Gene mapping and medical genetics

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Molecular genetics of human chromosome 21

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SUMMARY Chromosome 21 is the smallest autosome, comprising only about 1.9% of human DNA, but represents one of the most intensively studied regions of the genome. Much of the interest in chromosome 21 can be attributed to its association with Down's syndrome, a genetic disorder that afflicts one in every 700 to 1000 newborns. Although only 17 genes have been assigned to chromosome 21, a very large number of cloned DNA segments of unknown function have been isolated and regionally mapped. The majority of these segments detect restriction fragment length polymorphisms (RFLPs) and therefore represent useful genetic markers. Continued molecular genetic investigation of chromosome 21 will be central to elucidating molecular events leading to meiotic non-disjunction and consequent trisomy, the contribution of specific genes to the pathology of Down's syndrome, and the possible role of chromosome 21 in Alzheimer's disease and other as yet unmapped genetic defects.

Until recently, our knowledge of the organisation of chromosome 21 DNA was limited to the level of resolution afforded by cytogenetic approaches. Using a variety of staining techniques, the long arm has been subdivided into three bands, recognisable by their width and intensity of staining. With high resolution techniques applied to prometaphase chromosomes, some five to nine bands are distinguishable. By contrast, three distinct regions can be identified on the short arm of chromosome 21. The first is a highly fluorescent area near the telomere that exhibits extensive variation with quinacrine staining. Proximal to this fluorescent staining region is a secondary constriction that stains with silver and contains ribosomal RNA genes.^{1 2} Finally, there is a small area next to the centromere that is variable by C banding techniques and believed to contain highly repetitive DNA sequences.³

In the past few years, the necessary foundation for achieving a complete understanding of the structure of chromosome 21 at the molecular level has begun to emerge. The availability of chromosome specific recombinant DNA libraries has resulted in a rapid

expansion of the number of characterised DNA segments for this chromosome. Similarly, the construction of somatic cell hybrid lines containing translocated derivatives of chromosome 21 has facilitated the regional mapping of both genes and DNA markers. These advances have led directly to the construction of preliminary physical and genetic maps of the chromosome which promise to become increasingly detailed. As they evolve, collections of genetically and physically localised DNA segments will probably form the basis for attempts to develop a complete restriction map for chromosome 21, as a prelude to determining the entire DNA sequence of the chromosome. In parallel with these efforts, the knowledge gained from mapping approaches will facilitate investigations of the molecular mechanisms underlying the aetiology and pathogenesis of Down's syndrome and permit an evaluation of the potential role of chromosome 21 in numerous inherited disorders, particularly familial Alzheimer's disease.

Genes assigned to chromosome 21

As with the other human chromosomes,⁴ genes have been assigned to chromosome 21 by a number of

different approaches, particularly somatic cell genetics. In addition to conventional strategies, however, lymphocytes and fibroblasts from Down's syndrome patients have been used to show increased gene expression or enzyme activity for genes on chromosome 21 whose products can be quantified. To date, 17 genes have been assigned to chromosome 21 although 10 of these assignments remain provisional.

THE RIBOSOMAL RNA GENE CLUSTER

The ribosomal RNA gene cluster on chromosome 21 (RNR4),^{1,2} like those on the other four acrocentric chromosome pairs, contains approximately 30 tandemly repeated rRNA gene copies⁵ per chromosome and is located in the secondary constriction, or stalk region, of the short arm, 21p12⁶ (fig 1). The secondary constrictions are associated with cell nucleoli (nucleolar organising regions or NORs) and exhibit heritable,⁷ chromosome specific differences in affinity for ammoniacal silver stain.⁸ The staining

properties of the NOR also appear to be associated with an active functional state of the rDNA.⁹ A type of NOR variant, designated double NOR (dNOR) may play a role in promoting meiotic non-disjunction, thus predisposing subjects to producing offspring with Down's syndrome.¹⁰

Studies of species specific rDNA polymorphisms of man and ape¹¹ have led to the suggestion that genetic exchanges occur between ribosomal genes on non-homologous acrocentric chromosomes. This view is indirectly supported by evidence from cytological studies showing an association during mitotic metaphase of the satellite region of different acrocentric chromosomes,¹²⁻¹⁴ and by electron microscopic analysis of human meiotic oocytes showing that rRNA genes from different acrocentrics are structurally confined to the same nucleolar fibrillar centre.¹⁵ The sites of unequal homologous recombination leading to the 'concerted evolution' of the ribosomal gene family may be small DNA repeat elements found near the 28S rRNA gene.¹⁶

