New techniques to understand chromosome dosage: mouse models of aneuploidy

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Aberrations in human chromosome copy number and structure are common and extremely deleterious. Their downstream effects on phenotype are caused by aberrant dosage of sequences in the affected regions. However, we know little about why the abnormal gene copy number causes disease or why specific features result from deficits in specific chromosomes. Mice are the organism of choice to help us try to tease apart the complex relationships between genotype and phenotype in aneuploidy and segmental aneusomy syndromes. As new technologies such as chromosome engineering and the creation of transchromosomic mice become routine, these will help us identify individual dosage-sensitive genes that are causative in specific syndromes and will enable us to produce mouse models to accurately recapitulate human chromosomal disorders.

INTRODUCTION

To be euploid is to have the normal number and structure of chromosomes; as the readers of Human Molecular Genetics know well, the euploid number in humans is 46, comprising 22 pairs of autosomes and two sex chromosomes X and Y. Conversely, aneuploidy is having an abnormal number of chromosomes, such as an extra chromosome (trisomy) or one too few (monosomy), and thus having an abnormal copy number or ‘dose’ of the sequences on that chromosome. Incorrect copy number can also arise from structural abnormalities, such as duplications or deletions within an individual chromosome, which result in ‘segmental aneusomy’. Segmental aneusomy, ‘partial deletion and duplication’, ‘local deletion and duplication’, ‘segmental deletion and duplication’ are the terms found in the literature that refer to the same state of having a structural change in a portion of a chromosome, which alters the number of copies of that region from the euploid number.

So many terms referring to the same state tell us that dosage imbalance is well described and is a well known cause of human disease (Table 1). In humans, aneuploidy and segmental aneusomy are the leading known cause of pregnancy loss (1); it is estimated that >8% of clinically recognized conceptions have cytogenetically detectable numerical (~7%) or structural (~1%) abnormalities (2), and these chromosomal abnormalities occur in ~1% of live births (3). These figures will increase as higher resolution methods of detection, such as array comparative genomic hybridization (CGH), are used more widely (4,5).

The only known whole chromosome monosomy that survives until birth is monosomy X, which gives rise to Turner’s syndrome. In contrast, a number of whole chromosome trisomies survive until birth. These include the trisomies of the sex chromosomes (e.g. XXY resulting in Klinefelter’s syndrome and XXX resulting in triple X syndrome), as well as autosomal trisomies of chromosomes 13, 18 and 21 (1). It may be that increased numbers of sex chromosomes, and monosomy X, are tolerated reasonably well because all but one of the X chromosomes is inactivated, thus the normal state of X-linked gene dosage in most cells is ‘one dose’ (although a number of X-linked genes escape inactivation) (6); the Y chromosome carries relatively few genes and increased dosage (as in XY or XYYY) (7) is compatible with life. In contrast, whole chromosome trisomy of most autosomes is not viable and those trisomies that do survive after birth can have severe phenotypes. Trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) are...
characterized by a very short life expectancy (days and months, respectively), mental retardation and multiple developmental abnormalities. Trisomy 21 [Down’s syndrome (DS)] is the mildest of all the autosomal aneuploidies and is compatible with relatively long life, probably because this is the smallest autosome with the fewest genes (8), but it too results in a range of developmental abnormalities and is the most common known genetic cause of mental retardation (9).

Many regions throughout the genome have been described as causing disease phenotypes because of the partial duplication or deletion. Some segmental aneuploidies occur sufficiently frequently to have been given a name and a phenotype description, such as segmental monosomy of chromosome 5p, which results in the cri-du-chat syndrome [Table 1A, (10)], whereas the others are rare individual aberrations published as single case reports in the medical literature. A further complication of aneuploidy/segmental aneusomy is that the chromosomal regions involved are imprinted, the effects on phenotype will depend on the maternal or paternal origin of the mutant chromosome (e.g. Prader–Willi syndrome results from deletion of 15q11–13 on the paternal chromosome, resulting in segmental monosomy for the corresponding region on the maternal chromosome) (11). Aneuploidy/segmental aneusomy also occurs in somatic cells, and specific chromosome aberrations are often a defining feature of particular cancers (12).

