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Molecular Mechanisms in 22q11 Deletion Syndrome

Nigel M. Williams*

Department of Psychological Medicine and Neurology, MRC Centre in Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Cardiff, UK

*To whom correspondence should be addressed; tel: +44-(0)2920-687070, e-mail: williamsnm@cf.ac.uk

It is now well recognized that as well as having a characteristic facial dysmorphology and a range of congenital abnormalities, individuals with chromosome 22q11 deletion syndrome (22q11DS) have a greatly increased risk of developing psychosis, in particular schizophrenia. The majority of deletions span a large 3Mb region at 22q11. However, the presence of affected individuals carrying smaller deletions have not been sufficient to satisfactorily reduce the critical region for the behavioral phenotype beyond a ~1.5Mb region that contains at least 28 genes. By having a shared genetic variant that greatly increases risk to psychosis, individuals with 22q11DS are a relatively homogeneous population to study psychiatric disease. Despite this, the large volume of research performed over the last 15 years suggest that the mechanism by which haploinsufficiency at 22q11 increases risk to psychiatric illness is likely to be complex and it remains uncertain why individuals carrying identical 22q11 deletions can present with such a wide range of neuropsychiatric phenotypes. This review will therefore consider the ways in which deletions at 22q11 are expected to increase risk to develop psychiatric disease by summarizing the work that has been done to investigate three of the most likely disease causing mechanisms: (a) gene dosage sensitivity; (b) unmasking of recessive alleles or functional polymorphism; and (c) position effect.

Key words: 22q11DS/schizophrenia/psychosis/deletion/chromosomal abnormality

Introduction

Deletions at 22q11 have been associated with a heterogeneous range of clinical syndromes that include DiGeorge syndrome, Velo-cardio-facial syndrome, and conotruncal anomaly face syndrome.[1] It is however widely considered that these different diagnostic categories probably reflect variable outcomes from a single genetic mechanism,[1] and in this context, they can be grouped together under the collective term Chromosome 22q11 Deletion Syndrome (22q11DS). As would be expected from the product of this diverse range of clinical syndromes, the phenotype of 22q11DS is complex; nevertheless, it is now well established that people with 22q11DS have a greatly increased risk of developing psychosis and in particular schizophrenia.[2–6]

Microdeletion at chromosome 22q11 occurs in approximately 1 in every 4000 live births,[1] and while it is inherited from an affected parent in 5-10% of cases it occurs de novo in the remainder.[1] A range of molecular techniques have traditionally been used to identify the microdeletions at 22q11, ranging from fluorescence in situ hybridization (FISH) analysis, quantitative polymerase chain reaction (PCR) to the more recent application of comparative genome hybridization (CGH) and genome-wide arrays of single nucleotide polymorphisms (SNPs). The resolution with which these techniques are able to precisely define the breakpoints can vary considerably; however, it is accepted that approximately 87% of deletions include a common 3 Mb region, which includes at least 48 known genes, while around 8% span a smaller 1.5 Mb region (nested within the larger 3 Mb region),[7] which contains at least 28 genes. The relative homogeneity of the common deletions[8] is largely due to the presence of blocks of genomic sequence known as low copy repeats (LCR22s) at the breakpoints of each deleted region. The LCR22s are believed to act as targets for anomalous intrachromosomal homologous recombination during meiosis thereby generating the observed chromosomal rearrangements.

While it is evident that the common 3 Mb/1.5 Mb microdeletions are associated with 22q11DS, a number of cases have been reported with smaller atypical deletions at 22q11; however, these have not been sufficient to satisfactorily reduce the critical region for the neuropsychiatric phenotype to less than ~1.5 Mb. Screens of patients with neuropsychiatric disorders have also

identified increased rates of individuals carrying deletions at 22q11.[9,10] While microdeletions are by far the most common genomic variation associated with 22q11DS, it is important to note that other genomic instabilities that disrupt chromosome 22q11 have been reported in patients with the 22q11DS phenotype. In particular, a number of balanced translocations have been identified in individuals who display the typical 22q11DS phenotype[11,12] and individuals carrying the reciprocal microduplications mediated by the LCR22s have also been reported (reviewed in[13]). Clinical analysis of these duplication carriers has revealed a widely variable clinical phenotype that includes developmental cognitive impairments, including mental retardation, learning disabilities, and autism as well as dysmorphic phenotypes, which were similar to those correlated with deletions at 22q11.[13] The recent application of SNP and CGH arrays has detected duplications spanning 22q11 in unaffected control populations[9]; therefore, while the pathological relevance of duplications at 22q11 remains unclear, it is plausible that they convey risk to develop a somewhat milder behavioral phenotype albeit with reduced penetrance.

