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Mutations in the BLOC-1 Subunits Dysbindin and Muted Generate Divergent and Dosage-dependent Phenotypes

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Background: Genetic defects affecting subunits of protein complexes are presumed to generate identical diseases in mammals.

Results: Two mouse mutants in genes belonging to the BLOC-1 complex have divergent brain and pigmentation phenotypes.

Conclusion: Genetic defects affecting subunits of a complex manifest by partially overlapping clinical features.

Significance: Disease resulting from mutations in protein complexes may generate a wide range of clinically presentations.

Post-mortem analysis has revealed reduced levels of the protein dysbindin in the brains of those suffering from the neurodevelopmental disorder schizophrenia. Consequently, mechanisms controlling the cellular levels of dysbindin and its interacting partners may participate in neurodevelopmental processes impaired in that disorder. To address this question, we studied loss of function mutations in the genes encoding dysbindin and its interacting BLOC-1 subunits. We focused on BLOC-1 mutants affecting synapse composition and function in addition to their established systemic pigmentation, hematological, and lung phenotypes. We tested phenotypic homogeneity and gene dosage effects in the mouse null alleles muted (Bloc1s8sdy/sdy) and dysbindin (Bloc1s8sdy/sdy). Transcripts of NMDA receptor subunits and GABAergic interneuron markers, as well as expression of BLOC-1 subunit gene products, were affected differently in the brains of Bloc1s8sdy/sdy and Bloc1s8sdy/sdy mice. Unlike Bloc1s8sdy/sdy, elimination of one or two copies of Bloc1s8 generated indistinguishable pallidin transcript phenotypes. We conclude that monogenic mutations abrogating the expression of a protein complex subunit differentially affect the expression of other complex transcripts and polypeptides as well as their downstream effectors. We propose that the genetic disruption of different subunits of protein complexes and combinations thereof diversifies phenotypic presentation of pathway deficiencies, contributing to the wide phenotypic spectrum and complexity of neurodevelopmental disorders.

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Significance:

Genetic polymorphisms in the gene encoding dysbindin are risk factors for schizophrenia onset and associate with cognitive and neuroanatomical differences in normal individuals (1–13). Dysbindin (Bloc1s8) is a subunit of the cytosolic hetero-octameric referred to as BLOC-1 (the biogenesis of lysosome-related organelles complex 1). This complex consists of Bloc1s1–8 subunits. Analysis of dysbindin and its closely interacting BLOC-1 subunit mutants reveals a common set of autosomal recessive phenotypes in vertebrates and invertebrates. These phenotypes result from defective trafficking to lysosome-related organelles affecting systemic processes as well as pre- and post-synaptic neuronal compartments (13–16). For example, the sandy mouse null allele affecting dysbindin polypeptide expression, Bloc1s8sdy/sdy, impairs synaptic vesicle composition and function including glutamatergic and GABA-dependent neurotransmission (12, 13, 17–20). Similarly, dysbindin and Bloc1s1 mutants in Drosophila exhibit impaired neurotransmission, behavior and presynaptically abrogated glutamatergic synaptic homeostasis (21–24). Neuronal phenotypes have not been systematically explored in null mutations affecting other BLOC-1 complex subunits. In contrast, pigment dilution, pulmonary fibrosis, and bleeding diathesis, which constitute the core recessive systemic phenotypes of BLOC-1 mutations in mammals, are common to Bloc1s8sdy/sdy and four alleles affecting the expression of Bloc1s4 (reduced pigmentation), Bloc1s5 (muted), Bloc1s6 (pallid), and Bloc1s7 (cappuccino), respectively (14, 16, 25–30). The commonality of systemic phenotypes among these five mouse mutants is attributed to the idea long held by us and others in the field that each of these mutations exerts equal effects on BLOC-1 architecture, function, and downstream effectors (12, 14, 31).

Here we demonstrate that Bloc1s5 and Bloc1s8 mutations exert differential effects on BLOC-1 complex function and downstream effectors. We explored the type and magnitude of neuronal phenotypes associated with single and double copy BLOC1s5 muted or BLOC1s8 sandy null alleles present in mice of identical genetic background. We identified neuronal transcriptional phenotypes whose quality and/or magnitude differ
between these alleles. Transcriptional phenotypes were sensitive to the genetic dosage of mutant alleles. Our results support the concept that genetic mutations in dysbindin and its interacting BLOC-1 subunits generate only partially overlapping neuronal phenotypes in neurotransmitter systems implicated in the pathogenesis of schizophrenia.

