quantitative trait, single gene defects have been identified which can profoundly alter the phenotype. For example, the single gene pairs which determine albinism and Tay-Sachs disease can profoundly alter skin color and IQ respectively. However, the causes of less extreme variation can be exceedingly complex, resulting from the cumulative effects of many gene pairs and their interactions with each other and with the environment. Nevertheless, even if the effects of individual gene pairs cannot be identified, the source of the observed variation may often be inferred from an analysis of the phenotypic correlation of relatives of various degree. Twin studies have been widely used in the past to gain insight into the inheritance of quantitative traits, and with the support of a Program Project Grant from the National Institute of Maternal and Child Health, the Department of Human Genetics at the Medical College of Virginia has become a leader in the use of twins for biomedical research.

The basic idea of a classical twin study is to compare the differences between monozygotic (MZ) and dizygotic (DZ) twins. Since MZ twins arise from a single fertilized egg, they possess identical sets of nuclear genes, and differences between them are assumed to arise from environmental causes. DZ twins, on the other hand, are genetically no more similar than siblings, sharing, on the average, only half of their genes. The extent to which the intrapair differences of DZ twins exceed those of MZ twins provides a measure of the importance of genetic factors as a cause for variation in DZ twins. DZ twins are considered to be an appropriate comparison group because they are born at the same time and are assumed to share the same environmental similarities as MZ twins. Classical twin studies remain a very useful method for estimating the relative importance of genetic factors as a cause for variation in complex physical, physiological, or psychological traits. We have made extensive use of this approach to investigate the genetic control of bone mineral content, dental and cephalometric variables, electrocardiographic variables, amino acid metabolism, dermatoglyphic...
variables, and a variety of biochemical, physiological, and psychological traits. However, there are a number of limitations in the classical twin model. The method does not permit the recognition of maternal effects or an incisive resolution of the genetic effects, and the assumption that the environmental similarities of identical and fraternal twins are equivalent may be open to question for some traits. Also, in order to generalize the results, we must be willing to consider twins a random sample of the genotypes in the general population. This is probably a reasonable assumption for MZ twins; however, many genetic and environmental factors are known which profoundly influence the incidence of DZ twins and might, therefore, prevent direct extrapolation of the results of a twin study to the general population. Finally, both fraternal and identical twins may be exposed to unique intrauterine environmental effects which need to be better understood in order to evaluate their possible significance as a source of bias in classical twin studies.

In order to circumvent these problems, we have developed a powerful new model for the analysis of quantitative inheritance in man which exploits the unique relationships that exist within the families of identical twins. Because they have a genetically identical parent, the children of identical twins are related to each other in the same way as half-siblings (Fig 1). Genetically, the child of a twin is just as closely related to his twin aunt or uncle as he is to his twin parent. However, since the parents and their own children usually live in the same home, and are, for example, exposed to the same diet, we would expect any significant environmental similarities to make an additional contribution to the parent-offspring correlation. It is easy to see how a comparison of the correlations would permit a clear separation of genetic and environmental effects on the closely related half-sibs. Each family contains individuals who share one fourth of their genes (the half-sibs), one half of their genes (the full sibs), all of their genes (the MZ twins), and none of their genes (the spouses of the twins). These and other relationships permit the derivation of multiple linear equations in which an observed correlation, variance or covariance between family members is expressed as a linear combination of several unknown genetic and environmental variance components that we wish to estimate. Since the model yields more equations than unknowns, we can solve the system of equations and obtain the best fitting estimates of the unknown parameters. The calculations are quite complicated, particularly since we must allow for the fact that our observed correlations, variances, and covariances may not be independent of one another because they have been derived from individuals who are interrelated in a complex manner. However, the use of a high-speed computer makes solution of the equations technically feasible.

The entire process of parameters estimation can be summarized in matrix notation by the single equation, \( G = (M^T V^{-1} M)^{-1} M^T V^{-1} C \). \( G \) represents the vector of unknown genetic components we wish to estimate; \( M \) represents the matrix of coefficients derived from Table 1; \( C \) represents the vector of the observed mean squares; and \( V \) represents the variance/covariance matrix of the observed mean squares. Most physicians would probably find a detailed exposition of the mathematical analysis to be tedious and uninformative; however, the essence of the analysis is simple and intuitive, depending
as it does upon the concept developed in high school algebra, that if you have as many independent equations as you have unknowns, a solution can be obtained.

A unique feature of the model is that it permits the detection and estimation of genetically determined maternal effects. If intrauterine or postnatal maternal effects influence a trait to a significant degree, we would expect the offspring of identical female twins to be more similar than the offspring of male twins since the latter are born to genetically unrelated mothers. As suggested by Figure 1, the total variability or variance among the offspring may be divided by a statistical procedure known as a “nested analysis of variance” into within sibship, between sibship within half-sibship, and among half-sibship components. Each of these components may be expressed in terms of a series of constituent genetic and environmental subcomponents (Table 1). In this formulation, $V_A$ refers to the additive component of the genetic variance. Additive genetic effects are the ones responsible for the resemblance between parents and their offspring. The among half-sibship component of variance is equivalent to the covariance of half-sibs and since half-sibs share one fourth of their genes, this component includes one fourth of the total variation attributable to additive effects, the remainder being distributed as shown in Table 1. $V_{AA}$ refers to additive genetic effects that result from the interaction of genes at separate loci. The term $V_D$ refers to genetic effects that show dominance and arise from interactions between the two members of a gene pair (alleles). Dominance effects can contribute to the similarity only of individuals who are related to each other through both parents. For this reason, note that no dominance effects appear in the among half-sibship component. $V_{ER}$, $V_{ES}$, and $V_{EW}$ refer to environmental effects acting among half-sibships, between sibships within half-sibships, and within sibships respectively. Finally, maternal effects are designated by $V_M$. Since they contribute to the similarity of the half-sib offspring of female twins, $V_M$ appears in the among half-sibship component for female twins. In the case of male twins, on the other hand, the maternal effects arising from the genetically unrelated wives contribute to variation between sibships within each half-sibship. The five relationships shown in Table 1 provide five of the equations which can be used in the overall analysis described previously. If the analysis is confined to these relationships, it becomes possible to exploit the unique genetic relationship of monozygotic twins through observations of their offspring without incurring any of the methodologic liabilities that might be associated with the inclusion of data from the twins themselves.

Table 2 shows the results of an analysis of the birth weight of 254 children of 46 twin pairs. The data were collected by interviews and questionnaires, and prior to the analysis, the birth weights were adjusted for gestational age at the time of delivery. In all genetic models that fit the data well, there was evidence that genetically determined maternal effects made a highly significant contribution to the overall variation in birth weight. In fact, our results suggest that at least one third of the total variation in birth weight is actually fixed or determined prior to the time of conception by the genotype of the mother! Since birth weight is highly correlated with fetal survival, these findings raise the possibility that perhaps we should look more closely at the mothers of high and low birth weight infants; in future extensions of the present work, we would like to repeat the analysis

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<td>Analysis of the Birth Weight of 254 Offspring of 46 Pairs of Identical Twins</td>
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<table>
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<tr>
<th>Parameter</th>
<th>Estimate ± Standard Error</th>
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<tr>
<td>$V_{AA}$</td>
<td>0.014 ± 0.037</td>
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<td>$V_M$</td>
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<tr>
<td>$V_{ES}$</td>
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<td>$V_{EW}$</td>
<td>0.082 ± 0.028</td>
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<th>TABLE 3</th>
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<td>Distribution of Twins by Age, Sex, and Zygosity</td>
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<table>
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<th>Type</th>
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<td><strong>MONOZYGOTIC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-Male</td>
<td></td>
<td>15</td>
<td>11</td>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>Female-Female</td>
<td></td>
<td>14</td>
<td>3</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>29</td>
<td>14</td>
<td>16</td>
<td>59</td>
</tr>
<tr>
<td><strong>DIZYGO TIC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-Male</td>
<td></td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Female-Female</td>
<td></td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Male-Female</td>
<td></td>
<td>21</td>
<td>2</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>39</td>
<td>4</td>
<td>5</td>
<td>48</td>
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<tr>
<td><strong>GRAND TOTAL</strong></td>
<td></td>
<td>68</td>
<td>18</td>
<td>21</td>
<td>107</td>
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</table>
TABLE 4
Distribution of Newborn Twins by Race and Placenta Type

<table>
<thead>
<tr>
<th>Placenta type</th>
<th>Black</th>
<th>White</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td>MZ</td>
</tr>
<tr>
<td><strong>TWINS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monochorionic</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monoamnionic</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monochorionic diamnionic</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dichorionic, fused</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Dichorionic, separate</td>
<td>1</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td><strong>TRIPLET</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Monochorionic triamnionic</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trichorionic</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>10</td>
<td>21</td>
<td>2</td>
</tr>
</tbody>
</table>

Pedigrees of Familial Twinning

Fig 2—Pedigrees of 15 families showing a history of twinning in close relatives.