FIG 1 Location of genes and cloned DNA segments that are regionally assigned to chromosome 21. Genes and cloned DNA segments are designated by gene symbols assigned by the Human Gene Mapping Workshop Nomenclature Committee.¹⁷³ Cloned DNA segments are shown as numbers that make up the last part of the 'D alphanumeric' symbol (D21S...) used to identify the chromosome 21 origin of the DNA segments. Laboratory clone designations are used for DNA segments that have not yet been assigned 'D numbers'. Genes or DNA segments that detect polymorphisms are underlined. Brackets to the left of genes and DNA segments indicate the most likely location for the genes and DNA segments based on *in situ* hybridisation or somatic cell hybrid studies. In some cases, the assignment represents the shortest region of overlap that includes data from more than one study. See references 23, 35, 38, 65, 88, 109, 130, 137, 174, 175 for regional mapping genes or DNA segments by Southern analysis of somatic cell hybrids, or references 64, 83, 93 for localisation by *in situ* hybridisation studies.

tendency of the ribosomal genes to engage in non-homologous chromosome recombination has been proposed as a possible mechanism, via the formation of dicentric chromosomes, for promoting meiotic non-disjunction.¹⁷

SUPEROXIDE DISMUTASE 1

Two distinct forms of superoxide dismutase, the enzyme responsible for catalysing the removal of superoxide radicals, exist in eukaryotic cells: a mitochondrial manganese containing enzyme and a cytoplasmic copper/zinc containing enzyme. The locus (*SOD1*) for the cytoplasmic Cu/Zn form (*SOD1*) was one of the first gene assignments to chromosome 21.¹⁸ A *SOD1* cDNA clone has since been isolated,¹⁹ characterised,²⁰ and sequenced.²¹ Somatic cell hybrids were initially used to regionally map *SOD1* to band 21q22.²² The same approach has subsequently refined the localisation to 21q22.1²³ (fig 1). Because *SOD1* maps to the q22 band of the long arm, a region of the chromosome considered to be essential for the full expression of the Down's syndrome phenotype, it is one of the candidate genes considered for possible involvement in the pathogenesis of Down's syndrome. Subjects with Down's syndrome show an increase of about 50% in *SOD1* activity owing to higher levels of *SOD1* protein,²⁴⁻²⁷ while similar studies have shown that monosomy 21 patients have the expected half normal level of *SOD1* activity.²⁸ An RFLP at the *SOD1* locus has been used to achieve the first localisation of a functional gene on the genetic linkage map of chromosome 21²⁹⁻³¹ (fig 2).

ENZYMES FOR PURINE BIOSYNTHESIS

Phosphoribosylglycinamide synthetase (PRGS or GARS), phosphoribosylaminoimidazole synthetase (PAIS or AIRS), and glycinamide ribonucleotide transformylase (GARTF) are responsible for the catalysis of the third, fourth, and sixth steps, respectively, of the de novo purine biosynthetic pathway. Early somatic cell hybrid studies showed synteny for *SOD1* and PRGS activity.³² Human fibroblasts trisomic for chromosome 21 were then shown to have increased levels of PRGS activity.^{33 34} PRGS was regionally assigned to 21q22²² and eventually localised to the same sub-band as *SOD1*, q22.1 (fig 1), by dosage studies of partial monosomies and full and partial trisomies.³⁵ The assignment of *PAIS* to chromosome 21 is currently provisional. Evidence from somatic cell genetic studies suggests that PRGS and *PAIS* share a single locus.³⁶ Synteny for *PAIS* and *SOD1* in orang-utans and chimpanzees has also been demonstrated, providing additional support for the *PAIS* assignment.³⁷ The third enzyme of de novo purine

FIG 2 A genetic linkage map of the long arm of chromosome 21. The marker order is the most likely order based on an analysis of families that were jointly informative for at least three of the markers. The genetic distance (indicated as percent recombination) between the markers is based on the results of two point analysis using the computer programme LIPED.

biosynthesis, GARTF, is also encoded by a gene mapped to 21q11.2→q22.2 (fig 1) by studies that raise the possibility that the three enzymes PRGS, PAIS, and GARTF may constitute a multifunctional protein encoded by a single locus on chromosome 21.³⁸ Since PRGS has been shown to be raised in Down's syndrome cells, it is likely that PAIS and GARTF activities are similarly increased with possible consequences for the pathogenesis of Down's syndrome.

CELL SURFACE ANTIGENS AND RECEPTORS

Some of the first studies leading to the assignment of a gene to chromosome 21 explored the association of this chromosome with the expression of the cellular antiviral state induced by exposure to human interferon (HuIFN). Since the cellular response to IFN is species specific, for example,

