development from birth until 6 to 18 months of age, when they regress, losing speech and hand skills that they had acquired. Rett syndrome is progressive, and initial symptoms are followed by repetitive hand movements, loss of motor control, and intellectual disability. Often young girls will display symptoms indistinguishable from ASD early in the course of the syndrome, although social communication frequently improves later in childhood. Its prevalence is approximately 1 in 10,000 live female births.

Rett syndrome is an X-linked inherited disease caused by loss-of-function mutations in the *MECP2* gene, which encodes a transcriptional regulator that binds to methylated cytosine bases in DNA, regulating gene expression and chromatin remodeling. The gene product was initially thought to act predominantly as a transcriptional repressor, but studies of both the mouse model and human induced pluripotent stem cells have shown that overall gene expression is reduced when the gene is knocked out. Among the genes that have reduced expression in neurons is *BDNF*, encoding brain-derived neurotrophic factor. Studies in mouse models of Rett have found that overexpression of *BDNF* improves the knock-out phenotype. Other growth factors that increase gene expression but have more favorable neuropharmacological profiles, including insulin-like growth factor-1 (IGF-1), have also improved aspects of the mouse phenotype, leading to optimism about clinical trials of related compounds. Phase II human trials with both molecules are currently underway.

One might think that such a global abnormality in gene expression would lead to a very severe phenotype, but because females are mosaic, with approximately half of their brain cells expressing one normal copy of *MECP2* (due to random X-inactivation), they are viable but manifest the devastating Rett phenotype. Boys, who have a single X chromosome and thus a single copy of *MECP2*, typically die soon after birth or in infancy if they carry a loss-of-function mutation in *MECP2*.

The role of X-inactivation in the survival of female mutation carriers and the observation that favorable skewing (a shift toward preferential silencing of the mutant X) leads to a less severe clinical course have generated considerable interest in therapeutic strategies aimed at reactivating the normal but silenced X chromosomes in females with Rett syndrome. Although one can imagine considerable challenges resulting from the reactivation of many genes on a normally silenced chromosome, a recent study has reported a mouse mutation that leads to both alleles expressing MeCP2 without wholesale activation of genes on the X chromosome.

Interestingly, in 2005, duplications spanning *MECP2* were identified in males with severe intellectual disability. This condition, called *MECP2* duplication syndrome (MDS), includes autistic features, hypotonia, epilepsy, gait abnormalities, and recurrent infections. Like Rett syndrome, it has also been productively modeled in rodents. However, unlike Rett, the majority of identified cases are familial and not sporadic in nature. In these cases, female carriers are often healthy enough (due to favorable X-inactivation) to reproduce and transmit the duplication to boys with only a single X chromosome.

Williams Syndrome

Williams syndrome is caused by a segmental deletion of about 27 genes on the long arm of chromosome 7 and is characterized by mild to moderate intellectual disability, connective tissue abnormalities, cardiovascular defects, distinctive facies, and a behavioral phenotype characterized by increased sociability, preserved language abilities, affinity for music, and impaired visuospatial capabilities. The disorder occurs in 1 in 10,000 live births. The connective tissue and key cardiovascular symptoms have been attributed to the loss of the gene *ELN* (*elastin*), although no specific genes within the deleted interval have yet been definitively shown to result in the behavioral phenotype. Nonetheless, the social cognitive features of Williams syndrome are particularly intriguing: The degree of interest in social interaction is striking, leading to a nearly universal loss of reticence with strangers in children with the syndrome. In contrast to the almost complete absence of social anxiety, individuals with Williams syndrome have a high degree of general anxiety and isolated phobias. Finally, the affinity for and interest in music among a very large percentage of 7q11.23 deletion carriers, although less well characterized, are striking.

