

# Fragile X syndrome and autism at the intersection of genetic and neural networks

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**Autism, an entirely behavioral diagnosis with no largely understood etiologies and no population-wide biomarkers, contrasts with fragile X syndrome (FXS), a single-gene disorder with definite alterations of gene expression and neuronal morphology. Nevertheless, the behavioral overlap between autism and FXS suggests some overlapping mechanisms. Understanding how the single-gene alteration in FXS plays out within complex genetic and neural network processes may suggest targets for autism research and illustrate strategies for relating autism to more singular genetic syndromes.**

The diagnosis of autism depends on a 'triad' of deficits comprising impaired social interaction, impaired communication and restricted interests and repetitive behaviors. Although in some cases speech never develops fully or never develops at all, in other cases speech may be present but so inflexible and unresponsive to context that it is unusable in normally paced conversation. Speech is often limited to echolalia (parroted rote phrases or memorized scripts, or repetition of words just spoken by others), or confined to narrow topics of expertise where discourse can proceed without conversational interplay. The communicative impairment extends also to nonverbal signals such as gaze, facial expression and gesture. Social behaviors, too, are beset by a lack of flexibility and rapid coordination: children with autism do not coordinate attention between objects of mutual interest and the other people who may be interested in them, often engage in 'parallel play' at the edge of a group rather than joining in cooperative play, and do not in general engage in pretend play. Intense and narrowly focused interests tend to concentrate on systems that operate deterministically and repeatably according to tractable sets of rules, and behavior often is marked by motor stereotypes and compulsive actions. In addition to these diagnostic components, sensory hypersensitivity and motor incoordination are common features. Autism is the extreme of a spectrum of abnormalities whose milder variants include Asperger syndrome, where language is intact but social and communicative inflexibility and restricted and repetitive interests and behaviors remain, and the 'broader autism

phenotype' in which characteristic cognitive traits are present subclinically. The combination of this broad variation of phenotypes and a high rate of concordance in monozygotic twins suggests a large number of genetic and environmental biasing factors<sup>1</sup>.

FXS, in contrast, is caused by the silencing of a single gene (*FMR1*)<sup>2</sup> that codes for the fragile X mental retardation protein (FMRP), an RNA-binding protein normally produced in response to activation of group-1 metabotropic glutamate receptors<sup>3</sup>. Despite this well-defined genetic abnormality, the cognitive, behavioral and morphological symptoms of FXS are highly variable. Although the cardinal symptom of FXS is mental retardation, there are milder variants, especially in heterozygous females, in which the cognitive abnormality manifests only as learning disabilities or problems of emotion regulation. Physical abnormalities are often present and can include long face with prominent ears, arched palate, flat feet, hyperextensible joints, pectus excavatum, mitral valve prolapse, strabismus and macroorchidism<sup>4</sup>. Neurobehavioral symptoms can (but do not always) include social anxiety and gaze avoidance, sensory hypersensitivity and withdrawal from touch, stereotypic movements and behaviors such as hand flapping and rocking, poor motor coordination, delayed speech development and echolalia<sup>4,5</sup>. These neurobehavioral resemblances to autism in many cases of FXS may be reflected in neuroanatomical similarities: although findings have differed and a complete review is not possible within the space of this article, many (though far from all) studies suggest that at the level of groups but not individuals, both autism and FXS may involve an abnormally large caudate nucleus<sup>6–9</sup> and volumetric abnormality of the posterior cerebellar vermis<sup>10,11</sup>.

FXS differs from autism in that it can be defined in terms of a specific biomarker: an abnormally expanded sequence of CGG repetitions at the fragile X site at Xq27.3. There is, however, a wide range of individual differences in the length of this triplet expansion, the completeness of the resulting DNA methylation, the levels of transcription and translation into *FMR1* mRNA and FMRP, and (no doubt) the modulatory effects of polymorphisms in the many genes whose products FMRP regulates. The range of abnormal CGG expansion is divided into a 'full mutation' with 200 or more repetitions and an unstable, genetically anticipatory 'pre-mutation' with 55 or more<sup>12</sup>. The full mutation produces methylation of *FMR1*, little or no production of the corresponding mRNA and thus little or no FMRP. The pre-mutation, however, seems to decrease FMRP via a different mechanism, at translation rather than at transcription, rendering FMRP abnormally scarce despite an abundance of its mRNA. This large degree of fan-out from disruption of a single gene into an array of varying and sometimes opposite effects makes FXS itself something of a spectrum condition, that is, one featuring a range or even a continuum of severities—both biochemically and behaviorally.