There is still little clear understanding of how heterozygous microdeletions on chromosome 22q11 lead to this diverse spectrum of behavioral phenotypes and the elucidation of the complex pathogenic mechanisms conferred by chromosomal abnormalities warrants particular attention. This review will therefore consider the ways in which deletions at 22q11 are expected to increase risk to develop psychiatric disease by summarizing the work that has been done to investigate 3 of the most likely disease causing mechanisms: (a) gene dosage sensitivity, (b) unmasking of recessive alleles or functional polymorphism, and (c) position effect.

Analysis of gene dosage sensitivity in 22q11DS

Given that microdeletions account for the large majority of cases of 22q11DS, then the resulting phenotype is widely considered to be due to be haploinsufficiency of one or more dosage sensitive genes within the deleted region. Theoretically, it is expected that the expression of hemizygous genes spanned by deletions at 22q11 will correlate with the DNA copy number, where relative to the wild type, the level of expression of haploinsufficient genes would be reduced by half. The biological function of some loci is particularly sensitive to changes in gene dosage, and these are clearly candidates for the neuropsychiatric phenotypes associated with 22q11DS.[14]

The most straightforward approach to identify which genes are dosage sensitive is to analyze the expression of genes spanned by structural variants at 22q11. To date, probably due to the paucity of appropriate neural tissue from 22q11DS patients, there have been no investigations into the influence of hemizygosity on gene expression in patients with 22q11DS. The genomic sequence at 22q11 has a well-characterized syntenic region on mouse chromosome 16 (MMU16),[15] whereby most of the genes from within the 22q11 deleted region have a murine orthologue. Studies investigating the effects of dosage sensitivity in 22q11DS have therefore focussed the on the orthologous genes at MMU16.

Gene expression analysis of mouse embryos has revealed that many of the MMU16 genes are expressed during development and have a particularly dynamic pattern of expression across different developmental stages.[16,17] Direct relevance to 22q11DS has come from chromosome engineering experiments of the syntenic region at MMU16 which have created a number of murine models which carry different variations of long-range deletions as well as knockouts of individual orthologous genes in this region. These murine models have allowed researchers to identify which genes at MMU16 are most sensitive to haploinsufficiency. In the first such study, Lindsay and colleagues[18] deleted a 1.2 Mb segment (termed Df1) from MMU16 which encompassed 27 functional genes which have human orthologues within the human 1.5 Mb critical 22q11DS deleted region. While the mice carrying homozygous deletions exhibited early embryonic lethality, the heterozygous mice (Df1/+) were viable. Microarray based analysis of gene expression in the embryos of Df1/+ mice has revealed evidence for significantly reduced expression of genes spanned by the deletion indicating that at least in this murine model, a large number of the genes are dosage sensitive.[19] This together with reports that mice carrying hemizygous deletions at MMU16 have compromised neurogenesis and subsequent differentiation in the cerebral cortex demonstrates a potential biological defect incurred by dosage sensitive genes at this locus.[20] Measures of gene expression in the hippocampus of mice carrying the Df1 deletion have provided direct evidence that most hemizygous genes at MMU16 have a significant reduction in expression and are therefore likely to be dosage sensitive.[21,22] Gene expression analysis of single gene knockout models for *Tbx1*, *Gnb1l*, *Dgcr8*, *Zdhhc8*, *Prodh*, *Comt*, *Rtn4r*, and *Sept5* have also provided evidence that haploinsuffiency of each gene results in the expected reduction in expression.[23–28]

Murine models of 22q11DS have been able to reproduce many of the phenotypes associated with human 22q11DS, and this has been particularly fruitful in identifying which dosage sensitive gene underlies the cardiac abnormalities. Df1/+ mice present with cardiovascular abnormalities similar to those associated with human 22q11DS; however, the observation that mice that carried the Df1 deletion on one chromosome but had the complementary duplication on the other (termed Df1/Dp1 mice) did not present the abnormal cardiac phenotype seen in the Df1/+ mice, implied that the cardiac

abnormalities were due to hemizygosity of a dosage sensitive gene(s) within the deletion.[18] Subsequent studies in which a series of nested deletions and then single gene knockout models of the MMU16 locus were created, and these resulted in compelling evidence that haploinsufficiency of a single gene, *Tbx1*, is likely to be responsible for most, if not all, of the ''pharyngeal phenotype'' of 22q11DS.[29–31] *Tbx1*+/− mice have cardiovascular abnormalities that, like those in 22q11DS, appear to be due to defective development of the 4th pharyngeal arch artery. The relevance of these findings to the human disorder was supported by the subsequent identification of point mutations in *TBX1* in a series of patients who did not carry deletions at 22q11 but did present with the typical clinical features of 22q11DS.[32]