mouse cells are insensitive to HuIFN, somatic cell hybrids were used to identify the human chromosome responsible for coding the putative IFN binding site or receptor. These studies showed a correlation between induction of the antiviral state in hybrid cells after exposure to human alpha IFN (HuIFN-alpha) or beta IFN (HuIFN-beta) and the presence of chromosome 21.¹⁸⁻³⁹ A gene dosage effect for HuIFN-alpha in Down's syndrome lymphocytes,⁴⁰ and for HuIFN-beta in Down's syndrome fibroblasts,⁴¹ was subsequently demonstrated. Similar studies using cells from persons partially trisomic for chromosome 21 suggested that the locus encoding the IFN receptor maps in the region 21q21→qter⁴²⁻⁴³ (fig 1). Evidence for the existence of a cell surface component that mediated the response to HuIFN was initially provided by experiments showing that mice inoculated with mouse-human hybrid cells containing human chromosome 21 produced antibodies that could block the action of IFN-alpha and IFN-beta on human cells.⁴⁴ More recently, direct binding studies of radioiodinated HuIFN-alpha⁴⁵ and HuIFN-beta⁴⁶ to cell surface components of hybrid cells containing human chromosome 21 provided definitive evidence for the chromosome assignment and suggested a possible composite nature for the HuIFN-alpha/beta receptor (*IFNRA-IFNRB*). The same studies also showed absence of binding of HuIFN-gamma to the chromosome 21 hybrid cells, confirming that the gene coding for the HuIFN-gamma receptor resides on another chromosome. HuIFN-alpha⁴⁷ and HuIFN-beta⁴⁸ receptors have recently been isolated. The same receptor(s) apparently binds both HuIFN-alpha and HuIFN-beta, confirming earlier findings. The chemical and immunological characterisation that will follow receptor purification will eventually lead to the cloning of the gene and precise localisation of the chromosome 21 *IFNRA/IFNRB* locus.

Cell surface antigens that are recognised by monoclonal antibodies can also be assigned to human chromosomes by using interspecies somatic cell hybrids segregating specific human chromosomes together with species specific antibodies. Four genes coding for surface membrane proteins have been provisionally assigned to chromosome 21: *SI4*,⁴⁹ *MF13* and *MF14*,⁵⁰ and *MF17*.⁵¹⁻⁵² *MF17* has been identified as the gene for leucocyte adhesion glycoprotein (GP90), a 90 kd cell surface glycoprotein found on mononuclear leucocytes and granulocytes⁵³ and recognised by the monoclonal antibody 60.3.⁵² The mouse monoclonal antibody B2 that defines *MF13* and *MF14* displays a similar immune precipitation pattern to that observed with antibody 60.3 and, consequently, may define the

same glycoprotein complex.⁵² Antibody 60.3 inhibits in vitro cell aggregation of leucocytes,⁵⁴⁻⁵⁵ cell mediated cytotoxicity, T cell proliferative response, and migration of granulocytes.⁵³ Defective lymphocyte proliferation and polymorphonuclear leucocyte migration is associated with defects in the molecular complex encoded by *MF17*.⁵⁶⁻⁵⁸ It now appears that *MF17* encodes the same cell surface antigen as the common beta subunit shared by the members of a family of leucocyte cell adhesion proteins: lymphocyte function associated antigen 1 (LFA-1), macrophage antigen 1 (Mac-1), and p150,95.⁵⁹⁻⁶⁰ These proteins are not expressed in patients when the chromosome 21 encoded beta subunit is defective, resulting in a severe immunodeficiency disease characterised by profound defects in granulocyte, monocyte, and B and T lymphocyte function.⁶⁰⁻⁶¹ The expression of LFA-1, like the IFN receptor, is also increased in Down's syndrome patients.⁶² Both of these cell surface molecules may play a role in the immune system dysfunctions commonly associated with Down's syndrome.

ONCOGENES AND TUMOUR ASSOCIATED GENES

An unusually complex retrovirus, the avian erythroblastosis virus E26, is a transforming retrovirus that contains two distinct oncogenes, *v-myc* and *v-ets* surrounded by helper viral sequences. Although the *ets* proto-oncogene in the chicken constitutes a single functional domain, mammalian *ets* homologues are found dispersed in the genome with the 5' *ets* (*Huets-1*, *ETS1*) locus on human chromosome 11 and the 3' *ets* (*Hu-ets-2*, *ETS2*) locus on human chromosome 21.⁶³ Both loci are transcriptionally active. The chromosome 21 locus has been regionally assigned by in situ hybridisation to 21q22.⁶⁴ Analysis of somatic cell hybrids has confirmed this assignment and further localised *ETS2* to band 22-3 proximal to a breakpoint in this band found in a ring chromosome 21 hybrid⁶⁵ (fig 1). Synteny of this gene and other 21 markers in the cat and mouse on chromosomes C2 and 16, respectively, has been demonstrated in somatic cell hybrid studies.⁶⁴⁻⁶⁶ Furthermore, *Ets-2* and *Sod-1* are genetically linked in the mouse (5.0 ± 1.4 cM).⁶⁴ The genetic distance was estimated by scoring the segregation of polymorphisms for the two markers in crosses of inbred strains.

