181 Deletion of 7q11.23 Genes and Williams Syndrome

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Williams syndrome [WS; also Williams–Beuren syndrome (WBS)] is a neurodevelopmental condition that is caused by a hemizygous deletion of about 1.5–1.8 megabases, a region of chromosome band 7q11.23 that contains about 28 genes (**Ewart et al., 1993**; **Osborne, 1999**; **Osborne et al., 1999**). Found in 1 in 7500–20,000 live births (**Stromme et al., 2002**), persons with WS have characteristic facial features, congenital and adult cardiovascular disease, and distinctive cognitive and behavioral characteristics. WS is a compelling genetic model for understanding human cognition, chromosome organization, and evolution.

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Features of WS include small stature, a characteristic facial appearance, and cardiovascular abnormalities, most frequently involving supravalvular aortic stenosis (**Morris and Mervis, 2000**). Cognition is impaired with a typical full-scale IQ of 55 (**Bellugi et al., 2000**) and visuospatial functions that are relatively more affected than language skills. WS persons also exhibit hyperacusis (**Klein et al., 1990**), a drive to affiliative behavior (**Mervis et al., 2000; Jones et al., 2001**), increased nonsocial anxiety, and an emotional response to music (**Levitin et al., 2003**). The neurobiological basis of WS has been studied with in vivo structural and functional imaging, showing a distinctive neuroanatomical profile including smaller brain volumes, numerous localized differences in gray and white matter distribution, as well as variations in functional responses in visual–spatial, attentional, and emotional paradigms (**Galaburda et al., 2002**; **Reiss et al., 2004**; **Holinger et al., 2005**; **Thompson et al., 2005**; **Meyer-Lindenberg et al., 2006**). Genetic studies of the WS region indicate that the genomic region typically deleted is flanked by a mosaic of duplications that predispose to the deletion events and include families of repeated sequences, genes, and pseudogenes (**Korenberg et al., 2000**). However, individuals with atypical deletions have been reported, and they appear to exhibit a subset of cognitive and physical features. The findings in these individuals support the contribution of the *ELN* (*elastin*) gene encoding elastin to WS congenital heart disease and the contribution of the genes GTF2IRD1 and GTF2I to deficits in visuospatial function (**Korenberg et al., 1996, 1998**; **Tassabehji et al., 1999**; **Hirota et al., 2003**). These studies provide evidence for WS as a uniquely human model in which to study the genetic and developmental mechanisms that shape brain structure and adult cognition.

CLINICAL FINDINGS

Knowledge of the clinical aspects and molecular underpinnings of WS has grown dramatically in the 40 years since Dr JCP Williams posited that the combination of supravalvar aortic stenosis along with the "physical and mental characteristics here described may constitute a previously unrecognized syndrome" (**Williams et al., 1961**). In the following year, Dr Alois Beuren expanded the clinical spectrum of the disorder by reporting a dozen additional patients (**Beuren et al., 1962**). Therefore, the condition is referred to as WS or WBS (OMIM 194050). WS is now known to be a complex multisystem disorder usually caused by a microdeletion within chromosome band 7q11.23.

Findings in individuals with WS can be categorized into physical features, medical problems, growth and development problems, and cognitive/behavioral problems. Within each category, individual features have characteristic developmental trajectories. The expanding knowledge of WS features, combined with the development of human genetic tools, provides insight into the genes responsible for these features in the normal population.

Cardinal Features

Physical features of WS. The cardinal physical feature present in all persons with WS is their subtle but distinctive craniofacial dysmorphology. Infants with WS have periorbital puffiness, a small upturned nose, long philtrum, and often a small or recessed chin (Fig. 181–1). Facial features change during childhood with elongation of the face, more prominence to the nasal bridge, and development of full lips with macrostomia. Blue-eyed patients with WS have a lacy or stellate pattern in their irides, which generally cannot be seen in brown-eyed persons with WS. Most patients with WS are small at birth and continue to grow at approximately the third–tenths centile during childhood. WS-specific growth curves have been published.

Medical Problems of WS

The medical problems that can accompany WS are numerous, and only a subset will be described.