We have some idea of how aneuploidy arises: meiotic errors leading to germline aneuploidy, and early mitotic errors can result in aneuploidy in most cells of an individual (1): in tumors, DNA replication errors may result in clonal selection of cells with tumorigenic segmental aneusomies (12). However, we know little about why the abnormal gene dosage has such profound and often lethal effects on cell growth and on the development of the individual. As with all other human genetic disorders, the mouse is the model organism of choice for carrying out sophisticated manipulations to recapitulate (as far as possible) the human condition and start to understand the mechanisms by which dosage abnormalities affect the human biology. This review will focus on how genetically altered mice can be used to model human aneuploidy and segmental aneusomy. We provide examples from the investigation of human trisomy 21, which results in DS, as this is an area of research in which our laboratories collaborate to create and analyze mouse models.

ISSUES TO CONSIDER WHEN MODELING ANEUPLOIDIES IN MICE

What causes the phenotype?

In principle, the phenotypic effects of aneuploidy/segmental aneusomy could be caused by the increased or decreased expression of most of the genes in the aberrant region, irrespective of the nature of the genes themselves. Alternatively, phenotypes could arise from the altered dosage of specific genes. The latter possibility seems more likely because each different type of chromosomal anomaly results in a different phenotype with characteristics specific for that aneuploidy/segmental aneusomy.

If the phenotypes are caused by the altered dosage of specific genes/sequences, rather than aneuploidy/segmental aneusomy per se, then either only a few or a large number of genes in the aberrant region may be involved. Furthermore, aneuploidies/segmental aneusomies also affect all the non-protein-coding genes, microRNAs, regulatory regions, structural regions, etc., which lie within the deleted or ‘over-dosed’ region. For phenotypes arising from most aneuploidies/segmental aneusomies, the numbers of genes involved are unknown. However, for many genes, negative feedback mechanisms may exist to regulate the level of transcript or protein such that the altered gene dosage may not translate into the altered levels of protein. Indeed, in a mouse model of DS, only about half of the trisomic genes showed an increase in expression of 1.5-fold or higher (13–17). Furthermore, any particular tissue will only express a subset of the aneuploid genes. These considerations suggest that it is likely that specific phenotypes will be due to the altered dosage of only a small number of genes. In support of this, small regions of segmental aneusomy are increasingly implicated in disease, demonstrating that the altered dosage of only one or a few genes can cause a pronounced phenotypic change. For example, a recent study showed that the early onset Alzheimer’s disease was caused by duplication of a region of human chromosome 21 (Hsa21), which contains only four genes, including the amyloid plaque precursor protein, APP gene (18). A recent review of the whole genome array CGH noted that genomic deletions and duplications of between 1 kb and 10 Mb make up to 15% of all known mutations causing monogenic disorders (19).

Why model aneuploidies/segmental aneusomies?

The aim of modeling aneuploidies/segmental aneusomies in mice is to establish which gene(s) need to have altered

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**Table 1. Examples of aneuploidies and segmental aneusomies**

<table>
<thead>
<tr>
<th>Aneuploidy/Segmental Aneusomy</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Whole chromosome monosomy</td>
<td>Monosomy X, Turner’s syndrome</td>
</tr>
<tr>
<td></td>
<td>Having a single sex chromosome; congenital abnormality resulting in variable features including short stature</td>
</tr>
<tr>
<td>Whole chromosome trisomy</td>
<td>Trisomy 13, Patau syndrome</td>
</tr>
<tr>
<td></td>
<td>Rarely surviving until birth, which results in severe neurological and cardiac defects usually leading to death within a few weeks after birth</td>
</tr>
<tr>
<td></td>
<td>Trisomy 18, Edwards syndrome</td>
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<td></td>
<td>Severe developmental disorder, children usually die within the first few months after birth; occurs in about one in 6000 live births</td>
</tr>
<tr>
<td></td>
<td>Trisomy 21, DS</td>
</tr>
<tr>
<td></td>
<td>Occurs in about one in 700 live births, highly variable syndrome resulting in mental retardation and other abnormalities; life expectancy of &gt;50 years of age</td>
</tr>
<tr>
<td>Segmental monosomy</td>
<td>Segmental monosomy chromosome 5p, cri-du-chat syndrome</td>
</tr>
<tr>
<td></td>
<td>A relatively common deletion syndrome, occurring in between one in 20 000 and one in 50 000 live births. Characteristic craniofacial defects and severe mental retardation; death usually within the first few years of infancy</td>
</tr>
<tr>
<td></td>
<td>Segmental monosomy chromosome 22q11.2, Di George syndrome</td>
</tr>
<tr>
<td></td>
<td>Heart defects, moderate-to-mild mental retardation, hypocalcemia and other features</td>
</tr>
<tr>
<td></td>
<td>Segmental trisomy</td>
</tr>
<tr>
<td></td>
<td>Segmental trisomy 16p</td>
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<tr>
<td></td>
<td>Microcephaly, mental and growth retardation; variable features</td>
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dosage in order to give rise to individual aspects of a phenotype, to understand how such dosage imbalance gives rise to specific phenotypes and, ultimately, to identify potential therapeutic strategies.