Conversely, duplication of the identical region of chromosome 7, including the same 26 to 28 genes, is a significant risk factor for ASD and other neurodevelopmental syndromes apart from Williams syndrome. The observation of contrasting social phenotypes depending on whether there is loss or gain of a small region of the genome is fascinating. Whether social functioning in William syndrome is truly the opposite of that seen in ASD, as is sometimes argued, seems less interesting than the conclusion that this region of the genome must contain one or more genes that modulate social affiliation. Consequently, the molecular characterization of these deletion and duplication syndromes and intensive investigation of their impact on the development of molecular, cellular, and circuit properties in the central nervous system are particularly important.

Angelman Syndrome and Prader-Willi Syndrome

Angelman and Prader-Willi syndromes are paradigmatic examples of genetic syndromes that result from mutations in genes subject to parental imprinting. To understand these conditions, one must not only know the associated DNA lesion but also its parental origin.

For example, both syndromes most often result from the loss of the identical region of chromosome 15 (15q11-q13) but have readily distinguishable phenotypes. Angelman syndrome is characterized by severe intellectual disability, epilepsy, absence of speech, hyperactivity, and inappropriate laughter. In contrast, Prader-Willi is characterized by infantile hypotonia, mild to moderate intellectual disability, obesity, highly perseverative behavior, social disability, and diminished or absent satiety.

How these contrasting phenotypes result from the loss of the identical set of genes confounded medical geneticists until about the year 2000. The mystery was solved by the discovery that the chromosomal interval is imprinted. Specifically, within this region, multiple genes are expressed only on the paternally inherited chromosome (*maternal* imprinting), whereas at least two genes, *UBE3A* and *ATP10C*, are expressed only on the maternally inherited chromosome (*paternal* imprinting) (Figure 62–7).

This discovery, along with a series of studies that allowed for fine mapping of the interval, provided a parsimonious explanation for the clinical observations. If the deletion of proximal chromosome 15 involved the maternal chromosome, the patient would suffer the loss of the protein product of *UBE3A*, a ubiquitin-protein ligase that stimulates the degradation and turnover of other proteins, leading to Angelman syndrome. Alternatively, if the paternal chromosome carried the deletion, *UBE3A* would be expressed normally, but a series of other genes, including several strongly implicated in Prader-Willi syndrome, would be lost.