1. *Cardiovascular*: Approximately 50%–75% of patients with WS develop cardiovascular disease during their lifetime (Morris et al., 1988; Eronen et al., 2002). Abnormalities involve local or diffuse stenosis of any medium- or large-sized artery, most commonly in the ascending aorta above the aortic valve (the so-called supravalvar aortic stenosis) or in the pulmonary arterial tree. However, stenoses of the descending aorta, renal arteries, and intracranial arteries have been reported (Radford and Pohlner, 2000; Rose et al., 2001). The only risk factor identified to date is that males are more likely to have severe cardiovascular disease than females (Sadler et al., 2001). Intracardiac abnormalities are less common, although mitral valve prolapse, atrial septal defects, and ventriculoseptal defects have been reported (Morris et al., 1990; Kececioglu et al., 1993). Hypertension develops in approximately 50% of patients with WS, and the origin is unknown, although it is occasionally associated with renal artery stenosis (Morris et al., 1988; Deal et al., 1992; Broder et al., 1999). Neurovascular abnormalities are reported and may result in stroke (Ardinger et al., 1994; Soper et al., 1995; Cherniske et al., 2004). Sudden death is 25–100X increased in WS vs. the normal population (Wessel et al., 2004) and has been associated with stenosis of the coronary artery or its ostium (Bird et al., 1996). Point mutations or small intragenic deletions of ELN have been found in the autosomal-dominant disorder, familial supravalvar aortic stenosis (SVAS) (OMIM 185500). Deletion of the *ELN* gene appears to be associated with similar structural cardiovascular abnormalities in WS.
2. *Gastrointestinal*: Colic and a variety of feeding problems and gastrointestinal motility problems, including reflux and chronic constipation, are common (Morris et al., 1988). Recurring abdominal pain owing to diverticulitis and possibly abdominal arteriopathy can occur in adolescence and adulthood (Deshpande et al., 2005; Partsch et al., 2005).
3. *Endocrine*: The best studied abnormality is hypercalcemia, but both the etiology and the true prevalence are unknown (Jones, 1990; Kruse et al., 1992). Response to dietary restriction of calcium and administration of bisphosphonates (Cagle et al., 2004; Oliveri et al., 2004) may provide insight into the pathogenesis of hypercalcemia,

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Figure 181–1. Photographs of children with Williams syndrome (WS). (Used with permission.)

which is documented most often during infancy and young childhood. Thought not as well studied, abnormal glucose tolerance or overt diabetes mellitus is far more prevalent, detected in the majority of patients over 20 years of age by a standard 2-hour oral glucose tolerance test (Cherniske et al., 2004). The basis is unknown and may involve impaired insulin secretion, insulin resistance, or obesity. Subclinical hypothyroidism is common with an elevated thyroid-stimulating hormone (TSH) in the face of a normal thyroxine level (Cammareri et al., 1999; Bini and Pela, 2004; Cherniske et al., 2004; Stagi et al., 2005). Hypothalamic or pituitary dysfunction, as well as thyroid or other end-organ hypoplasia, detected in some patients with WS, may contribute to the pathogenesis.

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4. *Renal and Genitourinary*: Structural renal anomalies are found in 15%–20% of patients with WS (Pober et al., 1993; Pankau et al., 1996). Functional abnormalities are very common, including delayed toilet training, enuresis, and urinary frequency. Bladder diverticuli may be prevalent with advancing age, although there is no systematic study. Most preadolescents follow the normal sequence of pubertal development but have earlier pubertal onset, although not true precocious puberty, compared to population controls (Cherniske et al., 1999; Partsch et al., 1999). Females with WS have normal menstrual cycles, although information is inadequate on the timing of menopause. Both men and women with WS seem to be fertile, as indicated by reports of procreation (Mulik et al., 2004).
5. *Musculoskeletal*: Scoliosis (Osebold and King, 1994) as well as progressive joint contractures with gradual tightening of the heel cords and hamstrings can occur and result in a stiff and awkward gait, kyphosis, and lordosis by adolescence (Morris et al., 1988; Kaplan et al., 1989). Fine and gross motor function is impaired, leading to difficulty with tool use and handwriting at all ages.
6. *Dental:* Primary dentition in WS includes small and "peg"-shaped teeth and increased interdental spacing, congenital absence of one or more primary or secondary teeth, and anterior cross-bite malocclusion (Hertzberg et al., 1994; Axelsson et al., 2003). Secondary dentition shows many of the same abnormalities, although less severe (Axelsson et al., 2003).
7. *Ophthalmologic*: Strabismus, most often esotropia (Greenberg and Lewis, 1988), cataracts (Cherniske et al., 2004), and visual acuity problems such as hyperopia are reported in up to 50% of individuals with WS (Kapp et al., 1995; Roy, 1995).
8. *Ear, Nose, and Throat*: Recurrent middle ear infections and/or effusions complicate early childhood, and mild-to-moderate high-frequency sensorineural hearing loss occurs in most adolescents and adults with WS (Cherniske et al., 2004; Marler et al., 2005). Increased sensitivity to sound (hyperacusis) is common (90%), and individuals with WS report discomfort at 20 decibels (db) lower than controls (Gothelf et al., 2006). Many report specific phobias for certain sounds (Levitin, 2005). Most individuals have a hoarse or low-pitched voice; vocal cord abnormalities secondary to elastin deficiency may contribute (Vaux et al., 2003), although the voice is not hoarse in those with mutations of ELN in familial SVAS.
9. *Neurological*: Generalized hypotonia is found in infants with WS, although tone increases with age, with progression to spasticity (Chapman et al., 1996; Carrasco et al., 2005). Seizures are uncommon in WS. It is of interest that brain structure and function is abnormal in WS (see below) but that magnetic resonance imaging (MRI) scans of the brain are usually read as normal, without evidence of structural anomalies. Chiari malformation Type I is detected in up to 10% of scans (Pober and Filiano, 1995; Mercuri et al., 1997). Cerebellar signs in adults include ataxia and tremor (Pober and Szekely, 1999), and mild dystonias and apraxias are common.

