Regular review

Prenatal diagnosis and carrier detection in primary immunodeficiency disorders

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The individual forms of inherited immunodeficiency diseases are rare and the true incidence in the United Kingdom is not known. In countries where immunodeficiency registers have been established (Australia, Japan, Spain, and Sweden) an overall incidence of appreciable symptomatic antibody deficiency has been shown to be 1/11 000 live births, and that of severe combined immunodeficiency to be 14/million live births.¹ In the United Kingdom many of the severest forms are not diagnosed until several early deaths have occurred in a family, but with increasing awareness among paediatricians and with better laboratory facilities more of these children will be identified.

Despite rapid progress in the understanding of the underlying pathological mechanism of some of these disorders—for example, chronic granulomatous disease² and adenosine deaminase deficiency³—treatment is still far from adequate. Children with various forms of severe combined immunodeficiency will die of infections in early childhood unless bone marrow transplantation is undertaken. Bone marrow transplantation from a sibling with identical human leucocyte antigen is an option available only to less than 20% of cases, and mismatched HLA bone marrow transplantation is still associated with high morbidity and mortality.⁴ Patients with Wiskott-Aldrich syndrome and ataxia telangiectasia may survive into adulthood with appropriate support, but they have increasing morbidity and a much higher risk of developing lymphoreticular malignancy.⁵ With early diagnosis and aggressive treatment patients with chronic granulomatous disease are surviving into adulthood with varying degrees of chronic organ dysfunction, but many still die young of fungal infections, especially those caused by aspergillus.⁶⁻⁷ For patients with X-linked agammaglobulinaemia, immunoglobulin replacement treatment has prevented many of the infectious complications, but affected patients still develop chronic lung damage and may still be prone to fatal echoviral infection.⁸

In families who have already had a severely affected child, therefore, genetic counselling with the possibility of carrier detection and prenatal diagnosis is an indispensable part of good management. The severe inherited immunodeficiency disorders are listed in table 1 under either X-linked or autosomal recessive inheritance. With the recent unravelling of the underlying cellular, biochemical, or genetic defects of such disorders, prenatal diagnosis and carrier detection are becoming possible in many different ways.

Methods of prenatal diagnosis and carrier detection

GENE TRACKING

Direct analysis of the deoxyribonucleic acid (DNA) by Southern blotting⁹ has enormous potential for genetic prediction in diseases of strict Mendelian

Table 1 *Severe inherited immunodeficiency disorders*

Autosomal recessive inheritance
Severe combined immunodeficiency due to:
Adenosine deaminase deficiency
Purine nucleoside phosphorylase deficiency
Absence of HLA class I or II, or both (bare lymphocyte syndrome)
Reticular dysgenesis
Severe combined immunodeficiency in association with short limbed dwarfism
Leucocyte adhesion deficiency (leucocyte function antigen-1 deficiency)
Ataxia telangiectasia
Chronic granulomatous disease
Chédiak-Higashi syndrome
X-linked inheritance
X-linked agammaglobulinaemia
Severe combined immunodeficiency
Wiskott-Aldrich syndrome
Chronic granulomatous disease
X-linked hyperimmunoglobulinaemia M
X-linked lymphoproliferative disease
X-linked properdin deficiency
X-linked agammaglobulinaemia with growth hormone deficiency

inheritance. This permits prenatal diagnosis during the first trimester by analysis of chorionic villus biopsy specimens. The method used is called gene tracking.¹⁰ In most diseases, allelic genetic heterogeneity precludes elucidation of the mutation in all families, and gene tracking asks the question: 'Has the fetus or relative inherited the same relevant chromosomal region as a previously affected family member?'.¹⁰ Gene tracking requires a gene specific or closely linked DNA probe that detects a restriction fragment length polymorphism, which is used to distinguish each chromosome of the pair in key family members. It is independent of allelic genetic heterogeneity but only useful in families whose key members are heterozygous for that restriction fragment length polymorphism.

This approach is especially useful in diseases in which the underlying defect is unknown. As soon as a close linkage is established between the disease and a DNA probe, gene tracking may potentially be used in prenatal diagnosis and detection of carriers without knowing the defect involved. Recently, genetic linkage with restriction fragment length polymorphisms has been established for X-linked agammaglobulinaemia,¹¹⁻¹³ X-linked severe combined immunodeficiency,¹⁴ X-linked hyperimmunoglobulinaemia M,¹⁵ Wiskott-Aldrich syndrome,¹⁶ and X-linked lymphoproliferative disease¹⁷ (table 2).

