III. PRENATAL DIAGNOSIS PROGRAM

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,[1] followed in 1968 by Nadler.[2] 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.[3] 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[4] 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.

This is paper #41 from the Department of Human Genetics of the Medical College of Virginia and was supported in part by a Developmental Disabilities Grant from the Department of Mental Health and Mental Retardation of the Commonwealth of Virginia.

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TABLE 1
Relation of Maternal Age and Chromosome Abnormalities

Maternal Age	Distribution of total sample (%)	Distribution of mothers of children with chromosome abnormality (%)	Risk of chromosome abnormality by age group	
			N	Percentage
10–14	0.4	0	0/19	
15–19	11.0	5.3	1/490	0.20
20–24	34.4	42.4	8/1531	0.52
25–29	32.0	21.2	4/1423	0.28
30–34	14.3	5.3	1/635	0.16
35–39	6.2	21.2	4/277	1.44
40–44	1.4	5.3	1/62	1.61
45–49	.07	0	0/3	

Distribution of maternal ages. N = number of abnormalities divided by number in age group.

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 hemophilias, 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 recession 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%.[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 noninvasive 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)

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

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

TABLE 3
Medical College of Virginia Results of Antenatal Diagnosis
(9-1-73 to 7-1-77)

RESULTS	NUMBER
92XXXX	1
46XY	104
46XX	84
45XY-D-G+D/G	1
45XO	1
47XY+G	2
47XX+G	1
47XXX	1
Normal alpha-fetoprotein	21
Abnormal alpha-fetoprotein	1

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 (45XO) 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).

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Recent Advances in Cytogenetic Technology for Antenatal Genetic Testing

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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[1] and a method was developed[2] for the in vitro growth of peripheral blood leukocytes to yield metaphase chromosomes. The discovery[3] 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,[4] the 5M urea method,[5] and the acid-saline-Giemsa technique.[6] 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.[7] 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

This is paper #40 from the Department of Human Genetics of the Medical College of Virginia and was supported in part by a Developmental Disabilities Grant from the Department of Mental Health and Mental Retardation of the Commonwealth of Virginia.

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