Growth

Individuals with WS are short for their family background. Specific growth curves for WS are available (**Morris et al., 1988**). Failure to thrive is observed in 70% of infants, and the mean adult height is often below the third centile, with females being more affected than males. The growth pattern is characterized by prenatal growth deficiency, poor weight gain and poor linear growth in the first four years, and a brief pubertal growth spurt.

Life-Cycle Issues

WS is not a static condition. New medical problems arise, including progression of vascular stenosis, hypertension, or joint contractures. Medical monitoring throughout life is recommended, and medical monitoring guidelines for both children and adults with WS have been published. Mild accelerated physical and cognitive aging has been noted. Additional longitudinal studies will be required to resolve this and to identify potential therapeutic interventions.

Behavior

Some of the best studied features in individuals with WS pertain to their remarkable constellation of cognitive and personality traits. Early language acquisition is delayed, and although mild-to-moderate language impairments persist throughout life, the quality and affect of speech are relatively normal. Profound impairments in visual–spatial skills impact daily life, as seen in difficulty with handwriting and drawing, and these deficits can be readily elicited by standardized IQ testing. Patients with WS are very social and friendly, but the majority suffers from nonsocial anxiety, most acute of which is anticipatory anxiety for upcoming events be they positive or negative (i.e., comparable levels anxiety in anticipation of Christmas or in anticipation of a visit to the doctor's office, respectively). Although anxiety is the most prevalent behavioral abnormality, phobias, panic attacks, and depression also occur. Interest and enthusiasm for music are almost universal in WS, although the ability to perform music professionally is limited to a few exceptional individuals. **Levitin (2005)** has found increased activation in response to both music and noise in the right amygdala of persons with WS, suggesting a possible neuroanatomic correlation with musical interest in WS.

Clinical Management

Treatment includes early intervention programs, special education programs, and vocational training to address developmental disabilities, including speech/language, physical, occupational, and sensory integration therapies. Children and adolescents should have access to computers and be introduced to key-boarding skills in order to minimize lengthy handwritten tasks. Psychologic and psychiatric evaluation should guide therapy for the individual. Behavioral counseling and psychotropic medication are often used to manage behavior problems, especially attention-deficit disorder and anxiety. Surgery may be required for supravalvular aortic stenosis, mitral valve insufficiency, or renal artery stenosis. Referral to an endocrinologist is appropriate for management of persistent hypercalcemia and/or hypercalcuria. Children with WS should not be given multivitamins because all pediatric multivitamin preparations contain vitamin D. Treatment of hypercalcemia may include diet modification, oral corticosteroids, and/or intravenous pamidronate for refractory cases. Multisystem surveillance should be performed yearly, and specified annual monitoring guidelines have been published (**Cherniske et al., 2004**), and AAP Guidelines,

2001. Additional periodic evaluations are suggested in these guidelines and include serum concentration of calcium, thyroid function, hearing, and renal and bladder ultrasound examination; glucose tolerance test; cardiac evaluation; and ophthalmologic evaluation. In summary, WS is associated with specific phenotypic features that are uncommon in the normal population; this provides a unique opportunity to understand the genetic contributions to each of these features.