For X-linked severe combined immunodeficiency (a disease characterised by a block in early T cell differentiation) the gene has been mapped to Xq11-q13 by linkage analysis with restriction fragment length polymorphisms in nine families.¹⁴ Similarly, the gene for the Wiskott-Aldrich syndrome was mapped to the pericentric region of the X chromosome in 10 families.¹⁶ There is no evidence of non-allelic genetic heterogeneity in these two diseases so far,^{14 16} and the linked probes established in these studies (cpX73 (DXS159) for X-linked severe combined immunodeficiency and L1-28 (DXS7) for the Wiskott-Aldrich syndrome)

may be used for prenatal diagnosis and detection of carriers with the proviso that not all families are informative for the known probes. Furthermore, sporadic cases or pedigrees without key members for analysis may also present difficulties with this approach. In the past it has not been possible to detect carriers in these two disorders, though prenatal diagnosis has been possible for severe combined immunodeficiency by analysis of the lymphocyte subpopulation of fetal blood in mid-trimester.¹⁸

For X-linked agammaglobulinaemia (a disease characterised by a block in differentiation from pre-B cell to B cell development¹⁹) there are three studies mapping the gene to Xq21.3-22.¹¹⁻¹³ There seems, however, to be a degree of non-allelic genetic heterogeneity^{12 20} that has complicated the approach of gene tracking in prenatal diagnosis and carrier detection. Fortunately on the present evidence the proportion of unlinked families to Xq21.3-22 seems to be about 10%.²¹ A method has been developed for the calculation of genetic risk that takes non-allelic genetic heterogeneity into account.²¹ With this approach it is still possible to use the linked probes S21 (DXS17) and pXG12 (DXS94) for genetic counselling,¹³ and it has been used successfully to exclude the diagnosis of X-linked agammaglobulinaemia in a male fetus in the first trimester.²¹

For X-linked lymphoproliferative syndrome and X-linked hyperimmunoglobulinaemia M the linkage was established in only one family with each disease. The gene for X-linked lymphoproliferative syndrome was mapped to Xq24-27 and that for X-linked hyperimmunoglobulinaemia to Xq24-27 as well.^{15 17} More information will be needed in further families to establish whether there is non-allelic genetic heterogeneity, and until more is known the use of gene tracking for genetic counselling is probably not recommended in these two disorders.

For X-linked chronic granulomatous disease, the

Table 2 Severe inherited immunodeficiency disorders in which gene tracking may potentially be used in prenatal diagnosis and detection of carriers

Disease	Genetic localisation	Probes linked to disease
X-linked agammaglobulinaemia	Xq21.3-q22	S21 (DXS17) pXG12 (DXS94)
X-linked severe combined immunodeficiency	Xq11-q13	cpX73 (DXS159)
Wiskott-Aldrich syndrome	Xp11-p11.3	L1-28 (DXS7)
X-linked hyperimmunoglobulinaemia M	Xq24-q27	43-15 (DXS42)
X-linked lymphoproliferative syndrome	Xq24-q27	43-15 (DXS42)
X-linked chronic granulomatous disease	Xp21.1	Gene specific
Properdin deficiency	Xp21.1-ccn	L1-28 (DXS7)
X-linked agammaglobulinaemia with growth hormone deficiency	Unknown	Unknown

gene was mapped to Xp21 (22), cloned,²³ and identified as the β chain of cytochrome b 245,²⁴⁻²⁵ but no restriction fragment length polymorphism has been identified with the gene specific probe yet to make gene tracking possible. Only about 10% of the mutations of X-linked chronic granulomatous disease are caused by a gene deletion²³ that can be detected by the gene specific probe; hence only in such families would it be possible to use the gene specific probe for prenatal diagnosis in the first trimester.

MEASUREMENT OF GENE PRODUCT

There are four severe inherited immunodeficiency disorders, all of autosomal recessive inheritance, in which the underlying biochemical or cellular defects are known and the normal gene products can be measured. These are adenosine deaminase deficiency, purine nucleoside phosphorylase deficiency, bare lymphocyte syndrome, and leucocyte adhesion deficiency (leucocyte function antigen-1 deficiency). Adenosine deaminase deficiency results in severe combined immunodeficiency and accounts for about 25% of the autosomal recessive forms,²⁶ and purine nucleoside phosphorylase deficiency results in a predominantly T cell defect.²⁷ Both enzymes play a part in the purine degradation pathway and their activities in either fetal red cells or amniotic fluid cells can be assayed rapidly to permit prenatal diagnosis in the mid-trimester as their activities at this stage of gestation are the same as those found in children and adults.²⁸ In an affected fetus there will be little or no enzymatic activity with accumulation of the deoxynucleotides in the red cells, and immunological investigation of fetal blood would show profound lymphopenia, severely depleted T cells, and no phytohaemagglutinin induced proliferation.^{28, 29}

Recently it became possible to measure adenosine deaminase and purine nucleoside phosphorylase in chorionic villi, so permitting prenatal diagnosis of such deficiencies in the first trimester.^{29, 30} There is still a problem, however, with this approach in some cases in distinguishing between the homozygous affected state and heterozygous normal³⁰ because 10-15% of infants with severe combined immunodeficiency who are deficient in adenosine deaminase still have low but detectable adenosine deaminase activity in lymphoid cells.³¹ The most reliable prenatal diagnosis of adenosine deaminase deficiency is therefore still by analysis in mid-trimester of fetal blood in which enzyme activities, deoxynucleotides, T cell subpopulations, and proliferative responses may be measured.²⁸ Detection of carriers is also possible by finding intermediate activity of the enzyme in the subject concerned.

