CHAPTER

32

Developmental Disabilities, Autism, and Schizophrenia at a Single Locus:

Complex Gene Regulation and Genomic Instability of 15q11–q13 Cause a Range of Neurodevelopmental Disorders

N. Urraca, L.T. Reiter

University of Tennessee Health Science Center, Memphis, TN, USA

OUTLINE

Nomenclature

ARHGAP11B Rho GTPase activating protein 11B
ATP10A ATPase, class V, type 10A
C15orf2 Chromosome 15 open reading frame 2
CHRNA7 Cholinergic receptor, nicotinic, alpha 7
CTCF CCCTC-binding factor
CYFIP1 Cytoplasmic FMR1 interacting protein 1
Dube3a *Drosophila* ube3a
ECT2 Epithelial cell transforming sequence 2 oncogene
GABRA5 Gamma-aminobutyric acid (GABA) A receptor, alpha 5
Gabrb3 Mice GABA A receptor, subunit beta 3 gene
GABRB3 Gamma-aminobutyric acid (GABA) A receptor, beta 3 gene
GABRG3 Gamma-aminobutyric acid (GABA) A receptor, gamma 3
HBII52 Small nucleolar RNA, C/D box 115 cluster
Idic (15) Isodicentric chromosome 15
Int dup (15) Interstitial duplication chromosome 15
KLF13 Kruppel-like factor 13
MAGEL2 MAGE-like 2
MECP2 Methyl CpG binding protein 2
MKRN3 Makorin ring finger protein 3
MTMR10 Myotubularin-related protein 10

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MTMR15 Myotubularin-related protein 15
NDN Necdin homolog
NIPA1 Nonimprinted gene in Prader–Willi syndrome/Angelman syndrome chromosome region 1
NIPA2 Nonimprinted gene in Prader–Willi syndrome/Angelman syndrome chromosome region 2
OTUD7A OTU domain-containing 7A
PWRN1 Prader–Willi region non-protein-coding RNA 1
SmN Survival of motor neuron 1
snoRNAs Small nucleolar RNAs
SNRPN Small nuclear ribonucleoprotein polypeptide N
SNURF SNRPN upstream reading frame
TCF4 Transcription factor 4
TRPM1 Transient receptor potential cation channel, subfamily M, member 1
TUBGCP5 Tubulin, gamma complex associated protein 5
UBE3A Ubiquitin protein ligase E3A
Ube3a Mice Ube3a

32.1 COMPLEX GENOMIC CHARACTERISTICS AND GENE REGULATION AT THE HUMAN 15q LOCUS

The chromosome 15q locus is an enormously complex region of the human genome responsible for several devastating neuropsychological syndromes as well as a major causative locus in autism spectrum disorder (ASD) (see Chapter 34). Gene regulation in this region is extremely complex and is governed by a variety of molecular changes, including DNA methylation, antisense transcripts, microRNAs, and genomic rearrangements. The purpose of this chapter is to provide a broad introduction to some of the neurological disorders and causative genes in the 15q11–q13 locus.

Chromosome 15 is acrocentric; thus, it essentially has one long coding arm (q) and a short arm (p) containing mostly heterochromatin arrays of ribosomal RNA genes, satellite sequences, and other repetitive DNA sequences. The long q arm contains 2.9% of the coding genes in the human genome (Zody et al., 2006). A key structural feature of chromosome 15 is the high number of low-copy segmental duplications, with 8.8% of its euchromatin being inundated with low-copy repeats (LCRs) (Zody et al., 2006). These LCRs predispose genes in the region to dosage changes due to both deletion and duplication events mediated by nonallelic homologous recombination (NAHR) events and result in a spectrum of neurodevelopmental disorders (mechanism reviewed in Shaw and Lupski, 2004). This locus is complicated even further by a 2-Mb cluster domain of genes preferentially expressed from one parental allele or imprinted genes.

Imprinted genes are epigenetically marked in gametes to drive expression of that gene from a single parental allele in the offspring. An epigenetic mark (or imprint) is set depending on the gene and on the germ cell. In fertilization, somatic cells of the developing embryo contain both maternally and paternally inherited alleles; this epigenetic mark ensures that just one allele is expressed (Weaver et al., 2009). In the embryonic germ cells, the original imprint is erased and a new one is added depending on the sex and the gene. This parent-specific expression is directly due to these epigenetic modifications of the DNA (DNA methylation, histone modifications, and noncoding RNAs) and not due to changes in the primary sequence, thus altering the conformation of chromatin fibers and therefore the regulation of the expression of the nearby genes. Although these modifications can have dramatic effects on the phenotype, they do not alter the primary DNA sequence. The monoallelic expression of an imprinted gene may occur only in one of possibly several isoforms, or in particular tissues, or at particular stages of development. The untranslated mRNA H19 was the first gene shown to be imprinted in humans (Zhang and Tycko, 1992); as many as 40 such genes have now been identified, and another 156 new genes have been predicted by both computational and experimental approaches to be regulated in a parent-specific manner through epigenetic modification (Luedi et al., 2007).

The 15q11–q13 region contains several genes that show parent-of-origin-specific expression and others that show biallelic expression in the brain. Genes expressed exclusively from the paternal chromosome in the brain are *MKRN3*, *MAGEL2*, *NDN*, *SNRPN*, and a cluster of *snoRNAs*. *C15orf2* and *PWRN1* are genes that have shown monoallelic expression in human fetal brain (Buiting et al., 2007). Two genes show maternal-specific expression in the brain: *UBE3A*, an E3 ubiquitin ligase responsible for Angelman syndrome (AS) phenotypes that shows biallelic expression in most tissues except for a preferential expression of the maternal allele in human brains, and *ATP10A*, which also is preferentially expressed in the maternal chromosome in human brain as well as in fibroblasts (Herzing et al., 2001; Meguro et al., 2001). Recently it has been suggested that the *ATP10A* may be monoallelic in expression, dependent on sex and common genetic variation and not regulated by an imprinting mechanism (Hogart et al., 2008).

Recently, a large region between *Snrpn* and *Ndn* was found to be imprinted in the mouse brain, with several paternally expressed ncRNAs and a paternally expressed gene called *DOKist4* (Gregg et al., 2010). Although the phenotypic outcome of these additional paternally expressed genes and ncRNAs is still unknown, it clearly speaks to the complexity of this locus in terms of genotype to phenotype correlations.