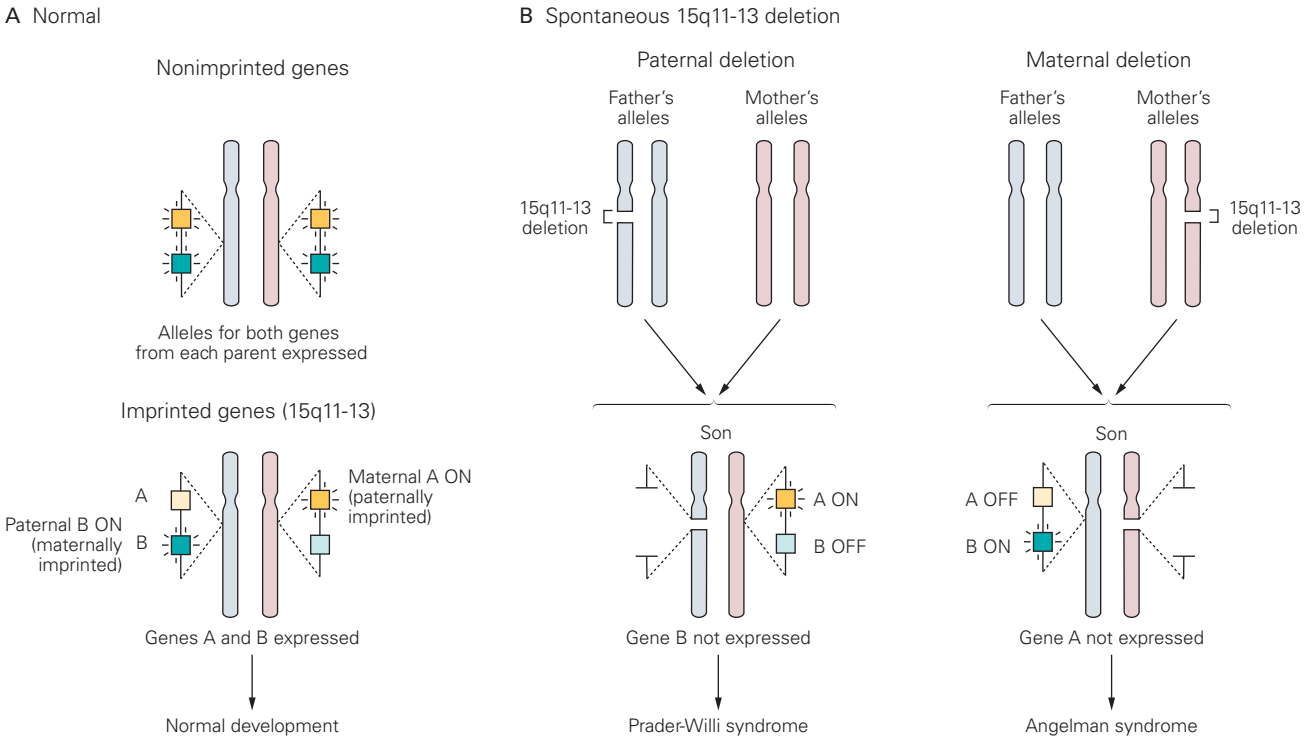

Figure 62–7 Imprinting in Prader-Willi and Angelman syndromes. Approximately 70% of Prader-Willi and Angelman syndrome patients inherit chromosome 15 from one parent with spontaneous (noninherited) deletions of the q11-13 interval. This interval contains imprinted genes with alleles that are either expressed or not depending on whether the chromosome was inherited from the father or mother. If the chromosome with the deletion is from the father, Prader-Willi syndrome occurs because maternally imprinted genes on the corresponding interval of the intact maternal chromosome (gene B, for example) are not expressed. If the chromosome with the deletion is from the mother, the gene for ubiquitin ligase (*UBE3A*) will not be expressed in offspring because of its normal inactivation on the paternal chromosome caused by imprinting; loss of expression of this gene leads to Angelman syndrome.

The solution to the phenotypic complexity seen in 15q11-13 deletion also led to a series of observations that revealed other previously unappreciated genetic mechanisms of behavioral pathology. For example, deletions on the maternal chromosome not directly involving the *UBE3A* gene were also observed in rare patients with Angelman syndrome, contributing to the identification of an Angelman syndrome *imprinting control* region mapping some distance from UBE3A but within the deletion interval. Similarly, the discovery of both Prader-Willi and Angelman syndromes in patients without deletions of any kind led to the recognition that in a small percentage of both conditions two copies of a chromosome from the same parent were present (with no representation from the other parent), a phenomenon called *uniparental disomy*.

Both syndromes have complex behavioral phenotypes. Social disability is characteristic of Prader-Willi; with Angelman syndrome, the overlap with ASD has been more difficult to establish because of the marked intellectual disability associated with the syndrome. Differentiating intellectual disability from ASD in individuals with very low IQ can be quite challenging. Nonetheless, there are multiple clear molecular and behavioral links with ASD. For example, duplications of the 15q11-13 region are a well-established risk factor for nonsyndromic ASD (see below), and functional de novo missense mutations in the gene *UBE3A* have been found in individuals with ASD without all of the features of Angelman syndrome.

Neurodevelopmental Syndromes Provide Insight Into the Mechanisms of Social Cognition

Although the fragile X, Rett, Williams, Angelman, and Prader-Willi syndromes collectively account for a small fraction of the burden of social disability in the population, studies of these disorders have contributed to major advances in the understanding of normal brain development, neurodevelopmental syndromes in general, and the mechanisms underlying social disability in particular. A number of biological processes identified in the study of these disorders—including the contribution of epigenetic mechanisms and chromatin dynamics, synaptic dysfunction, and the role of aberrant local protein synthesis—have all turned out to be important initial clues to the biological and developmental mechanisms underlying nonsyndromic forms of ASD. Moreover, characterization of the genetics underlying certain neurodevelopmental syndromes provided some of the earliest examples of a phenomenon that is now well accepted in ASD—either losses or gains of identical risk genes or regions may lead to neurodevelopmental disorders, sometimes with overlapping and sometimes contrasting phenotypes.