In contrast to the pharyngeal phenotype, clearly there is no rodent model that can globally recapitulate the neuropsychiatric disorders seen in patients with 22q11DS. Therefore, while it is more challenging to associate dosage sensitivity to behavioral deficits in murine models of 22q11DS, it has been possible to examine a series of cognitive functions that can also be objectively tested in individuals with 22q11DS. Deficits in cognitive tasks associated with activity in the prefrontal cortex and hippocampus, including attention, working memory, executive function, and short-term verbal memory have been reported in 22q11DS patients. Murine models have therefore been assessed for these component phenotypes, and these studies have demonstrated that Df1/+ mice have deficits in sensorimotor gating, working memory, and fear conditioning phenotypes which have been implicated as endophenotypes in schizophrenia as well as other severe psychiatric disorders that have reported in 22q11DS patients.[23,24,33] This suggests that dosage sensitive genes from the deleted region modulate these behaviors.

While the analysis of murine models with large deletions at MMU16 are necessary to recapitulate the potentially complex functional consequences of a contiguous gene deletion, studies of single gene knockout models have provided a more precise insight into the correlation between dosage imbalance and specific behavioral phenotypes. Single gene knockouts have not been created for all of the 22q11 orthologs present at MMU16; however, they have revealed that haploinsufficiency of numerous MMU16 genes can confer an abnormal behavioral response. For example, while haploinsufficiency of *Dgcr8* and *Zdhhc8* results in mice with impaired prepulse inhibition (PPI),[24,25] *Comt*, *Rtn4r*, and *Sept5* knockout models reveal negligible deficits in sensorimotor gating when compared with wild type.[26–28]

Studies of *Prodh* and *Comt* murine knockouts have provided an insight into the potential functional complexity conferred by the haploinsufficincy of multiple dosage sensitive genes. Paterlini and colleagues[34] observed that while mice carrying a heterozygous knockout of the *Prodh* gene had the expected reduction in gene expression of *Prodh* transcripts, the expression of *Comt* transcripts and proteins in the prefrontal cortex was significantly increased. Studies of behavioral phenotype supported this interaction, where mice that are haploinsufficient for either *Comt* or *Prodh* had no detectible deficit in tests of working memory, while mice with a combined deficient of both genes had significant deficits in working memory.[34] Given that 22q11DS involves the deletion of a series of contiguous genes, this observation provides an insight to the potential complexity when multiple 22q11 genes acting on common cellular processes are haploinsufficient.

Given the evidence implicating *TBX1* with the 22q11DS pharyngeal phenotype, it is not surprising that researchers have assessed whether it also increases risk to the behavioral disorders. Paylor and colleagues[23] demonstrated that hemizygous deletion of either *Tbx1* or its adjacent gene *Gnb1l* can cause deficits in tests of sensorimotor gating. Interestingly, none of the 5 patients with point mutations in *TBX1* reported by Yagi and colleagues[32] had mental retardation, and psychiatric disorders were not described, although only one case was adult and therefore in the common age of risk of psychosis. Nevertheless, in other cohorts of individuals who do not carry 22q11 deletions, functional mutations in *TBX1* have been identified in individuals with Asperger syndrome[23] and unspecific cognitive deficits.[35]

Investigations into the impact of large deletions on gene expression have revealed that a minority of genes has a negative correlation between gene copy number and gene expression.[36] This, together with the potential that duplications at 22q11 can confer neuropsychiatric phenotypes similar to those associated with deletions have led some researchers to investigate the potential gain of function incurred by overexpression of genes at MMU16.[37–39] In one of these studies, Stark and colleagues[39] used engineered Bacterial Artificial Chromosome (BAC) constructs to generate 2 transgenic murine models, one overexpressing the genes *Prodh* and *Vpreb2* (Tg-1) and another overexpressing the genes *Zdhhc8*, *Ranbp1*, *Htf9c*, *T10*, *Arvcf*, and *Comt* (Tg-2). Behavioral analysis revealed that relative to the wild-type mice, the Tg-1 murine model presented with a significant increase in measures of PPI. When taken together with studies of Prodh knockout mice which show significantly reduced PPI, these findings are particularly interesting because they suggest that Prodh has a key role in modulating the degree of sensorimotor gating in mice and that this effect is dosage sensitive. By contrast, no significant differences were found in PPI testing of the Tg-2 line.[39]