This region is one of the most unstable regions in the human genome (Knoll et al., 1993) because of a number of complex LCRs that predispose the region to misalignment during meiosis I, which leads to unequal NAHR events involving both sister chromatid and interchromosomal exchanges (Lupski, 1998; Robinson et al., 1998).

In addition, there is a high rate of recombination in women in this locus (Robinson and Lalande, 1995). 15q11–q13 homologous pairing is an epigenetic regulatory mechanism disrupted by genomic rearrangements such as duplications and deletions. CCCTC-binding factor (*CTCF*) controls the process of X chromosome pairing as well as pairing at the 15q11–q13 locus (Meguro-Horike et al., 2011). At least five recurrent breakpoints (BPs) have been identified containing LCRs involved in microdeletions, microduplications, inversions, as well as isodicentric chromosomes (see Figure 32.1).

Severe phenotypic consequences at the 15q locus can be caused by deletions, duplications, mutations, or defects on a single active allele, be it maternal or paternal. In rare instances, two copies of chromosome 15 can be inherited from a single parent (uniparental disomy), causing the same severe phenotypic consequences as loss of function for one 15q allele. The two primary genomic disorders that cause neuropathology in this region are the Prader–Willi syndrome (PWS) and Angelman syndrome (AS), whose phenotypes result from loss of the paternal or maternal contribution of the 15q11–q13 genomic region, respectively. The first nine individuals with PWS were described in 1956 by the endocrinologists Prader, Labhart, and Willi (Clarke and Boer, 1995). By 1976, it was apparent that a recurrent microdeletion could be visualized by high-resolution chromosome analysis in patients with PWS and AS (Ledbetter et al., 1981). In 1989, it was established that AS is a direct result of maternal deletion, whereas PWS results from a paternal deletion of the same region on chromosome 15 (Knoll et al., 1989). This 2-Mb domain is often referred to as the PWS/AS critical region. In 95% of PWS/AS patients, there are two main types of deletions: class I deletions, with breakpoints at BP1 (proximal) to BP3 (distal), and class II deletions, with breakpoints from BP2 (proximal) to BP3 (distal) (see Figure 32.1). The remaining 5% of PWS/AS patients have the distal breakpoint at BP4. Patients with smaller deletions from BP1 to BP2 have been identified, but these individuals do not present with classic PWS or AS and may represent a distinct neurological syndrome unrelated to the primary causative genes for PWS and AS (Doornbos et al., 2009). As with most genomic disorders, these LCRs mediate not only deletion events, but also reciprocal duplication events. The same LCRs and therefore the same breakpoints in addition to BP5 can mediate inverted dup(15) marker chromosomes and some cases of interstitial duplications and triplications of chromosome 15q11–q13 (Roberts et al., 2002). The clinical presentation associated with deletions tends

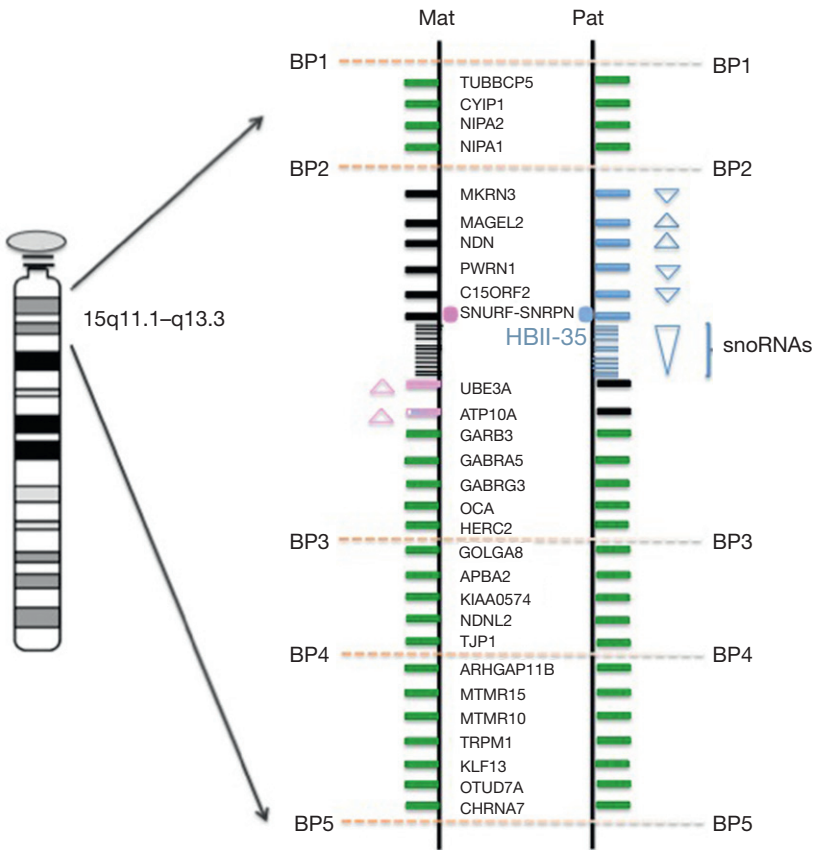

FIGURE 32.1 15q11.1–q13.3 region. Maternal and paternal alleles are shown separately. Maternally expressed genes are in pink, paternally expressed in *blue*, biallelically expressed genes in green, and the genes silenced on a particular allele are in *black*. *ATP10A* varies among populations, so its pink/blue. Dots pink and blue refer to AC-IC and PW-IC, respectively. The five different common breakpoints for both duplications and deletions in this region appear as dotted lines.

to be more uniform, and commonly includes dysmorphology, which facilitates diagnosis, while duplications present with a more subtle phenotype that often includes autism when maternally inherited, but may also include anxiety and other neurological effects in paternally inherited duplications (Hogart et al., 2010). The widespread use of array comparative genomic hybridization (array CGH) has increased the frequency of detection of submicroscopic interstitial 15q duplications, as well as smaller, possibly pathogenic deletions in this region. Smaller deletions and duplications found in neurodevelopmental disorders such as schizophrenia and autism will be described later in this chapter.