ETS2 is the first potential oncogene sequence mapped to chromosome 21. A non-random chromosomal translocation involving chromosome 21, t(8;21)(q22;q22), is associated with a subgroup (M2) of acute myelogenous leukaemia (AML).⁶⁷ *ETS2* is in the region of chromosome 21 involved in the translocation.⁶⁵ Whether *ETS2* or sequences near it are directly involved in the translocation or possibly

associated with at least some of the cases of subtype M2 AML remains to be determined. *ETS2* is now localised to a region of chromosome 21 (21q22) that is apparently required for the Down's syndrome phenotype.⁶⁸⁻⁷⁸ Since these patients are at an increased risk for leukaemia,⁷⁹ a possible physiological role for this gene in the development of Down's syndrome or in the increased incidence of acute leukaemias in these patients should be evaluated.

Another gene on chromosome 21 that is potentially associated with malignancy is an oestrogen regulated gene (*BCEI*) cloned from the MCF-7 human breast cancer cell line.⁸⁰⁻⁸¹ *BCEI* was assigned to chromosome 21 by analysis of somatic cell hybrids⁸² and localised to band q22.3 by in situ hybridisation⁸³ (fig 1). The RFLP associated with the *BCEI* cDNA⁷⁰ should permit its genetic map location to be determined. It will also be a useful marker in family studies testing the association between polymorphisms at the *BCEI* locus and susceptibility to breast cancer in families with a high incidence of this type of cancer.

LIVER TYPE PHOSPHOFRUCTOKINASE

Three different tissue specific subunit types of the glycolytic pathway enzyme 6-phosphofructokinase exist: muscle type (PFKM), platelet type (PFKP), and liver type (PFKL). The structural loci coding for the different types are dispersed in the human genome, with *PFKM* assigned to chromosome 1,⁸⁴ *PFKP* on chromosome 10,⁸⁵ and *PFKL* on chromosome 21.⁸⁶ Type specific and species specific monoclonal antibodies were used together with somatic cell hybrids to assign *PFKL* to chromosome 21.⁸⁶ Similar methods were used to regionally map *PFKL* to q22²² although this result is in disagreement with an alternative assignment of 21q21→21pter based on gene dosage studies of erythrocytes from patients with chromosome 21 monosomies and partial trisomies.⁸⁷ Recent analysis of somatic cell hybrids with translocation derivatives of chromosome 21 confirmed the q22 assignment and further localised *PFKL* to the distal part of band q22.3⁸⁸ (fig 1). Given that gene mapping with well characterised somatic cell hybrids is significantly more accurate than gene dosage studies, the q22.3 assignment is more likely to be correct. As the key rate limiting enzyme of glycolysis with a structural locus in the obligate Down's syndrome region of chromosome 21, *PFKL* is another gene candidate for a role in the developmental pathology of this syndrome. This suggestion is strengthened by the finding that *PFKL* activity is expressed in the brain throughout prenatal and postnatal human development.⁸⁹

CYSTATHIONINE β SYNTHASE

The most common cause of homocystinuria, an autosomal recessive inborn error of metabolism, is deficiency of cystathionine β synthase (CBS), an enzyme required for sulphur amino acid metabolism. Mapping of *CBS* to chromosome 21 marks the first assignment of a discrete genetic disease locus to this autosome.⁹⁰ In this case, the recessive inheritance pattern and the biochemical function of the gene have been well established. A CBS dosage effect detected in chromosome 21 monosomies and partial trisomies led to the regional assignment of q21→q22.1⁹¹ (fig 1). An analysis of CBS activity in chromosome 21 trisomies resulting from translocations involving chromosome 21 excluded 21pter→q11, supporting the previous assignment.⁹² Finally, in situ hybridisation experiments using a CBS cDNA probe localised *CBS* to 21q22.⁹³ It has been speculated that an increase in CBS synthesis in Down's syndrome patients may cause overproduction of cystathionine and cysteine, thereby perturbing specific methylation mechanisms and contributing to the pathology of Down's syndrome.⁹¹

OTHER GENES PROVISIONALLY ASSIGNED TO CHROMOSOME 21

Two early gene assignments to chromosome 21 were based on metabolic studies of Down's syndrome patients. Reductions in specific enzyme substrates presumably reflect increased enzyme activity that correlates with the additional gene copy on chromosome 21. Serum 5-hydroxytryptamine (5-HT) levels are reduced in these patients, suggesting a locus on chromosome 21 for the putative 5-hydroxytryptamine oxygenase regulator (*HTOR*) that mediates the metabolism of 5-HT.⁹⁴ Similarly, lower levels of urinary taurine excretion in Down's syndrome patients, along with the competitive inhibition of taurine by beta alanine, have led to the proposal that an enzyme responsible for a beta amino acid transport system (*AABT*) is coded by a gene on chromosome 21.⁹⁵ Evidence for polymorphism in the *AABT* system⁹⁵ should allow an assessment of its potential linkage to polymorphic chromosome 21 DNA markers.