Analysis of genotypes on the haploinsufficient chromosome in 22q11DS

Chromosomal abnormalities, in particularly deletions, are clearly the primary mutational mechanism in

individuals with 22q11DS, and this has inevitably resulted in gene dosage imbalance being the central working hypothesis in our attempts to understand the increased risk to neuropsychiatric illness in 22q11DS. Nevertheless, it is well recognized that for many genes, the level of the haploinsufficient gene product does not change compared with those of disomic cells,[14] implying that the expression of such genes are presumably under tight homeostatic regulation that somehow compensates for gene dosage imbalance. It is plausible that by removing one allele, deletions at 22q11 may unmask the deleterious effect of a remaining recessive or functional allele now present in a hemizygous state. In this scenario, functional alleles present in both dosage sensitive and insensitive genes could potentially influence the clinical phenotype associated with 22q11DS. This mechanism of ''unmasking'' of functional alleles on one chromosome by a deletion on the other is often invoked but rarely proven; however, there are now a number of examples where this form of compound heterozygosity has resulted in disease phenotypes.[40] One clear example has been described in patients with common Sotos syndrome (SoS), which can be a result of microdeletions that span the causative gene NSD1. The variable size of the deletion can result in some spanning the adjacent plasma coagulation factor 12 (*FXII*) gene which in turn carries a functional polymorphism termed 46C/T. SoS patients who are hemizygous for the hypoactive 46T allele can be further compromised, presenting with additional features associated with FXII deficiency,[40] while those hemizygous for the 46C allele have no FXII deficiency.

Given the complex clinical phenotype of 22q11DS and the large number of genes spanned by the deletions, it is feasible that these mechanisms may well play a role in clinical variability. Indeed, given that haploinsufficiency is common to most 22q11DS patients, it is reasonable to hypothesize that the background genetic variation that remains on the haploinsufficient chromosome could well play an important role in conferring risk to variable phenotypes such as neuropsychiatric disorders. To date, no study has systematically screened the nondeleted chromosome in 22q11DS patients for the presence of additional rare functional variants. Instead there have been a number of studies that have tested common variants for association with the expression of the neuropsychiatric phenotype in 22q11DS patients, testing the hypothesis that at a given 22q11 gene, common risk variants for psychiatric illness would be expected to show much higher penetrance in subjects whose gene function is already compromised by hemizygosity.

Catechol-O-methyl transferase. Located within the ~1.5 Mb region deleted in 22q11DS, the Catechol-O-methyl transferase (*COMT*) gene is a strong candidate for schizophrenia because it encodes an enzyme that degrades catecholamines, including dopamine. The COMT protein occurs as 2 distinct isoforms: a soluble form found in the cell cytoplasm (S-COMT) and a longer membrane bound form (MB-COMT). In most assayed tissues, the S-COMT form predominates, accounting for around 95% of total COMT activity; however, MB-COMT is the more prevalent species in brain.[41] *COMT* contains a nonsynonymous G > A polymorphism that produces a valine-to-methionine substitution at codons 108 and 158 in the S-COMT and MB-COMT transcripts, respectively (Val(108/158)Met). The amino acid change results in altered COMT activity in both S-COMT and MB-COMT, whereby the Val form of COMT is reported to have higher activity than the Met.[42] Although expressed widely, COMT appears to be a minor player in dopamine clearance compared with neuronal synaptic uptake by the dopamine transporter and subsequent monoamine oxidase metabolism.[42] However, one region where this may not apply is the prefrontal cortical where dopamine transporter expression is low, and the importance of COMT appears to be greater.[26]

Based upon the role of COMT in dopamine catabolism, it follows that individuals with 22q11DS who are hemizygous for the low-activity Met allele may be expected to have increased brain dopamine levels, which in turn confers an increased risk to develop psychosis.[43] A number of studies have now directly tested this hypothesis by genotyping the *COMT* Val(108/158)Met variant in 22q11DS patients and comparing the frequency in those that have developed psychiatric disorders to those who have not. Gothelf and colleagues[44] performed a longitudinal analysis of 24 adolescents with 22q11DS, of which 7 had experienced psychotic illness. In accordance with their primary hypothesis, psychotic symptoms were more severe in 22q11.2DS subjects who were hemizygous for the low-activity Met allele (40.5 vs 29.2 on the Brief Psychiatric Rating Scale) at 18 years of age. This particularly promising result was however compromised by the small sample size and studies of larger samples of 22q11DS patients have not supported these findings. In one study of 48 adults with 22q11.2 deletions, of which 12 had schizophrenia, there was no significant evidence for an association between the *COMT* Met allele with psychosis.[6] Moreover, there was no significant difference in symptom severity as measured by a schizotypy scale between individuals with Met or Val alleles.[6] Similarly, in an independent sample of 73 Caucasian adults with 22q11DS, of which 33 had schizophrenia, the lower activity Met allele was again not significantly associated with schizophrenia.[45]