In modeling aneuploidy/segmental aneusomy, the phenotypes that arise are usually complex, and, like any other genetic disorder, depend on the content of the affected DNA, including individual alleles, as well as its interaction with the rest of the genome (genetic background) and the environment (8). However, the essential criteria for modeling aneuploidy/segmental aneusomy in mice are to accurately re-create the human disorder not only in terms of the genetic content but, most importantly, to get the correct gene dosage.

How to model aneuploidies? Transgenesis and chromosome engineering

At the DNA level, there is a problem of both scale and expression. The simplest approach to model segmental trisomy might be to generate mice bearing individual transgenes, in which a candidate gene of choice is expressed from an exogenous promoter; however, this is unlikely to re-create the correct overexpression dosage or the correct spatial and temporal patterns of expression. A further complication is that most transgene constructs use cDNAs and thus do not recapitulate the full set of splice isoforms expressed by many genes. A better approach is to use BAC or YAC transgensics, as here the gene(s) will be expressed from an endogenous promoter and are thus more likely to faithfully mirror expression (and splice variants) of the endogenous genes. However, even the largest YAC can only carry up to 1–2 Mb of DNA, and many well described partial trisomies are an order of magnitude larger than this.

The solution to these problems of scale and expression is to use Cre–loxP based methods for chromosome engineering of mouse chromosomes to create large-scale deletions or duplications mimicking those seen in the human conditions (chromosome engineering reviewed recently in 20). All genes in these modified chromosomes continue to use their endogenous promoters and the changes can encompass regions from <1 Mb to many megabase pairs in length. However, even the precise genome manipulation of chromosome engineering can encounter another problem in modeling aneuploidy/segmental aneusomy: there is no 1:1 correlation between human and mouse chromosomes. Large regions have been moved around during the 80 million years, which separate our species, and even the smallest human chromosome, Hsa21, has homologous regions on three different mouse chromosomes (Mmu10, 16 and 17) (21). Thus, it may be difficult to recapitulate a complete phenotype for a human disorder of interest (especially if it involves very large regions or whole chromosomes) by engineering of mouse chromosomes alone.

An alternative approach that solves this problem is to work with the human chromosomes themselves, inserting them into the mouse, using the process of chromosome-mediated gene transfer (22–25) to manipulate the content of mouse embryonic stem (ES) cells as, for example, we have recently done with Hsa21 to create a transchromosomal mouse model of DS (26,27).

Mosaicism

Many aneuploidy syndromes also entail some degree of mosaicism. For example, trisomy chromosome 8 is sufficiently well recognized to have a named syndrome associated with it, Warkany syndrome (28,29), but is only ever present in live births when the individuals are mosaic for euploid:trisomy 8 cells. This illustrates an important point about the phenotypic variability in aneuploidy syndromes: it is thought that phenotypes tend to be less severe if greater numbers of euploid cells are present in mosaic individuals. It would be possible to model such mosaicism through, for example, aggregation chimeras or by using Cre–loxP-mediated recombination to generate deletions or duplications in somatic tissues (20).

Is it better to use human or mouse genes to model duplications?