The α -A2 polypeptide subunit of crystallin (*CRYA1*), a major component of the structural proteins of the mammalian lens, was mapped to human chromosome 21 using a bovine cDNA clone and a panel of somatic cell hybrids.⁹⁶ There is a lack of synteny between the related α , β , and γ families of crystallin proteins with β (*CRYB1*) on chromosome 17,⁹⁷ and the γ genes (*CRYG1*, *CRYG2*, *CRYG3*, *CRYG4*) on chromosome 2.⁹⁸⁻⁹⁹ A surprising finding is that *CRYA1* is on mouse chromosome

17,⁶⁶ since all previous human chromosome 21 genes that have been mapped in the mouse have been assigned to mouse chromosome 16.⁶⁶ This is the first evidence for a possible break in the region of homology between human chromosome 21 and mouse chromosome 16. Four human genes from chromosome 21 have counterparts on mouse chromosome 16: *SOD1*, *PRGS*, *IFNRA/IFNRB*, and *ETS2*. The human genes, with the possible exception of the IFN receptor which has not been regionally assigned, all map in 21q22, the region implicated in the pathogenesis of Down's syndrome. An animal model for Down's syndrome has been proposed that uses mice that are trisomic for mouse chromosome 16 (MMU 16).^{100 101} The finding that mouse chromosome 17 also shares homology to human chromosome 21 may have important consequences for this model. The regional localisation of *CRYA1* on human chromosome 21 might in fact help to define further the critical region involved in Down's syndrome, in addition to opening the possibility of the involvement of other regions of the mouse genome in the model of the syndrome.

Chromosome 21 aneuploidy

DOWN'S SYNDROME

Down's syndrome is the most common cause of mental retardation associated with a chromosomal abnormality. The observation, first published in 1959, of the presence of a third chromosome 21 (trisomy 21) in the cells of those with Down's syndrome has special historical significance, since it was the first practical application of the then relatively new field of cytogenetics.¹⁰² It is now known that although 95% of Down's syndrome patients have three complete copies of chromosome 21 per cell, 5% of cases studied result from Robertsonian translocations involving chromosome 21, while reciprocal translocations account for less than 1%.¹⁰³ Down's syndrome cases exhibiting partial trisomy 21 resulting from translocations have been studied in order to define the critical region of the chromosome required for the full phenotypic expression of the syndrome. This region appears to be limited to the 21q22 band since patients lacking a duplication of this band do not have Down's syndrome,^{71 72 74 76} while those that have an additional 21q22 band as a result of translocation display the Down's syndrome phenotype.^{68 71 73-75} There is some disagreement as to whether specific sub-bands of 21q22 (22-1, 22-2, 22-3) are required for Down's syndrome. Although some have excluded band 21q22-3 from the critical region,⁷³ others have observed the syndrome in patients that are trisomic only for 21q22-3-qter.^{77 78} With the exception of the

ribosomal RNA genes, all of the genes that have been regionally mapped (*SOD1*, *PRGS*, *CBS*, *PFKL*, *BCE1*, *IFNRA/IFNRB*, *ETS2*) potentially lie within the region of chromosome 21 associated with Down's syndrome and may therefore contribute to the phenotype. Polymorphic DNA markers that have been physically and genetically mapped to these subregions can be used to define further the pathogenetic region of the chromosome, circumventing many of the difficulties of cytogenetic interpretation.

The majority of cases of Down's syndrome result from non-disjunction of chromosome 21 during formation of the gametes at meiosis I (MI) or meiosis II (MII) in either of the parents. Chromosome analysis by Q banding and other staining methods can detect heritable differences associated with the short arm region of chromosome 21. The parental origin of the extra chromosome and the meiotic stage of error can therefore be determined, provided the parents are informative for the heteromorphic markers. A compilation of studies¹⁰⁴ shows that 80% of cases of Down's syndrome result from non-disjunction that is of maternal origin while 20% is paternal in origin. In the mother, 80% of the meiotic errors occur during MI and 20% during MII and in the father, 60% are MI errors and 40% are MII. Although the correlation of increasing maternal age and risk for having Down's syndrome offspring is generally accepted,¹⁰⁵ not all studies are in complete agreement.¹⁰⁶ There is also disagreement regarding an apparent excess of paternal meiosis I errors in trisomy 21 relative to other trisomies.^{107 108}

Studies of the parental origin of non-disjunction should be significantly enhanced by the recent availability of polymorphic DNA probes. One study has already shown the efficiency of using DNA markers.¹⁰⁹ With an appropriate set of DNA probes, most parents will be informative for at least one or more of the DNA markers. Evidence for recombination can also be obtained, while probes specific for the centromere and pericentromeric regions of the chromosome will help to define further the contribution of DNA sequences potentially involved in non-disjunction.

Some factors that appear to be associated with chromosome 21 non-disjunction have also been identified. Satellite associations involving chromosome 21,¹¹⁰ active NORs revealed by positive silver staining of the ribosomal cistrons,¹¹¹ and premature centromere division¹¹² have all been proposed as contributing factors. The best evidence for a possible causal factor comes from studies of double NOR (dNOR) variants in Down's syndrome families where the parental origin of non-disjunction could

be determined. In these families, there was a clear association between a dNOR and the parental chromosome involved in non-disjunction.¹⁰ Additional population and family studies will, however, be required before the validity of the dNOR variant as a risk factor for Down's syndrome can be fully evaluated.

