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Down syndrome genetics: unravelling a multifactorial disorder

Diana Hernandez and Elizabeth M. C. Fisher*

Neurogenetics Unit, Department of Biochemistry and Molecular Genetics, Imperial College School of Medicine at St. Mary's, Norfolk Place, London W2 1PG, UK

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Down syndrome is a common disorder affecting many tissues both during development and later on in adult life; the principle feature of all cases is a specific form of mental retardation, which is combined with a range of variable traits. Down syndrome is an aneuploidy syndrome that is caused by trisomy for human chromosome 21. While the phenotype is most likely due to a subtle increase in gene dosage of only a small minority of the estimated 500–800 genes that are present on this chromosome, the molecular genetics of Down syndrome remains speculative. However, recent advances on a number of fronts, including chromosome studies, gene identification and mouse modelling, are giving us the tools to dissect this multifactorial gene dosage disorder.

INTRODUCTION

Down syndrome (DS) is one of the few genetically determined disorders that the general public have heard of and can recognise, because of its striking characteristic facial features and remarkable frequency. Awareness of individuals with DS is not a modern phenomenon—Olmec artefacts from Mexico and Saxon remains from the UK have been documented to include likely DS individuals, as have European paintings of the Renaissance (1). However, the clinical and scientific definition of this common syndrome only began in the middle of the last century (1–3), and the definitive description of DS came in 1866 when John Down described a series of patients with what we now call Down syndrome (4,5). As early as 1932, some authors were suggesting that DS may be due to a chromosomal aberration arising from non-disjunction (1), and in 1959 the cause of the phenotype was found to be trisomy of chromosome 21 (6,7). We now know that up to 1 in 600 individuals are born with DS, making trisomy 21 the most common known genetic cause of mental retardation (8). In addition, it is estimated that up to 1 in 150 of all pregnancies involve a trisomy 21 fetus (9).

PHENOTYPIC FEATURES OF DOWN SYNDROME

Trisomy 21 gives rise to a variety of traits, all of which have variable penetrance and expressivity within the DS population, except for the specific type of mental retardation and neonatal hypotonia that are seen in nearly 100% of cases (10,11). The DS phenotype, such as the classic facial appearance, is well documented, and includes certain clinically important features that are particularly associated with DS. For example, congenital heart defects of the endocardial cushion type are present in ~60% of DS individuals, but these cases account for 70% of all such defects (11–13). The syndrome gives rise to a 10- to 30-fold increased risk of acute leukaemia: almost 1% of DS children will be affected, up to 50% of whom will have acute non-lymphocytic leukaemia subtype M7 during their first 4 years of life (8,14). Transient leukaemia occurs almost exclusively in DS newborns and is characterised by megakaryoblasts in the blood which disappear 1–3 months after birth (8,14).

The mental retardation associated with DS is increasingly well described as large age-controlled studies are undertaken. It is typified by developmental delay, language and memory deficits and cognitive abnormalities of auditory–verbal processing (11,13,15,16). Measurements of DS IQ vary widely from below 20 to at least an IQ of 69; this range of results reflects the age and environment of the individual as well as genotypic factors. Generally in DS, IQ appears to decline during childhood and continues decreasing in adolescence and adulthood (17). Psychiatric disorders, such as depression, are not unusual in the DS population (1).

Although many organs are affected by the DS phenotype, the central nervous system is a focus of study, partly because mental retardation is a key feature of the syndrome and partly because we know so little about the genetic causes of mental retardation even though ~1–2% of the population are affected in different ways (18). Classical anatomical studies, combined with new neuroimaging techniques of live individuals, are characterising the nervous system in DS, including the small cerebral and cerebellar hemispheres and brain stem (19). Histopathological studies show that the main difference between DS and normal brains appears to be in neuronal organisation and number (20), including subtle alterations in different cortical layers, particularly reductions in cell number in layers 2 and 4 (20,21). Also in DS, the dendritic trees, which continuously expand in normal early growth and development, appear to become relatively atrophic 4 months after birth (20). Abnormal pre- and postnatal synaptic parameters have been reported, including

*To whom correspondence should be addressed

possibly fewer synapses in DS and other changes that could lead to reduced efficiency of synaptic transmission (20,21). A general delay in myelination and altered electrophysiological membrane properties have also been described for DS neurons (20,21).