32.2 EPIGENETIC CONTROL OF GENE EXPRESSION ACROSS 15q11–q13

Imprinted gene expression on 15q is coordinately controlled in *cis* by an imprinting center (IC), a genetic element functional in germline and/or early postzygotic development that regulates the establishment of parental specific allelic differences in replication timing, DNA methylation, and chromatin structure. In other words, the IC's function is to reset the maternal and paternal imprints. A mutation at the IC sets the imprint permanently, and the parental imprint will be fixed on the chromosome on which the mutation arose, resulting in a heritable form of the imprinting disorder (Saitoh et al., 1996). The IC regulates imprinting in the whole region and is located in the 5′ untranslated region of the small nuclear ribonucleoprotein polypeptide N (*SNRPN*) gene. The PWS/AS IC has two functional components: a 4.3-kb DNA segment at the promoter of *SNRPN* involved in PWS (PWS-IC) and a 0.9-kb element ~35 kb upstream of *SNRPN* which regulates the gene responsible for AS (AS-IC) (Buiting et al., 1995). AS-IC acquires a primary imprint during gametogenesis, thus establishing the maternal epigenotype. The inactive maternal IC is marked by CpG methylation and by histone H3 Lys 9 di-methylation, while the active paternal IC is marked by histone H3 Lys4 methylation (Soejima and Wagstaff, 2005). The unmethylated maternal allele of the AS-IC most likely binds a *trans*-acting factor that confers methylation on the PWS-IC maternal allele after fertilization. It is assumed that, once established, the PWS-IC paternal epigenotype spreads across the entire PWS/AS domain in the soma.

32.2.1 Epigenetic Control Across the 15q Region

Histone acetylation and chromatin structure are influenced by DNA methylation, resulting in the production of compacted chromatin that is refractory to transcription. In the PWS/AS region, *MKRN3*, *NDN*, and *SNRPN* are silenced by maternal-specific CpG methylation of the promoter/exon I region (Driscoll et al., 1992; Glenn et al., 1996; Jay et al., 1997).

The *SNRPN* gene is a bicistronic-imprinted gene that encodes two polypeptides, the SNURF (SNRPN upstream reading frame) and the SmN (splicing factor involved in RNA processing) (Gray et al., 1999). This gene has 10 exons: exons 1–3 encode the SNURF protein, and exons 4–10 encode the SmN. This SNRP–SmN transcript unit also hosts the genes to encode multiple noncoding RNAs (snoRNAs), and it serves as the start site for the *UBE3A* antisense transcript (Runte et al., 2001). Because the CpG island of the promoter is hypermethylated on the maternal chromosome and hypomethylated on the paternal chromosome, the gene is transcribed exclusively from the parentally inherited chromosome and shows a high level of expression in the brain and the heart. Different methods have taken advantage of the differentially methylated *SNRPN* promoter for the diagnosis of PWS and AS. Our group recently utilized this differentially methylated region to develop a high-resolution melting curve assay to determine the parent of origin of the duplicated chromosome in individuals with 15q duplication (Urraca et al., 2010).

32.3 GENES KNOWN TO CAUSE DISEASE IN THE REGION

32.3.1 The E3 Ubiquitin Ligase Gene (*UBE3A*)

The *UBE3A* gene product is the canonical HECT domain E3 ubiquitin ligase enzyme known as E6-associated protein (E6-AP). Although originally identified as a host protein that interacts with viral E6 protein during papillomavirus infection (Huibregtse et al., 1993), the primary role of this enzyme is thought to be the monoubiquitination of protein substrates that are destined for recycling by the ubiquitin proteasome system or are tagged for trafficking to various cellular compartments. In addition to this enzymatic function, E6-AP can also function as a transcriptional co-activator of steroid hormone receptor genes (Nawaz et al., 1999). Maternal deficiency for *UBE3A* results in the neurodevelopmental disorder Angelman syndrome (Kishino et al., 1997). This gene exhibits maternal allele-specific expression in most regions of the brain in mice and humans, with the strongest expression restricted to hippocampal and cerebellar neurons (Albrecht et al., 1997; Dindot et al., 2008). The discovery of maternally inherited loss-of-function mutations in *UBE3A* has clearly established *UBE3A* as the causative gene in AS (Kishino et al., 1997; Matsuura et al., 1997). Although a handful of putative UBE3A substrates have now been identified, most notably the Arc protein which regulates synaptic AMPA receptors (Greer et al., 2010) and ECT2

protein which is a key regulator of the actin cytoskeleton (Reiter et al., 2006), it is still unclear if the key proteins responsible for the AS phenotype are regulated by UBE3A at the protein or transcriptional level, or both.

Cytogenetic abnormalities in the PWS/AS region have been described in 1–3% of autistic individuals. Consistent with a role for *UBE3A* in autism are studies that have demonstrated copy number variants not only at the *UBE3A* locus, but also at other loci regulating the ubiquitin–proteasome pathway in autistic individuals (Glessner et al., 2009). The *UBE3A* gene itself has been proposed as a candidate gene for autism susceptibility, although linkage and association studies have been somewhat inconsistent (Cook et al., 1998; Guffanti et al., 2010; Nurmi et al., 2001).

32.3.2 SnoRNA Cluster

Small nucleolar RNAs (snoRNAs) are noncoding RNAs located in the nucleolus which are involved in rRNA modifications. Pre-rRNA maturation includes endonucleolytic and exonucleolytic cleavages plus modifications such as methylation or pseudouridylation. There are two main classes of snoRNA: the C/D box snoRNAs, which are associated with methylation, and the H/ACA box snoRNAs, which are associated with pseudouridylation (Bachellerie et al., 2002). There is a cluster of C/D box snoRNA genes encoded in the 15q11–q13 region that are processed from introns of the paternally expressed *SNURF-SNRPN* sense *UBE3A* antisense transcript (see Figure 32.1). *HBII52* has 47 copies, *HBII85* has 24 and the others genes (*HBII-36*, *HBII-13*, *HBII-437*, *HBII-238A*, and *HBII-438B*) one copy each. These snoRNAS are highly expressed in the brain (Cavaille et al., 2000). Recent studies demonstrate that chromatin de-condensation of MBII/HBII snoRNAs plays a significant role in the regulation of nucleolar size during neuronal maturation (Leung et al., 2009). Reports on balanced translocations affecting the paternal copy of 15q11–q13 and three microdeletions in the snoRNA region (15q11.2) have now implicated the *HBII-85* snoRNA as the key paternally expressed causative gene(s) for the PWS phenotype (De Smith et al., 2009; Duker et al., 2010; Gallagher et al., 2002; Sahoo et al., 2008).