Although some have proposed models involving specific genes that promote non-disjunction, there is currently little direct evidence for this possibility. The postulated existence of an autosomal recessive gene that causes meiotic non-disjunction in the homozygous parent,¹¹³ or a gene that induces mitotic non-disjunction in the homologue fertilised ovum,¹¹⁴ are examples of such models. It has been proposed that a specific DNA haplotype defined by the polymorphic markers *D21S1/D21S11* may be associated with a predisposition to non-disjunction.¹¹⁵ However, the recent genetic map assignment of the marker loci indicates that at least 18% recombination must exist between the marker pair and DNA sequences at the centromere (fig 2). Further studies with polymorphic markers at or near the centromere, such as CW21p,¹¹⁶ will help to evaluate these findings. RFLPs associated with double or active NORs would also be especially helpful in providing a direct molecular marker for some of the potential predisposing factors associated with non-disjunction.

MONOSOMY 21 AND RING 21

Complete monosomy 21, either homogeneous or mosaic, occurs rarely if at all. A small number of cases have been described with varying uncertainty of the cytogenetic diagnosis.¹¹⁷⁻¹²² Most cases of proximal monosomy 21 (21pter→q21) result from the inheritance of a parental translocation.^{123 124} However, two cases of de novo partial monosomy 21 (with loss of the centromere) studied by high resolution banding techniques have been reported.^{125 126} Polymorphic DNA probes are now being used to define further this type of chromosomal abnormality. The enhanced resolution gained with molecular probes has resulted in the reclassification of some cases of monosomy 21 originally identified by cytogenetic evaluation.¹²⁷

Partial monosomy 21 also occurs after the formation of a ring chromosome with the end segments deleted, resulting in a partial monosomy for these segments. Although usually associated with microcephaly and multiple malformations, some cases have been described where the ring chromosome 21 carriers are phenotypically normal.^{128 129} A somatic cell hybrid that contains a ring chromosome 21 has been characterised using DNA probes. DNA markers have been localised proximal and distal to the

breakpoints in the ring chromosome bands 21q22:3 and 21p11.¹³⁰ One of the DNA probes (*D21S3*) contained the breakpoint junction¹³¹ providing the first DNA segment whose position can be compared directly on the cytogenetic, genetic, and physical maps of the chromosome (fig 1).

Physical molecular map of chromosome 21

CHROMOSOME 21 LONG ARM

The molecular genetic map of chromosome 21 is rapidly evolving as advances are made in the development of methods for the selection and characterisation of somatic cell hybrids, high resolution chromosome banding, the construction of chromosome specific libraries, and the efficient analysis of linkage data using highly informative pedigrees and sophisticated computer programmes. A number of chromosome 21 specific DNA libraries have been made from chromosomes isolated by sedimentation,¹³² or flow sorting,¹³³ or from somatic cell hybrids containing chromosome 21 as the only human chromosome.^{134 135} Cloned DNA segments that detect RFLPs have been characterised and catalogued at the biennial Human Gene Mapping Workshops. More polymorphic DNA markers currently exist for this chromosome than any other autosome with the exception of chromosome 4.⁴ A recent report describes the regional assignment of a number of these markers by hybridisation to panels of somatic cell hybrids containing translocation derivatives of chromosome 21.¹³⁰ Some of the same markers were also localised by in situ hybridisation.⁹³ Although the resolution of mapping of hybrids with specific breakpoints in chromosome 21 should be higher than that obtained by in situ hybridisation, the former approach is limited by the availability of chromosome 21 translocation hybrids containing appropriately distributed breakpoints along the chromosome. Preliminary data from studies of hybrids made with x ray irradiation, combined with characterisation of the resulting clones containing different terminal deletions of the chromosome with existing molecular markers, may provide a suitable panel of chromosome 21 derivatives for mapping studies.¹³⁶ Nevertheless, initial attempts at ordering markers on the long arm of chromosome 21 have met with success, especially in the 21q22:3 region where a specific probe fortuitously marks the breakpoint junction in a hybrid cell line derived from a ring chromosome 21.¹³¹ Three of the six genes regionally mapped to 21 are within this well defined region of the chromosome.

CHROMOSOME 21 SHORT ARM AND CENTROMERE

The short arm region of chromosome 21 is the site

for the tandemly clustered repetitive satellite DNA sequences associated with the proximal short arm³ and the rRNA gene family located in the short arm stalk.^{1,2} Other short arm DNA sequences that are not associated with satellite or rDNA sequences have also been identified. Most of these appear to be members of DNA sequence families that have members on other chromosomes. pUNC724, pi220, and pi2F4, localised to 21q13 (fig 1), are members of a complex, interspersed DNA family designated 724 that has a unique organisation on each acrocentric chromosome.¹³⁷ The 724 family also contains members that map to the pericentromeric region of chromosome 21.