NANCE: TWINS IN BIOMEDICAL RESEARCH

after dividing the families into high, low, and intermediate birth weight categories. We are currently trying to identify additional fertile adult MZ twins who would be suitable for inclusion in our MZ half-sib studies. The project involves the collection of a large amount of medical and genetic data from the families during an out-patient visit, and the participants are informed of any abnormalities that are detected in any of the blood, urine, or physiologic screening tests.*

**Biology of Twinning**

Since the new program of twin research was initiated at MCV, 107 twin pairs have been evaluated

* Twins may be referred to the study by calling the Project Coordinator, Phyllis Winter, at 804/770-4645.
and enrolled in the Twin Panel (Table 3). The sample includes 40 pairs of newborn twins and two sets of triplets ascertained from the MCV Obstetrical Service. Systematic placental examinations have been performed in these twins (Table 4). It is now clear that monozygotic twins may have either two separate placentas or a single placenta. The former are all dichorionic while the latter include dichorionic pairs and monochorionic twins that may either be diamnionic or rarely monoamnionic. These differences in placentation are thought to reflect differences in the stage of embryonic life at which the twinning process occurred. One goal of the newborn twin study is to determine whether these striking placental differences are reflected in phenotypic differences between the twins. Preliminary evidence suggests that the intra-pair differences in cord blood cholesterol in dichorionic twins are more than five times as great as those observed in monochorionic pairs.9

The twins studied to date have included 15 families in which there was a history of twinning in a close relative (Fig 2). A genetic predisposition to dizygotic twinning has long been recognized, presumably mediated by neuroendocrine differences in some women. For MZ twins, however, it is much less clear whether or not the occasional occurrence of familial aggregation indicates a genetic effect, and the cases shown (see Fig 2) do little to resolve this uncertainty. In family #1885, the birth of two sets of MZ twins to a woman by different fathers strongly suggests a maternal influence, whereas family #1836, in which MZ twins were born to a member of a male MZ pair, suggests patrilineal inheritance. Finally, in family #1642, placental examinations were performed on both twin pairs, revealing monoamnionic placentation in one and diamnionic monochorionic placentation in the other—a finding which suggests that familial occurrence is not confined to twins of a single placenta type.

Summary

The MCV Twin Panel was begun in 1976 and includes twins and higher order multiple births of all ages. Twin studies offer many opportunities for innovative research, and it is intended that the Panel be a research resource for clinical investigators at the Medical Center.

REFERENCES


Genetic Studies of Deafness and of Retinitis Pigmentosa

JOANN A. BOUGHMAN, Graduate Assistant, Department of Human Genetics
FREDERICK R. BIEBER, M.S., A.D. Williams Predoctoral Fellow, Department of Human Genetics
WALTER E. NANCE, M.D., PH.D., Professor of Human Genetics, Pediatrics, and Medicine

In experimental animals where the generation time is short and matings can be controlled experimentally, it is a relatively simple task to determine whether a trait is genetic, how it is inherited, and where the causal gene pair is located. However, in human genetics, inferences must be drawn by pooling observations on many small families in which the trait of interest has occurred. The condition may be etiologically heterogeneous, resulting from environmental causes in some families and showing variable patterns of inheritance in others. Hereditary deafness and retinitis pigmentosa (RP) provide instructive examples of the problems involved in the genetic analysis of family data in man.

Hereditary Deafness

It is now clear that the interactions of literally hundreds of genes are required to provide the information needed to form a normal ear, and that a defect in any one of many genes can result in deafness.1 Well over 100 specific syndromes have been described showing dominant, recessive, and sex-linked patterns of genetic transmission in which hearing loss is a major finding.2 On the other hand, many environmental causes of deafness are known, such as rubella, prematurity, and ototoxicity, and in a given case, in the absence of a recognizable syndrome or a positive family history, it may be difficult to be sure whether the cause is genetic or nongenetic.

The problem is complicated by the fact that human families are so small that with recessive deafness (the most common genetic type) there may be only one affected child in the family. Table 1 shows the expected proportion of families that would have no affected children, one affected child (simplex families), and more than one affected child (multiplex families), with a recessive trait. The multiplex families are the "obvious" genetic cases, and the task of the geneticist is to estimate what proportion of the simplex families are, in fact, genetic cases in which by chance only one affected child has occurred. The remaining simplex cases are sporadic; they arise from nongenetic causes and are not associated with an appreciable recurrence risk. The proportion of nongenetic or sporadic cases is designated by the letter x.

A second problem in the analysis of data from human families relates to ascertainment biases. In the case of a recessive trait, a large proportion of the families at risk will have no affected children (Table 1). Since there may be no way for us to discover these families, we must base our conclusions about the expected proportion of affected children on an incomplete or truncated sample of families which has been ascertained because there has been at least one affected child in the family. In order to accurately estimate the recurrence risk, we must allow, in an appropriate manner, for the families with no affected children that we had no way of discovering.

The parameter $\pi$ is the probability that an affected individual will be independently discovered by the sampling procedure. The value of $\pi$ can vary from nearly zero to one and is a measure of the completeness of the sampling procedure. Finally, we wish to estimate $p$, the recurrence risk or segregation ratio. If the estimate of $p$ is close to 0.25, we might conclude.
that the data agreed satisfactorily with the hypothesis of recessive inheritance.

The process by which the three parameters $\pi$, $x$, and $p$ are estimated is known as *segregation analysis*.

With the use of a high-speed computer, we can estimate what values of $x$, $\pi$, and $p$ provide the best explanation for any given set of data containing information on the number of affected and normal individuals and index cases in each of a large series of families.

The results of segregation analysis of data from 11,968 families of deaf children are shown in Table 2. In the first line we see that in the case of 86 consanguineous matings, the hypothesis of recessive inheritance with no sporadic cases (that is, $p=0.25$ and $x=0.0$) provides a satisfactory explanation for the data. The relatively low $X^2$ values in the last two columns indicate a good fit of the hypothesis to the data. This means that whenever we elicit a history of consanguinity, it is safe to assume that we are dealing with recessive deafness even if there is only one affected child in the family. This result is not surprising since parental consanguinity is the hallmark of recessive inheritance. When present, it may indicate that the affected child has inherited two copies of the same rare recessive gene carried by one of the common ancestors of the parents.

In the second and forth lines, we see that the hypothesis that all of the children in 11,900 nonconsanguineous matings have recessive deafness is resoundingly rejected as indicated by the enormous $X^2$ values. However, when we allow $x$ to assume the best fitting value of 0.6 in the families with a negative family history, the hypothesis that the remaining cases are recessive (that is, $p=0.25$) fits very well indeed (line three). This tells us that among these nonconsanguineous families, 40% are genetic and probably recessive while 60% are nongenetic. It is of considerable interest that among families in which there was a remote history of deafness, in a grandparent, aunt, uncle, or a cousin for example, the estimated proportion of sporadic or nongenetic cases was much smaller ($x=0.2$), as shown in line five. Similar analysis can be performed for deaf children arising from deaf by normal matings, which presumably are attributable to dominant inheritance, as well as for affected children arising from deaf by deaf matings, which may include both dominant and recessive cases.

---

**TABLE 1**

<table>
<thead>
<tr>
<th>NUMBER OF CHILDREN IN FAMILY</th>
<th>FAMILIES WITH NO AFFECTED CHILDREN (%)</th>
<th>FAMILIES WITH ONE AFFECTED CHILD (%)</th>
<th>FAMILIES WITH MORE THAN ONE AFFECTED CHILD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
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<td>5</td>
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<td>6</td>
<td>18</td>
<td>35</td>
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<tr>
<td>7</td>
<td>13</td>
<td>31</td>
<td>56</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>27</td>
<td>63</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>MATING TYPE AND HYPOTHESIS TESTED</th>
<th>NUMBER OF SIBSHIPS</th>
<th>NUMBER OF CHILDREN</th>
<th>$X_p^2$</th>
<th>$X_x^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearing X hearing consanguineous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_o$: $p = 0.25$, $x = 0$</td>
<td>86</td>
<td>150</td>
<td>148</td>
<td>2.1</td>
</tr>
<tr>
<td>Negative family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_o$: $p = 0.25$, $x = 0$</td>
<td>10,509</td>
<td>12,712</td>
<td>28,739</td>
<td>5,289.6</td>
</tr>
<tr>
<td>$H_o$: $p = 0.25$, $x = 0.6$</td>
<td>10,509</td>
<td>12,712</td>
<td>28,739</td>
<td>1.9</td>
</tr>
<tr>
<td>Positive family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_o$: $p = 0.25$, $x = 0$</td>
<td>1,391</td>
<td>2,142</td>
<td>3,496</td>
<td>97.0</td>
</tr>
<tr>
<td>$H_o$: $p = 0.25$, $x = 0.2$</td>
<td>1,391</td>
<td>2,142</td>
<td>3,496</td>
<td>0.05</td>
</tr>
</tbody>
</table>
cessive phenotypes. Table 3 provides a tally sheet for the segregation analysis of a large sample of 16,471 deaf children. Although there were multiple affected children in only about 6% of the families, it can be seen that our best estimate is that almost exactly half of the cases are in fact genetic in etiology. Most educators and physicians who work with the deaf find this estimate to be surprisingly high. Few otolaryngologists consider themselves to be geneticists in spite of the fact that half of the young children they see with profound hearing loss are deaf because of genetic reasons. The frequency of patients with simply inherited genetic traits in this group is actually larger than that observed among children who are referred to a typical human genetics clinic for evaluation and counseling.