Perhaps the most striking neurological feature of DS is the Alzheimer disease (AD)-like neurohistopathology that is found in close to 100% of individuals by the time they are 35 years old (13,22). By the age of 40, up to 30% of DS individuals may have AD-like dementia (23), and even those who do not dement appear to show a distinctive pattern of age-related deficits (24). The amyloid plaque precursor gene (*APP*) on chromosome 21 is mutated in some forms of early onset familial AD. It has been suggested that three copies of *APP* may cause the AD-like pathology seen in DS (25). Data relevant to this hypothesis are starting to appear from the very rare older DS people with partial trisomy 21; for example a 78 year old woman with a 21q partial trisomy that excludes the *APP* gene is described as having atypical DS and no dementia or MRI evidence for AD-like pathology (26).

AD-like dementia seems to present up to 20 years after the appearance of AD-like plaques and tangles, which is thought to be a slower lag time for AD symptoms than occurs in the general population; this is unexpected given that the DS brain has fewer neurons than normal and therefore could be more susceptible to damage (27). The exact mechanism of neurodegeneration in DS remains unknown, but recently it has been found that DS cortical neurons in culture degenerate and undergo apoptosis at a time when normal neurons remain viable. Possibly this is due to a defect in the metabolism of reactive oxygen species that causes apoptosis and thus neurodegeneration in DS (28).

DOWN SYNDROME CHROMOSOME ABERRATIONS

Large-scale studies combined with cytogenetic and molecular assays now show that ~95% of DS is due to 'full trisomy 21' in which an entire extra chromosome 21 is present (29). In these cases, ~86% of the extra chromosomes are maternally derived, mainly due to non-disjunction in meiosis II (75%) rather than meiosis I (25%). Approximately 9% of full trisomy 21 is due to paternal meiotic error, with non-disjunction occurring equally in paternal meiosis I and II. Less than 5% of full trisomy 21 is caused by mitotic errors (29–31).

All full trisomies are strongly associated with rising maternal age, and this is most likely attributable to maternal meiotic non-disjunction increasing with age, not to age-related changes in the uterine environment—mean maternal age is higher than that of control populations for both maternal meiosis I and II chromosome 21 non-disjunction, but not for paternal or mitotic errors (29–32). One major factor that was thought to be implicated in the maternal age-related increase in trisomy and non-disjunction was reduced genetic recombination. This was indeed shown to be the case in maternal trisomy 21 when recombination along 21q was found to be diminished between the non-disjoined chromosomes, correlating with increasing maternal age (29). This effect is seen almost exclusively in meiosis I cases (29). Thus age-related reduced recombination may be a major cause of non-disjunction, and could provide a partial explanation for the increase of trisomy 21 with maternal age (29,31).

In one North American population, it is estimated that at least 56% of women >35 years old have prenatal diagnosis for DS, and 90% of those with a trisomy 21 fetus will terminate the pregnancy (31). In this population, the DS birth prevalence rates for women over 35 are approximately half that reported prior to the widespread use of prenatal testing (31). Such effects can result in an underestimation of risk in older mothers.

The remaining ~5% of cases of non-full trisomy 21 are accounted for by: (i) mosaicism between a full trisomy 21 cell line and usually a euploid cell line (<5%); (ii) chromosome 21 translocations including Robertsonian translocations (<5%). These are mainly 21q isochromosomes that are both dicentric and monocentric in about equal proportions (33,34). A very small percentage of rearrangements are true Robertsonian translocations of chromosome 21q partnered mostly with chromosome 14q; rarely, chromosomes 13 or 15 are involved instead (35); and (iii) partial trisomy 21 (<<1%).