The advantage of using human chromosomes or fragments of them to model human trisomies and partial trisomies is that the added human genetic sequences contain all the relevant human genes, including their regulatory elements, and thus may model the disease more closely than does the equivalent mouse sequences. However, human genes may not behave in a mouse environment in the same way as they do in the human—they may show changes in the quantity and in the spatial and temporal patterns of transcription. There may also be differences in translation and posttranslational modification, and the human proteins may not have exactly the same activity as their mouse homologs. If a transgenic protein (i.e. within an engineered chromosome context) works suboptimally in a mouse, this will affect the subtle dosage imbalance that we are trying to model, or worse, create a neomorphic function. At present, there is no clear answer to the question of whether it is better to try and model these diseases with human or mouse genes; researchers will need to empirically establish which works best for their system.

IMPORTANT STEPS IN MODELING ANEUPLOIDIES IN MICE

The euploid chromosome number in mice is 40; of the aneuploidies, only monosomy X, XXXY and XYY and, very rarely, trisomy 19 are known to survive beyond birth in mouse (30,31) [there is also one recorded case of an XXXX mouse (32)]. However, breeding schemes using Robertsonian translocations that can be used to produce aneuploidy and study whole chromosome gene dosage effects on the embryo are well known to classical mouse geneticists (30,33,34).

Random mutagenesis

Ionizing radiation and specific chemicals (sometimes called ‘aneugens’) such as chlorambucil (35) can induce a high rate of chromosome rearrangements, which may result in partial aneuploidies (30). These agents can be used to directly treat mice or mouse ES cells, which may then be used to create mouse strains carrying segmental aneusomies (36). However, both aneugens and radiation produce essentially random changes [but see (37)], and although helpful in
defining the biology of many genes and systems, and informing us as to how some aneuploidies arise in the first place (38,39), they have not generally been used to model specific human syndromes. A notable exception to this is the Ts65Dn mouse that arose in a radiation mutation experiment (40), which carries a small extra chromosome consisting of the centromere of Mmu17 and a small portion of Mmu16 that has homology to Hsa21 (41); the mouse is aneuploid and has 41 chromosomes. The Ts65Dn mouse is an important and informative model of DS, as it is trisomic for 136 of the ~247 genes on Hsa21 (42,43).

Occasional mutants have arisen unexpectedly during transgenesis and gene-targeting experiments, where manipulation of the genome has caused unexpected changes unrelated to the transgene insertion. Again, these are random changes that do not generally model human segmental aneuploidies. However, another notable exception to this is provided by the Ts1Cje mouse, which arose during a gene-targeting experiment (of the Sod1 locus on Mmu16) giving rise to an apparently balanced translocation such that a portion of Mmu16 is translocated onto Mmu12 (44). Mice carrying this chromosome have a normal chromosome number but are trisomic for 105 Mmu16 genes that have orthologs on Hsa21 (42) and thus Ts1Cje is another important model of DS.

Transgenesis

Since the 1990s, single gene and YAC or BAC transgenic mice have been specifically created to model aspects of human trisomy or segmental trisomy syndromes. Single gene transgenics almost never recapitulate the dosage effects of individual genes (even when the precise mRNA and protein levels in tissues of interest are known), for the reasons stated earlier, i.e. inappropriate control regions such as promoters. Therefore, BAC or YAC transgenics may be considered more useful. One successful example of their application again comes from DS research: a set of overlapping YAC transgenics was created using human chromosome 21 YACs, to pinpoint the location and therefore identity of genes involved in cognition in the transgenic mice, and thus potentially in human DS (45). This study implicated trisomy of DYRK1A (minibrain) in learning defects in mice that may in turn correlate with such defects in humans (46,47).

Chromosome engineering

More recently, Cre-LoxP technology has been used to design and engineer specific mouse chromosomes in order to both model aneuploidy and to identify the important dosage-sensitive genes (20,48). These new strains are giving insight into the mechanisms by which dosage imbalance produces phenotype. Reeves and co-workers (42) generated a mouse strain, Ts1Rhr, carrying a partial duplication in Mmu16, encompassing the genes homologous to the so-called DS critical region on Hsa21. This region contains about 33 genes and is believed to contain critical genes whose trisomy leads to two of the cardinal features of DS, mental retardation and hypotonia. Comparison of Ts1Rhr with the other larger partial trisomies Ts65Dn and Ts1Cje allowed the authors to conclude that there was a complex relationship between trisomic gene content and one particular phenotype—craniofacial dysmorphism (42). This work shows the future of aneuploidy/segmental aneusomy studies: as chromosome engineering will enable the creation of lines of mice carrying overlapping trisomic/deleted regions to pinpoint individual genes of importance out of hundreds that may be implicated in a single syndrome.