Single copy polymorphic markers have not yet been identified for the short arm of chromosome 21. One polymorphic short arm marker, *D21S5* (fig 1), also has loci on two or three other chromosomes, although these have not yet been unequivocally identified.¹²² *D21S5* is only weakly linked to proximal markers on the long arm.¹³⁸ A polymorphic single copy DNA sequence designated CW21pc (fig 1) has been isolated from a ring chromosome 21¹¹⁶ and assigned to the proximal long arm, providing a genetic marker for the pericentromeric region of the long arm.¹³⁹ Other centromere based DNA sequences that are potentially polymorphic are alphoid DNA sequences,¹⁴⁰ repetitive DNA sequence families found at the centromere of all human chromosomes. Alphoid sequences are organised in a chromosome specific manner. A method to identify chromosome specific RFLPs associated with alphoid DNA probes has been used successfully to identify polymorphisms on the X chromosome and chromosome 17.¹⁴¹ Since this approach can be generally applied, cloned alphoid DNA probes from chromosome 21¹⁴² should eventually provide genetic markers for the centromere. A centromere based linkage map could therefore be established, and, together with other polymorphic short arm markers, be used to evaluate the influence of recombination in the short arm region of chromosome 21 on meiotic non-disjunction.

Genetic linkage on chromosome 21

GENETIC LINKAGE MAP FOR 21q

The combination of physical mapping approaches described above with genetic linkage mapping of polymorphic genes and DNA markers should result in a detailed molecular map for chromosome 21. Physical and genetic map length along the chromosome could then be compared directly. A linkage analysis of the polymorphic markers in a large Venezuelan pedigree¹⁴³ has produced a genetic map of chromosome 21q.^{138 143 144} Fig 2, compiled from

data recently reported at HGM8, illustrates the genetic linkage relationships of selected markers from a pairwise two point analysis assuming no sex difference in recombination. The relative order of the markers was first determined by a three point cross analysis. The map is in remarkably close agreement with data obtained from the physical map (fig 1). *D21S13* has been assigned to the long arm of chromosome 21 by analysis of somatic cell hybrids (M Van Keuren, 1986, personal communication). Therefore, the map extends from a marker (*D21S3*) defining a physical breakpoint in band q22.3 to marker presumably in either proximal q21 or possibly q11. The precise physical localisation of *D21S13*, the marker at the top of the linkage map, will require additional hybrids with breakpoints near the centromere.

Cloned DNAs exist for the *CBS*, *SOD1*, *BCE1* and *ETS2* genes that have been regionally assigned. *SOD1* is polymorphic and has already been placed on the genetic map.^{31 138} An RFLP detected by *Bce* will enable its genetic locus relative to the *D21S3*/*D21S23*-*D21S17* loci to be determined. *ETS2* map also yield an RFLP, thus confirming its assignment and defining its genetic location.

The goal of future genetic mapping studies is to obtain a map sufficiently detailed that any new gene assigned to chromosome 21 can be rapidly localised once a suitable polymorphism is found. New methods are also being applied that result in the direct isolation of new genes from chromosome 21.¹⁴⁵ This approach should identify additional genes that may also play a role in the pathogenesis of Down's syndrome. Furthermore, as long as the appropriate families exist for a chromosomally undefined genetic disease (for example, familial Alzheimer's disease), a subset of highly informative markers can be used to exclude the chromosome from consideration rapidly or, alternatively, to locate the disease gene with high precision. For this purpose, we have established chromosome 21 specific cosmid libraries and have subsequently isolated and subcloned a number of RFLP detecting markers.¹³⁵ The potential thus exists for increasing the informativeness of selected cosmid marker loci by additional screening of flanking subclones for RFLP detection.

Multipoint analysis of the existing genetic map, including additional markers at the telomere of the long arm, confirms the marker order shown in fig 1 and lends statistical support for this order as well as providing evidence for increased recombination near the telomere and for sex specific differences in the recombination frequency along the chromosome.¹⁴³ The direct comparison of physical to genetic distance will be facilitated by more precise

methods of physically assigning markers to the chromosome. Electrophoretic methods capable of fractionating large DNA fragments (>100 kb) generated by restriction enzymes that cut infrequently in mammalian DNA are gaining wide application. These methods should bridge the gap between molecular and cytogenetic levels of resolution. Probes for *D21S8* and *D21S56* have been localised to specific large fragments, establishing a minimum distance between the loci and confirming the expected separation of the markers based on the previous localisation by somatic cell genetics.¹⁴⁶ The construction of a complete macro-restriction map of chromosome 21 by pulsed field electrophoresis will lay the foundation for the eventual DNA sequencing of the entire chromosome.

A ROLE FOR CHROMOSOME 21 IN ALZHEIMER'S DISEASE?