32.3.3 Gamma-Aminobutyric Acid Genes

The 15q11–q13 region contains a cluster of $GABA_A$ receptor subunits genes: *GABRB3*, *GABRA5*, and *GABRG3*, which encode for the gamma-aminobutyric acid (GABA) receptor subunits β3, α5, and γ3, respectively. During development, $GABA_A$ receptors play a role in proliferation, migration, and differentiation of precursor cells that direct the development of the embryonic brain (Owens and Kriegstein, 2002). GABAergic dysfunction in the brains of autistic individuals has been reported and particular attention has been devoted to the GABRB3 subunit (Pizzarelli and Cherubini, 2011). It has been observed that this gene is normally biallelically expressed in the brain, but in autistic individuals monoallelic expression has been found in combination with a reduction in GABA receptor protein (Hogart et al., 2007). Additional studies indicate that *GABRB3* expression is reduced in the parietal cortex cerebellum of subjects with autism (Fatemi et al., 2009). It has been shown that a rare coding variant of the GABRB3 gene is associated with autism when transmitted maternally (Delahanty et al., 2011). This same variant was identified in two independent families segregating with childhood absence epilepsy (Tanaka et al., 2008).

32.3.4 *CYFIP1* and Other Autism-Related Genes

More severe behavioral problems (autism, ADHD, and obsessive–compulsive disorder) appear in patients with type I deletions (BP1–BP3) than in patients with type II deletions (BP2–BP3); this could be influenced by the genes between BP1 and BP2: *TUBGCP5*, *NIPA1*, *NIPA2*, and *CYFIP1*. The latter three of these are widely expressed in the central nervous system, while TUBGCP5 is expressed in the subthalamic nuclei. Patients with a microdeletion at 15q11.2 between BP1 and BP2, which includes the four genes, present delayed motor and speech development, dysmorphisms, and behavioral problems (Doornbos et al., 2009). It seems that the haploinsufficiency of *NIPA1* does not cause any disease, as PWS/AS patients do not exhibit progressive spastic paraplegia compared to autosomal dominant hereditary spastic paraplegia. In terms of autism, more attention has been focused on *CYFIP1* since Prader–Willi phenotypes have been observed in fragile X syndrome (FXS) patients (see Chapter 33), and it was found that the fragile X associated protein (FMRP) acts in concert with CYFIP1 protein to regulate mRNA translation in neurons (Napoli et al., 2008). In patients with this FXS sub-phenotype that show PWS overlap, C*YFIP1* mRNA levels are generally reduced by two- to fourfold as compared to normal controls or individuals with FXS without PWS features (Nowicki et al., 2007).

32.4 CONTIGUOUS GENE DELETION/DUPLICATION SYNDROMES ON 15q

32.4.1 Deletion Syndromes

32.4.1.1 PWS (OMIM #176270)

PWS is a contiguous gene syndrome caused by the loss of function in those genes situated within the 15q11–q13 region. The syndrome is the direct result of loss of a paternally expressed gene or genes in the 15q

region. The molecular events resulting in PWS include interstitial deletions (70%), uniparental disomy (UPD) (25%), imprinting center defects (<5%), and, on rare occasions, chromosomal translocations (<1%).

The syndrome has a prevalence of 1/15,000–1/30,000, and the main clinical features include an initial failure to thrive, followed later in life by an obsession with food and other behavioral problems, including intellectual disability (for a detailed review of phenotypic features and management, see Cassidy and Driscoll, 2009; Goldstone et al., 2008, respectively). There are also significant phenotypic differences between patients with 15q deletions as opposed to maternal UPD. In UPD patients, the facial features and the hypopigmentation are less frequent than in patients with the deletion; in contrast, sleep disorders and behavioral abnormalities such as psychosis and autism are more common (Cassidy, 1997). Seizures are present in half of the individuals with a deletion and in less than 10% in UPD cases (Varela et al., 2005). Individuals with class I deletions have a more severe phenotype than those with class II deletions, including self-injurious behavior, deficits in adaptive behavior, obsessive–compulsive behavior, and difficulties in visual-motor integration (Butler et al., 2004). Cardiovascular and respiratory disorders related to the obesity that results from obsessive food compulsion are the most frequent causes of death in these patients. In 1993, a group of clinicians established the clinical diagnostic criteria for PWS (Holm et al., 1993). With the availability now of molecular laboratory testing, when there are clinical findings pointing to the disorder the revised criteria recommends to test for PWS at different ages depending on the phenotypes observed (Gunay-Aygun et al., 2001). The most widely used laboratory test is based on the paternal differences in DNA methylation from the 5′ end of the *SNRPN* gene. PWS deletion patients have only a maternal methylated allele. Since the phenotypic outcomes can be somewhat variable depending on the size and character of a genomic defect, it is important to determine the molecular class of the loss of function for genetic counseling purposes. Chromosomal analysis can identify large deletions and translocations, *SNRPN* fluorescence *in situ* hybridization (FISH) probes detect deletions, and DNA polymorphisms from the patient and parents are useful in the diagnosis of UPD cases. MS-HRM has also been shown to be an efficient and cost-effective method for the identification of PWS or AS deletions (Hung et al., 2011; White et al., 2007).