Phosphatidyl-inositol-4-kinase-catalytic-α. Jungerius and colleagues[46] initially identified significant evidence for association with SNPs and haplotypes at the Phosphatidyl-inositol-4-kinase-catalytic-α (*PIK4CA*) locus in a sample of 310 schizophrenic cases and 880 control

from the Netherlands. In that study, 3 common SNPs (minor allele frequency > 0.19) located at the *PIK4CA* locus (rs2072513, rs165862, and rs165793) showed significant association with schizophrenia. This finding obtained some support from the genome-wide association study of the International Schizophrenia Consortium[9] which reported nominally significant association at one of these SNPs (rs165862, $P < .0067$) in a sample of white individuals of European origin but not in a much smaller sample from a different ethnic background.[47] Given that the *PIK4CA* gene is located within distal half of the 3 Mb region, the commonly deleted in 22q11DS Vorstman and colleagues[48] followed up this work by testing the possibility of an interaction effect between *PIK4CA* and deletions at 22q11 by analyzing SNPs at *PIK4CA* in Canadian adults with 22q11DS, comparing those with schizophrenia to those without. This study identified significant evidence for association with rs165793 ($P = .006$) supporting the hypothesis of an enriched effect size in the 22q11DS cohort (OR = 9.47 [1.16–77.56]). In an attempt to replicate these findings, Ikeda et al[49] performed association analysis in a sample of 83 individuals with molecularly confirmed 22q11 deletions, of which 24 has experienced episodes of psychosis. No evidence for association was obtained for either SNPs or haplotypes, thus failing to provide confirmation of a large increased risk to schizophrenia at the *PIK4CA* locus.

G-protein beta-subunit-like/T-Box1. In following up the reports that haploinsufficiency at the T-Box1 (*TBX1*)/G-protein beta-subunit-like (*GNB1L*) locus contributes to the behavioral deficits observed in murine models of 22q11DS,[23] Williams and colleagues[50] initially identified significant evidence for genotypic association at the *TBX1*/*GNB1L* locus in a sample of nondeleted schizophrenics and controls. While the significant evidence for excess homozygosity was strongest at markers rs5746832 and rs2269726 and was apparent in the full sample, post hoc analysis revealed that it was much stronger in the male patients.[50] Given the absence of any nonsynonymous variants in *TBX1* and *GNB1L* exons that could to explain the significant results, evidence was sought to identify whether cis-acting changes in gene expression could provide a mechanism for the observed genotypic association. The expression of both *TBX1* and *GNB1L* were demonstrated to be under the influence of cis-acting variation, but the SNPs associated with schizophrenia correlated only with *GNB1L* expression. Thus, the 'T' allele at rs2269726 was associated an average of 20% increased expression relative to that of 'C'.[50]

Given that excess of both the TT and CC homozygotes in schizophrenics (OR = 1.28 and 1.36, respectively), the authors concluded that risk to psychosis reflects dosage sensitivity, with risk being related to overexpression or underexpression of *GNB1L* relative to heterozygotes. According to this model, it would be expected that risk for psychosis in males with 22q11DS would be related to low expression of *GNB1L*. In order to test this hypothesis, rs2269726 was then typed in a sample of 83 22q11DS adult patients, of which 22 had experienced episodes of psychosis. Significant evidence for association with psychosis was again identified and again it was selectively in males ($P = .018$).[50] The association was however opposite to that predicted; alleles that were correlated with increased *GNB1L* expression were associated with psychosis in 22q11DS males, an observation that is apparently incompatible with a simple dosage sensitivity model. One possible, albeit speculative, explanation of these findings is that hemizygosity for low expression *GNB1L* alleles results in levels of expression that are sufficiently low to induce compensatory mechanisms, whereas hemizygosity for high expression variants does not. Alternatively, the results could represent a gene dosage imbalance between *GNB1L* and an interacting gene also located within the 22q11 deletion.[51] The exact function of *GNB1L* is unknown; however, it clearly contains a WD40 repeat domain, which is known to mediate protein-protein interactions. It therefore follows that other WD40 repeat containing genes at 22q11, such as *HIRA*, would be obvious candidates to explore such a mechanism.

Analysis of genes outside of the deleted region

By removing or altering a key regulatory gene or sequence, copy number variants (CNVs) can have a direct effect on gene expression of a nearby gene.[14] This is supported by clear evidence that the variable gene expression in a large proportion of dosage sensitive genes are the direct result of CNVs that lie adjacent to the genes coding sequence and these "position effects" have been reported for genes that are located over 2 Mb away from the CNV boundaries.[36] It is therefore plausible that structural variants located at 22q11 can have a direct effect on regulatory genes or sequences located within the deletion which then in turn influence the expression of dosage sensitive genes that lie elsewhere on the same chromosome (in *CIS*) or potentially on other chromosomes (in *TRANS*).

Studies of global gene expression in the Df1/+ murine models of 22q11DS have identified evidence that genes located outside the Df1 deletion are differentially expressed.[19,21] However, while there is synteny between human 22q11 and MMU16, there are also major differences in the genomic order of the genes as well as the sequences flanking deletions between the 2 species. These differences can make it difficult to translate evidence for positional effects seen in murine models to humans. In addition, the large range of clinical phenotypes associated with 22q11DS mean that it is difficult to pinpoint which changes in differential expression are relevant to the increased risk to neuropsychiatric phenotype. Further investigations are therefore necessary to establish the underlying cause of the altered expression of these genes.