Unfortunately, it is not always possible to identify those simplex cases that are genetic. As an aid to diagnosis in these cases, we have begun to establish a Genetic Registry of Hereditary Deafness. The Registry is based upon pedigree data from a sample of about 5,000 matings among the deaf that were collected by E. A. Fay, a professor of Gallaudet College in Washington, D.C., before the turn of the century. The Registry contains detailed information on about 30,000 individuals that was collected by Fay and is supplemented by data on current pedigrees. In about 7% of patients with genetic deafness, we find that it is possible to establish a genealogic linkage with someone listed in the Registry. Thus in some cases, use of the Registry can help establish the genetic nature of the disorder in situations where it might not be apparent from the clinical evaluation or the immediate family history.

The Registry should also promote the recognition of genetic heterogeneity which, as previously mentioned, is known to be extensive in hereditary deafness. An autosomal recessive form of hereditary deafness known as Usher syndrome provides an excellent example of heterogeneity even within a single clinical entity. In this condition affected persons suffer from progressive blindness due to retinitis pigmentosa (RP) in addition to sensorineural hearing loss. Our studies suggest that the classic delineation of Usher syndrome with early-onset, severe sensorineural deafness and RP may have to be modified.

We are collecting data on affected individuals using two different methods of ascertainment. The first involves ophthalmologic screening of students in schools for the deaf. About 4% to 6% of this group have an associated RP. Since these students tend to have severe hearing loss, our ascertainment is biased with regard to degree of hearing loss. To circumvent this problem we are also documenting the hearing status of a population of affected patients identified through the National Retinitis Pigmentosa Foundation. Our preliminary data indicate that considerable clinical and genetic heterogeneity exist in these families. Of great interest is the disparity in degree of hearing loss in probands and their affected sibs in the multiplex sibships. Table 4 shows that in 29 sibships

### Table 3

Summary Results of Segregation Analysis in 12,661 Informative Families with Deaf Children.

<table>
<thead>
<tr>
<th>Mating Type</th>
<th>Number of Families</th>
<th>Number of Deaf Children</th>
<th>Children with Sporadic Deafness</th>
<th>Children with Genetic Deafness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Recessive</td>
<td>Dominant</td>
</tr>
<tr>
<td>Hearing X hearing</td>
<td>11,986</td>
<td>15,004</td>
<td>8,126</td>
<td>6,650</td>
</tr>
<tr>
<td>Deaf X hearing</td>
<td>254</td>
<td>478</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deaf X deaf</td>
<td>421</td>
<td>989</td>
<td>0</td>
<td>451</td>
</tr>
<tr>
<td>Total</td>
<td>12,661</td>
<td>16,471</td>
<td>8,126</td>
<td>7,101</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td></td>
<td>49.3</td>
<td>43.1</td>
</tr>
<tr>
<td>% of Genetic</td>
<td></td>
<td></td>
<td>85.1</td>
<td>14.9</td>
</tr>
</tbody>
</table>

### Table 4

Hearing Loss in Affected Sibs of Probands

<table>
<thead>
<tr>
<th>Degree of Hearing Loss in Proband</th>
<th>Degree of Hearing Loss in Affected Sibs of Probands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>None</td>
</tr>
<tr>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

* numbers refer to sibships
the probands had either mild or severe hearing loss, but the sibs affected with RP had no hearing loss; and in six sibships the probands reported severe hearing loss while the sibs had only a mild loss.

Thus Usher syndrome may represent only part of a broad clinical spectrum of disability involving these two sensory modalities, and our Registry should not only promote the recognition of such heterogeneity but improve the quality of medical, genetic, and educational research on such diseases by providing rosters of affected individuals who are etiologically homogeneous.

Retinitis Pigmentosa

The term retinitis pigmentosa refers to a group of genetic disorders in which there is a progressive loss of vision associated with a characteristic pigmentary degeneration of the retina, nyctalopia, and progressive loss of peripheral vision, often leading to blindness. The genetic heterogeneity of this group of disorders is well documented. First, it is known that RP may be inherited in all three Mendelian modes: recessive (80% to 90% of cases), dominant (5% to 10%), and X-linked (1% to 5%). Second, several specific genetic syndromes, of which RP is a part, have been identified (Table 5). More evidence for genetic heterogeneity comes from the study of many animal models for RP which indicate that the phenotype seen in these degenerative disorders may be produced by various primary lesions.

In collaboration with the National Retinitis Pigmentosa Foundation, we are conducting a nationwide survey in order to clinically and genetically characterize a sample of probands with RP. Systematic questionnaire data on family history, age of onset and progression of the symptoms, and associated abnormalities, have been analyzed on 670 individuals, forming a data base of 12,348 members of families including 1,390 affected individuals.

Nyctalopia was the most frequently noticed first symptom, especially in the younger age groups. The most common extraocular finding was hearing loss, reported by 30.4% of the probands, indicating their loss was severe. This finding in our large proband sample reemphasizes the association between hearing loss and RP.

Segregation analysis has been performed on 405 informative proband sibships with no recognizable syndromes. Table 6 summarizes the results of these analyses, showing estimates of the segregation frequency and proportion of sporadic (nongenetic) cases. The estimate of penetrance in the case of the dominant mode of inheritance was 0.58. The finding of a low segregation ratio for both the recessive and dominant forms of RP is not surprising in view of the natural history of these disorders. The decreases in these ratios reflect the fact that the age of onset may not have been reached by many sibs, the median onset age for probands being nearly 15 years. Perhaps

### Table 5

<table>
<thead>
<tr>
<th>Name</th>
<th>Mode of Inheritance</th>
<th>Some Typical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usher</td>
<td>AR</td>
<td>Profound deafness; RP</td>
</tr>
<tr>
<td>Refsum</td>
<td>AR</td>
<td>Neurupathies; EKG abnormalities; ichthyosis; deafness; RP; hyposmia</td>
</tr>
<tr>
<td>Bassen-Kornzweig</td>
<td>AR</td>
<td>Abetalipoproteinemia; RP; acanthocytosis; gastrointestinal disease</td>
</tr>
<tr>
<td>Laurence-Moon-Bardet-Biedl</td>
<td>AR</td>
<td>Mental retardation; obesity; polydactyly; hypogonadism; RP</td>
</tr>
<tr>
<td>Cockayne</td>
<td>AR</td>
<td>Dwarfism; mental retardation; hearing loss; unusual facies; RP; dermatitis</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Mating Type</th>
<th>Number of Sibships</th>
<th>Segregation Ratio</th>
<th>Proportion of Sporadic Cases</th>
<th>Penetrance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal X Normal Nonconsanguineous</td>
<td>312</td>
<td>0.17 ± 0.06</td>
<td>0.11</td>
<td>—</td>
</tr>
<tr>
<td>Normal X Normal Consanguineous</td>
<td>18</td>
<td>0.45 ± 0.12</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Normal X Affected</td>
<td>71</td>
<td>0.29 ± 0.04</td>
<td>0</td>
<td>0.58</td>
</tr>
</tbody>
</table>
the most significant finding from the segregation analysis is the evidence it provides that a small proportion of the cases are nongenetic in etiology.

In conclusion, genetic registries, such as the one described in this article, could have a nationwide impact on the diagnosis of hereditary disease and on genetic counseling for affected individuals and their families. Our research applies an innovative technique for the diagnosis of genetic diseases that could serve as a prototype to demonstrate the practical value of categoric genetic registries. This research will almost certainly lead to the recognition of new forms of hereditary deafness and retinitis pigmentosa which could be the first step in the development of specific therapies.

REFERENCES


The Genetic and Environmental Effects on Diabetes in Humans and Animals: An Overview

ROGER M. LORIA, PH.D., Assistant Professor, Departments of Microbiology and Pathology
LINDA A. COREY, PH.D., Assistant Professor of Human Genetics

Despite intense scrutiny the precise etiology of diabetes mellitus remains unclear. There appear to be two major forms of diabetes: juvenile-onset or insulin-dependent diabetes, and late-onset or insulin-independent diabetes. The late-onset form, in itself, may be etiologically heterogeneous. Either form may occur at any age, with a clear distinction between the two often being difficult to make. Juvenile-onset diabetes, representing 5% to 10% of all cases, is charac-
characterized by abrupt onset, clinical manifestation of hyperglycemia and ketoacidosis, and generally by a requirement for exogenous insulin; in maturity-onset diabetes plasma levels of insulin are usually normal or elevated and the abnormality in glucose metabolism results from a decrease in the number of insulin receptors rather than a deficiency of the hormone itself.

Genetics of Diabetes in Man

It is generally accepted that there is an hereditary predisposition to diabetes, but there is little agreement as to its mode of inheritance. Early investigators concluded that diabetes was inherited in a simple Mendelian fashion, probably as an autosomal recessive trait. Subsequent studies, however, suggest that diabetes may be controlled by a number of genes whose final expression is influenced to varying degrees by the environment. It is still impossible to resolve the multifactorial versus monogenic controversy from the data and analytical methodologies now available. Diabetes is undoubtedly heterogeneous and this fact, as well as the late age of onset in many cases, greatly complicates genetic analysis. For example, striking differences in concordance rate among identical twin pairs who have late-onset diabetes (92%) compared with identical twins with early-onset diabetes (52%) provide strong evidence that the two forms are etiologically distinct. Concordant juvenile-onset pairs also had a positive family history of diabetes more frequently than discordant pairs, suggesting that some cases are predominantly genetic, and others environmental, in etiology.

