Indications for Cytogenetic Studies: An Update

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Abstract

Evaluation of chromosomes has now become a widely accepted diagnostic tool in many areas of clinical medicine. Many physicians appear to equate chromosome analysis with genetic diagnostics without realizing that only a fraction of genetic diseases are associated with chromosome aberrations. Moreover, cytogenetic tests are complicated, time consuming and expensive, and most laboratories have the capacity to handle only a limited number of specimens. This paper provides a list of current indications for requesting cytogenetic studies.

Key Words: *cytogenetic studies, indications, prenatal, clinical*

It is estimated that chromosome aberrations occur in about 10% of human concepti (90% of which are eliminated as spontaneous abortions), 40% of first trimester abortions, and 5% of stillbirths and perinatal deaths. In liveborn the incidence of major chromsome abnormalities is estimated to be one in 156;[1] those with abnormal number of chromosomes (numerical aberrations) constitute 55% of cases; the remainder are due to structural changes in the chromosomes (structural aberrations). Among children with severe mental retardation and congenital malformations, as many as 10 to 15% have chromosomal abnormality.

Steady progress in methods of culturing cells from blood, bone marrow, solid tissues, amniotic fluid, and recently from chorionic villi, and advances in the staining techniques have permitted precise definition of the structure of individual chromosomes and identification of even small deletions and rearrangements. This has resulted in a phenomenal acceptance of cytogenetic studies not only in evaluation of genetic conditions but in many other areas of clinical medicine.

Cytogenetic laboratories generally are located in the genetics departments of larger universities, although smaller hospitals and even centralized commercial laboratories have begun providing this service. This testing procedure itself is complicated, labor intensive and expensive, and most laboratories have capacity to handle only a limited number of samples. A routine cytogenetic analysis of peripheral lymphocytes takes a minimum of 3-4 days, and that of skin or amniotic fluid takes 2-4 weeks. In severely malformed newborns in whom life sustaining surgical and medical measures are being contemplated, bone marrow cultures may be used to provide chromosomal diagnosis within 3-4 hours in order to facilitate the decision making process.

Indications for chromosomal study are considered below. Included are those related to patients and their relatives (clinical), and those for diagnosis of fetus (prenatal).

Clinical indications (Table 1)

1. Confirmation of clinical diagnosis of a known chromosomal syndrome

Cytogenetic confirmation is needed even in classic

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Table1
Clinical indications for Cytogenetic Analysis

1. Confirmation of a clinical diagnosis
2. Multiple congenital anomalies
3. Parents and siblings of individuals with structural aberrations.
4. Offspring of individuals with balanced structural rearrangement
5. Mental retardation
   a. Unknown etiology
   b. X-linked
6. Anomalous sex development
   a. Intersex; ambiguous genitalia
   b. Female with growth and/or abnormal primary and secondary sexual development
   c. Female or male infertility
7. Mendelian conditions
   a. Predisposing to increased chromosome breaks
   b. Roberts-SC phocomelia syndrome
8. Malignancies
9. Multiple spontaneous abortions
10. Products of conception
    a. Abortuses and stillbirths
    b. Hydatidiform moles

trisomies such as Down's syndrome, where clinical diagnosis is unequivocally established by the clinical and dermatoglyphic criteria. Cytogenetic studies are needed to distinguish a known chromosomal syndrome from a phenocopy, i.e., a condition which clinically mimics the phenotype of a known chromosomal syndrome. Additional indications include ruling out the presence of a mosaicism or a chromosomal translocation. A proband with mosaicism has a better prognosis for physical and mental development. In the latter event similar studies on parents and other relatives will be needed to detect a carrier status.

2. Multiple congenital anomalies

One begins the evaluation of an individual with multiple congenital anomalies by excluding a known non-chromosomal (monogenic, multifactorial or teratogenic) condition. A further step would be to carry out chromosome analysis. Although some chromosomal syndromes can be readily identified on clinical grounds, in a large proportion of cases the pattern of malformations may not be typical enough to be associated with a known chromosomal aberration. In fact it may have been produced by a different chromosomal abnormality. Clinical features such as low birth weight, poor postnatal growth, delayed psychomotor development, malformations in different organ systems, dysmorphic facies, abnormal palmar creases and dermatoglyphics are generally associated with abnormal chromosome constitutions, but these features lack specificity. If karyotype analysis of peripheral lymphocytes is normal and there is a lingering clinical suspicion of a chromosomal anomaly, analysis of another tissue (e.g., skin) is indicated to rule out possibility of mosaicism.