Probably fewer than 50 cases of partial trisomy 21 have been documented, and these have been the basis of studies aimed at associating a portion of the variance of a specific DS trait with a particular region of chromosome 21. Thus attempts have been made to define a 'Down syndrome chromosome region' or 'Down syndrome critical region' for certain traits (for example, see 11–13, 36–38). These associations are extremely difficult to find owing to the incomplete penetrance of most DS traits and the small sample size. In addition, there are discrepancies between the phenotypes/trisomic segments of some individuals and the consensus critical regions, therefore the terms DCR and DSCR have been dropped currently (6th International Workshop on Chromosome 21, Cold Spring Harbor, 1996). Nevertheless, detailed phenotypic assessment of partial trisomy 21 individuals indicates that a high percentage of the variance of some features, such as congenital heart disease, may be associated with trisomy for localized regions of the chromosome (11). However, it is likely that several genes along the length of chromosome 21 account for the total variance of each trait. It appears unlikely that gametic imprinting is involved in DS to any great extent (39).

CHROMOSOME 21 AND THE IMPACT OF THE HUMAN GENOME PROJECT

Like all other chromosomal aneuploidy syndromes, Down syndrome is most likely due primarily to aberrant gene dosage, in this case from three doses of chromosome 21 genes. For most genes, the presence of an extra copy probably has no phenotypic consequences because of the regulation of genes and their products. The genes that cause DS appear to be exceptions, and we have no standard human genetic approaches, such as linkage analysis, to isolate these genes directly. However, other routes can be followed: for example we can assay whether the phenotypic variation in DS correlates with allelic variation, to determine if a particular genotype gives rise to a particular trait (40,41). In this way, we may be able to map regions that are important for determining specific DS traits.

All molecular genetic investigations depend on the genomics resources that are available and, fortunately for those researching the molecular genetics of DS, chromosome 21 is a paradigm for mapping and cloning studies. Thanks to the efforts of many laboratories around the world, a dense genetic map has existed for some time and the physical map is well established (Human Genome Database). Chromosome 21q was one of the first human chromosomes for which an almost complete YAC contig was published (42,43). Since then, an almost complete YAC minimal tiling path and set of cosmid 'pockets' has been created for 21q,

and new BAC and PAC contigs have also been isolated in localized regions (for example, see 44–55). A complete *Not*I restriction map exists of 21q (56) and local maps for other enzymes are being determined (for example, see maps in 46,48,55,57–59).

As the maps of chromosome 21 grow more dense, the research emphasis has shifted to gene isolation and to sequencing the chromosome (at least 21q), and possibly its mouse homologues. A complete transcription map of chromosome 21 will be helpful for indicating candidate genes (i) that map to regions associated with variance for a particular trait, and (ii) that can be overexpressed, for example in transgenic mice, to determine the effects of aberrant dosage. It is estimated that chromosome 21 carries up to 800 genes (60) but currently only ~10% of these are entered as full-length sequences in the Human Genome Database. However, various groups have undertaken gene isolation protocols, such as cDNA selection, exon trapping and genomic sequencing with software trapping, resulting in the cloning of large numbers of expressed sequences—although generally only limited information is available for each clone (for example, see 60–66). Through the generosity of these groups and the genome-wide EST efforts, several hundred likely chromosome 21 partial gene sequences have been made available for further investigation in databases such as dbEST. Therefore, the number of genes officially mapping to chromosome 21 is rising rapidly (for example, see 67–73,90), including genes known to be involved in neuronal development in *Drosophila* (for example, see 74–76).