Genetics of Spontaneous Diabetes Mellitus in Animal Models

A variety of animal models with spontaneous diabetes mellitus have been reported in the literature (Table 1). Apparently many different mutations can lead to spontaneous diabetes. Simple autosomal recessive inheritance is displayed in db/db, ob/ob, and others.

<table>
<thead>
<tr>
<th>Animal Name</th>
<th>Mutation &amp; Chromosome number</th>
<th>Inheritance</th>
<th>Obesity</th>
<th>Hyperglycemia</th>
<th>Elevated Serum Insulin</th>
<th>Ketosis</th>
<th>Changes in Islet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes C57Bl/Ks</td>
<td>db/db 4</td>
<td>Autosomal recessive</td>
<td>++</td>
<td>+++</td>
<td>transient+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Obese C57B1/6</td>
<td>ob/ob 6</td>
<td>Autosomal recessive</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Yellow A5/a</td>
<td>Autosomal dominant</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>KK mouse</td>
<td>Polygenic</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NZO</td>
<td>Polygenic</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>PBB/Ld</td>
<td>Polygenic (?)</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C3Hf × 1</td>
<td>Hybrids (fi)</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Spiny mouse</td>
<td>Polygenic</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Sand rat</td>
<td>Polygenic</td>
<td>+</td>
<td>+++</td>
<td>transient</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Zucker rat</td>
<td>fa/fa Autosomal recessive</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chinese hamster</td>
<td>Polygenic (4 genes)</td>
<td>-</td>
<td>+++</td>
<td>transient</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>S. African hamster</td>
<td>Polygenic</td>
<td>-</td>
<td>+++</td>
<td>(?)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
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</table>
and fa/fa rodents; autosomal dominant A\(^y\) inheritance is apparent in the yellow mouse; and finally, polygenic inheritance has been invoked for the KK, NZO, PBB/Ld mice strains, rats, and hamsters.

The clinical syndromes expressed by these rodent models will in most cases involve obesity and/or hyperglycemia as seen in the db/db, ob/ob mice, and the fa/fa rat with or without ketosis; hyperglycemia without obesity is evident in the Chinese and South African hamster; while marked obesity without overt diabetes was displayed in ob/ob, A\(^y\), NZO, PBB/Ld mice, and the Zucker rat. This wide range of symptoms illustrates the difficulty in determining whether genetic, environmental or maternal factors are the primary determinants in this disease.

It now seems clear that even where diabetes is associated with a specific mutation, that is, db/db in the C57B1/KsJ mouse, other genetic and/or environmental factors, such as genetic modifiers or viral infection, may be crucial in initiating this disease. The ob/ob (obese) mutation in the C57B1/6J inbred mouse leads to obesity without diabetes. Transfer of this mutation to the C57B1/Ks inbred mouse strain resulted in a C57B1/Ks ob/ob animal with a metabolic disorder strikingly similar to the diabetic mutant (db/db) itself. These results clearly indicate that two separate mutations which are known to be located on different chromosomes can give rise to indistinguishable phenotypes when placed on the appropriate genetic background.

Environmental Factors in Diabetes Mellitus

Environmental factors such as dietary intake,\(^{10}\) gross obesity,\(^{11}\) pregnancy, increased levels of estrogen in females,\(^{12}\) and other endocrine changes have been correlated with onset of diabetes mellitus. Finally, infectious agents such as congenital rubella, group B coxsackieviruses, and mumps have also been shown to be associated with this syndrome.\(^{13}\) There is good reason to believe that congenital rubella may lead to diabetes mellitus in up to 20% of children who have been infected in utero.\(^{14}\) Similarly, mumps virus has long been recognized as a cause of pancreatitis in man, and scattered reports suggest that persistent diabetes mellitus may sometimes appear one to eight weeks after infection.\(^{14,15}\)

Presently, three experimental models for virus-induced diabetes have been reported in the literature. The first involves infection by an unknown virus in the guinea pig;\(^{16}\) the second model involves the experimental infection of mice with encephalomyocarditis (EMC) virus which specifically destroys B cells of the pancreas.\(^{13,17-19}\) Susceptibility appears to depend on genetic factors; some inbred strains are highly susceptible and others are not. These data clearly demonstrate that a viral agent belonging to the picornavirus group, can induce diabetes mellitus in susceptible animals. The third model system involves coxsackievirus B infection of the diabetic mutant mouse. Presently the best candidate for a causative viral agent of diabetes mellitus in man is coxsackievirus B. This agent has pancreatropic properties both in mice and humans\(^{20}\) and coxsackievirus B4 has been demonstrated in both the exocrine and endocrine sections of the pancreas in human newborns with encephalomyocarditis.\(^{21}\) In addition, extensive epidemiological studies have established a correlation between coxsackievirus B4 infection and acute-onset diabetes mellitus.\(^{22}\)

Recent studies carried out by one of us have shown that the db mutation in the mouse causes a significant increase in susceptibility to coxsackievirus B4, and a dose-effect correlation between the virus and the diabetes mutation was evident. These observations were well supported by histopathological findings of the pancreata of animals infected with this virus (Table 2).\(^{23}\)

These two animal models (EMC and coxsackievirus B4) involving picornavirus infection demonstrate how genetic factors can interact with the environment to cause diabetes. In the EMC virus model the nature of the genetic predisposition of the host is unknown, but is thought to be a recessive trait involving more than one gene.\(^{19,20}\) In contrast, the findings in the mouse demonstrate that a single mutation at the db locus is responsible for the increased susceptibility to diabetes in coxsackievirus B4 infection.

Histocompatibility and Immune Factors in Diabetes

Renewed support for the hypothesis that immune mechanisms may have an etiological role in diabetes mellitus has been provided from the association between the histocompatibility antigens HLA-B8, W15, CW3, and HLA-D with diabetes mellitus.\(^{10,24-27}\) The possibility that HLA-genes predispose the host to virus infection, resulting in B cell destruction and insulin-dependent diabetes has been suggested. Alternatively, the HLA-genes may be involved in the induction of autoimmune responses, which again may have been triggered by virus infection.\(^{2,16,27}\) The latter alternative is supported by the observation that the diabetogenic HLA-D8 marker
TABLE 2
Coxsackievirus B4 Edwards Infection in the Inbred Diabetic and Normal C57B1Ks Mouse

<table>
<thead>
<tr>
<th>Animal Genotype</th>
<th>Virus Dose in PFU Animal</th>
<th>% Mortality of CB4 Infected Animal</th>
<th>Histopathological Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^4</td>
<td>10^5</td>
<td>10^6</td>
</tr>
<tr>
<td>db/db</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>db/+</td>
<td>10</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>+/+</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

1 CB4E = Coxsackievirus B4 Edwards.
2 PFU = plaque forming units.
3 C57B1Ks inbred mice with the specific mutation were used. The db/db animals displayed only chemical diabetes; food intake was monitored and regulated.

has also been associated with an increase in autoimmune diseases.\textsuperscript{28} Finally, the evidence for, and significance of, autoimmunity in diabetes is still highly debatable and little information on the regenerative properties or repair mechanisms of B cells is available.

In summary, the evidence presented argues for genetic, environmental (viral), and immunological factors in diabetes mellitus. The complexity of the genetic factors in this syndrome is further emphasized by the heterogeneous nature of the disease itself, which may represent many different genetic entities. Insulin-dependent and insulin-independent diabetes mellitus may each be etiologically heterogeneous. Understanding of the interaction between genetic and environmental factors will permit the evaluation of their respective roles in the etiology of this syndrome.

Table 1 is adapted from Renold et al\textsuperscript{1} and Hunt et al.\textsuperscript{5}

REFERENCES
VI. CASE REPORTS

Syndrome Identification

What's in a name? This question is often asked of a genetic counselor when a syndrome is newly delineated. The brief case reports that follow demonstrate the importance of establishing precise diagnoses. They also emphasize that many of these syndromes are recognizable only after careful physical examination of the proband and family members, consultation with other subspecialties (for example, neurology, radiology, orthopedics, dentistry, pathology), and a review of the medical literature.

While it is true that there usually is no treatment for the basic condition, complications can often be anticipated and serious consequences averted. Thus, the diagnosis of Pierre Robin anomalad forewarns
the physician of the risk of airway obstruction in the prone position and of feeding difficulties in early infancy. Knowledge of cervical spine anomalies in chondrodystrophy alerts the neurologist to central nervous system signs or symptoms that may result from basilar brain compression.

A second benefit of syndrome identification is that a better prognosis regarding such important factors as longevity, morbidity, and ultimate adult height and mentality may be made. The dysmorphic infant with Saethre-Chotzen syndrome is not at substantial risk for mental retardation. Recognition of the earlier signs and dominant nature of the familial form of amyotrophic lateral sclerosis enables better counseling regarding longevity and morbidity.

Knowledge of the mode of inheritance provides risk figures for recurrence so that family planning can be practiced. It is not sufficient to state that the patient with a dominant condition has a 50% risk that each offspring will inherit the disorder; as demonstrated in the families with Pierre Robin anomalad and Saethre-Chotzen syndrome, some persons have minimal manifestations, thus reducing the significance of the existence of the condition. In the case of sex-linked diseases, as in the family with chondrodystrophy, identification of female carriers can establish a 50% risk that male offspring will inherit the disorder. Further studies in the family with ring 9 chromosome abnormality should establish whether the proband, her mother, or other family members are at risk for bearing anomalous offspring.