COGNITIVE-BEHAVIORAL PROFILE AND NEUROBIOLOGY

Cognition

A hallmark of WS is the dissociation between a relative strength in language and a profound impairment in spatial cognition. Nonetheless, there are consistent cognitive deficits in WS; generally, standard IQ scores range from 40 to 90, with means being around 60 (**Bellugi et al., 2000**; **Searcy et al., 2004**). Aspects of general problem solving are often impaired, and many individuals are not able to live independently or to balance a checkbook (**Udwin and Yule, 1990**). Complex expressive language abilities and auditory processing are relatively strong, despite decreased hearing and hyperacusis. Spatial cognition is disproportionately impaired, particularly at the level of global organization, and is characterized by a fractionated attention to detail, although face processing abilities are a relative strength. From studies across different populations, a characteristic WS cognitive profile is emerging (**Bellugi et al., 2000**; **Jones et al., 2000**; **Mervis and Klein-Tasman, 2000**; **Mervis et al., 2000**).

Language Processing

One striking aspect of the WS profile is the remarkably strong language abilities in adolescence and adulthood, in contrast to, overall, the impairment seen in cognitive abilities. Although in the earliest stages of language development children with WS show significant delay, once language is acquired, this ability tends to become a relative strength in the cognitive profile (**Singer and et al., 1997**). Adolescents with WS score significantly higher on measures of receptive vocabulary than measures of general cognitive functioning. On a word fluency test, in which participants are required to "name all the animals you can in one minute," scores of adolescents and adults with WS are similar to standardized norms for their chronological age and show better performance on a wide variety of grammar probes reversible passives, negation, tag questions, conditionals, sentence repetition, sentence completion, sentence correction, etc. (**Reilly et al., 1990**; **Bellugi et al., 1994**; **Rossen and al, 1996**).

Referential pointing emerges after the onset of expressive language (Mervis and Bertrand, 1992), and exhaustive categorization abilities are delayed well beyond the vocabulary spurt (Mervis and Bertrand, 1997). Thus, children with WS, unlike typically developing children and children with other neurodevelopmental disabilities that result in delayed but sequentially typical developmental milestones, follow an alternate developmental path in acquiring language skills. Short-term memory for speech sounds or "phonological working memory," a form of memory relevant to language learning and comprehension, is relatively preserved (**Wang and Bellugi, 1994**; Jarrold et al., 1999; Mervis et al., 1999, 2004; Vicari et al., 2004).

Structural Language Abilities Versus Language Use: Contrasting sharply with their deficits in structural language, a unique facet of the language abilities of individuals with WS is their ability to use heightened linguistic skills to socially engage others. Many individuals with WS display a strong impulse toward social contact and affective expression. The intersection of language and affect in individuals with WS has been investigated through a series of narrative tasks in which participants are asked to tell a story from a wordless picture book (**Reilly et al., 1990**, 2004; Losh et al., 2000). Findings have consistently shown that individuals with WS of all ages employ significantly more social-evaluative devices to capture the listener's attention than do comparison participants, whereas their mastery of morphosyntax lags behind typical development until approximately the age of 15 years. Interestingly, this effect has also been observed across different languages and cultures (Reilly et al., 2005a, 2005b).

The most telling distinction between individuals with WS and typical individuals at any age or other contrast groups (e.g., DS, language impairment, and early focal brain lesions; Reilly et al., 2004) is their increased linguistic affect. In summary, in adolescents and adults with WS, expressive language is typically a strength and is used effectively (and sometimes effusively) in social situations. In fact, perhaps the prime characteristic of this syndrome is a strong impulse toward social contact and affective expression.

Visuospatial Processing

Spatial Cognition: In studies examining spatial cognition, subjects with WS are impaired across all age ranges examined (**Bellugi et al., 1994**; **Harrison et al., 1995**; **Reilly et al., 1995**), performing more poorly than even children with frank right hemisphere lesions (**Courchesne et al., 1995**). Difficulties seem to be especially acute with respect to the global rather than local level and are most apparent in tasks involving visual–spatial construction, such as copying (e.g., the Developmental Test of visual-motor integration (VMI; Beery and Buktenica, 1967), "block construction" (e.g., Pattern Construction subtest of the Differential Abilities Scale, DAS; Elliot, 1990), and mental rotation (**Bellugi et al., 1994**; Farran et al., 2001, 2003). Lack of cohesion, or gestalt organization, are typical in drawings by individuals with WS, (Karmiloff-Smith et al., 1995; Reilly et al., 1998). For example, a person with WS might draw an elephant with its tail or trunk as separate entities from the elephant itself, lacking global organization (Fig. 181–2). On block design tasks, individuals with WS are typically unable to organize blocks into a global pattern (Bellugi et al., 1996), have difficulties integrating simple shapes, and fail to reproduce the global form (Karmiloff-Smith et al., 1995; **Bellugi et al.,** 1996, **2000**). Face processing appears to be an area of relative strength in WS, which is in stark contrast to other visual–spatial construction abilities (**Bellugi et al., 2000**).