3. Parents and siblings of individuals with structural aberrations

Once a patient is identified to have a structural aberration (e.g., deletion, translocation, inversion, ring chromosome, etc.), chromosomes of both parents should be analyzed to determine if the abnormality is inherited. If one of the parents is a carrier of the balanced form of rearrangement, the investigations are extended to siblings and other relatives. If both parents have normal karyotypes, the patient's chromosomal abnormality most likely originated *de novo,* and additional family members need not be studied.

4. Offspring of individuals with balanced structural rearrangements

An individual with a balanced chromosomal rearrangement is at a considerably high risk to bear an offspring with an unbalanced chromosome complement. The risk will depend on (1) sex of the carrier parent, (2) the type of rearrangement, e.g., Robertsonian and reciprocal translocation, inversion, etc., and (3) the specific chromosome and the breakpoints. The risks are generally higher if the carrier individual is a female with Robertsonian tranlocation involving D (chromosomes 13-15) and G (chromosomes 21-22) groups chromosomes. Chromosome studies are indicated in all children and first degree relatives. In case of a reciprocal structural translocation the chance of having an offspring with unbalanced chromosome constitution is 11.7%, irrespective of sex of the carrier parent.[2]

5. Mental retartation

Chromosome abnormalities such as balanced structural rearrangements and sex chromosomal aneuploidies (XXX and XXY) could be reasons for mental retardation in patients who present with few or no dysmorphic features. Appropriate laboratory and other evaluations must be performed to rule out an inborn error of metabolism, developmental defect of the nervous system, or other specific mental retardation syndrome before requesting chromosomal analysis.

A marker chromosome called fragile X chromosome was described in males with mental retardation and macroorchidism.[3] The unusual X chromosome appears to have a constriction near the end of the long arm (XQ27.3)[4] producing a thin stalk extending from the main portion of the chromosome.

A culture medium deficient in folic acid induces the fratile site.[5] The X chromosome appears to be fragile in the area of the constriction and often breaks at this point. The abnormality reportedly occurs in 0.3 to 0.92% in general population,[6] in 2 to 10% of male mental retardates,[7] and in 30 to 50% of families with a history of X linked mental retardation.[8]

6. Abnormalities of sex development

Examples of abnormal sex development include ambiguous genitalia, primary or secondary gonadal and growth failure, and infertility. When a sex chromosomal abnormality is suspected in a patient, one may begin with determination of the sex chromatin (Barr body), a relatively inexpensive and less time consuming test. It is usually done by examining stained epithelial cells of the buccal mucosa and counting cells which show a densely staining mass of DNA in the interphase nuclei.

a) Ambiguous genitalia

Ambiguous genitalia are observed in intersexual conditions which are classified as either true hermaphrodite (individuals with both testicular and ovarian tissue), or pseudohermaphrodite (individuals with only one type of gonad, either testis or an ovary). In pseudohermaphrodites the chromosomal sex corresponds with the sex of the internal gonad (XY in testicular feminization and XX in adrenogenital syndrome). By contrast, true hermaphrodites may be sex chromosomal mosaics (e.g., XX/XY), or with normal XX or XY karyotypes. It is necessary to examine at least two different tissues (e.g., blood and skin) before accepting the diagnosis of XX or XY true hermaphroditism.

b) Abnormalities of growth or of primary or secondary sexual development

Female patients with proportionate short stature, absence of secondary sexual characters, and primary amenorrhea, occuring together or individually, should be investigated to rule out Turner's syndrome. Similarly, secondary amenorrhoea of undetermined etiology could be due to a sex chromosome mosaicism, an additional indication for cytogenetic studies.

c) Male or female infertility

Male infertility may be associated with chromosomal abnormalities. Chandley[9] reported a 2.2% incidence of chromosome abnormality in a survey of men with subfertility. About 7% of infertile men with sperm count below 10 million/ml have abnormal karyotypes.[10] Reduced fertility or infertility in females may be associated with 45, X mosaicism and strutural abnormality of X chromosome.[11]

7. Mendelian conditions

Cytogenetic analysis provides an additional tool for diagnosis of individuals with automosomal recessive conditions such as Fanconi anemia, Bloom syndrome, ataxia telangiectasia, xeroderma pigmentosum which predispose to increased tendency for chromosome breaks and rearrangements.[12] The evidence suggests that interference with the normal processes of the DNA repair system may explain the underlying defect in these conditions.