Often patients seen in our clinic have been evaluated elsewhere for one or more components (for example, short stature, dysmorphic features, mental retardation, congenital malformations) of their disorder, but a precise diagnosis has not been made. Identifying the syndrome provides a diagnosis and permits a proper focusing on the disease process. While evaluation is often time-consuming, the reward is a family gratified by the establishment of a diagnosis that will allow a better understanding of the condition.

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A Report of Familial Ring (9) Chromosome

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Ring chromosomes originate in the simultaneous occurrence of two breaks at opposite ends of the chromosome and the subsequent reuniting of the free ends to form a ring. They may be compatible with normal life, as only a fractional loss of genetic material has occurred, or they may lead to spontaneous abortion or to an offspring with severe physical and mental handicap attributable to significant genetic alterations.

The dearth of published case reports underscores
the rarity of familial ring chromosomes. De novo ring chromosomes have been described in all chromosome groups in man, but familial transmission of rings has been limited to chromosome 17, chromosome 18, chromosome 21, Palmer et al and by E. Engel, MD (oral communication, May 1977), and a G-group chromosome not identified by banding analysis. Since the formation of a ring chromosome results in the loss of genes, it is not surprising that the phenotype of the individual possessing the ring can resemble the phenotype resulting from isolated short or long arm deletions, a combination of both, or duplications of the short or long arm due to recombination during meiosis.

Case Reports

Case I [Figure (top left, III.5) and (top right)]: P.S. was the product of a 28-week gestation of a 24-year-old para 1, gravida 1, abortion 0 mother who developed severe toxemia accompanied by a sudden decrease in estriol level. Because of fetal distress, a Caesarian section was performed, and a 1,360 gm female was delivered on May 1, 1974. The baby sat alone at eight months, walked at 18 months, and had a vocabulary of three to four words at 18 months. In September 1975, she was referred by a pediatrician to the Medical College of Virginia for evaluation of her failure to thrive. Her length was 28 cm, weight 6,914 gm, and head circumference 39 cm (all below the third percentile); she showed slight bilateral epicanthal folds and normal ears. Other tests revealed a bone age of 12 months, normal skull films, 10/10 arches on dermatoglyphic analysis, normal intravenous pyelogram, normal sweat chloride, and a normal Denver Development Test.

The patient was reexamined in the Genetics Counseling Clinic on March 10, 1976. At that time her developmental age on the Denver Development Test was 14 months (8 months under chronological age), her vocabulary still contained only four words, and she still was not toilet-trained. Examination revealed an alert, active female child with microcephaly, proportionate small size, large but not simple or low-set ears, pointed teeth, prominent nose, wide down-
turned mouth, slight epicanthal folds, and no congenital heart defects. Her skull bones were fused.

The patient returned to the Genetics Counseling Clinic at MCV on April 15, 1977, when she weighed 9.525 gm, with a height of 85 cm. Her vocabulary had increased to about 12 words and one three-word sentence. Thyroid studies showed T₄, T₃, and serum-free thyroxin to be within normal limits.

Case 2 [Figure (top left, 11,5) and (top right)]: C.S. is a 27-year-old female and the mother of Case 1. Her medical history includes an appendectomy at age 12 years, a kidney infection at 16 years, and scarlet fever at 17 years. Following the birth of her first child, she elected to have a tubal ligation. On examination in April 1977, she appeared to be of average intelligence, had been graduated from high school, and was working as a processor at an electrical equipment plant. She was 149.87 cm tall and weighed 58.9 kg. Dermatoglyphics were normal.

Cytogenetic Studies
Case 1: Peripheral blood lymphocytes were cultured in vitro using a slight modification of the method used by Moorhead et al. Metaphase chromosomes were stained with the trypsin-Giemsa chromosome banding technique of Seabright. Twenty randomly selected metaphases were counted, and a modal number of 46 chromosomes was established. Five cells were photographed and karyotyped and a large ring chromosome identified as chromosome 9 was present in all cells. Chrome chromosome analyses established the breakpoints at region 2, band 4 in the short arm (p24) and region 3, band 4 (q34) in the long arm, indicating that the two breaks were near the terminus of the short and long arms, 46,XX,r(9)(p24;q34). A representative karyotype is shown (Figure, bottom left).

Case 2: At her own request, cytogenetic studies were done on the mother of the proband (11,5), the maternal grandparents (I, I and 2), and the proband’s father (II,6). Normal karyotypes were documented for the maternal grandparents and the proband’s father; however, a ring chromosome (9) was found in all cells scored from duplicate peripheral blood cultures from the proband’s mother. Chromosome banding analysis revealed her karyotype to be 46,XX,r(9)(p24;q34). A partial karyotype is shown (Figure, bottom right).

Discussion
Two aspects of these cases are of particular interest to the clinical geneticist and cytogeneticist. The first is the de novo origin of a ring chromosome in a mother with few, if any, phenotypic abnormalities; and second, the transmission of what appears, cytogenetically, to be the same ring chromosome—structurally unaltered—to her daughter whose major physical defects are short stature and microcephaly associated with mental retardation. One could argue that the ring chromosome is responsible for the phenotype of the affected daughter, or that the ring (9) in the daughter and the associated phenotype are unrelated because the mother possesses the same ring.

Ring chromosomes present the clinical geneticist with a challenging counseling problem because many aspects of their behavior during meiosis remain obscure. The way in which the ring chromosome participates in genetic recombination during meiosis, the occurrence or not of sister chromatid exchanges within the ring, and the subsequent genetic constitution of the ring will all determine whether a balanced or unbalanced gamete results. Although chromosome banding analysis allows the definitive identification of the involved chromosome and the accurate determination of the points of breakage, genetic determinations await the refinement of the human gene map.

It is possible that there is a tissue distribution difference of the ring (9) in the mother and daughter. Although only peripheral blood has been examined to date and no evidence of chromosome mosaicism documented, tissue mosaicism cannot be ruled out.

We are aware of two other patients possessing a de novo ring (9) with break points identical to those in our family, (p24;q34), reported by Fraisse et al, and by Y. Nakagome, MD et al (written communication, March 1976). There are many similarities between our patient and Nakagome’s patient: proportionate short stature, prominent nose, microcephaly, mental and motor retardation, and poor speech development. The dermatoglyphic findings in our patient are of interest in that the finger pattern showed 10/10 arches, similar to the case report of Fraisse et al, but in contrast to Nakagome’s patient in whom there was a high palmar triradius only.

It is not possible to make definitive phenotype-karyotype correlations based on three clinical cases, but the following observation can be considered. In our family, the proband’s phenotype could result from genetic alterations associated with the ring chromosome and the normal phenotype of the proband’s mother from a yet-to-be identified tissue mosaicism, or the contrasting phenotypes could be the result of the hemizygous expression of paternal recessive alleles. It is also possible that only minor genetic changes occurred at the time of de novo ring formation which the mother inherited. Finally, the potential influence of ascertainment biases in a family of this type must always be considered. If, for whatever reason, the ring chromosome is consistent with phenotypes ranging from normal to severely affected, one would be much more likely to observe severely affected sporadic cases and mildly affected parents of...
familial cases than to find severely affected parents with normal children.

REFERENCES


Dominantly Inherited Amyotrophic Lateral Sclerosis (Motor Neuron Disease)

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The term amyotrophic lateral sclerosis (ALS) was first introduced by Charcot to describe cases with mixed upper and lower motor neuron signs without sensory impairment. Later, the syndromes of progressive bulbar palsy (PBP) and progressive muscular atrophy (PMA) were recognized to be variations of the same pathological process, and ALS was used as an inclusive term to refer to these syndromes as well. Although some authors reserve the term ALS for the specific syndrome of mixed upper and lower motor neuron lesions and use the term “motor neuron disease” to refer to the constellation of syndromes, most cases...
of the literature on familial cases uses ALS as a generic title. We will adhere to this convention.

Initially, the disease was thought to be sporadic, with a stable incidence of 1 in 100,000 population per year. In the early 1950s, reports appeared of a high incidence of ALS in the Chamorro Micronesians of Guam and on the Kii Peninsula in Japan. Studies suggested that at least some of the cases of ALS among Chamorros might be familial, and at about the same time familial cases began to be reported in Caucasian populations.

**Case Report**

**Proband:** The proband (Fig 1, III-2) is a 55-year-old white female with known maturity-onset diabetes, hypothyroidism, hypertension, and hepatosplenomegaly diagnosed as cirrhosis with fatty infiltration on liver biopsy, who presented with a three-year history of progressive weakness in her legs, causing difficulty in walking upstairs and rising from a chair. For one year, weakness increasingly impaired her ability to pick up objects, and her writing had become illegible. There had been no episodes of acute exacerbation or remissions.

On examination, wasting and fasciculations of the tongue were noted, and her speech was slow and slurred, and the jaw jerk was hyperactive. There was weakness and wasting in the legs, and all reflexes were brisk with sustained ankle clonus, bilateral Hoffman signs, and bilateral positive Babinski responses. Sensation was normal in all modalities. VDRL, serum B₁₂, cerebrospinal fluid (CSF), myelogram, and computerized axial tomography (CT) scans were all normal. An electromyogram (EMG) showed changes consistent with ALS, especially in the C₆ and T₁ nerve root distributions.