32.4.1.2 *Angelman syndrome (OMIM 105830)*

Angelman syndrome (AS) was first recognized in 1965 by physician Harry Angelman (pronounced as if a "male angel"), who described three unrelated subjects all presenting with a similar curious phenotype, including inappropriate laughter, happy demeanor, and a "puppet-like" dangling of the arms (Angelman, 1965). The estimated prevalence of AS is between 1:1000 and 1:20,000 (Williams, 2005). AS is a neurogenetic disorder characterized by severe intellectual disability, ataxia, seizures, EEG abnormalities, bouts of inappropriate laughter, absent speech, autistic-like behavior, sleep disorder and postnatal microcephaly, macrostomia, maxillary hypoplasia, and prognathia (Williams et al., 2006). AS is difficult to detect in infancy, as hypotonia and developmental delay are fairly nonspecific features that can occur in infancy for a number of reasons. The characteristic phenotypes become clearer after age one. Seizure onset is often between 1 and 3 years. Many seizure types have been reported, but atypical absence and myoclonic seizures are most frequent. The most common EEG finding is the presence of triphasic delta activity with a maximum over the formal regions (Conant et al., 2009). The Angelman phenotype becomes less striking in adulthood, the sleep problems improve, but most patients continue to have seizures (for further review and diagnostic approaches, see Van Buggenhout and Fryns, 2009). It has been proposed that, to rule out AS, *SNRPN* methylation assay tests should be performed on any patient with developmental delay, severe intellectual disability, speech impairment, and happy disposition (Varela et al., 2004).

The molecular causes of AS include interstitial deletions of the maternal chromosome (70%), paternal UPD in 3–5% of the cases, imprinting center mutations in 10%, and maternally inherited *UBE3A* mutations in 5–10%, leading to the absence of the gene product. Of the ~10% of AS patients who do not have chromosome 15q defects, it appears that at least some of these individuals may have mutations in potential UBE3A pathway genes such as *MECP2* and the transcription factor *TCF4* (Takano et al., 2010; Watson et al., 2001). Maternally derived deletions and *UBE3A* mutations result in a more severe phenotype than uniparental disomy or imprinting defects, both of which still retain two intact copies of *UBE3A*. These maternal loss-of-function individuals often have severe microcephaly, greater delay in developmental milestones, more severely impaired communication skills, and more severe seizures, in addition to hypopigmentation in the deletion cases (Lossie et al., 2001). The nonimprinted gene responsible for this pigmentation loss is the *OCA* gene, which also causes type II oculocutaneous albinism and is located in the distal portion of 15q11–q13 within the common class I and class II deletion boundaries (Fridman et al., 2003). Comparing the main deletion classes, there are no major phenotypic differences, except for the absence of speech in class I patients (Varela et al., 2004) and perhaps an increased risk for ASD in class I individuals (Peters et al., 2004).

32.4.2 Duplications Syndromes

As many as 3–5% of individuals with broad ASD have copy number changes at the 15q locus (Hogart et al., 2010). Two types of 15q duplications have been identified that result in an ASD phenotype. These duplications can occur as either simple interstitial duplications containing fewer than 18 genes or more complex extrachromosomal isodicentric duplications containing >60 genes.

32.4.3 Isodicentric Chromosome 15q Duplications

A marker chromosome is a structurally abnormal, unidentified extra piece of chromosomal material. Marker chromosomes usually occur in addition to the normal chromosome complement and are thus also referred to as supernumerary chromosomes. When a marker chromosome occurs in the PWS/AS region, the isodicentric chromosome 15 [idic(15)], it is formed by the inverted duplication of proximal 15q resulting in an additional small di-centromeric chromosome detectable by cytogenetic and FISH analysis (Battaglia, 2005). This [idic(15)] is one of the most common supernumerary marker chromosomes in humans (Webb, 1994). Idic (15) marker chromosomes that do not include the PWS/AS critical region have no obvious clinical effect (Huang et al., 1997), whereas most of those including this region are maternally derived, arise *de novo*, and lead to a neurobehavioral phenotype (Battaglia, 2008). Most idic (15) chromosomes arise through BP3:BP3 or BP4:BP5 recombination events (see Figure 32.1). The clinical features of idic (15) syndrome include moderate to profound developmental delay/intellectual disability, autism or autistic-like behavior, central hypotonia, minor dysmorphisms, and seizures (Battaglia, 2008). Tetrasomy of this region is associated with a more severe phenotype than trisomy, suggesting that there is a dosage effect for a gene or genes duplicated in this region. In particular, there is an apparent correlation between the frequency and severity of seizures in these individuals and the increase in copy number, clearly implicating the duplicated GABA receptor gene cluster as a contributor to the increased seizure risk and the difficult seizure management.

32.4.4 Interstitial 15q11–q13 Duplication

This submicroscopic duplication is almost impossible to detect by traditional karyotype analysis; however, with the widespread use of array CGH in the clinic, it is now becoming a frequent cause of ASD where chromosomal rearrangements are involved. Most int dup (15) patients with an interstitial duplication 15q11–q13 share the common deletion breakpoints of PWS/AS and are the result of the reciprocal NAHR event that forms the common PWS/AS deletions. In most cases, the duplication is *de novo*, but there are a few familial cases, which have been critical to understanding the difference in phenotype associated with maternal versus paternal inheritance of the duplication. There is only one report of an interstitial duplication that expanded during meiosis when transmitted from a carrier mother to her son; otherwise, the duplication is stable (Gurrieri et al., 1999). There is a wide range of severity in the developmental disabilities; however, most individuals with this smaller duplication present with a mild, somewhat sociable form of autism combined with moderate-to-severe anxiety disorder. The severity of the phenotype does not show an obvious correlation between the duplicated region sizes. There has been one report of an adult with a maternally derived int dup (15) who had a severe phenotype characterized by intractable epilepsy (Orrico et al., 2009). However, in the authors' own cohort of 15 interstitial duplication 15q subjects, just two individuals present with severe seizures (manuscript in review).

Like the deletions, there is also a parent-of-origin effect for the duplications containing the PWS/AS critical region. Maternally transmitted 15q duplication is most frequently detected, because it is consistently associated with autistic features with variable degrees of developmental delay. Unfortunately, genotype–phenotype correlations have not consistently demonstrated that these duplications are the sole cause of ASD in these cases. Boyar et al. reviewed the literature and described the phenotype in 31 confirmed maternal cases, with intellectual disability, developmental delay, learning disabilities, language impairment, and autism/autistic-like behavior as the most common finding, followed by hypotonia, repetitive behavior, hyperactivity, poor attention, and clumsiness. Less frequent are seizures, echolalia, aggression, and hypopigmentation (Boyar et al., 2001).