**MATERIALS AND METHODS**

**Reagents**—Mouse anti-pallidin was a gift from Dr. Esteban Dell’Angelica (UCLA, Los Angeles, CA) (29). Rabbit anti-VAMP-2 and VGAT were purchased from Synaptic Systems (Göttingen, Germany). VAMP7 monoclonal antibody was a generous gift of Dr. Andrew Peden (University of Sheffield, UK). Dysbindin 1A and 1C were detected with the antibody PA3111 (32) Mouse mutants have been previously described (17, 33, 34). Bloc1S5(mu/mu) mice in CHMU background (CHMU/LeJ, stock number 000293) were backcrossed by at least six generations with C57B6 mice obtained from The Jackson Laboratory (Bar Harbor, ME). Bloc1S5(mu/mu) were a gift of Dr. R. Swank (Roswell Park Cancer Institute, Buffalo, NY). Bloc1S8(dy/sdy (sandy) and Bloc1S6(mu/pa) were also in C57B6 genetic background. Sandy mice were previously described (35). Mouse genotyping was performed by PCR of genomic DNA with the primers forward muted (ctatgaagagtgacgagctgt) and reverse muted (agcagtagtgacagctg) and reverse muted (agcagtagtgacagctg).

**Mouse and Human Subjects**—All mice were bred in-house following institutional animal care and use committee-approved protocols. Human post-mortem tissue derived from samples of U.S. citizens autopsied at the Hospital of the University of Pennsylvania as approved by the institutional review board at that university. Autopsy consent from next of kin or legal guardian was obtained in all cases. For most cases, consent was granted in writing before death and always confirmed after death. Ethics committee at the University of Pennsylvania approved the consent procedures. To keep post-mortem delays to a minimum when written consent had not been obtained before death, verbal consent was obtained as witnessed by a third party and documented by the physician making the request. Written records of the consent for autopsy were archived. These procedures for written and verbal consent are standard medical practice in the United States.

**Brain Sections, Immunohistochemistry, and Microscopy**—Detailed procedures for mouse tissue preparation, indirect immunofluorescence microscopy, and quantification procedures were described in our previous work (17, 33, 36). Briefly, brains were obtained from mice 6–8 weeks postnatal. Animals were anesthetized with ketamine and then transcardially perfused with Ringer’s solution followed by fixative (4% paraformaldehyde for 12–18 h followed by sectioning on a vibratome into 60–4% paraformaldehyde for 12–18 h followed by sectioning on a vibratome into 60-

**RESULTS**

**Bloc1S5 Muted Mouse Models Reveal Unique Dysbindin and Pigment Dilution Phenotypes**—Our studies were prompted by the identification of unexpected differences in dysbindin polypeptide composition in brains of mice carrying null alleles of Bloc1S5 (muted, mu) and Bloc1S8 (sandy, sdy). Dysbindin immunoblot detected two dysbindin immunoreactive polypeptides of ~35 and ~50 kDa in human and wild type mouse brain (Fig. 1). We have termed these bands dysbindin 1A and 1C (Fig. 1) (32). The identity of these immunoreactive bands as Bloc1S8 polypeptides was confirmed by their absence in dysbindin null Bloc1S8(dy/sdy) brain tissue (Fig. 1A, lanes 5 and 6 versus lanes 3 and 4 from samples of wild type littermates). Similarly, we observed a drastic reduction of dysbindin 1A in mouse brain lacking the BLOC-1 subunit muted (Bloc1S5(mu/mu)). In contrast, dysbindin 1C expression remained unaffected in Bloc1S5 null mice (Fig. 1A, compare lanes 7 and 8 with lanes 9

**Melanin Measurements Procedure**—Procedures were performed according to Hoyle et al. (37). Briefly, a solution containing a (9:1 ratio of Solune 350 (PerkinElmer Life Sciences) and water was added to hair samples at a ratio of 250 μl/mg of hair. Samples were vortexed for 1 min, heated to 95 °C for 30 min, cooled, vortexed for 2 min, heated to 95 °C for 15 min and then brought to room temperature. Next, 400 μl of the sample were transferred to a tube containing 600 μl of Solune 350/ water mixture. Samples were again vortexed and heated to 95 °C for 15 min. Then they were centrifuged for 10 min at 13,000 × g to remove debris. Absorbance at 500 nm was measured for each sample. The standard curve was obtained by dissolving purified melanin from Sepia officinalis (Sigma-Aldrich). Sepia melamin was processed as hair samples. Dilutions were made from the stock solution using Solune 350/water solution.