Cognitive Development

Trajectories in Cognitive Domains: Questions about the relationships between the cognitive deficits and areas of relative sparing emerge from the findings on WS. Do the various cognitive abilities depend on one another or can they be dissociated? Do they change throughout development? What are the underlying brain systems for these peaks and valleys of abilities in cognitive domains? In WS, there are clearly different trajectories across the three domains reported (lexical knowledge, spatial cognition, and face processing). In a lexical knowledge task, children with WS begin very low, but show a sharp increase with age. On a standard drawing task, individuals with WS are consistently below the levels found for Down syndrome (Reiss et al.) at all age levels and plateau early in development. On a facial processing

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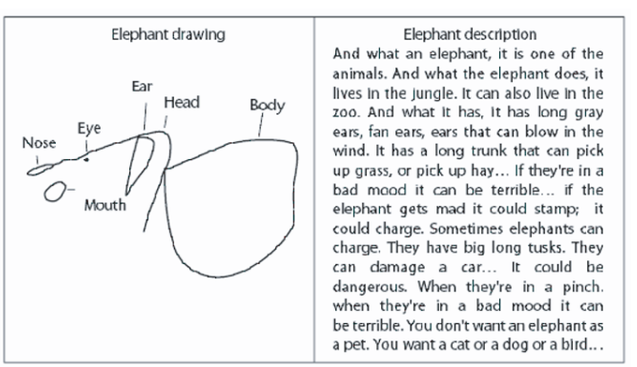

Figure 181–2. Contrasts of drawing and description of an elephant by a teenager with Williams syndrome (WS). The dissociation between language and spatial cognition in WS is evident (full-scale IQ of 49, verbal IQ of 52, and performance IQ of 54).

task, the individuals with WS tend to perform well even at a relatively early age and continue doing well throughout development (**Harrison et al., 1995**; Fig. 181–3).

Social Behavior

Persons with WS have been frequently described as being overly friendly, hypersociable, and unusually attracted toward strangers (e.g., Gosch and Pankau, 1994, 1997; **Jones et al., 2000;** Doyle et al., 2004). In addition to the unusually heightened sociability, individuals with WS also appear highly empathetic and have been shown to exhibit enhanced emotional empathy compared to individuals with other developmental disabilities (Tager-Flusberg and Sullivan, 2000). Clear differences in temperament and affiliative drive are seen from early childhood throughout development (**Jones et al., 2000**; Mervis et al., 2003). At the same time, individuals with WS show substantial problems in social adjustment, including difficulties in forming and sustaining relationships with peers (Gosch and Pankau, 1994, 1997), and have been suggested to lack social judgment (Einfeld et al., 1997; Gosch and Pankau, 1997). An abnormally positive assessment of unfamiliar faces closely reflects real-life social behaviors in WS (**Jones et al., 2000;** Doyle et al., 2004). Analyses indicate that poor ability to recognize facial affect may be related to willingness to approach strangers in individuals with WS (Järvinen-Pasley et al., 2006). This suggests a dissociation between social perception and social expression. Although persons with WS are socially fearless, they nevertheless show significant anxiety (Pober and Dykens, 1996) that has, however, been suggested to be "nonsocial" in nature and in particular relate to new situations (Dykens, 2003; Layfer et al., 2006).

In sum, a large body of evidence shows that some key measures do converge on uncovering distinctive aspects of the WS social-affective phenotype that appear to be present already in infancy. The distinctiveness of the social behavior in WS appears to be specifically linked to their interactions with and approachability toward unfamiliar people (e.g., **Jones et al., 2000**; Doyle et al., 2004). This may later manifest as compromised understanding of others' mental states and deficits in social-perceptual abilities (Gagliardi et al., 1003; Karmiloff-Smith et al., 1995; Tager-Flusberg et al., 1998; Tager-Flusberg and Sullivan, 2000; Plesa-Skwerer et al., 2005, 2006).