Roberts-SC phocomelia syndrome, characterized by tetraphocomelia, cleft lip and cleft palate and other multiple congenital abnormalities, is inherited as an autosomal recessive trait. The chracteristic occurrence of premature centromere separation in karyotypes of patients[13] offers a very useful laboratory marker in diagnosis of this condition.

8. Malignancies

It is now well recognized that many malignancies are associated with specific chromosomal aberrations. The aberrations are mostly balanced reciprocal translocations in leukemias and lymphomas in contrast to deletions and sometimes trisomis in solid tumors.[14] Chronic myelogenous leukemia and Burkitt's lymphoma are associated with reciprocal translocations between 9 and 22, and 8 and 14 chromosoms, respectively. The aniridia-Wilms tumor syndrome is associated with a deletion of the short arm of chromosome 11 (band p13), some cases retinoblastoma involve deletion of the long arm (q14) of chromosome 13, and in neuroblastoma the segment p31--36 of the short arm of chromosome 1 is deleted. Additional examples of malignancies associated with chromosomal aberrations include acute and chronic lymphocytic leukemia, acute nonlymphocytic leukemia, small cell lung carcinoma, and meningioma. Cytomenetic studies are important for genetic counseling (e.g., in retinoblastoma), and provide prognostic clues in other situations (e.g., better chemotherapeutic response of individuals with Philadelphia chromosome).

9. Multiple spontaneous abortions

About 8% couples experiencing two or more spontaneous abortions show cytogenetic abnormality;[15] there is an apparent positive relationship between the frequency of chromosome aberrations and the number of sponteneous abortions. Couples with a stillborn or malformed infant have much greater frequency of chromosomal abnormalities than those without such history. A chromosomal translocation or inversion in one of the parent may be repsonsible for the recurrent abortions. Cytogenetic studies of such couples allow for more precise genetic counseling and monitoring of future pregnancies.

10. Products of conception

a) Abortions and stillborns

About 40% of the fetuses (primarily first trimester spontaneous abortions) are found to have chromosome abnormality which include autosomal

Table 2

Prenatal Indications for Cytogenetic Studies

1. Advanced maternal age
2. Previous pregnancy with non-inherited chromosome anomaly
3. Abnormal parental karyotype.
4. Parent with balanced structural rearrangement
5. Mendelian conditions
6. Fetal sex
7. Multiple miscarriage and stillbirths
8. Fragile X syndrome
9. Uncertain and dubious indications
   a. Radiation exposure
   b. Elevated maternal antithyroid antibodies
   c. Double NORs
   d. Maternal anxiety
10. Low levels of maternal serum AFP levels

trisomies (52%), 45,X (19%), polyploids (22%), and 7% with other anomalies such as structural aberrations, mosaicism, or other monosomies.[1] Perinatal deaths (stillbirths and neonatal deaths) also show an overall frequency of 6.31% chromosome abnormality;[1] the chromosome abnormalities are similar in type of those found in newborns.[16] Karyotype analysis should be performed on all spontaneously aborted fetuses, stillborns and newborns, whether malformed or phenotypically normal. It is also required that all products of abortions induced because of prenatally diagnosed chromosomal anomaly should be studied to confirm the abnormality.

b) Hydatidiform moles

Hydatidiform moles represent an abnormal growth of the trophoblast. They occur about once in 1500 recognized pregnancies in Caucasian women, but more frequently in certain Far East populations (e.g., 1 in 200 in Taiwan). The partial moles contain an abnormal embryo with triploid chromosomal constitution, the triploidy resulting from fertilizations of an egg by two sperms.[17] Complete moles do not have an embryo and are thought to arise through fertilization of an empty egg by an X bearing sperm, and a subsequent doubling of the paternal chromosome set giving it a 46, XX chromosome complement.[18] A minority of moles have other chromosome constitution including trisomies and monosomis. More than 50% of choriocarcinomas arise from moles; about 2% of the partial and 10% of complete moles become malignant.[19]

Prenatal Indications (Table 2)

Cytogenetic tests are now being performed on fetal materials obtained by means of an amniocentesis in the second trimester or by a chorionic villi biopsy in the first trimester of a pregnancy, providing correct diagnosis in over 99.5% of cases. Because of the invasive nature of the procedures for obtaining fetal materials, prenatal diagnosis is indicated only for pregnant women with an increased risk having an affected child which can be detectd at a stage when termination of pregnancy, if needed, is still possible and permitted by law.