In the bare lymphocyte syndrome the deficient gene products are the HLA molecules—either class I or II—which were shown to be secondary to defective synthesis³² associated with an abnormal transactive regulatory gene outside the major histocompatibility complex.³³ This syndrome results in a combined immunodeficiency with repeated bacterial and viral infections. Two affected fetuses have been diagnosed in mid-trimester by membrane immunofluorescence on blood lymphocytes and monocytes using specific monoclonal antibodies for HLA class I and II molecules.³⁴ Leucocyte adhesion deficiency presents as delayed cord separation and fulminating bacterial infection. The basic genetic defect is the result of the absence of synthesis of the mature β chain subunit of the leucocyte function antigen-1 heterodimer.³⁵ As the β chain is essential for assembly of the α/β molecule on the cell surface, it should be possible to perform prenatal diagnosis in the mid-trimester using monoclonal antibodies for leucocyte function antigen-1 α and β chains. This has not yet been attempted. It is not possible to detect carriers of either bare lymphocyte syndrome or leucocyte function antigen-1 deficiency as they express the HLA and leucocyte function antigen-1 molecules normally.

CELL FUNCTION OR DYSFUNCTION

The knowledge that the superoxide generating system in granulocytes is already functionally complete by 16 weeks' gestation enables one to diagnose X-linked chronic granulomatous disease prenatally in mid-trimester using the nitroblue tetrazolium test, which measures superoxide production.³⁶⁻³⁷ Normal fetal granulocytes function as well as normal adult granulocytes, and there are clear cut separations in these functions among an affected, a heterozygous, and a normal subject.³⁶ This is still the most reliable method of prenatal diagnosis of this disease except in cases where gene deletion can be shown by the gene specific probe. Even when a restriction fragment length polymorphism is found in the future, there will still be families in which gene tracking is not possible because the key members are not heterozygous for that restriction fragment length polymorphism and the simple functional nitroblue tetrazolium assay for prenatal diagnosis will still be useful.

X-linked lymphoproliferative syndrome is characterised by increased susceptibility to fatal Epstein-Barr virus infection, acquired agammaglobulinemia, and lymphoma.³⁸ Asymptomatic female carriers of X-linked lymphoproliferative syndrome have abnormal antibody responses to Epstein-Barr virus³⁹ and serial monitoring of antibody titres over a year or more may be of predictive use in

evaluating female subjects at risk of being carriers.

Another condition, ataxia telangiectasia, has been diagnosed prenatally by showing cellular dysfunction.⁴⁰ The diagnosis of an affected fetus was based on increased spontaneous chromosomal breakage in the amniotic cells and the presence of an abnormal clone with a 14/5 translocation. Prenatal exclusion has also been performed by analysis of chromosomal breakage of lymphocytes after small doses of irradiation.⁴¹

PRENATAL DIAGNOSIS BY SHOWING ABNORMALITIES OF A CELL SERIES

Having defined the ontogeny of various lymphocyte subpopulations in fetal life (table 3) it is possible to diagnose immunodeficiency disorders with absence of certain lymphocyte series prenatally.^{28 42-43} The technique depends on the ability to analyse small amounts of fetal blood obtained in mid-trimester, and was initially perfected for analysing mononuclear cell populations stained with fluorescent antibody on a fluorescent activated cell sorter.⁴² More recently this had been adapted to allow enumeration on slides after peroxidase staining (Linch and Levinsky, unpublished observations). It is primarily useful in the prenatal diagnosis of severe combined immunodeficiency syndrome irrespective of its cause, as long as the abnormality in the lymphocyte cell series has been well defined in an affected child in the family seeking prenatal diagnosis.^{28 42-43}

It is not advisable to use this technique for the prenatal diagnosis of X-linked agammaglobulinaemia because of the variability in the numbers of B cells in a normal fetus in mid-trimester.⁴² If normal numbers of B cells were seen, however, this would be sufficient to exclude the diagnosis.⁴³ In the Chédiak-Higashi syndrome the neutrophils have large inclusion bodies in the cytoplasm⁴⁴ and as

enough neutrophils may be found in fetal blood at 18-20 weeks' gestation, prenatal diagnosis of this autosomal recessive condition should be possible and it has already been excluded in three fetuses.⁴³

CARRIER DETECTION BY EXPLOITING THE EFFECTS OF LYONISATION

The Lyon hypothesis states that permanent inactivation of one of the two X chromosomes occurs at random in every somatic cell in the female subject early in embryogenesis.⁴⁵ The pattern of inactivation of X chromosomes is then transmitted in a stable fashion to all progeny cells. Therefore in a female carrier of an X-linked immunodeficiency disorder there will be two distinct populations of mature cells if the gene is not essential for development of that cell type, but there will only be one population of mature cells with the active normal X-chromosome that confers selective advantage if the gene is essential for development of that cell type.