In spite of these limitations, analysis of the murine Df1/+ model has revealed evidence that the gene *Crkl* that flanks the deleted region shows reduced expression relative to the wild-type mice.[19] In addition, a series of studies have revealed that haploinsufficiency of *DGCR8* can result in potentially important and widespread positional effects. The *DGCR8* gene is a key component of the "microprocessor" complex that is essential for miRNA production.[24] *DGCR8* is deleted in 22q11DS and analysis of single gene knockout models of the murine orthologue at MMU16 (*Dgrc8*) has revealed that haploinsufficiency results in alterations in the biogenesis of brain miRNAs, which in turn resulted in the downregulation of a specific subset of mature miRNAs.[24] Given that miRNAs have a key role in regulating gene expression (primarily by inhibiting mRNA translation or stability), it is not surprising that the authors also detected evidence for the subsequent upregulation of a number of transcripts in the murine brain. Stark and colleagues[24] were not able to identify the entire set of gene targets, but the potentially large scale effects on gene expression could be a major positional effect incurred by deletions at 22q11 that is likely to influence the expression of genes both in *CIS* and in *TRANS*. Recent work by Fénelon and colleagues[52] has suggested that these widespread effects could have an impact on the underlying biology of 22q11DS whereby mice haploinsufficient for *Dgcr8* demonstrated deficits in behavioral responses[24] and had abnormalities in synaptic properties of cortical neurons,[52] implicating altered short-term plasticity as a potential mechanism underlying the cognitive dysfunction in 22q11DS. However, the mechanisms underlying short-term plasticity are complex and diverse, and the precise role of *DGCR8* in these processes remains to be determined.[52]

Discussion

Individuals with 22q11DS have a significantly increased risk to develop neuropsychiatric illness, which as the result of having a shared genetic risk factor makes them an important population to study. Understanding the molecular mechanism with which deletions at 22q11 are likely to increase, this risk will enable us to disentangle the complex behavioral phenotype associated with 22q11DS.

Most studies have followed the expected hypothesis that the complex neuropsychiatric phenotype of 22q11DS reflects reduced expression of multiple dosage sensitive genes located at 22q11 which are not just prevalent during brain development but also in the adolescent and adult brain. Research into dosage sensitivity has focussed on murine models of 22q11DS, which have revealed that numerous orthologous genes are indeed dosage sensitive and some have been shown to be responsible for a number of the phenotypes that are most replicable in murine models.[29–31] In analyzing the neuropsychiatric disorders associated with 22q11DS, some potentially important studies have demonstrated that dosage imbalance at a number of genes is sufficient to cause deficits in a number of behavioral responses in mice. However, despite reports that 22q11DS patients also have abnormal responses in behavioral paradigms analogous to those tested in mice, their relevance to the neuropsychiatric disorders in humans with 22q11DS remains unclear. It therefore remains uncertain why individuals carrying identical 22q11.2 deletions can present with such a wide range of neuropsychiatric phenotypes. Evidence from the murine models of 22q11DS supports the notion that pleiotropic[23] and epistatic effects[34] may contribute to this phenotypic complexity. Moreover, it is plausible that an individual's genetic background, both on the remaining haploinsufficient chromosome and elsewhere in the genome, is likely to contribute to the variable behavioral phenotype of 22q11DS. It is therefore likely that 22q11DS may reflect the combined effect of reduced dosage of multiple contiguous genes acting in concert with background genetic variation and positional effects to manifest the clinical phenotype presented.

In order to understand the genetic and ultimately the biological mechanisms that lead to the complex neuropsychiatric phenotype in 22q11DS, it is important that the work performed on murine models is complemented by transcriptomic and proteomic analyses in adult and postnatal tissues from 22q11DS human subjects. The paucity of appropriate neural tissue available from 22q11DS patients has made these studies prohibitive however the next wave of molecular studies may well be able to utilize the approaches pioneered by Takahashi andYamanaka[53] in generating induced pluripotent stem (iPS) cells from terminally differentiated cells (eg, fibroblasts). In this way, iPS cells generated from 22q11DS patients could potentially be reprogrammed to generate human neuronal cell lines that maintain both the primary deletion at 22q11 and the individuals genetic background. While it is accepted that this technology currently has a number of caveats, it does allow the possibility of performing molecular analysis of 22q11DS neuronal cell lines. Such studies will potentially result in an unprecedented investigation into the genetic and biological mechanisms that lead to the complex neuropsychiatric phenotype seen in 22q11DS patients.