Importantly, in addition to the first clues regarding molecular mechanisms, recent studies of a number of Mendelian syndromes have challenged conventional wisdom by highlighting, in model systems, the potential reversibility of developmental phenotypes, even into adulthood. These observations, particularly with regard to Rett, Angelman, MDS, and fragile X syndromes, defied the long and generally held belief that the deficits associated with these types of severe syndromes are unchangeable. Moreover, the relevant studies have underscored the fact that a range of manipulations—from genetic, to pharmacological, to the more recent use of antisense oligonucleotides (in the case of *MEC2* duplication and Angelman syndromes)—have all been successful in reversing phenotype.

These findings provide not only an avenue forward for the development of rational therapies in humans but also a critical antidote to the penchant for nihilistic views of therapeutics development in neurodevelopmental disorders. In short, these findings have collectively, and now repeatedly, reinforced the notion that rationally designed therapies may reverse key symptoms long after initial pathology has begun to unfold in brain development. The question of how much of the core symptomatology seen in nonsyndromic ASD is a consequence of ongoing functional derangements, versus what would more traditionally be considered developmental pathology, remains to be clarified. One should note, however, that even with the limited treatments available, the observation that some children improve years after the onset of symptoms suggests that aspects of ASD pathology are not entirely static and may ultimately yield to the development of novel biologically driven treatment approaches.

The Complex Genetics of Common Forms of Autism Spectrum Disorder Are Being Clarified

The recent discovery of genes causing idiopathic ASD—once a scientific quagmire—has been among the most dramatic success stories in the field of human genetics. The combination of high-throughput genomic technologies—including the ability to assay common and rare variations in both the sequence and structure of DNA—the consolidation of large patient cohorts, and considerable investment in ASD research has transformed the field.

Initial breakthroughs can be traced to studies of the genes encoding the family of neuroligins—cell

adhesion molecules found at postsynaptic densities of glutaminergic synapses (Chapter 48). At the beginning of this century, the group led by Thomas Bourgeron, a geneticist at the Pasteur Institute, first identified putatively deleterious coding mutations in the genes coding for neuroligin 4X (loss-of-function) and neuroligin 3X (missense). About 6 months after the initial report on the loss-of-function mutation in *NLGN4X*, a nearly identical loss-of-function mutation in the same gene was found linked to both intellectual disability and ASD in a large pedigree. The relevance of the neuroligin 3X mutation to ASD has taken longer to clarify. Contemporary studies provide statistical evidence that *NLGN3X* is a probable, but not yet definitive, ASD risk gene. Additional studies of large cohorts will clarify this question.

In retrospect, these findings were prescient. The two papers on neuroligins pointed to the importance of loss-of-function heterozygous mutations leading not only to ASD but to a wide range of neurodevelopmental phenotypes and highlighted a role for synaptic proteins at the excitatory synapse. Moreover, in addition to being a harbinger of the contributions of both rare and de novo mutations (Chapter 2), the reported findings from Bourgeron's group also hinted, in retrospect, at a female protective effect as well as a paternal origin of de novo point mutations. In the initial report, the unaffected mother carried a de novo loss-of-function mutation on her paternally inherited X chromosome, which she passed to two affected sons.