In X-linked chronic granulomatous disease the gene is not essential for the development of the neutrophils, but pivotal in enabling effective killing of bacteria. There are therefore two populations of neutrophils in a female carrier, one that can reduce nitroblue tetrazolium and one that cannot.³⁶ This is the basis of carrier detection of X-linked chronic granulomatous disease by the nitroblue tetrazolium test. The female carriers are prone to a lupus like syndrome,⁴⁶ and severe lyonisation can lead to a clinical picture of chronic granulomatous disease in some cases.⁴⁷

In X-linked agammaglobulinaemia the gene is essential for the development of B cells. There is therefore only one population of mature B cells with the active normal X chromosome in a female carrier.^{48 49} This was first established by finding mature B cells in female carriers of X-linked agammaglobulinaemia who were heterozygous for

Table 3 *Lymphocyte ontogeny in humans*

<i>T cells</i>	<i>No of weeks after conception</i>	<i>B cells</i>
Epithelial thymus	5	
	6	
	7	
Thymus becomes lymphoid	8	Cμ ⁺ pre-B cells in liver
	9	
Corticomedullary differentiation in thymus	10	Sμ ⁺ B cells in liver
	11	Sγ ⁺ B cells in liver
	12	Sα ⁺ B cells in liver
Hassall's corpuscles appear	13	Most μ ⁺ B cells have δ
	14	
T cells in spleen	15	B cells in blood
T cells in blood		and spleen

glucose-6-phosphate dehydrogenase and who only expressed one of its isoenzymes, though their T cells and granulocytes expressed both isoenzymes.⁴⁸ Using recombinant DNA probes, which simultaneously detect restriction fragment length polymorphisms and patterns of methylation of X-chromosome genes, Fearon *et al*⁴⁹ showed the pattern of random X-inactivation in T cells and granulocytes in female carriers of X-linked agammaglobulinaemia, but it did not occur in B cells where only one of the two X chromosomes was preferentially active. Using the same technique to examine X-chromosome inactivation patterns female carriers of X-linked severe combined immunodeficiency and of the Wiskott-Aldrich syndrome can be detected by examining their T cells, which show patterns of non-random X-inactivation.⁵⁰⁻⁵¹ This strongly supports the hypothesis that T cells have the intrinsic defect in both disorders, in which the genes are essential for the development of T cells. This technique is, however, limited to about 50% of the female subjects who are heterozygous for the DNA probes used, which are from the 5' end of the genes encoding the phosphoglycerate kinase and hypoxanthine phosphoribosyltransferase.

Conclusion

The severe immunodeficiency disorders listed in table 1 are all 'single gene' disorders that are rare, and therefore prenatal diagnosis and detection of carriers will be limited to families which have already had an affected child. When further progress is made in elucidating the basic biochemical defect in these disorders, precise prenatal diagnosis, or carrier detection, or both, will become possible by measurement of the absent or aberrant protein or enzymes. Before that is possible, however, the strategy for diseases in which the underlying defect is unknown remains mapping of the gene by linkage study using restriction fragment length polymorphism; this would permit prenatal diagnosis and detection of carriers. There are, however, constraints associated with analysis of restriction fragment length polymorphism and there is always less than 100% certainty if gene specific probes are not used.

The purpose of the detection of carriers should be to assist informed decision making before pregnancy. Accurate information about possible risks will permit some couples to avoid pregnancies that are at high risk, while other couples may decide to embark on pregnancies that they would, without this knowledge, have avoided.⁵²

The purpose of prenatal diagnosis is to provide the option of not continuing with an abnormal pregnancy, but should the mother wish to continue with the pregnancy the prenatal diagnosis allows the family time to prepare to care for an affected child⁵² or to attempt appropriate treatment as soon as possible after birth. This is particularly relevant for those with severe combined immunodeficiency in which both matched and mismatched bone marrow transplants are curative, and the results are successful in about 90% of HLA matched, and 50-60% of HLA haploidentical, transplants.⁴

Although an increasing number of these conditions are amenable to diagnosis in the first trimester by analysis of chorionic villus biopsy specimens, at present most can only be diagnosed by analysing fetal blood taken at 18-20 weeks' gestation. Legislation to limit the time of abortion to the eighteenth week would deny such families the option of prenatal diagnosis.

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