On the other hand, very few cases of paternally transmitted 15q duplication have been reported, and most of these individuals appear neurotypical, as one can see from familial cases in which unaffected mothers have transmitted paternally derived duplication chromosomes to their affected children (Cook et al., 1997; Roberts et al., 2002). However, there are a few paternal cases reported with speech delay and behavior problems. Mohandas et al. reported a patient with nonspecific developmental delay and partial agenesis of rostral corpus callosum; Mao et al. described a patient with global developmental delay, depression, obesity, food-seeking behavior, and self-injurious tendencies; and Roberts et al. presented a series of 16 cases in which only one patient had paternal duplication, and his phenotype included developmental delay and behavioral disorder (Mao et al., 2000; Mohandas et al., 1999; Roberts et al., 2002).

The maternal bias of duplication-associated risk in autism suggests the requirement of a maternally expressed transcript for greatest ASD risk, clearly implicating the maternally expressed *UBE3A* gene in the ASD phenotype in 15q duplication individuals.

32.5 COMPLEX DISEASES

32.5.1 Microdeletion 15q13.3

The use of array CGH in patients with developmental delay, intellectual disability, and/or dysmorphic features has resulted in the recognition of a recurrent 15q13.3 microdeletion (Ben-Shachar et al., 2009; Sharp et al., 2008). The critical deletion spans 1.5 Mb and arises through NAHR between LCR sequences on BP4 and BP5, telomeric to PWS/AS region.

The first description of recurrent 15q13.3 microdeletion was by Sharp et al., who screened for idiopathic intellectual disability using whole-genome array CGH or quantitative polymerase reaction (PCR) and found the microdeletion with a frequency of 1/350. Interestingly, in their series 7 out of 9 had epilepsy and/or abnormal EEG findings (Sharp et al., 2008). Incomplete penetrance (65–70%) has been reported and some relatives of 15q13.3 microdeletion individuals were reported as neurotypical, while others had intellectual disability, epilepsy, and/or neuropsychiatric disorders (Ben-Shachar et al., 2009; Shinawi et al., 2009).

Based on the findings of Sharp et al., a case-control study was conducted in patients with idiopathic generalized epilepsy, and there were 12 cases with the 15q13.3 microdeletion (12/1223) and none in the controls (0/3699). However, in these 12 cases, 9 cases did not have dysmorphic features or intellectual disability. Dibbens et al. proposed the segregation of the microdeletion as a susceptibility variant, because they analyzed families and they found that the microdeletion did not account for all the epilepsy risk in their families (i.e., there were multiple affected individuals without the microdeletion as well as unaffected individuals with the deletion (Dibbens et al., 2009)). Finally, there is a report of a patient carrying a homozygous microdeletion, inherited from both parents, with severe epileptic encephalopathy, retinopathy, autistic features, and choreoathetosis (Masurel-Paulet et al., 2010).

The chromosomal region for the 15q13.3 microdeletion encompasses seven genes: *ARHGAP11B*, *MTMR15*, *MTMR10*, *TRPM1*, *KLF13*, *OTUD7A*, and *CHRNA7* (see Figure 32.1). The PWS-IC seems to regulate *CHRNA7* levels with MECP2 as the link between epigenetic mark and transcriptional regulation (Yasui et al., 2011). The *CHRNA7* gene is highly expressed in the brain and is considered a good candidate gene, at least for the epilepsy phenotypes in 15q13.3 deletion individuals. *CHRNA7* encodes a synaptic ion channel protein that mediates neuronal signal transmission, and mutations in other members of the nicotine receptor subunit gene family cause the autosomal dominant nocturnal frontal lobe epilepsy.

A smaller deletion (~680 kb) that includes the *CHRNA7* gene has been identified with a frequency of 1 in 2960, but the phenotype is quite similar to the larger deletion of 1.5 Mb (Shinawi et al., 2009). In some ethnicities, the BP4–BP5 region can be inverted (Sharp et al., 2008), which predisposes this region to the smaller deletion by NAHR between CHRNA7-LCR copies on the normal and inverted chromosomes (Shinawi et al., 2009).

32.5.2 Schizophrenia and Behavioral Abnormalities

Schizophrenia is a severe mental disorder characterized by hallucinations, delusions, cognitive deficits, and apathy. Like other common diseases, it is a complex genetic disorder with high heritability (Lichtenstein et al., 2009). There is no doubt that genetic factors play a role in the pathogenesis of the disorder, but there is no single major gene that confers the risk for the whole phenotype. It is likely that common alleles of small effect and some rare alleles with relatively large effects are combining to increase susceptibility to the development of schizophrenia (Wang et al., 2005). Nowadays, it is known that there are copy number variations (microdeletions/microduplications) that contribute to the etiology of schizophrenia. Genome-wide scans for structural variants have identified deletions on chromosomes 1q21.1, 3q29, 15q11.2, 15q13.3, and 22q11.2 and duplications on chromosomes 16p11.2 and 16p13.1 that increase the risk of schizophrenia (Consortium, 2008; Ingason et al., 2011; Mccarthy et al., 2009; Mulle et al., 2010; Stefansson et al., 2008). Maternal 15q11–q13 duplication has also been found to be a risk factor for schizophrenia, with an odds ratio of 7.3 (Ingason et al., 2011).

Interestingly, a genome-wide linkage analysis showed that an endophenotype found in schizophrenia, P50 auditory gating deficit, is linked (LOD score = 5.3) to chromosome 15q13–q14, the locus for the *CHRNA7* gene (Freedman et al., 1997). This gene is considered a candidate gene for schizophrenia based on positive association studies (Freedman et al., 2001; Stephens et al., 2009).

32.5.3 Microduplication 15q13.3

Recently, a few individuals with the reciprocal duplication between BP4 and BP5 have been reported. This duplication originates by NAHR between BP4 and BP5 LCRs and presents with no recognizable phenotype as

in the microdeletion cases with developmental delay/intellectual disability and psychiatric disease, but not epilepsy. There is also inter- and intrafamilial variability suggesting incomplete penetrance, since other family members with the duplication appear clinically neurotypical (Van Bon et al., 2009). Interestingly, a smaller duplication (358–680 kb) that includes the *CHRNA7* gene has been reported with the same clinical outcomes as the larger BP4–BP5 duplication. These cases are all inherited and some family members also present with a psychiatric disease that can be due to a very low penetrance or could be explained as a risk factor for alcoholism and psychiatric or behavioral disorders (Szafranski et al., 2010). Thus, the only reports of this smaller duplication differ in the duplication size, but all include the *CHRNA7* gene (Szafranski et al., 2010). Therefore, studies to date show a remarkably variable expressivity for this 1.5-Mb deletion/duplication on chromosome 15q13.3, which includes neurotypical phenotype, intellectual disability, autism, seizures, bipolar disorder, and schizophrenia.