**Other Family Members:** It can be seen from the pedigree (Fig 1) that the proband's mother (II-3), maternal aunt (II-4), cousin (III-10), niece (IV-2), and two brothers (III-1, III-8) have all been diagnosed as having ALS. The age at onset has been variable, as has the duration of the disease in the different family members.

The offspring of the proband are presently under investigation. The eldest son (IV-3) has definite upper motor neuron signs in the legs with brisk reflexes and bilateral Babinski responses which, combined with the family history, are highly suggestive of ALS. Two offspring (IV-4 and IV-5) show minimal signs of muscle wasting and slightly brisk reflexes. The diagnosis of ALS here is less certain, but the possibility exists that these two brothers are also affected. The two youngest children (IV-6 and IV-7) show no sign of the disease. EMG studies may help establish a definite diagnosis in those family members at risk.

**Discussion**

**Evidence for Heterogeneity in ALS**

There are three recognizable clinical forms of classical, sporadic ALS. In progressive bulbar palsy (PBP), muscles innervated by the medulla are primarily affected. The presenting symptoms are dysarthria and dysphagia of insidious onset. The tongue atrophies and shows conspicuous fasciculations. Palatal involvement is indicated by progressive speech difficulties and regurgitation of food through the nose. Whereas PBP is the result of a lower motor neuron (LMN) lesion, it is frequently accompanied by upper motor neuron (UMN) involvement of the cranial nerves leading to "pseudobulbar palsy." Here the tongue is again small but tends to be spastic, and the jaw, palatal, and pharyngeal reflexes are exaggerated. In addition, there is marked emotional lability with attacks of inappropriate laughing or crying.

In a second form of the disease, muscles in-
nervated by the spinal nerves are primarily affected and UMN signs accompany those resulting from the lesions of the LMNs. Isolated UMN lesions are indicated by spasticity and brisk reflexes with bilateral Babinski responses, whereas isolated LMN lesions present with muscle wasting, weakness, and fasciculations with depressed or absent reflexes. The actual physical signs depend on the relative preponderance of UMN or LMN degeneration.

In a third form, LMN lesions predominate resulting in progressive muscular atrophy, but UMN lesions may occasionally be present. The symptoms and signs of LMN lesions are as described above and usually start in the hands and forearms.

Although these three clinical presentations can be distinguished, all are thought to be variations of the same process. Sensory impairment is not found, although patients may complain of muscle cramps or abnormal sensations resulting from muscle fasciculations.

The diagnosis is usually made on clinical grounds, although the EMG shows characteristic patterns of denervation with fibrillation, fasciculation, and giant action potentials indicating spontaneous activity.

Pathologically, extensive neuronal loss is evident with astrocytic gliosis of the UMNs and LMNs as well as the lower cranial nuclei. Cytoplasmic and nuclear shrinkage occurs with excessive accumulation of lipofuscin. In Guamanian cases, additional features include neurofibrillary degeneration of the orbital gyrus, Ammon horn, and basal ganglia. Granulovascular bodies are almost always present in the pyramidal cells of Ammon horn where eosinophilic crystalloid inclusion bodies are also seen.

Although reports of familial adult-onset ALS constitute only about 5% to 10% of the reported cases, at least 30 Caucasian families have been described in which there appears to have been a dominant mode of inheritance.

Among the Chamorros, nearly 50% of all reported cases are familial; however, extensive studies have failed to define a clear pattern of inheritance. It is possible that there may be a genetic predisposition to an environmental agent.

It is still uncertain whether these three entities, sporadic, Guamanian, and familial ALS, represent one or several etiologically different diseases. All three present with indistinguishable symptoms of muscular atrophy, lateral sclerosis, and bulbar palsy. Dementia, often seen in affected Chamorros, also occurs in both familial and sporadic cases. Involvement of the posterior columns has been described in a small number of inherited ALS cases but not with enough consistency to be distinctive.

Other parameters may permit a clear distinction of the three types (Table 1).

Sporadic ALS typically has a late onset in the early 50s; the first signs usually appear in the upper extremities, and the disease has a short duration leading to death within five years. In contrast, a review of 30 published Caucasian pedigrees indicates that familial cases more often have an onset in the early forties beginning in the lower extremities with a longer duration. Chamorro cases resemble familial ALS in onset and duration but are similar to sporadic

| TABLE 1 | Comparison of Sporadic, Guamanian, and Familial ALS |
|-----------------|-----------------|-----------------|
| Parameter value | Number of cases | Parameter value | Number of cases |
| Sporadic | Guamanian | Familial |
| Age of onset, yrs | 52.7 ± 2.7 | 46.1 ± 13.1 | 42.7 ± 11.1 |
| Onset under 30 yrs | 1% | 8% | 13.3% |
| Duration, yrs | 2.5 | 4 | 4.3 |
| Duration > 5 yrs | 8.1% | 34.3% | 36.5% |
| Initial Site | | | |
| Bulbar | 25% | 13% | 18% |
| Upper extremity | 41% | 64% | 26% |
| Lower extremity | 30% | 20% | 56% |
| Other | 4% | 3% | 0% |
| Sex ratio: male:female | 1.86:1 | 1.94:1 | 1.13:1 |
ALS in sex ratio and site of initial involvement.\textsuperscript{17,20} The Chamorro population on Guam appears to be intermediate in many respects between the familial and sporadic types. However, until the etiology of ALS is known, it will not be possible to classify these diseases with certainty.

\textit{Genetic Counseling}

In this family, the pedigree suggests autosomal dominant transmission with complete penetrance, but in the absence of male-to-male transmission, a sex-linked dominant inheritance cannot be excluded. The genetic advice given to family members (consultands) will depend on the presence or absence of signs of the disease and their relationship to individuals known to be affected.

For example, a 47-year-old man, whose father has been diagnosed as having ALS, wants to know if he too will become affected and what is his risk of having an affected child. His a priori risk of having inherited the gene from his father is one half because of the dominant transmission of ALS. From a review of published familial cases, ALS appears to be a strongly penetrant trait with nonpenetration occurring in only 3\% of the cases.\textsuperscript{18,21} Figure 2 depicts a cumulative distribution of the age of onset of familial ALS in 52 males and 46 females culled from the literature. Patients who do carry the gene have only a one-fourth probability of reaching the age of 47 without manifesting the disease. The posterior or final probability that the consultand, age 47 with an affected father but no symptoms himself, will develop ALS is one fifth or 20\% (Table 2).\textsuperscript{22} The practical implication for genetic counseling is that the longer the consultand lives and is symptom free, the lower will be his risk of carrying the gene.

\textbf{Conclusion}

Familial conditions, such as ALS, Huntington chorea, dystrophia myotonica, polyposis coli, and the presenile dementias, in which individuals who carry the abnormal gene may not develop symptoms until late in the reproductive period, present difficult problems for the genetic counselor. Modification of the a priori probabilities by consideration of the age of the consultand, as described above, can make a considerable difference to risk estimates.

\textbf{REFERENCES}


A Case of Saethre-Chotzen Syndrome

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Saethre-Chotzen syndrome was described independently by the Norwegian psychiatrist, Saethre,\(^1\) and the German psychiatrist, Chotzen,\(^2\) in the 1930s; since that time many cases have been reported, some using the terms acrocephalosyndactyly, type III, and craniooculodental syndrome. Clinically, the syndrome is characterized by premature closure of the cranial sutures, low-set hairline, nasal septum deviation, brachydactyly, and ptosis.\(^3\) It is inherited as an autosomal dominant with complete penetrance and...
great variability in expression. Because of this variable in expressivity, the syndrome is difficult to diagnose in the less severe form without a positive family history.

Recently, an infant was admitted to the Medical College of Virginia newborn nursery who had many of the features of Saethre-Chotzen syndrome, as well as other findings which have rarely been reported in association with the syndrome.

Case Report

The patient is a black female infant who was born five weeks prematurely to a 17-year-old para 1, gravida 1 mother and a 23-year-old father. She weighed 1,830 gm and had a 27.5 cm head circumference at birth. At four weeks, she was transferred to the MCV Hospital with Group B-Beta streptococcal sepsis which was successfully treated with a ten-day course of penicillin and gentamicin. On admission, she was noted to have facial asymmetries, ptosis, hypertonia and a probable ventricular septal defect; she was referred for a genetics consultation.

Her family was evaluated for stigmata of Saethre-Chotzen syndrome. The child's father was unavailable for examination but was reported as having a face that was slightly asymmetrical with an under-developed left cranium and the left eye smaller than the right. These asymmetries were said to be more pronounced in childhood. The mother was examined and did not show any stigmata of the syndrome. No other family members were known to be affected.

The physical examination of the child revealed an acrocephalic skull with left-sided malar, frontal and anterior parietal bone hypoplasia, and microcephaly. Her face was asymmetric with left-sided ptosis; she had dystopia with generally small palpebral fissures, the left slit being shorter than the right. Her nasal bridge was flat, her nose had a broad tip and a long prominent philtrum, and her mouth was thin and down-slanting. The left ear was lower than the right and posteriorly rotated, but both ears were well formed. Her neck was short and displayed a decreased range of motion, though it appeared normal on x-rays. Her hips also displayed a 20 degree decrease in motion. She had two cafe-au-lait spots; one 2 x 3 cm on her back and one 2.5 cm in diameter in the groin area. There was a sacral dimple on her lower back. She had a grade IV/VI systolic murmur, probably caused by a ventricular septal defect.