1. Advanced maternal age

The maternal age group of 35 years and greater is currently the single most numerous category receiving prenatal cytogenetic diagnosis. The incidence of Down's syndrome as well as other autosomal trisomies in the newborn has been observed to increased with the maternal age. The risk of having a fetus with chromosome abnormality is 1-2% for maternal age of 35-39 years, 2-5% for an age of 40-44 years, and 5-10% for an age of 45 years and greater.[20] Although some studies have implicated paternal age of 40 years and greater with increased incidence Down's syndrome,[21] most major studies have not found evidence for a positive paternal age effect.[22]

2. Previous pregnancy with non-inherited chromosome abnormality

Parents of a baby with a trisomy have an increased risk (1.42%) of a second non-disjunctional event,[23] not necessarily involving the same chromosome. The predisposition for another non-disjunctional event seems to be greater for younger women; a woman under the age of 30 years has approximately 20 times higher risk for a chromosome abnormality than a woman of the same age without such history. The increased recurrence risk may be attributed to (1) parental mosaicism, (2) a structural chromosome rearrangement, (3) a Mendelian gene producing a higher risk of non-disjunction, or (4) exogenous factors.

3. Abnormal parental karyotype

Individuals with sex chromosome aneuploidy such as XYY[24] and XXX[25], and those mosaic for sex and autosomal chromosomal trisomies are at an increased risk for bearing offspring with abnormal karyotypes. There have been several instances of women with Down's syndrome who have delivered infants with Down's syndrome. A high priority for prenatal diagnosis should be given to such situations.

4. Parent with a balanced chromosomal rearrangement

Currently less than 5% of prenatal diagnoses are performed because one of the parents is a carrier of either a reciprocal structural rearrangement or a Robertsonian translocation. Regardless of sex, a carrier of a reciprocal structural translocation has 11.7%[2] risk of having an offspring with an unbalanced chromosome constitution. The risk is greater if the length of the involved chromosomes is smaller, and when one of the involved chromosomes is an acrocentric chromosome. A couple with a D/D

Robertsonian translocation appears to carry a low risk, while a female carrier of a Robertsonian translocation involving D and G group chromosomes has 10-15% risk; the risk appears almost negligible for a carrier male.[2] For a parent carrier of a balanced inversion (except INV(9)QH) the risk for an offspring with an unbalanced karyotype is 4% for a carrier father and 7.5% for a carrier mother.

5. Mendelian conditions

Cytogenetic methods alone permit prenatal diagnosis of Fanconi anemia,[26] ataxia telangiectasia,[27] Bloom syndrome[28] and xeroderma pigmentosa,[29] the autosomal recessive conditions with increased tendency for chromosome breaks. They can also provide *in utero* diagnosis of Roberts SC phocomelia syndrome by observation of characteristic premature centromere separation in amniotic fluid chromosomes.[12]

6. Fetal sex determination

Fetal sex determination is indicated when a mother is an obligate carrier or at a high risk of being a carrier of an X-linked disease which cannot be diagnosed prenatally. It is always done by karyotype analysis and not by examination of sex chromatin. Parental desire for a sex determination without any medical indications is not an indication for undertaking an amniocentesis.

7. Multiple miscarriages and stillbirths

Couples with a history of stillbirths or a malformed infant, and those with history of infertility or subfertility carry an increased risk of bearing a fetus with abnormal chromosome and should be considered for prenatal diagnosis services. Couples with a history of repeated abortion but no stillbirth or malformed infant are not considered at higher risk.

8. Fragile X syndrome

The fragile X chromosome has been demonstrated in amniotic fluid cultures[30] and in chorionic villus samples.[31] Amniotic fluid cell culture appears to be about 92% reliable for detection of fetuses with the fra (X) chromosome; therefore prenatal diagnosis of fragile X should be regarded as an experimental procedure.[32] Improved cytogenetic methods and/or DNA studies will in the future provide a reliable diagnostic technique of fragile X chromosome.

9. Uncertain and dubious indications

There are several conditions (e.g., accidental radiation exposure, elevated maternal antithyroid antibodies, double NORs in parental karyotypes) where the association with fetal chromosomal abnormality has not been unequivocally established and should be given a lower priority. In others the studies have ruled out such an association (e.g., exposure to cancer therapy and other mutagens, parental alpha-1-antitrypsin (P1) types, and more frequent satellite associations in parents). In some countries like Norway, no consideration is given for maternal anxiety in providing prenatal diagnosis, while in Switzerland almost 25% of amniocenteses are performed for that reason alone.

10. Low levels of maternal serum alpha-fetoprotein

There is growing evidence to suggest that a low level of meternal serum alpha-feto-protein (MSAFP) in the second trimester is associated with birth of trisomic offspring.[33] This is emerging as an indication for amniocentesis.[34]

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