32.6 ANIMAL MODELS OF 15q REGION DISORDERS

Perhaps no other single region of the human genome has been the inspiration for such a variety of transgenic mouse models as the 15q region. One clear advantage of studying the 15q region in mice is that the entire 15q11.2–q13 locus from BP1–BP5 is syntenic to the mouse chromosome 7qC region, making it feasible to study not only single-gene disorders, but also the effects of multiple-gene deletions and duplications in the genomic disorders that occur at 15q in humans.

Several mouse models have now been produced for the single-gene imprinting disorder AS through both knock-out and knock-in strategies (Jiang et al., 1998; Miura et al., 2002). As in the human conditions, these mice only exhibit phenotypes when the *Ube3a* deficiency is passed though the maternal germline. These models have provided a system for the study of ataxia, motor control, and long-term potentiation (LTP) defects observed in AS patients. They have also been used to identify novel phenotypes due to loss of *Ube3a* related to cerebellar controlled lick rhythm (Heck et al., 2008) and experience dependent maturation of cortical neurons of the visual cortex (Kashiro et al., 2009; Yashiro et al., 2009). In addition, the recent construction of a YFP–*Ube3a* fusion mouse has revealed not only that *Ube3a* is maternally expressed in most regions of the brain, but also that the paternally inherited allele is not completely silent, as previously thought (Dindot et al., 2008). In addition, GFAP-positive neuronal precursors in the vermus are not subject to imprinted regulation and show bi-allelic expression of *Ube3a* (Dindot et al., 2008). Mice with a maternal deletion from *Ube3a* to *Gabrb3* had increased seizure activity, altered ultrasonic vocalization, and impairment in learning and memory tasks (Jiang et al., 2010).

Two recently developed mouse models have revealed details about the function of the PWS/AS imprinting center as well as the molecular mechanism of PWS phenotypes. HBII-85 snoRNAs sequences are highly conserved between humans and mice. Mice lacking the MBII-85 snoRNA ortholog show postnatal growth retardation, delayed sexual maturation, motor learning deficit, and hyperphagia, as well as other features seen in PWS patients (Ding et al., 2008). The snoRNAs at this locus and a neuron-specific long antisense UBE3A transcript (Rougeulle et al., 1998) that initiates at the *SNRPN* promoter (see Figure 32.1) are both involved in the complex orchestration of allele-specific repression that results in the *UBE3A* gene being expressed from the maternal allele and the MBII-85 snoRNA from the paternal allele. The development of a mouse with a 35-kb targeted deletion of the PWS-IC has greatly accelerated the understanding of the epigenetic and transcriptional changes that must take place to cause allele-specific expression (Yang et al., 1998). This mouse model of PWS accurately recapitulates neonatal phenotypes including feeding difficulties, failure to thrive, and small size. However, it has proven difficult to utilize this model for behavioral studies in adult mice due to its early lethality in a C57BL/6 genetic background. Chamberlain et al. were able to solve this dilemma by moving the PWS-IC^{del} mutation to a variety of genetic backgrounds until they were able to rescue this lethality, which appears to be unrelated to the mutation at the PWS-IC (Chamberlain et al., 2004). This adult viable PWS-IC^{del} mouse was used to identify cognitive abnormalities as well as to assay imprinted expression for genes in the 15q/7C region, with particular emphasis on *Ube3a*, which is known to be maternally expressed in neurons, as well as for two genes that have been the subject of some debate in the literature, *ATP10A* and the *Gabrb3* cluster, which were both shown to be biallelically expressed in neurons in this mouse model of PWS (Relkovic et al., 2010). In an effort to understand how the snoRNA cluster, and specifically the PWS-associated *HBII-85* gene, is regulated in neurons, Leung et al. looked for changes in chromatin decondensation and nucleolar size in the brains of PWS-IC^{del} animals and compared them to brain tissue from both AS and PWS *SNRPN>UBE3A* deletion individuals (Leung et al., 2009). In both the animal model and the human brain samples, they identified a large region (~888 kb) from the *Snrpn* gene to the *Ube3a* gene (see Figure 32.1) that undergoes dramatic chromatin decondensation in neurons on the paternal allele only, thus providing an open chromatin conformation and presumably

transcriptionally active zone around the paternal *Snrpn* promoter, which transcribes the *HBII-85* snoRNA gene as well as the antisense-*Ube3a* transcript (Leung et al., 2009). Most individuals with PWS are also deleted for additional 15q genes or have maternal uniparental disomy for this region, resulting in overexpression of the maternal *UBE3A* gene. Although loss of paternal *HBII-85* transcription alone may not account for all aspects of the Prader–Willi phenotype, the use of this imprinting center mouse model to reveal the complex mechanisms of imprinted expression and regional epigenetic regulation on 15q is just now becoming evident.

32.6.1 Additional Mouse Models for Genes Deleted or Duplicated in the 15q11–q13 Region

One complication in the phenotypic analysis of AS, PWS, and duplication 15q autism is that most of these individuals are deleted or duplicated for a region encompassing some 18 genes, including several genes that, when deleted in mouse models, cause phenotypes on their own. The *Gabrb3* null mice, for example, display epilepsy, learning and memory deficits, as well as defects in social behavior (Delorey et al., 1998). There is also an interesting relationship between the *p* gene and the *GABRA5* and *GABRB3* subunits genes. Deletion of both *p* alleles causes rearrangements of Gabaα5 and Gabaβ3 receptors, producing a peculiar phenotype characterized by ataxia, jerky gait, and seizures (Nakatsu et al., 1993). Obviously, the duplication of this cluster in humans contributes to the difference in severity of phenotypes observed between interstitial duplication 15q individuals and the more severely affected isodicentric 15q duplication cases, where as many as 6 copies of the *GABRB3* cluster may be present (Hogart et al., 2007).