**Quantitative Real Time PCR**—Control and mutant cortical and hippocampal regions were dissected from young adult animals between postnatal days 42 and 52 sacrificed by CO2 narcosis. Tissue was flash frozen and TRIzol-extracted (Invitrogen), and isolated RNA was reverse transcribed into cDNA using SuperScript III first strand synthesis (Invitrogen). PCR amplifications were performed on a LightCycler480 real time plate reader using LightCycler 480 SYBR Green reagents (Roche).

**Statistical and Bioinformatic Analyses**—Statistical analyses performed with a KaleidaGraph v4.03 (Synergy, Reading, PA).

**RESULTS**

**Bloc1S5 Muted Mouse Models Reveal Unique Dysbindin and Pigment Dilution Phenotypes**—Our studies were prompted by the identification of unexpected differences in dysbindin polypeptide composition in brains of mice carrying null alleles of Bloc1S5 (mutated, mu) and Bloc1S8 (sandy, sdy). Dysbindin immunoblot detected two dysbindin immunoreactive polypeptides of ~35 and ~50 kDa in human and wild type mouse brain (Fig. 1). We have termed these bands dysbindin 1A and 1C (Fig. 1) (32). The identity of these immunoreactive bands as Bloc1S8 polypeptides was confirmed by their absence in dysbindin null Bloc1S8(dy/sdy) brain tissue (Fig. 1A, lanes 5 and 6 versus lanes 3 and 4 from samples of wild type littermates). Similarly, we observed a drastic reduction of dysbindin 1A in mouse brain lacking the BLOC-1 subunit muted (Bloc1S5(mu/mu)). In contrast, dysbindin 1C expression remained unaffected in Bloc1S5 null mice (Fig. 1A, compare lanes 7 and 8 with lanes 9
and 10). We performed quantitative real time PCR to examine the expression of dysbindin 1C by an alternative approach. The only AceView-predicted transcript encoding a Bloc1s8 dysbindin polypeptide with a putative molecular mass of dysbindin 1C is bSep07 (Fig. 1B). The bSep07 mRNA was proportionally reduced in hippocampus from single or double copy loss of Bloc1s8 (Fig. 1C). In contrast, the bSep07 mRNA remained unaltered in Bloc1s5 muted hippocampus. Thus, the dysbindin 1C polypeptide and the bSep07 mRNA expression are spared in the Bloc1s8 muted hippocampal formation.

We hypothesized that this difference in dysbindin polypeptide expression between Bloc1s5mumu and Bloc1s8dydy could reflect wider differences in molecular and systemic phenotypes among BLOC-1 null mutations. To test this hypothesis, we generated mice carrying the Bloc1s5mumu and Bloc1s8dydy alleles on identical genetic backgrounds. These mutant alleles were originally isolated in CHMU and DBA/2J backgrounds, respectively (16, 28). We confirmed the presence of mutant alleles by sequencing PCR fragments encompassing the ETn transposon insertion in the Bloc1s5 (Fig. 2, A and B) and the deletion in the Bloc1s8 loci in C57B mouse strains (data not shown). Addition-
Phenotypic Heterogeneity of BLOC-1 Null Mutations

It is well established that BLOC-1 subunit genetic defects decrease polypeptide expression of the other BLOC-1 complex constituents (16, 25–29). This effect is thought to result from degradation of unassembled BLOC-1 polypeptides. Thus, we tested whether palladin protein reductions observed in Bloc1s5+/mu and Bloc1s8sdy/sdy hippocampi were due to only post-translational mechanisms or whether altered transcript levels could account for the similar reduction of palladin protein levels. We measured Bloc1s6 palladin transcripts in the adult hippocampal formation as well as the cortex of Bloc1s5+/mu and Bloc1s8sdy/sdy mice by quantitative real time PCR (Fig. 3, A and B, rows 1 and 2). Palladin Bloc1s6 transcript levels were reduced in the hippocampal formation of both Bloc1s5+/mu (Fig. 4A, rows 1 and 2) and Bloc1s8sdy/sdy mice (Fig. 4B, rows 1 and 2), whereas we saw no changes in the expression of dysbindin Bloc1s8 mRNA. Similarly, palladin Bloc1s6 transcript content was half of control adult cerebral cortex in Bloc1s5+/mu (Fig. 4C, rows 9 and 10) and Bloc1s8sdy/sdy mice (Fig. 4D, rows 9 and 10). Bloc1s5 muted mRNA was 50% of the wild type levels in Bloc1s5+/mu cortex (Fig. 4A, rows 7 and 8) and was undetectable in Bloc1s5sma/mu cortex (Fig. 4C, rows 7 and 8). Thus, mRNA levels precisely matched the genotype of Bloc1s5 mutant mice, demonstrating the fidelity of mRNA determinations.