CHROMOSOME 21 GENES AND THEIR OVEREXPRESSION

The current chromosome 21 transcription map provides us with some interesting sequences with which to investigate the consequences of overexpression. Such studies invariably take place with transgenic mice so that the effects on the whole body can be evaluated. Mice are not human and, therefore, we can only use them to model dosage effects, rather than DS. Nevertheless, mouse models allow us to dissect biological pathways and mechanisms even though the outcome of overexpression might be different from what occurs in humans (77). In the future, other model systems, such as fly or yeast, are likely to become important to functional studies of human aneupoloidy, especially as many partial trisomies are known in these organisms.

Transgenic mice that overexpress chromosome 21 genes, such as *APP* (78–80), *SOD1* (81), *PFKL* (82) and *S100b* (83,84) exhibit a range of neurological, behavioural, anatomical and other deficits. While these mice allow us to study the overexpression of single genes, they do not mimic the possible interactions that occur from multiple genes on a trisomic chromosome. In addition, overexpression from a single transgene is often significantly greater than the subtle 3:2 dosage difference seen between DS and normal individuals; one recent exception is provided by transgenic mouse lines in which the *Ets2* transcription factor is overexpressed at levels comparable with that seen in human DS. These mice develop skeletal abnormalities similar to those found in DS individuals (85).

DOWN SYNDROME AND MOUSE MODELS

Mice other than the single gene transgenics also contribute to our understanding of the molecular genetics of DS and gene dosage effects from particular genomic regions. One of the first mice to be studied extensively in relation to DS was the trisomy 16 mouse, because a large portion of mouse chromosome 16 is syntenic to a large portion of human chromosome 21 (86). However, these mice do not survive postnatally and mouse chromosome 16 contains many genes which do not map to human chromosome 21. A more helpful model for DS research has been provided recently by the Ts65Dn mouse that is trisomic for the telomeric portion of mouse chromosome 16, which is syntenic to a portion of human chromosome 21 (87). These mice survive into adulthood and have learning and behavioural deficits. Thus cognitive deficits can be attributed to gene dosage effects from the trisomic region, and this mouse model provides the substrate for a new round of genetic experiments, such as breeding the trisomic chromosome onto mice with individual gene knockouts, to compare dosage for specific genes from the region, or breeding the trisomic region onto different genetic backgrounds to map modifier loci.

Another new and important model is provided by an '*in vivo* library' of mice which are transgenic for a set of human YAC and P1 clones that span a 2 Mb region of 21q22.2 (88). At least one region of chromosome 21 that is contained within these vectors appears to give rise consistently to a specific behavioural deficit in different lines of mouse. Thus a dosage-sensitive effect can be mapped to a critical region, leading to the analysis of candidate genes (Smith, pers. comm.) and, again, these mice can be used for other genetic breeding experiments.

DOWN SYNDROME: THE FUTURE

This review does not contain a section heading called 'the molecular genetics of Down syndrome', because the bulk of such a section would be almost exclusively speculation at the moment. However, a number of routes for investigation are emerging that will provide new data for our understanding of why trisomy 21 arises, and why it causes Down syndrome.

Approaches in the human population can be taken to establish why the constant and variable features of DS occur—is trisomy for a region enough to have an effect? or are particular allelic combinations important? and do epistatic effects (such as modifier loci) and environmental effects come into play? (11,13,40,41). If we can work out these relationships, they are likely to have clinical relevance in establishing, for example, who is most likely to succumb to leukaemia or dementia.

Mouse models clearly have a role to play in helping our understanding of dosage sensitivity of chromosome 21 genes. Recent advances in Cre-*loxP* technology allow us to tailor specific chromosome duplications in mice (89), and this, in combination with other forms of transgenesis, will help dissect the effects of modifier loci through quantitative trait analysis. Such genes are likely to play a role in at least some of the phenotypic variability of human DS.

In the long term, probably the very long term, we can speculate about treating aspects of DS pathology, or even ameliorating the DS phenotype completely, for example by targeting an inactivation centre such as *Xist* into the extra chromosome 21.

It took almost 100 years to get from the phenotypic description of DS to the cytogenetic explanation. With the pace of current progress, it should take considerably less time to get to the molecular genetic definition.

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