Alzheimer's disease is a progressive dementia of unknown aetiology that exhibits some remarkable relationships to Down's syndrome.¹⁴⁷⁻¹⁵⁴ It is a neuropathological disorder characterised by plaques and neurofibrillary tangles in the cerebral cortex and hippocampus with granulovascular degeneration in the hippocampus.^{155 156} In the normal population, approximately 20% of people over 80 years of age develop Alzheimer's disease, whereas virtually 100% of patients with Down's syndrome over 35 years of age develop pathological changes in the brain that are diagnostic for Alzheimer's disease.¹⁵⁷⁻¹⁶⁰

There are a number of neurochemical and genetic similarities displayed by the two disorders, in addition to the specific brain pathology described above. Decreased concentrations or activities of neurotransmitters, especially those involved in cholinergic transmission, such as choline acetyltransferase and acetylcholinesterase, are typically found in brains of both Down's syndrome and Alzheimer's disease patients.^{148 153 161 162} Both diseases are associated with abnormalities in cerebral metabolic rates of glucose utilisation measured by positron emission tomography using glucose labelled with radioactive fluorine.^{153 163}

A possible genetic association between Down's syndrome and Alzheimer's disease is suggested by an apparent increased risk for Down's syndrome in families with a high prevalence of Alzheimer's disease.^{153 164-167} This form of the disorder, which has been termed familial Alzheimer's disease, typically shows a younger age of onset for the disease,¹⁵³ reminiscent of the accelerated ageing seen in Down's syndrome patients. One approach to evaluate the genetic role of chromosome 21 is to examine the activity of proteins coded by genes on chromo-

some 21 in patients with familial Alzheimer's disease. Preliminary studies suggest that SOD1 activity is significantly increased in Alzheimer's fibroblasts when compared to normal controls.¹⁶⁸ Molecular probes for *SOD1* and other cloned chromosome 21 genes can be used to establish further the role of chromosome 21.

A possible biochemical link for these two diseases is provided by the identification of an identical component (amyloid β protein) from neuritic plaques in the brains of both Down's syndrome and Alzheimer patients.¹⁶⁹ Amyloid-like structures, associated with the proteinaceous infectious agents (prions) that cause scrapie in sheep and goats and Creutzfeldt-Jakob disease, kuru, and Gerstmann-Straussler syndrome in humans, are similar to those found in the brains of Down's syndrome and Alzheimer's disease patients. A defect in immune surveillance possibly associated with genes on chromosome 21 could result in increased susceptibility to these agents. However, the recent mapping to chromosome 20 of a human cDNA homologue to a hamster scrapie prion protein rules out any direct link between the human prion gene and Down's syndrome or Alzheimer amyloid.¹⁷⁰ Although there are biochemical similarities of senile plaque amyloid and structural similarities of the neurofibrillary tangles found in both Down's syndrome and Alzheimer brains, significant differences in plaque morphology have also been reported.¹⁷¹

Whether a single chromosome 21 gene or possibly a cryptic trisomy¹⁷² of a critical sub-region of the chromosome causes familial Alzheimer's disease is not clear, but the cumulative evidence suggests some role for chromosome 21 in the pathogenesis of the disorder. Studies are now being focused on specific gene candidates as they are assigned to this chromosome, or on using molecular probes to test for chromosome 21 imbalance in the tissues of Alzheimer's disease patients. Using the recently completed genetic map, conventional linkage studies can now be employed to test for the association of specific genes on chromosome 21 and Alzheimer's disease. Given a sufficiently informative set of families, a multipoint approach to linkage analysis with a complete set of polymorphic markers should lead to identification of a chromosome 21 genetic locus associated with Alzheimer's disease or exclusion of a primary role for this chromosome in the aetiology of the disorder.

Conclusion

Molecular genetic analysis of chromosome 21 is rapidly building the framework for a complete understanding of the primary structure of this

chromosome. Since it is the smallest human autosome, chromosome 21 could be the first region of the genome for which a complete physical and genetic map is available, leading ultimately to determination of its complete DNA sequence. The number of functional genes assigned to the chromosome still represents a small fraction of those it must contain. It is likely that, as in the past, the identification of new loci will continue to result from attempts to define the causes of the Down's syndrome phenotype. The efforts to discover new genes and to analyse their function may further accelerate if familial Alzheimer's disease can be genetically assigned to chromosome 21.

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Note added in proof

Several investigators have recently identified the gene responsible for the amyloid component of plaques found in the brains of Alzheimer's disease patients. The gene has been physically and genetically mapped to chromosome 21 (Goldgaber D, et al. Characterisation and chromosomal localisation of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* 1987;235:877-80. Tanzi R, et al. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer

locus. *Science* 1987;235:880-4. Kang J, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 1987;325:733-6). Another report describes positive genetic linkage between the familial Alzheimer's disease locus and polymorphic DNA markers on chromosome 21 (St George-Hyslop P, et al. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science* 1987;235:885-9). These studies support a primary role for a gene or genes on chromosome 21 in the aetiology or pathogenesis of both of Alzheimer's disease.