The unexpected effect of the Bloc1s5 muted allele on the expression Bloc1s6 palladin messages prompted us to test whether the Bloc1s5 muted allele affected the expression of other BLOC-1 subunit mRNAs. Bloc1s5 and 6 were the only transcripts whose expression was reduced in Bloc1s5sma/mu brain tissue (Fig. 4C). In contrast, we observed a significant decrease in the content of six of the eight BLOC-1 complex subunits, Bloc1s2, 3, 5, 6, and 7 (Fig. 4D, rows 7–10) in the hippocampal formation as well as the cortex of Bloc1s6 paleolin (pa) mice. Unlike the double copy Bloc1s5 muted mutation, double copy null Bloc1s6 palladin (pa) and Bloc1s8 sandy (sdy) had no effect on neuronal Bloc1s5 muted and Bloc1s6 palladin mRNA expression (Fig. 5). These results indicate that mutations in loci encoding BLOC-1 subunits differ in molecular phenotypes related to the expression of transcripts encoding BLOC-1 subunits. Moreover, these findings uncover a hitherto unknown effect of mutations in protein trafficking complexes in the levels of their own subunit encoding transcripts.

FIGURE 3. Bloc1s5 muted affects the expression of the BLOC-1 complex polypeptide palladin. A, hippocampal sections from wild type, Bloc1s5+/mu and Bloc1s5sdy/sdy mice were stained with antibodies against the BLOC-1 subunit palladin and the synaptic vesicle marker VAMP2. The right panels present look-up tables (LUT) to highlight differences in palladin expression among genotypes. B, a quantitation of palladin immunoreactivity expressed as ratios between palladin and VAMP2. Each dot represents an independent staining performed in four wild type and Bloc1s5sma/mu hippocampi or two Bloc1s5+/mu hippocampi. C and D, immunoblot determinations of palladin and their quantitation in mouse hippocampi (n = 3 animals and 3 independent determinations; one-way analyses of variance followed by Dunnett’s multiple comparison was used in B and D). IB, immunoblot.
NR1 subunit and NR2 isoforms in Bloc1s5mu/mu, Bloc1s5+/mu, Bloc1s8dy/dy, and Bloc1s8+/dy hippocampal formations.

Similar to previous reports of NR1 transcripts in sandy prefrontal cortex, we found that NR1 mRNA is decreased in Bloc1s8dy/dy hippocampus (Fig. 6B, rows 1 and 2) (18). In addition, mRNA levels of all NR2 isoforms were significantly reduced in Bloc1s8dy/dy as compared with wild type hippocampal formations (Fig. 6B, rows 3–10). In contrast, Bloc1s8+/dy hippocampal formations had increased in NR2A and NR2B mRNAs without effects on other receptor subunits (Fig. 6A, rows 3–6). Vamp2 transcript levels encoding the synaptic vesicle protein synaptobrevin 2 were used as mRNA loading controls in all Bloc1s8dy/dy and Bloc1s8+/dy studies (Fig. 6, A and B, rows 11 and 12). We contrasted the NMDA receptor transcriptional signature observed in Bloc1s8dy/dy and Bloc1s8+/dy hippocampi with that of Bloc1s5mu/mu and Bloc1s5+/mu (Fig. 6, C and D). Bloc1s5mu/mu tissue altered the expression of all NMDA receptor mRNAs tested (Fig. 6D, rows 1–10). However, phenotypic overlap between Bloc1s5+/mu and Bloc1s8dy/dy was restricted to only two of the five NMDA receptor subunits analyzed, NR2C and D (Fig. 6D, rows 7–10). Transcripts encoding these two subunits were reduced both in Bloc1s5mu/mu and Bloc1s8dy/dy (Fig. 6D, rows 7–10). Furthermore, and in contrast to Bloc1s8+/dy hippocampi, Bloc1s5+/mu did not affect expression of any receptor subunit mRNAs (Fig. 6C). Syp transcript levels encoding the synaptic vesicle protein synaptophysin were used as controls in all Bloc1s5+/mu and Bloc1s8dy/dy determinations (Fig. 6, C and D, rows 11 and 12). These results demonstrate that mutations to different BLOC-1 subunit genes differentially affect NMDA receptor subunit expression in the hippocampus, with marked changes in Bloc1s8 mice but moderate effects in Bloc1s5 animals.