Her extremities were slightly asymmetrical with the right arm and leg 0.5 cm longer than the left. She had two dimples on either side of each elbow and knee, a peculiar long longitudinal skin crease along both inner calves, and mild hallux valgus.

A dermatoglyphic analysis revealed an excess of whorls (six), one of which was a radial loop with a central pocket. She had an intermediate triradius. Both the a-b ridge count and the total finger ridge count were within normal limits.

X-rays of her skull and hips in the neonatal period were read as normal except for a normal variant of a lückenschädel deformity of the skull. Repeat x-rays of the skull at 6 months showed 1) cranial asymmetry with the left orbit smaller than the right, 2) coronal sutures that were patent but narrower than the other cranial sutures, and 3) a left temporal bone that was higher than the right (Figure-a). X-rays of the cervicothoracic spine at that time were normal. Chromosome analysis showed a normal female karyotype, and her serum calcium of 11 mg/100 ml was normal at 6 months.

A follow-up examination at age 8½ months showed all her physical measurements to be below the 3rd percentile with a length of 63 cm, weight of 13 lb 12 oz, and head circumference of 39.5 cm. She was generally hypertonic and
had poor head control. However, her motor milestones were not delayed; she was able to crawl and transfer objects from hand to hand by 8 months and sit without support at the age of 9 months.

From these physical and radiological findings the diagnosis of Saethre-Chotzen syndrome was made.

Discussion

The physical findings of 120 cases of Saethre-Chotzen syndrome were compared by O. A. Pantke et al. In facial appearance the patients were strikingly similar to each other and to our patient. More than 75% had ptosis, deviated septum, acrocephaly, and low-set frontal hairline. The facial asymmetries and unusual cranial contours in the syndrome probably result from premature closure of the coronal or sphenobasilar sutures. This may also account for the tear duct stenosis and the mild mental retardation which are occasionally found. Skull x-rays like the ones in this case are characteristic, showing premature closure of the sutures as well as asymmetries in bone and orbit size. Hypertelorism and dystopia canthorum are often observed. Optic atrophy may be an associated finding.

The ears are frequently small and low-set, with folded pinnae. Conductive hearing loss is an occasional finding. The palate is either high-arched or clefted. Dental anomalies such as missing teeth and peg-shaped or anomalous maxillary lateral incisors are important in differentiating this syndrome from the other acrocephalies.

The extremities may show mild syndactyly; brachydactyly is common. Clinodactyly and hallux valgus are sometimes noted.

The cardiac defect our patient exhibits is rarely found in Saethre-Chotzen syndrome; however, it was reported in Chotzen's original cases and in several individuals of another kindred.

The multitude of associated findings in this syndrome can not be explained on the basis of premature closure of the sutures alone, though this does account for many of the facial features. Other malformations such as the cardiac defect, dental anomalies, and defects of the extremities indicate that one gene has varied effects on many organ systems.

Our case further documents the association of cardiac defects with Saethre-Chotzen syndrome while also demonstrating many of the characteristic findings. The child's father probably represents a very mild expression of this dominantly inherited condition, demonstrating the wide variability of expression seen in this disorder.

REFERENCES


A New Familial Chondrodystrophy Simulating Parastremmatic Dwarfism

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Recent developments in tissue culture and enzyme analysis have made it possible to classify more precisely some of the skeletal dysplasias and to understand their pathophysiology; thus almost all seven clinical types of mucopolysaccharidoses are due to separate single enzyme deficiencies—one type, the Sanfilippo syndrome, has three subtypes, each with a different enzyme deficiency. The majority of the skeletal dysplasias have no definable biochemical abnormality and are classified on the basis of clinical and radiological findings and the mode of inheritance. The purpose of this report is to present a family with an apparently new type of chondrodystrophy.

Case Report

Proband: An 18-year-old male with a diagnosis of Morquio disease was brought to the Medical College of Virginia Genetic Counseling Clinic by his mother and sister. He was the product of a normal, uncomplicated pregnancy, labor, and delivery, and at birth was considered normal. At age 3 to 4 months his mother states that he appeared to be behind in his development, although he was said to have had a three-word vocabulary at 5 months and at 10 months could put three words together in a sentence. He was evaluated at 3½ years and found to have mild kyphosis of the dorsolumbar region, moderate genu valgus, and slight restriction of internal and external rotation, and extension, of both hips; the sternum was noted to be particularly prominent. Radiographic examination showed slight diffuse changes of chondrodystrophy throughout, with the changes in the spine appearing more marked (Figure, b, f). He was unsuccessfully fitted with braces in an effort to make him ambulatory.

The skeletal changes progressed and when first seen at the MCV Clinic in 1976, the patient was markedly dwarfed (length 36½ inches), with coarse facies showing hypertelorism, a flat, wide nasal bridge, anteverted nostrils, a lack of cartilage in the nose and ears, heavy eyebrows, strabismus, and searching nystagmus. There was no corneal clouding. There were marked contractures of the limbs with limited movement in all directions of the knees and hips. The wrists were thickened but otherwise normal. The fingers were hyperextensible and the metacarpal joints appeared enlarged. Extension of the left elbow was nearly normal, while the right elbow had a 20 degree limitation of extension. All of the joints appeared knobby and enlarged, accentuated by the thinness of the extremities (Figure, e). There was severe kyphosis with mild thoracolumbar scoliosis and the sternum protruded anteriorly to a very marked degree (Figure, d). Also noted were signs of trunk and upper-extremity ataxia. While in the hospital he showed marked difficulty in breathing, with wheezing and congestion. There was no hepatosplenomegaly nor an abnormality of the palate.

There appeared to be moderate psychomotor retardation with disproportionate speech impairment, but mentation was difficult to assess due to the difficulty in speech and the lack of formal education.

The skeletal survey revealed marked kyphoscoliosis of the entire spine with a universal platyspondyly without anterior beaking of the vertebral body. The end plates were markedly irregular with lacelike ossifications. No evidence of rhizomelia could be demonstrated. There were marked changes about the knees with flaring of the metaphyses and marked irregularities of the ossification centers, both metaphyseal and epiphyseal, with the same lacelike ossification seen in the spine (Figure, g). Films of the pelvis revealed shortening of the ilium bilaterally, with the same irregular lacelike ossification bordering the iliac bones and around the joint spaces (Figure, c). Marked deformities were present in the feet. All of the actively growing centers showed the same type of changes as the knees. There was no significant bowing of the long bones. The skull showed a marked thickening of the calvarium, both over the convexities of the
skull and at the base. A midline tomogram revealed the presence of basilar impression. These findings were felt to be inconsistent with a diagnosis of Morquio disease or any other spondylometaphyseal dysplasia.

Blood studies on the proband showed no unusual findings except for mild borderline anemia and an increase in platelets. An SMA 6 was within normal limits. SMA 12 was normal except for an increase in alkaline phosphatase. Urinalysis was within normal limits. An electrocardiogram showed a generalized grade I dysrhythmia, but the study was reported to be a technically difficult recording due to movement. Hearing sensitivity was within normal limits, with a bilateral 20-decibel hearing loss. A pulmonary lab report noted the presence of severe hypoxemia and moderate metabolic acidosis which was uncompensated.

An enzyme analysis of skin fibroblasts, (courtesy Dr. Thaddeus Kelley), showed normal levels of fucosidase, mannosidase, \( \beta \)-galactosidase, hexosaminidase, arylsulfatase, and \( \alpha \)-iduronidase. On two runs of radioactive sulfate accumulation the cultures from the proband were similar to the controls.

Other Family Members: An investigation of the family history revealed two other relatives, a brother and a maternal uncle, who appear to have had the same disease as the proband (Figure, a). Further, it appears that the proband’s mother is mildly affected, and a maternal aunt and maternal grandmother may show minor signs of the disease.

The proband’s brother was born in 1954 after a normal, uncomplicated pregnancy, labor, and delivery. He sat alone at 6 months, but walking was delayed until 18 months and even then he had trouble with his balance. A diagnosis of rickets was made at about 1 to 2 years of age and he was admitted to a children’s hospital in 1958 for evaluation of his deformities. Physical examination revealed severe kyphosis in the low dorsal and upper-lumbar spine and marked genu valgus with enlargement of the knee joints. Motion through the hip joints was slightly restricted in external and internal rotation and in extension. The radiologist’s report stated that there was “generalized disease of cartilaginous development,” and that the changes seen were consistent with a diagnosis of Morquio disease. The patient was seen again in 1961 at which time he was ambulatory; however, the deformities about the knees had increased as had the kyphosis. He was attending school and was reported to be doing well. A physical examination following admission to a hospital for respiratory stridor in 1970 noted him to be a grossly skeletaly deformed dwarf (height 40 in; weight 60 lb), with coarse facies, ocular hypertelorism, flaring of the alae nasee, saddle nose, pectus carinatum, and marked kyphoscoliosis. There was darting nystagmus but no corneal clouding. He was treated unsuccessfully with
aminophylline and died three days after admission, the cause of death being listed as terminal cardiorespiratory failure with interstitial pneumonia of viral etiology.