Several years later, two key findings further ushered in the modern age of reliable and reproducible genetic studies in ASD. First, papers in 2006 and 2007 reported on the observation of rare de novo heterozygous copy number variations (Chapter 2) in children with ASD and intellectual disability. These studies focused specifically on idiopathic, nonsyndromic ASD and on families with only a single affected individual (simplex families). Both papers reported high rates of relatively large copy number variations among individuals with both intellectual and social disability. Second, it was not clear if individuals with ASD simply had more chromosomal abnormalities than those without. However, this question was soon answered by studies from multiple laboratories. De novo copy number variations did not appear to be distributed randomly throughout the genome but tended to cluster in distinct regions of the genome, suggesting that the increased rate in such cases was a consequence of an accumulation of specific risk events. Moreover, as higher-resolution genomic assays began to be applied, similar results emerged: Only certain subsets of mutations (eg, point mutations that disrupt gene function) proved to be elevated in individuals with autism, pointing to the aggregation of causal mutations in affected individuals, not hypermutability, as an explanation for the excess rate(s) of de novo events in affected individuals.

A considerable investment in studying copy number variations in simplex families has resulted in a steadily expanding list of copy number variations that clearly and dramatically increase the risk for ASD. At present, about a dozen genomic intervals reach genome-wide significance based on genome-wide screening of cases for de novo mutations (Figure 62–8). As a result, the American College of Medical Genetics now considers screening for copy number variations the standard of care for an individual presenting with ASD of unknown etiology.

Studies of de novo mutations have advanced throughout the second decade of this millennium, leading to the discovery that, similar to de novo copy number variations, de novo changes in the sequence of DNA—both single nucleotide variants and insertions or deletions (indels)—also contribute to ASD risk and can similarly be used to identify specific risk genes. Recent reports have now leveraged this approach to include more than 100 genes carrying large-effect single nucleotide variants and indel mutations that disrupt the function of the encoded protein (ie, likely gene-disruptive [LGD] mutations) (Chapter 2).

Several associated findings deserve mention here. First, although the contribution of de novo mutations to the risk for ASD in the total population is quite small (in the neighborhood of 3%), the proportion of individuals with large-effect de novo mutations who are seen in clinical settings and recruited for genetic studies is quite significant, as high as 40% of girls. The reason for this apparent contradiction is that most of the risk to the population writ large is carried in small-effect common variations that in most individuals are not sufficient to result in them crossing a diagnostic threshold for ASD. In short, most individuals carrying some degree of risk in the population never show overt social impairment and do not come to clinical attention. Conversely, individuals with large-effect de novo copy number variations, single nucleotide variants, and indels are much more likely to have significant clinical manifestations and seek medical attention.

Second, studies of de novo single nucleotide variants and indels in ASD using exome sequencing have found that the rate of de novo mutations increases with the father's age. Consistent with this observation is the finding that the vast majority of deleterious de novo sequence mutations in ASD cases are present on the paternally inherited chromosome. Although the