Bloc1s5mu/mu and Bloc1s8dy/dy Mouse Hippocampi Diverge in GABAergic Transcriptional Profiles—Homeostatic plasticity maintains circuit excitability in face of perturbations. Set point is restored by changes in gene expression in functionally opposing neurotransmitter systems (48, 49). Thus, we reasoned that the NMDA receptor phenotypic divergence between Bloc1s5mu/mu and Bloc1s8dy/dy should be accompanied by divergent and proportional changes in GABAergic neurotransmission markers. Parvalbumin levels are reduced in GABAergic interneurons of the Bloc1s8dy/dy hippocampal formation (19). We measured parvalbumin and a panel of GABAergic interneuron subpopulation markers by quantitative real time PCR using (Fig. 7). We observed a reduction in the mRNA level for parvalbumin in Bloc1s8dy/dy hippocampal formations, an observation consistent with the reported reduced density of parvalbumin positive cells in Bloc1s8dy/dy tissue (Fig. 7A, rows 3 and 4). Similar results were obtained with markers of GABA-positive interneurons such as the glutamate decarboxylase of 65 kDa (Gad2), GABA transporter 2 (Slc6a13), vesicular GABA transporter (VGAT, Slc32a1), the neuropeptide somatostatin (Sst), or interneuron enriched transcription factors such as Arx, Npas1, and Lhx6 (Fig. 7A, rows 11–16) (50–52). In contrast with Bloc1s8dy/dy hippocampi, transcript levels of GABA interneurons remained unaltered in Bloc1s5mu/mu tissue except for Arx and Npas1 transcripts, which were less pronouncedly reduced (Fig. 7B, rows 11–14). There were no significant changes in transcript levels in Bloc1s8+/dy and Bloc1s5+/mu hippocampi (data not shown). mRNA loading controls were similar across genotypes
as measured by transcripts encoding the synaptic vesicle protein synaptophysin (Sphysin; Fig. 7, A and B, rows 17 and 18). We confirmed these differences in GABAergic phenotypes by measuring the protein levels of the synaptic vesicle GABA transporter (VGAT; Fig. 7, C–E). Similar to what we observed with VGAT mRNA levels, the VGAT polypeptide was drastically reduced in Bloc1s8sdy/sdy and Bloc1s5mu/mu mutants (Fig. 7, C–E).
Our results provide evidence that markers, a phenotype that diverges from mutations in demonstrating that the Bloc1s8 sdy/sdy contrast, VGAT polypeptide expression in in (Fig. 7). These findings demonstrate that mutations affecting different subunits of a protein complex (53, 54).

The mechanism by which mutations in dysbindin and interacting BLOC-1 subunits generate common as well as divergent phenotypes is presently unknown. However, we speculate that phenotypes common to all BLOC-1 complex subunits mutations require its normal quaternary structure. In contrast, divergent phenotypes may be caused by remnants of the BLOC-1 complex left after uneven protein down-regulation of the octamer. As such, BLOC-1 down-regulation remnants should have different composition/stoichiometry among mutations affecting the dysbindin-BLOC-1 complex subunits to account for phenotypic divergence. The extent of degradation of BLOC-1 complex subunits seems divergent among mutations affecting different BLOC-1 complex subunits, although this has not been quantitatively assessed (16, 25–30). Our data support the remnant-derived phenotype hypothesis as exemplified by the effects that the Bloc1s5mu/mu and Bloc1s8sdy/sdy mutants exert upon the expression of dysbindin 1C in mouse brain. The remnant-derived phenotype hypothesis makes two predictions. First, it suggests that some mutation-associated phenotypes could be semidominant or partially expressed in single copy loss mutations. This is the case for the hippocampal palladin transcript decrease and pigmentation reduction in Bloc1s5mu/mu, as well as the effect of Bloc1s8sdy/sdy on NR2A and B transcripts. Second, it predicts the counterintuitive notion that phenotypes in a null mutation affecting one BLOC-1 complex subunit could be ameliorated by genetic deficiencies in a second subunit of the complex. Additionally, divergent phenotypes may reflect loss of function of roles played by individual dysbindin-BLOC-1 subunits occurring outside the octameric dysbindin-BLOC-1 complex. This is the case of Bloc1s7 snapin that engages in molecular interactions independent of dysbindin-BLOC-1 complex (55–59). The complexity of the divergences in transcriptional phenotypes extends beyond differences in just the mRNAs affected. NR1 and NR2B are decreased in Bloc1s8sdy/sdy, yet these same mRNAs are up-regulated in Bloc1s5mu/mu. This suggests different pathways affecting these messages in these two BLOC-1 null phenotypes. The molecular determinants defining the type of transcript affected and whether they are up- or down-regulated in BLOC-1 null alleles remains to be explored.