A mouse model of paternal uniparental disomy showed high incidence of failure to thrive with spontaneous death in the first month, similar to both the *HBII-85*-deficient and PWS-IC^{del} models of PWS. Survivors developed obesity, hyperactive behavior, ataxic gait, and electroencephalograph with high-amplitude delta rhythmic activity (Cattanach et al., 1997).

In an effort to reflect the interstitial duplication 15q syndrome in mice more accurately, Nakatani et al. used chromosomal engineering techniques to duplicate the entire 7qC region syntenic to typical human class II duplication breakpoints in interstitial duplication 15q cases (Nakatani et al., 2009). It was somewhat surprising that the animals with paternally but not maternally inherited duplications of 7qC showed poor social interaction, behavioral inflexibility, abnormal ultrasonic vocalizations, and anxiety. However, the animals with maternal duplication did trend toward significance on several tests, and, more importantly, an accurate test for autism assessment in mice has not yet been developed; so, in the future, these animals may exhibit subtle autistic features, like their human counterparts. That being said, as more interstitial duplication 15q cases are identified using array-CGH methods, there has been an increase in the number of paternal 15q duplication cases, who typically present with both anxiety and sleep problems but generally do not have cognitive defects (L. Reiter and N.C. Schanen, unpublished observations). In the end, this mouse model of int dup(15) may be a better reflection of the actual phenotypes observed in a large cohort of int dup(15) from both maternal and paternal origins.

32.6.2 *Drosophila* Models of 15q Syndromes

A recent avenue of research has been the construction of human disease models in the well-studied and easily manipulated genetic model organism *Drosophila melanogaster* (Doronkin and Reiter, 2008; Pfleger and Reiter, 2008). A model for Angelman syndrome revealed motor defects, LTP deficits, and circadian rhythm abnormalities in flies lacking the *UBE3A* ortholog *Dube3a* (Wu et al., 2008). Flies lacking Dube3a or even overexpressing Dube3a in the nervous system did not show any gross morphological changes in brain structure of neuronal connections as predicted by phenotype-observed mouse models of AS. Additional studies revealed that both overexpression and loss of Dube3a can affect the number and complexity of dendritic arbors in the fly peripheral nervous system (Lu et al., 2009), suggesting that Dube3a may control more subtle aspects of synaptic connectivity and is perhaps not involved in nervous system development, *per se*.

Our group has capitalized on the mis-expression methodologies available in the *Drosophila* system, primarily the GAL4/UAS system, which allows for overexpression of a given construct in a variety of specific tissue types or under the control of temporal regulators such as heatshock induction (Duffy, 2002). Using a proteomics approach, the authors of this study have identified over 80 unique protein or transcription targets of Dube3a (manuscript in revision). The first is a Rho-GEF involved in actin cytoskeletal remodeling, which the authors identified as a protein that decreased in intensity when they overexpressed human UBE3A in fly heads (Reiter et al., 2006). This protein, known as Pebble in flies and ECT2 in mammals, physically interacts with both fly and human UBE3A proteins in 293T cells. In addition, in a mouse *Ube3a* loss-of-function model of AS changes in Ect2 expression is observed in both the hippocampus and cerebellar regions of the brain (Reiter et al., 2006). More recently, the authors identified a protein called Punch in flies and GTP cyclohydrolase I in mammals using

overexpression of fly Dube3a in heads. The regulation of Punch, however, appears to be through the transcription co-activation function of Dube3a, as the ubiquitin ligase function is not required for increased Punch transcript levels, increased dopamine levels, or hyperactivity observed in these flies (Ferdousy et al., 2011).

32.7 CONCLUSIONS

Human chromosome 15q11–q13 is a complex genomic region subject to regulation by parental imprinting. Several LCRs in this region predispose 15q to genomic rearrangements, causing parent-specific dosage changes that lead to a variety of neurodevelopmental disorders, including autism. The widespread use of array-CGH technology to detect CNVs in the 15q region has made it a focal point for the study of PWS/AS autism and even certain forms of schizophrenia. In addition, animal models of 15q disorders run the gamut from flies to mice and include not only single gene knock-outs, but also chromosomally engineered models duplicating or deleting the entire region of the mouse genome syntentic to 15q11.2–q13. With such a wide array of resources available in both human and model organism genetics, it is only a matter of time before someone unlocks the mechanisms of gene regulation, methylation, imprinting, and genomic recombination that cause these neurological disorders with the hope of eventually designing therapeutics for the treatment of these syndromes.

Glossary

Array-CGH: A microarray method commonly used to identify deleted and duplicated segments of the genome.

C57BL/6 C57 black 6: A common inbred strain of laboratory mouse.

Endophenotype: Psychiatric concept that refers to a biomarker (neurophysiologic, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological) that is associated with the phenotype in the populations, is heritable, is illness independent (manifests in individuals whether or not illness is active), co-segregates with illness, and is found in non affected family members at a higher rate than in the general population (Gottesman and Gould, 2003).

Epigenetic: Refers to DNA and chromatin modifications that can affect gene function without change in the genotype.

Epigenotype: DNA-exclusive states of gene expression and epigenetic modification; the maternal state and the paternal state.

HBII: Nomenclature used for human genes of the snoRNA cluster; there are different genes (as HBII-13, HB-36, HBII-85, etc.).

HRM: High-resolution melting curve analysis; a method that can quickly distinguish short DNA fragments which differ by as little as a single nucleotide without the need for DNA sequencing.

GAL4/UAS: Bipartite expression system used in *Drosophila* that was derived from the yeast GAL4 gene promoter.

Imprinting: A difference in gene expression that depends on the parent of origin of the allele.

Imprinting center (IC): A region of the DNA usually marked by differential methylation that regulates the imprinted expression of a gene or genes in the region.

Knock-in mouse: Insertion of a protein-coding cDNA sequence at a particular locus in the mouse within the gene of interest.

Knock-out mouse: One or more genes turned off through a targeted mutation in the mouse.

Ortholog: Genes in different species that are similar in DNA sequence and also encode proteins with the same function.

Penetrance: The proportion of individuals with a genotype known to cause disease and present clinical symptoms.

Segmental duplication: Regions bigger than 1 kb that are not high-copy repeats and have more than 90% identity to another region in the genome.

Syntenic: Gene loci that are in sequence on a chromosome between two species.

Uniparental disomy: Two copies of a specific chromosome, both inherited from one parent.

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