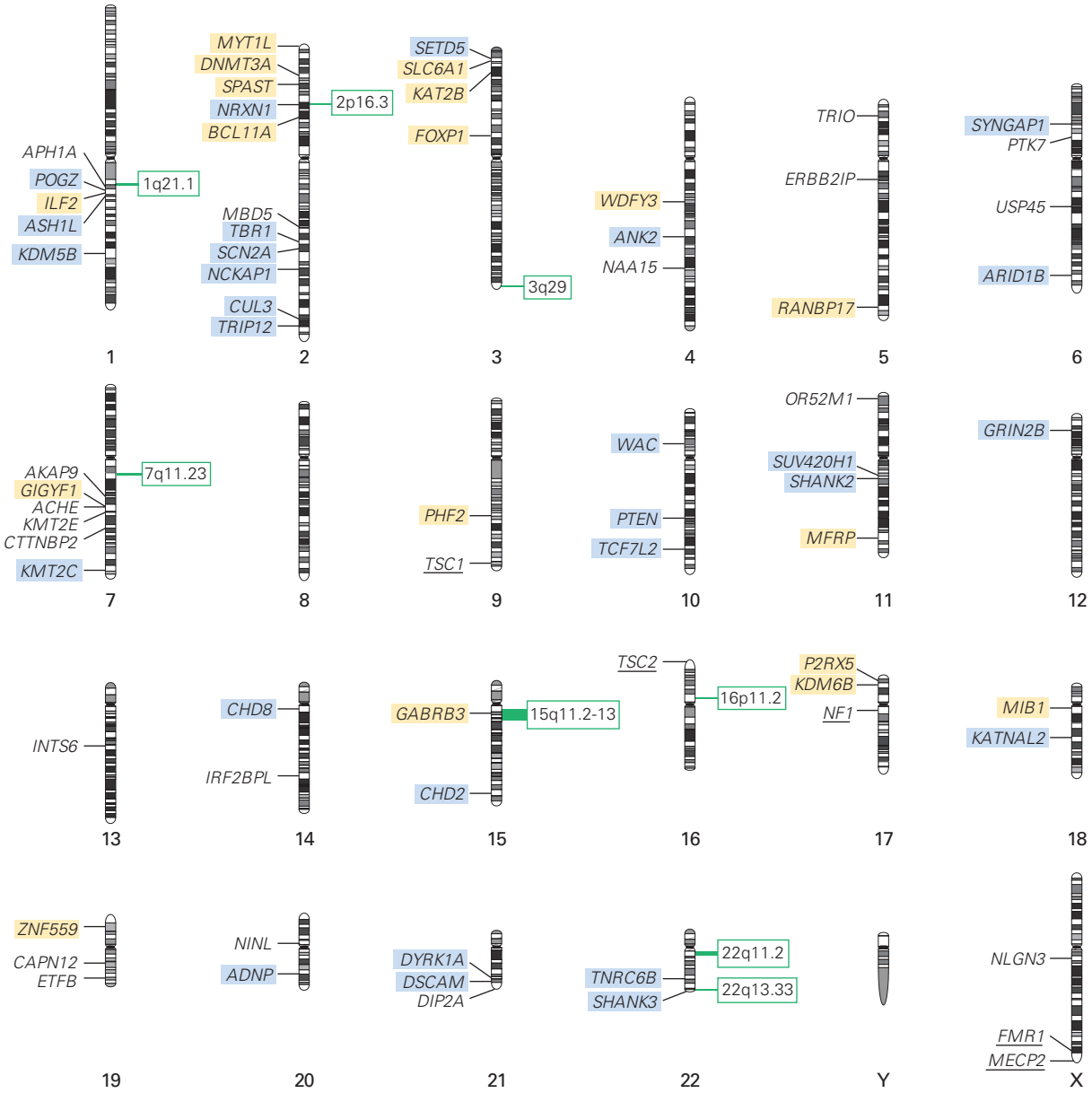

Figure 62–8 **Multiple genes and copy number variations that have been strongly associated with idiopathic risk for autism spectrum disorders (ASDs).** The figure identifies 71 genes and copy number variations (CNVs) associated with risk for ASDs based predominantly on the recurrence of de novo mutations. Abbreviations in **blue shading** denote genes with a false discovery rate (**FDR**) less than 0.01; abbreviations in **yellow shading** denote an FDR between 0.01 and 0.05; and abbreviations with **no shading** denote an FDR greater than 0.05 and less than 0.1. **Green bars** identify CNVs with an FDR less than 0.05. Data from Sanders et al. 2015. Statistical analysis was performed using the methods described in Sanders et al. 2015. Five additional genes with names underlined cause the syndromic forms of ASD discussed in the chapter text. Gene identification in ASD is continuing at a rapid pace. Up-to-date lists of associated genes and genomic regions can be found at https://gene.sfari.org.

absolute increase in risk with age is small, this observation nonetheless provides a conceptual framework for understanding secular increases in ASD prevalence. It also sets the stage for further studies of the impact of environmental factors in increasing de novo mutations and thereby potentially increasing the true incidence of clinical ASD cases.

The relationship of de novo large-effect mutations to intellectual disability has been the subject of considerable discussion, with some contending that de novo