Our findings also have implications for neurodevelopmental disorders such as schizophrenia. Dysbindin protein expression is decreased in the brain of 80% of schizophrenia patients tested thus far (32, 38). The cause(s) of this dysbindin down-regulation remains unknown. However, it is unlikely that DTNBP1 polymorphisms associated with schizophrenia risk alone may explain dysbindin protein reduction. The frequency of these

**Phenotypic Heterogeneity of BLOC-1 Null Mutations**

**FIGURE 8. Summary of transcriptional phenotypes in Bloc1s8sdy/sdy and Bloc1s5mu/mu brains.** Diagram depicts the transcriptional phenotypes in BLOC-1 null brains. Bloc1s8 denotes the putative dysbindin 1C transcript bSept07. Colored boxes depict no changes (gray), down-regulation (blue), or up-regulation (orange) as compared with wild type C57BL/6J tissue.
Phenotypic Heterogeneity of BLOC-1 Null Mutations

DTNBP1 disease-associated small nucleotide polymorphisms is too low to account for the highly penetrant dysbindin protein down-regulation phenotype observed in schizophrenia populations (60). Dysbindin levels depend on protein expression of other BLOC-1 complex subunits, a mechanism considered post-translational. Likely, ubiquitin ligases like TRIM32 may down-regulate dysbindin in the absence of other BLOC-1 subunits (61). This untested model is analogous to the mechanism that accounts for the degradation of the adaptor complex AP-3 after one of its subunit encoding genes is mutated (62). Protein complexes frequently respond with multiprotein down-regulation of many of their constituents after genetic mutation of only one of their components (62–65). En bloc degradation of the BLOC-1 complex does not consider transcriptional contributions. Our results add an unsuspected and novel layer of complexity as BLOC-1 subunit transcripts encoded by nonmutated genes are modified by specific mutations in BLOC-1 subunits. We demonstrate down-regulation of four BLOC-1 transcripts, other than Bloc1s5 mRNA, in Bloc1s5/*+/*+ brain tissue. Most notably, there is a concurrent and similar drop in pallidin transcript and polypeptide levels in both single and double copy Bloc1s5 muted mutants. Pallidin transcript levels are normal in Bloc1s8 mutants irrespective of gene dosage. However, phenotypes associated with loss of one gene copy are not restricted to Bloc1s5/*+/*+ brain. They at least include pigment dilution in Bloc1s5/*+/*+ skin and NR2A-B transcripts in Bloc1s8/*+/sdy brain. Thus, allele and gene dosage-specific effects in a protein complex such as BLOC-1 could be a general mechanism to generate phenotypic diversity among individuals with mutations in different loci belonging to a pathway. Single copy losses of either one gene or chromosomal segment are important genetic risk factors for diverse neurodevelopmental disorders ranging from schizophrenia to autism spectrum disorder (66–70). These genetic defects are also genetic modifiers of human cognition (71). The phenotypic spectrum in these single copy loss variations is quite wide. On one hand, the same genetic locus (72–74). Mechanisms by which these single copy losses generate neurodevelopmental and cognition defects are not understood. However, it is likely that multiple molecular mechanisms may account for their pathogenic effect. We propose that common single copy loss of genetic loci associated to schizophrenia (44, 45), such as chromosome 22q11 deletion syndrome (75), could expand and diversify their clinical presentation spectrum by altering the stability of messages and/or polypeptides belonging to macromolecular complexes.

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