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As neither autism nor even FXS has an invariant phenotype, comorbidity is difficult to assess. Although most cases of autism are not associated with FXS (prevalence 4% or less), the converse is not necessarily true: estimates of the prevalence of autism in FXS have ranged from 5% to as much as 60% (ref. 13). This wide variation probably stems from differences in diagnostic thresholds within the autism spectrum, as well as variations in ascertainment and sampling. Recent studies using comprehensive diagnostic instruments have yielded prevalence estimates for autism in the FXS population between 18% and 33% (refs. 14,15), and most of the variance between autistic and non-autistic FXS subgroups seems to be within autism's social and communicative dimensions, rather than the dimension of repetitive behaviors and restricted interests<sup>16</sup>. Social and communicative symptoms of autism are continuously distributed within the FXS and FXS pre-mutation populations<sup>17</sup>, and an account of shared symptoms that considers this spectrum offers more information than one that applies some arbitrary diagnostic threshold to separate 'autistic' from 'non-autistic' FXS. Even simple thresholding, however, yields a significant comorbidity of autism in both the full mutation and the pre-mutation subgroups of FXS (ref. 17).

A consistent neuropathological observation in FXS, in both humans and knockout mice, is high numbers of abnormally long, thin, tortuous dendritic spines<sup>18</sup>. This immature dendritic morphology, along with exaggerated synaptic plasticity, susceptibility to epilepsy and anxiety and other behavioral symptoms, is consistent with impaired repression of response to activation of group-1 metabotropic glutamate receptors<sup>19</sup>. A similar surfeit of dendritic spines is observed in autism, but only in a low-IQ subgroup<sup>20</sup>—a result that needs further study and replication as more postmortem tissue becomes available. This similarity, if borne out, suggests that low IQ in FXS and in some cases of autism may arise from a common mechanism, with a specific syndrome of mental retardation arising from abnormal synaptic plasticity, and the general syndrome of autism arising from the cumulative effect of this synaptic abnormality and other modifying factors on the connectivity properties of neural networks. Other neuropathological observations of autism, not associated with FXS, have identified abnormalities in neuronal density and number<sup>21</sup> and minicolumn structure and extent<sup>22</sup>, which may produce like perturbations at the network level via different mechanisms at the cellular level. These and other findings have supported the conjecture that the final common pathway in autistic brain development may be a perturbation of neural connectivity<sup>1</sup> in which abnormalities of information processing within local networks may interfere with the development of appropriate long-range connections between brain regions.

Psychophysics too suggests partial overlap between the neural perturbations in autism and in FXS. In FXS (ref. 23) and in autism<sup>24</sup>, perceptual thresholds for second-order (texture contrast, multiplicative signal) stimuli are abnormally elevated in comparison to thresholds for first-order (luminance contrast, additive signal) stimuli. People with FXS additionally have elevated thresholds for first-order stimuli specific to the magnocellular pathway (low spatial frequencies)<sup>25</sup>, whereas the first-order performance of people with autism is normal or even superior<sup>24</sup>. The magnocellular layers of the lateral geniculate nucleus normally contain high levels of FMRP, and in FXS their structure is selectively disrupted<sup>25</sup>. This combination of a primary processing abnormality specific to FXS and a secondary processing abnormality common to FXS and autism again suggests that the neuronal abnormality in FXS is but one of many possible routes to the network-level perturbations that produce autism<sup>24</sup>. Notably, perceptual deficits for complex motion in autism correlate with superior performance on the Embedded Figures Test (ref. 26), suggesting that the same neural property that perturbs perceptual processing also influences the development of the autistic cognitive emphasis on localized details.

### The significance of network interactions

Both the genetic and the neural properties of FXS point out the importance of considering the network level, not just the level of individual genes or individual neurons. Although FXS is in one sense a single-gene disorder, it is more proximally the result of disruption in regulatory networks via the many genes whose transcripts FMRP binds, and probably in many cellular processes. This very complexity is what gives FXS the power to disrupt brain development so pervasively. Similarly, the heterogeneity of neuropathological and genetic observations in autism suggests that autism's essential characteristic may not be any specific cellular pathology, but rather a perturbation of the network properties that emerge when neurons interact. Thus even though FXS and autism differ starkly at the level of single-gene dysfunction, they may bear a great deal of similarity in terms of network dysfunctions and the combinational effects of genetic, epigenetic and environmental modifying factors on these network dysfunctions. Disorders whose analyses can be confined within single genes or isolated cells are, comparatively speaking, easy to understand. Network problems are hard, but solving them promises profound insights into learning and memory and the development of cognition.