The proband's mother is a 51-year-old woman with coarse features and difficulty in walking. Her height is 62 in. Radiographs in 1976 showed mild degenerative changes in the distal phalanges of the hand and in the body of T-12 as well as partial collapse of the latter. Accentuated lordosis of the lumbosacral spine was noted with anterior lipping and mild degenerative changes. There was deformity of the feet with pes cavus bilaterally. The long bones showed early degenerative changes about the hips. The iliac bones were shortened and there was flattening of the acetabular roof. These changes were similar to, but much less severe than, those found in her son.

The proband's mother reported that she had had a brother, born in 1929, who was affected similarly to her two sons. He appears to have followed the same clinical course and died in 1950, reportedly from the same type of illness as his deceased nephew.

Also noted in the family history is a maternal aunt who walks with difficulty, although she is reported to be of normal height; the maternal grandmother has the same type of walking difficulty. The proband's two sisters, who have two different fathers, are of normal height and appearance and present a striking contrast to their mother.

The family history is very suggestive of X-linked inheritance, with the males being affected more severely than the females.

Discussion

On the basis of radiographic, biochemical, and ophthalmologic findings, Morquio disease and the other mucopolysaccharidoses were eliminated from the diagnosis. Of the spondyloepiphyseal dysplasias (SED), SED congenita, and SED tarda and SDT tarda brachyolmia type were considered. SED congenita is an autosomal dominant in which the deformities are present at birth and it is further differentiated from this case by its characteristic presence of rhizomelia and retinal detachment. SED tarda is a relatively mild form of X-linked recessive dwarfism in which the age of onset is between 5 to 10 years of age. SDT brachyolmia is also a mild form of dwarfism in which the skeletal deformities are not consistent with the radiographic findings in this case. None of the mucopolysaccharidoses nor the SEDs show the striking lacelike ossification described in our patient.

Parastremmatic dwarfism was considered because of the distinctive lacelike ossification of the bones; however, in a written communication, in February, 1977, with Dr. Langer, who first described parastremmatic dwarfism, it was noted that our patient did not have the classic twisting or bowing of the long bones. The essentially normal configuration of the proximal femora and the type of changes seen in the vertebral bodies differentiate this case from parastremmatic dwarfism which is considered to be an autosomal dominant disease.

We feel that this patient represents an X-linked form of dwarfism, not previously described, which is characterized by coarse facies, severe skeletal deformities involving nearly all bones, and a distinctive lacelike ossification of the iliac bones, the end plates in the spine, and the epiphyses and metaphyses of the knees and elbows. In such cases neurologic and cardiopulmonary complications lead to demise in late adolescence or early adulthood.

REFERENCES

Familial Occurrence of Pierre Robin Anomalad

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The Pierre Robin anomalad is a congenital defect consisting of micrognathia, glossoptosis, and cleft palate. While these three stigmata represent the classical triad of anomalies first described by Pierre Robin in 1923, less severe forms of the anomalad are seen with a variable expression of the microsigns of the disease. Dennison suggests that the determination of exactly what constitutes a mild degree of Pierre Robin anomalad is a subjective matter. While cleft palate is commonly present, it may sometimes be replaced by a high-arched palate. He reports that radiography shows that there is a short mandibular body joining the ramus at a more acute angle than normal. The more severe cases can lead to respiratory obstruction and death. Anatomically, the defect is quite clear; the body of the mandible is posteriorly displaced causing the tongue to block normal respiratory and alimentary pathways.

No mode of inheritance has yet been clearly established. While the majority of cases of this rare disorder (1:50,000 births) appear to be sporadic, there have been several reports of familial occurrence; cases of affected brothers have been presented and an X-linked subvariety exhibiting persistence of the left superior vena cava and atrial septal defect has been described. A family having maternal half-siblings with the Pierre Robin anomalad is reported here. The mother bore a single affected male and four normal females by her first husband and two affected females by her second husband. Strong evidence exists for dominant transmission through the mother, who herself exhibits mild characteristics of the disorder. We therefore propose that this family represents an autosomal dominant form of the Pierre Robin anomalad with variable expressivity.

Case Report

The proband (Fig 1, III,1) was born on July 22, 1976, of a 26-year-old mother and a 25-year-old father. She was the 8 pound 10½ ounce full-term product of a pregnancy complicated by maternal vaginal discharge which was treated with a cream and resolved in the second trimester, and leaking of amniotic fluid with spontaneous resolution from the fourth through the sixth months. The patient was born by a normal vaginal delivery, cried immediately, and did not need resuscitation. Midline cleft palate and micrognathia were noted at birth, with the lips intact. Other than two left preauricular skin tags and possible bilateral high frequency hearing loss, no other abnormalities were present. She breathed rapidly and became cyanotic when allowed to lie flat on her back with resultant backward displacement of the tongue. Postnatal jaundice was treated with phototherapy. She was discharged with special feeding instructions, but readmitted a month later due to poor weight gain. At two months she was noted to have a grade I
to II/VI systolic ejection murmur, but chest x-ray, electrocardiogram, and a cardiology consultation suggested that there was no organic heart disease present. The patient was hospitalized many times during the first months of life due to multiple episodes of pneumonia and the inability to maintain adequate nutrition. At age 7 months a tracheostomy was done which resulted in improved breathing and weight gain. At age 11 months the patient was admitted for bronchoscopy and removal of the tracheostomy tube; she was unable to breathe adequately without it, and the tube was reinserted. She died suddenly at the age of one year, but no postmortem examination was permitted.

The proband's sister (Fig 1, III,2), also affected with Pierre Robin anomalad, died two months after birth as a result of poor weight gain and a terminal bout of aspiration pneumonia. Their half-brother (Fig 1, III,3), who died 28 days after birth, had severe congenital heart disease, including persistent left superior vena cava, tricuspid atresia, atrial septal defect, right-sided aortic arch, and descending aorta in addition to the Pierre Robin anomaly. Of the four normal half-sisters of the proband (Fig 1, III,4,5,6,7), two have slightly high, narrow palates and small chins. The mother has a very high-arched palate and micrognathia. A lateral x-ray of the mother's jaw demonstrated that one mandibular ramus was smaller and narrower than the other, presenting as asymmetrical appearance, and the mandibular angle was steeper than normal (Fig 2).

Discussion
We have presented a family in which three children (1 male and 2 females) who had two different fathers were affected with the Pierre Robin anomalad. The mother exhibits some of the characteristic stigmata of this syndrome which strongly suggests that an autosomal dominant form of the Pierre Robin anomalad exists with variable expressivity.

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Normally palpable organs:
the edge of the liver descending, on inspiration, below the costal margin (A); the lower pole of the right kidney (B); the abdominal aorta (C); the descending colon and the sigmoid (D); the ascending colon (E); and occasionally the bladder (though rising of this organ beyond the pubis does not necessarily indicate disease).

Impossible to outline, unless diseased, distended or enlarged: the gallbladder, pancreas, stomach, small intestine, transverse colon and spleen.

The A. H. Robins G.I. Series consists of six booklets, designed to provide a quick, yet comprehensive review of basic procedures and practices in G.I. medicine—with particular emphasis on the physical examination as performed in the office or at bedside. If you have teaching responsibilities, limited quantities are available: Part 1—Inspection, Part 2—Palpation, Part 3—Percussion, Part 4—Auscultation, Part 5—Abdominal Pain and Part 6—Differential Diagnosis of Abdominal Disorders. Write to: The Medical Department, A. H. Robins Company, 1407 Cummings Drive, Richmond, Virginia 23220.
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Excerpted from Volume 2 of the G.I. Series on physical examination of the abdomen:

Normally palpable organs:

- the edge of the liver descending, on inspiration, below the costal margin (A);
- the lower pole of the right kidney (B);
- the aortic arch (C);
- the descending colon and the sigmoid (D);
- the ascending colon (E); and occasionally the bladder (though rising of this organ beyond the pubis does not necessarily indicate disease).

Impossible to outline, unless dissected, are the duodenum, the gallbladder, pancreas, stomach, small intestine, transverse colon and spleen.
Spasm reactor?

Donnatal!

each tablet, capsule or 5 ml (tsp.) of elixir (23% alcohol)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tr>
<td>Phenobarbital</td>
<td>(1/4 gr) 16.2 mg (warning: may be habit forming)</td>
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<tr>
<td>Hyoscyamine sulfate</td>
<td>0.1037 mg</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td>0.0194 mg</td>
</tr>
<tr>
<td>Hyoscine hydrobromide</td>
<td>0.0065 mg</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Each Donnatal No. 2 Tablet</th>
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<tbody>
<tr>
<td>Phenobarbital</td>
<td>(1/2 gr) 32.4 mg</td>
</tr>
<tr>
<td>Hyoscyamine sulfate</td>
<td>0.1037 mg</td>
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<tr>
<td>Atropine sulfate</td>
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</tr>
<tr>
<td>Hyoscine hydrobromide</td>
<td>0.0065 mg</td>
</tr>
</tbody>
</table>

**Indications:** Based on a review of this drug by the NAS/NRC and/or other information, FDA has classified the following indications as possibly effective: adjunctive therapy in the treatment of peptic ulcer, the treatment of the irritable bowel syndrome (irritable colon, spastic colon, mucous colitis) and acute enterocolitis. Final classification of the less-than-effective indications requires further investigation.

**Brief summary.** Contraindicated in patients with glaucoma, renal or hepatic disease, obstructive uropathy (for example, bladder neck obstruction due to prostatic hypertrophy) or a hypersensitivity to any of the ingredients. Blurred vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur at higher dosage levels, rarely at the usual dosage.

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