On a larger scale, we recently published a paper in which we transferred almost the whole of human chromosome 21 into a mouse, using a combination of chromosome-mediated gene transfer and ES cell culture (24,27). The resultant mouse strain, Tc1, is trisomic for about 227 genes (92% of the genes on Hsa21) and shows multiple phenotypes that resemble human DS, including defects in learning, synaptic plasticity, brain and heart development and craniofacial morphology.

IDENTIFICATION OF GENES CAUSING PHENOTYPES IN ANEUPLOIDIES

Phenotypic analysis

Once a mouse model is available for an aneuploidy or segmental aneusomy syndrome, the next step is to determine which genes are important for which aspects of the phenotype, as a first process in distinguishing primary effects of aberrant dosage from downstream effects [see (8) for discussion]. This depends critically on robust, reproducible and biologically meaningful assays for phenotype. Mouse phenotyping is an area that is rapidly expanding and being refined (49). Several programs are underway to develop comprehensive standardized tests of multiple systems. The Eumorphia project has created the EMPReSS screen (50); the Phenome project is gathering baseline data using standardized tests on inbred lines (51,52). An aim of these programs is to produce standardized operating procedures so that, as far as possible, results from different laboratories are comparable.

However, mice are not human and clearly there are physiological and other differences in the presentation of phenotypes between mice and humans. An intriguing area of research is in behavioral analysis. Mice are largely nocturnal prey animals and, as a result, have evolved behaviors that are not found in humans. Nevertheless, there are clear parallels between mouse and human behavior, and comparisons of these are now starting to be made (53). Phenotype testing in mouse models can, on occasion, reveal previously unknown aspects of the original human condition. Reeves and colleagues determined that the Ts65Dn mouse model of DS has a reduced number of cerebellar neurons compared with euploid controls. Researchers then went back to study the cerebellum from individuals with DS and found that the same reduction was also present in humans (51).

Methods for finding dosage-sensitive genes

Once a robust assay shows that a particular phenotype is evident whenever a particular chromosomal segment is present (trisomy) or absent (monosomy), then the ease of manipulating the mouse genome to produce mice carrying reduced or increased gene copy numbers comes to the fore.
For example, to identify genes responsible for features of DS, the appropriate mouse models (Tc1, Ts65Dn and Ts1Cje) can be crossed to mice bearing deletions in segments of mouse chromosomes syntenic to Hsa21 to pinpoint the segment containing the relevant gene(s) (Fig. 1). Further breeding to ever smaller deletions and eventually to single gene knockouts may ultimately identify dosage-sensitive genes that produce an assayable phenotype. The fact that such an approach is feasible is elegantly demonstrated in a recent publication, which identifies that it is three copies of the App gene in the Ts65Dn and Ts1Cje models of DS, which is responsible for the degeneration of cholinergic neurons that may underlie the early onset Alzheimer’s disease in DS (54).

NEW TECHNOLOGIES DRIVE OUR UNDERSTANDING OF ANEUPLOIDIES

Aneuploidy and segmental aneusomy syndromes are common and complex. Furthering our understanding of their molecular genetic basis will be highly informative about many aspects of functional genomics from chromosome architecture (56) and gene regulation to the effects of altering the stoichiometry of the proteome. Investigations of the genetic basis for these disorders inform our basic biological understanding as well as knowledge of individual aspects of each syndrome. This is relevant not only to individuals with aneuploidy syndromes but also to the euploid population. Individuals with DS have an increased rate of leukemias and an earlier onset Alzheimer’s disease compared with the euploid population, and an understanding of these disorders in DS will be informative for similar disorders in euploid humans. With the advent of new gene therapies for single gene dominant disorders (such as RNAi-based approaches) and recessive disorders, there is real hope that identifying key dosage-sensitive genes may open up genetic therapies that at least tackle the non-developmental aspects of some syndromes. Small molecule therapeutic approaches may also be possible, once key pathways have been identified (57). As with every other human disease under study, mouse models are critical for our understanding, and without such models, the difficult task of unraveling the causes of aneuploidies and segmental aneuploidies would be impossible.

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