In this regard, these relations of emergence between individual genes and gene networks, and between individual neurons and neural networks, are analogous to the developmental relations between individual brain regions or cognitive subsystems on the one hand, and networks of brain regions and emergent cognitive capacities on the other. Historically, the approach in many studies has been to attempt to dissect developmental disorders as though they were lesions, a missing locus or capacity in an otherwise normal, fully developed brain in which all other factors have somehow been held constant. This approach is inappropriate to the study of developmental disorders because it assumes that the disorder is a function of a localized module, rather than an emergent property of developmental interactions among many brain regions and functions<sup>27</sup>. The genetic and neural analog of the lesion study is the study of a single candidate gene, or of a single neurotransmitter or protein. These approaches are valuable in that they can identify points of entry to the relevant genetic or neural networks. A full understanding, however, requires approaching the problem at the network level.

It is often tacitly assumed that the relation between gene expression and cellular phenotype, or the relation between individual neuronal properties and emergent neural phenotype, is monotonic and independent. That is to say, we assume that (i) an abnormal loss of function in a gene or in a cellular process ought to produce a phenotype opposite to that found in the case of an abnormal gain of function, and (ii) this relation between dosage and phenotype is the same regardless of the individual's genetic, environmental or developmental context. We make these assumptions of monotonicity and independence for the same practical reason that a physicist posits a frictionless surface, a statistician contrives a stationary process, or a novelist invents thematic characters and plots: they simplify complex relationships for which we have no exact models, and they are often close enough to reality to make useful predictions about real-world processes. They are, however, fictions.

For counterexamples to such assumptions, we can look to pharmacology, where the classic dose-response curve is the strongly non-monotonic 'inverted-U' surrounding an optimal dosage, and where a drug's kinetics and therapeutic effect can depend strongly on competitive or synergistic factors arising from other drugs or from individual variation. Careful characterization of neurodevelopmental disorders suggests similar dose-response relations between genes and developmental processes. Such relations are especially likely to exist, and to evoke profound effects, in the case of genes that regulate the activity of large networks of genes or proteins. Several instances of such genes are relevant to autism.

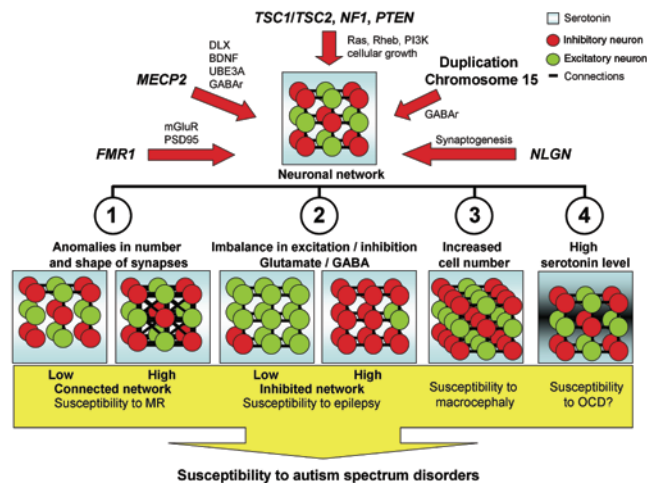
The tumor suppressors *TSC1/TSC2* and *NF1* are GTPase-activating proteins with widespread effects on cell survival, cell structure and cell function, whose disruption causes tuberous sclerosis<sup>28</sup> and type-1 neurofibromatosis<sup>29</sup>, both of which are comorbid with autism. Knocking out *Kras* in *Nf1*-deficient mice (an *Nf1*<sup>+/-</sup> and *Kras*<sup>+/-</sup> double-knockout) restores the wild phenotype<sup>30</sup>, illustrating the importance of interactions at the network level. Both *NF1* and the *TSC* complex negatively regulate the phosphoinositide-3 kinase pathway, as does the tumor suppressor *PTEN*. Mutations in *PTEN*, a regulator of cell size and number, have been identified in people with autism and macrocephaly<sup>31</sup>, and *PTEN* knockouts produce anxiety behaviors, deficits in social behaviors and increased spine density<sup>32</sup> reminiscent of the FXS phenotype. These cases illustrate the crucial nature of appropriate gene dosage in establishing optimal numbers of neurons and synapses during development.