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References

1. Scambler PJ. The 22q11 deletion syndromes. *Hum Mol Genet*. 2000;9:2421–2426.

2. Bassett AS, Hodgkinson K, Chow EW, et al. 22Q11 Deletion Syndrome in Adults With Schizophrenia. *Am J Med Genet*. 1998;81:328–337.
3. Pulver AE, Nestadt G, Goldberg R, et al. Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. *J Nerv Ment Dis*. 1994;182:476–478.
4. Shprintzen RJ, Goldberg R, Golding-Kushner KJ, Marion RW. Late-onset psychosis in the velo-cardio-facial syndrome. *Am J Med Genet*. 1992;42:141–142.
5. Papolos DF, Faedda GL, Veit S, et al. Bipolar spectrum disorders in patients diagnosed with velo-cardio-facial syndrome: does a hemizygous deletion of chromosome 22q11 result in bipolar affective disorder? *Am J Psychiatry*. 1996;153:1541–1547.
6. Murphy KC, Jones LA, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry*. 1999;56:940–945.
7. Shaikh TH, Kurahashi H, Saitta SC, et al. Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum Mol Genet*. 2000;9:489–501.
8. Dunham I, Shimizu N, Roe BA, Chissoe S. The DNA sequence of human chromosome 22. *Nature*. 1999;402:489–496.
9. The International Schizophrenia Consortium.Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature*. 2008;455:237–241.
10. Stefansson H, Rujescu D, Cichon S, et al. Large recurrent microdeletions associated with schizophrenia. *Nature*. 2008;455:232–236.
11. McGoey RR, Lacassie Y. Paternal balanced reciprocal translocation t(9;22)(q34.3;q11.2) resulting in an infant with features of the 9q subtelomere and the 22q11 deletion syndromes due to 3:1 meiotic segregation and tertiary monosomy. *Am J Med Genet A*. 2009;149A:2538–2542.
12. Shuib S, Abdul Latif Z, Abidin NZZ, Akmal SN, Zakaria Z. Inherited t(9;22) as the cause of DiGeorge syndrome: a case report. *Malays J Pathol*. 2009;31:133–136.
13. Wentzel C, Fernström M, Ohrner Y, Annerén G, Thuresson A-C. Clinical variability of the 22q11.2 duplication syndrome. *Eur J Med Genet*. 2008;51:501–510.
14. Inoue K, Lupski JR. Molecular mechanisms for genomic disorders. *Annu Rev Genomics Hum Genet*. 2002;3:199–242.
15. Puech A, Saint-Jore B, Funke B, et al. Comparative mapping of the human 22q11 chromosomal region and the orthologous region in mice reveals complex changes in gene organization. *Proc Natl Acad Sci U S A*. 1997;94:14608–14613.
16. Maynard TM, Haskell GT, Peters a Z, et al. A comprehensive analysis of 22q11 gene expression in the developing and adult brain. *Proc Natl Acad Sci U S A*. 2003;100:14433–14438.
17. Amati F, Biancolella M, Farcomeni A, et al. Dynamic changes in gene expression profiles of 22q11 and related orthologous genes during mouse development. *Gene*. 2007;391:91–102.
18. Lindsay EA, Botta A, Jurecic V, et al. Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature*. 1999;401:379–383.
19. Prescott K, Ivins S, Hubank M, et al. Microarray analysis of the Df1 mouse model of the 22q11 deletion syndrome. *Hum Genet*. 2005;116:486–496.
20. Meechan DW, Tucker ES, Maynard TM, LaMantia A-S. Diminished dosage of 22q11 genes disrupts neurogenesis and cortical development in a mouse model of 22q11 deletion/DiGeorge syndrome. *Proc Natl Acad Sci U S A*. 2009;106:16434–16445.
21. Sivagnanasundaram S, Fletcher D, Hubank M, et al. Differential gene expression in the hippocampus of the Df1/+ mice: a model for 22q11.2 deletion syndrome and schizophrenia. *Brain Res*. 2007;1139:48–59.
22. Meechan DW, Maynard TM, Wu Y, et al. Gene dosage in the developing and adult brain in a mouse model of 22q11 deletion syndrome. *Mol Cell Neurosci*. 2006;33:412–428.
23. Paylor R, Glaser B, Mupo A, et al. Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. *Proc Natl Acad Sci U S A*. 2006;103:7729–7734.
24. Stark KL, Xu B, Bagchi A, et al. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nat Genet*. 2008;40:751–760.
25. Mukai J, Liu H, Burt R a, et al. Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. *Nat Genet*. 2004;36:725–731.
26. Gogos J a, Morgan M, Luine V, et al. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Natl Acad Sci U S A*. 1998;95:9991–9996.
27. Hsu R, Woodroffe A, Lai W-S, et al. Nogo Receptor 1 (RTN4R) as a candidate gene for schizophrenia: analysis using human and mouse genetic approaches. *PloS one*. 2007;2:e1234.
28. Suzuki G, Harper KM, Hiramoto T, et al. Sept5 deficiency exerts pleiotropic influence on affective behaviors and cognitive functions in mice. *Hum Mol Genet*. 2009;18:1652–60.
29. Lindsay EA, Vitelli F, Su H, et al. Tbx1 haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature*. 2001;410:97–101.
30. Jerome LA, Papaioannou VE. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. *Nat Genet*. 2001;27:286–291.
31. Merscher S, Funke B, Epstein JA, et al. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell*. 2001;104:619–629.
32. Yagi H, Furutani Y, Hamada H, et al. Role of TBX1 in human del22q11.2 syndrome. *The Lancet*. 2003;362:1366–1373.
33. Paylor R, McIlwain KL, McAninch R, et al. Mice deleted for the DiGeorge/velocardiofacial syndrome region show abnormal sensorimotor gating and learning and memory impairments. *Hum Mol Genet*. 2001;10:2645–2650.
34. Paterlini M, Zakharenko SS, Lai W-S, et al. Transcriptional and behavioral interaction between 22q11.2 orthologs modulates schizophrenia-related phenotypes in mice. *Nat Neurosci*. 2005;8:1586–1594.
35. Torres-Juan L, Rosell J, Morla M, et al. Mutations in TBX1 genocopy the 22q11.2 deletion and duplication syndromes: a new susceptibility factor for mental retardation. *Eur J Hum Genet*. 2007;15:658–663.
36. Stranger BE, Forrest MS, Dunning M, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. *Science*. 2007;315:848–853.
37. Hiroi N, Zhu H, Lee M, et al. A 200-kb region of human chromosome 22q11. 2 confers antipsychotic-responsive behavioral abnormalities in mice. *Proc Natl Acad Sci U S A*. 2005;102:2–7.
38. Suzuki G, Harper KM, Hiramoto T, et al. Over-expression of a human chromosome 22q11.2 segment including TXNRD2, COMT and ARVCF developmentally affects incentive learning and working memory in mice. *Hum Mol Genet*. 2009;18:3914–3925.