In addition to mutations in dosage-dependent genes themselves, the absence of upstream regulatory factors also can destabilize gene dosage. *FMRP*, which couples activation of group-1 metabotropic glutamate receptors to modifications of mRNA translation in dendritic spines, is a case in point. Another is *MECP2*, a methyl-CPG-binding protein that regulates expression of genes including *UBE3A* and *GABRB3* (ref. 33) and whose disruption causes Rett syndrome<sup>34</sup>, a developmental disorder with which autism bears substantial phenotypic overlap.

All of these genes, via downstream effects on neuron numbers, neuronal and synaptic structure or neurotransmission, have the potential to induce fundamental alterations in network properties. Consideration of this network level may explain how it is that autism symptoms in each of these conditions are so variable: whereas dysfunctional *FMR1*, *MECP2*, *TSC1*, *TSC2*, *NF1* or *PTEN* alone may produce mental retardation and other symptoms, the combination of this insult with genetic and environmental modifiers of network properties may translate into more or less severe phenotypes, including autism. Neurodevelopmental disorders in general perturb such complex relations of gene expression and development that variable penetrance and variable phenotype are likely to be the rule rather than the exception.

### Many routes to dysfunction

In contrast to these cases of phenotypic overlap or comorbidity with disorders involving single regulatory genes, in some cases a strong single-gene association within an individual pedigree has not been replicated in large-scale association studies, or has yielded inconsistent results. The most well known and current of these cases is that of the neuroligin genes *NLGN3* and *NLGN4*. Neuroligins are postsynaptic cell adhesion molecules that contact presynaptic neuroligins. Neuroligins 3 and 4 in particular localize to glutamatergic synapses, and loss-of-function mutations in these genes segregate in certain pedigrees with a phenotype of variable penetrance, including mental retardation, autism and Asperger syndrome<sup>35,36</sup>. Though such mutations have been associated with a few sporadic cases of individuals with autism spectrum conditions<sup>37</sup>, this relationship is not robust enough to yield statistically significant association in large-scale studies of genetic linkage<sup>38-41</sup>. Nevertheless, even a sporadic association with a single gene can yield crucial information on the synaptic abnormalities possibly present in autism. *In vitro* studies suggest a major role for neuroligins in synapse formation, as their postsynaptic expression induces fully functional presynaptic terminals in contacting axons. Functional studies of the *NLGN3* R451C and *NLGN4* D936X mutations clearly indicate defective trafficking and synapse induction properties in the mutated proteins<sup>42,43</sup>. Furthermore, the association of neuroligins with scaffolding proteins such as PSD-95 regulates the balance between numbers of excitatory and inhibitory synapses<sup>43</sup>, and in turn, the activity-dependent translation of PSD-95 is regulated by *FMRP* (ref. 44). Thus, although neuroligins may have little in common with syndromic autism-



**Figure 1** Modifications of neuronal networks that increase susceptibility to autism spectrum conditions (ASC). 1. Inappropriate numbers and/or morphologies of synapses may lead to a too weakly or too strongly connected network. Such abnormal synaptic connectivity is frequently observed in mental retardation (MR), a condition present in 75% of individuals with ASC. 2. An imbalance between GABA and glutamate may lead to an abnormal inhibition or excitation associated with epilepsy, a condition present in 30% of individuals with ASC. 3. An increased number of neurons may cause macrocephaly, observed in 30% of individuals with ASC. 4. High levels of serotonin are observed in at least 25% of individuals with ASC. This abnormal neuromodulation may alter network properties, as observed in patients with OCD and mutations of the serotonin transporter.

related genes such as *FMR1* in terms of immediate function, their roles in synaptic plasticity point to a common pathway at the level of networks of genes whose effects interact to determine the properties of networks of neurons. The influence of this pathway on the balance between excitation and inhibition is an important one for autism, as it may help to explain not only autism's comorbidity with epilepsy but also autism's abnormalities of neural information processing<sup>1,45</sup>.

The serotonin membrane transporter *SLC6A4* is another case of a genetic association that has been inconsistently replicated. *SLC6A4* is of interest because of several reports of its association with autism and because of the nearly even split of these association studies between findings of preferential transmission of the long or the short alleles of the *SLC6A4* promoter<sup>46</sup>. Efforts to make sense of these apparently contradictory observations again implicate the relationship of gene dosage to genetic and environmental modifiers—for instance, the role of *SLC6A4* may differ within the subpopulation of people with autism (and their relatives) in whom platelet serotonin is elevated. A liability to autism conferred by *SLC6A4* may require functional interaction of variants in several coding and non-coding regions<sup>47</sup>. Notably, rare *SLC6A4* functional alleles, some resulting in a gain of function, have been identified in neuropsychiatric disorders, including obsessive-compulsive disorder (OCD), anorexia and Asperger syndrome, pointing toward dimensional (pertaining to quantitative, incidental properties that may be shared in distinct disorders), rather than categorical (qualitative, essential properties unique to a particular disorder), roles for *SLC6A4* gene variants in stereotypic behaviors<sup>48</sup>.