39. Stark KL, Burt RA, Gogos JA, Karayiorgou M. Analysis of prepulse inhibition in mouse lines overexpressing 22q11.2 orthologues. *Int J Neuropsychopharmacol*. 2009;12: 983–989.
40. Kurotaki N, Shen JJ, Touyama M, et al. Phenotypic consequences of genetic variation at hemizygous alleles: Sotos syndrome is a contiguous gene syndrome incorporating coagulation factor twelve (FXII) deficiency. *Genet Med*. 2005;7:479–483.
41. Tenhunen J, Salminen M, Lundström K, et al. Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters. *Eur J Biochem*. 1994;223:1049–1059.
42. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75: 807–821.
43. Dunham I, Collins J, Wadey R, Scambler P. Possible role for COMT in psychosis associated with velo-cardio-facial syndrome. *Lancet*. 1992;340:1361–1362.
44. Gothelf D, Eliez S, Thompson T, et al. COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. *Nat Neurosci*. 2005;8:1500–1502.
45. Bassett AS, Caluseriu O, Weksberg R, Young DA, Chow EWC. Catechol-O-methyl transferase and expression of schizophrenia in 73 adults with 22q11 deletion syndrome. *Biol Psychiatry*. 2007;61:1135–1140.
46. Jungerius BJ, Hoogendoorn MLC, Bakker SC, et al. An association screen of myelin-related genes implicates the chromosome 22q11 PIK4CA gene in schizophrenia. *Mol Psychiatry*. 2008;13:1060–1068.
47. Kanahara N, Iyo M, Hashimoto K. Failure to confirm the association between the PIK4CA gene and schizophrenia in a Japanese population. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B:450–452.
48. Vorstman JAS, Chow EW, Ophoff RA, et al. Association of the PIK4CA schizophrenia-susceptibility gene in adults with the 22q11.2 deletion syndrome. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B:430–433.
49. Ikeda M, Williams N, Williams HJ, et al. Failure to confirm association between PIK4CA and psychosis in 22q11.2 deletion syndrome. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B:980–982.
50. Williams NM, Glaser B, Norton N, et al. Strong evidence that GNB1L is associated with schizophrenia. *Hum Mol Genet*. 2008;17:555–566.
51. Birchler JA, Riddle NC, Auger DL, Veitia RA. Dosage balance in gene regulation: biological implications. *Trends Genet*. 2005;21:219–226.
52. Fénelon K, Mukai J, Xu B, et al. Deficiency of Dgcr8, a gene disrupted by the 22q11.2 microdeletion, results in altered short-term plasticity in the prefrontal cortex. *Proc Natl Acad Sci U S A*. 2011;108:4447–4452.
53. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676.