Mutations in genes such as *FMR1*, *TSC1/2*, *NF1* or *NLGN3/4* that control the growth and maturation of cells and synapses increase risk for autism, but are not strict determinants of autism. Modifying factors—genetic, epigenetic or environmental—must therefore be involved in autistic development<sup>49</sup>. Among the candidates for such modifiers are

genes with more direct or specific effects on excitatory and inhibitory processes, such as the glutamate receptor genes *GRIN2A* and *GRIK2* and multiple GABA receptor genes<sup>49</sup> identified by linkage and association studies, along with genes that confer abnormal serotonergic or other neuromodulation. On the basis of all these associations, we suggest that autism may arise from various combinations of three cumulative and overlapping factors (Fig. 1): (1) an anomaly in synapse formation or maintenance, (2) an imbalance between excitation and inhibition<sup>45</sup>, (3) abnormal cell number and (4) abnormal neuromodulatory function affecting network properties. Within this framework, a defect in synapse formation or maintenance alone may have no consequences or could be associated with isolated mental retardation. An imbalance in excitation and inhibition alone may have no consequences or could be associated with epilepsy. An increase or decrease in cell number or size could be associated with macro- or microcephaly. Abnormal modulation of neural networks alone could have no consequences or could be associated with other neuropsychiatric diseases, such as obsessive-compulsive disorder, more frequently observed in autism relatives as compared to the general population<sup>50</sup>. In all these cases, abnormal expression within complex networks of genes translates into abnormal function in complex networks of neurons.

In networks of interacting neurons, just as in networks of interacting genes, dosage effects are crucial, and more is not always better. The balance between excitatory and inhibitory neural processes depends on expression of many genes directly or indirectly involved in neurotransmission, including receptors, membrane transporters, enzymes for neurotransmitter synthesis or degradation, cytoskeletal and vesicular proteins, signaling and effector proteins, and regulators of transcription and translation. All of these genes participate in setting a level of bias within a continuum of neural connectivity, establishing a sort of 'neural dosage' analogous to gene dosage. Optimizing this neural dosage is crucial in optimizing the representational capacity of neural networks. At the extreme of a completely connected network in which any input activates every unit, network entropy is zero because no two inputs can be distinguished from each other. This zero-entropy condition is informatically equivalent to the opposite extreme of a completely unconnected network in which no input activates any unit, where again no two inputs can be distinguished. To phrase it another way, at the extreme of complete connectivity so much noise is propagated that the signal is masked, and at the other extreme of zero connectivity, not enough signal is propagated to overcome the noise. In either case, the outcome is a noisy network, a poverty of neural representation and disruption of further activity-dependent development. Only in an intermediate range of connectivity can local network entropy be maximized, and only with properly patterned local activity can appropriate long-range connections develop into a network of high information-theoretic complexity in which information can be both efficiently represented and efficiently propagated. Thus either extreme in the dosage of a neuronal property may produce the same neural abnormality<sup>1</sup>. This realization may be crucial to making sense of autism's multifactorial genetics and divergent endophenotypes. We suggest that neural network properties arising from patterns of neuronal connectivity are a strong candidate locus of convergence for autism's multiple causal factors, as well as a locus of divergence into autism's many and various associated phenotypes.

Considering autism at the network level may lead to a useful dissociation of therapy from pathology, in that normalizing influences may be applied to the network via mechanisms entirely distinct from those that have disrupted it. Indeed, such a dissociation between pathological processes and therapeutic targets has already been suggested in animal models of FXS, where treatment with an mGluR5 antagonist<sup>19</sup> does nothing to reverse the pathologically exuberant response to receptor

activation, but instead prevents these receptors from being activated to such a degree in the first place. In the case of autism, drugs that act at the synapse to alter excitatory or inhibitory bias may prevent further development of pathology. As a single-gene disorder whose analysis illuminates networks of interacting genes and networks of interacting neurons, FXS opens for us a route to understanding the complexity of autistic development and a possibility of producing targeted therapies.

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#### COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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