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Neurobiology

The neurobiological profile of individuals with WS is being revealed through studies of brain structure with 3D computer-graphic analyses of MRI and brain cytoarchitectonics in autopsy brains. Functional studies with event-related potentials (ERPs) and functional MRI (fMRI) have documented abnormal activations in restricted regions of the parietal, temporal, and frontal cortices when tested by verbal, visuospatial, face processing, emotion, and attention-related tasks (**Meyer-Lindenberg et al., 2004, 2005a, 2005b**; **Mobbs et al., 2004**).

Neuroanatomy

Morphometric studies reveal a smaller overall brain volume (by 11%–13%) in WS compared to control groups plus numerous localized differences in gray matter distribution (particularly in the left hemisphere) and in the size of particular subregions of the cerebral cortex; particularly reductions of the parietal and occipital gray matter volume and increases in the gray matter density of the orbitofrontal, anterior cingulate, insular, and superior temporal gyrus (**Reiss et al., 2004**) preserved cerebellar cortex and subcortical structures (**Wang et al., 1992**; **Jones et al., 2002**). The reduction in subcortical white matter (18%) markedly exceeds that of gray matter (**Thompson et al., 2005**), which may be related to the increased gyrification (**Schmitt et al., 2002**). In addition, an increased average cortical thickness has been reported for a broad region of the occipital and temporal cortex almost exclusively in the right hemisphere, along with bilateral differences in "fractal complexity" of cortical folding (**Thompson et al., 2005**). Altered cell size and density in primary visual and primary auditory cortices have also been described (**Galaburda et al., 2002**).

Brain Function

fMRI: Using fMRI neuroimaging, **Meyer-Lindenberg et al. (2004, 2005b) and Eckert et al. (2005)** identified hypoactivation in WS in the parietal region associated with the dorsal visual stream and structural abnormality in the immediately adjacent region of the parieto-occipital/intraparietal sulcus, providing evidence linking these in WS. Further, **Mobbs et al. (2004)** suggested a strong trend toward being less accurate in determining the direction of gaze with activation in the right fusiform gyrus (FuG). Overall, neural imaging results from multiple groups point to abnormal structure and function in regions of the posterior parietal–occipital cortices that are implicated by WS performance in a subset of visual–spatial construction deficits.

ERPs: ERPs have also been useful in assessing the timing and organization of the neural systems that are active during cognitive and linguistic processing in WS (**Wang et al., 1992**; Neville et al., 1994; **Hickok et al., 1995**; Mills et al., 1997, 1998;). Electrodes are placed on the scalp over specific brain areas while subjects are processing information, which then reflect the time course of neural activation on a millisecond to millisecond basis. Studies of brain-wave activity during face-processing and language paradigms have shown distinct ERP patterns that differentiate WS from normal controls (Mills et al., 2000). It is important to point out that face recognition in WS is normal

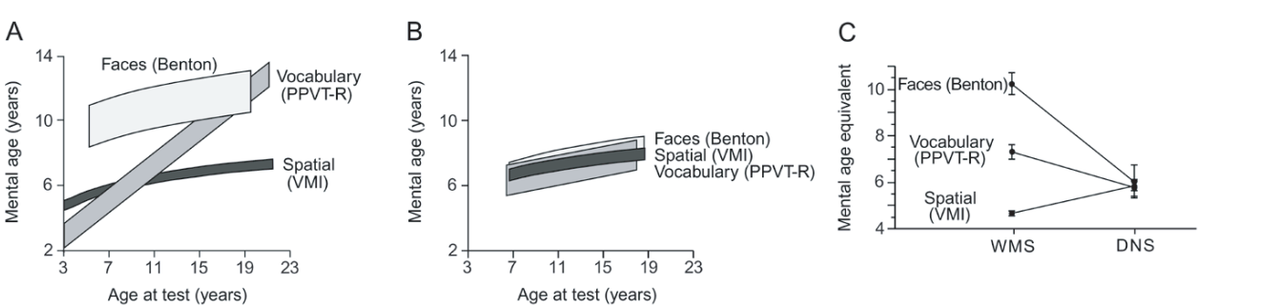

Figure 181–3. Distinct trajectories in cognitive domains in Williams syndrome (WS) but not in DS. Developmental trajectories of contrasts between language, face, and space processing in WS are shown. *(A)* Individuals of all ages with WS show distinctly different trajectories in three domains: lexical knowledge, spatial cognition, and face processing. On a standardized test of vocabulary, individuals with WS start with low scores and then show a sharp increase with age. On a spatial task that involves copying geometric shapes, the performances of participants with WS are consistently below those with DS and plateau at an early age. On a task of face processing, individuals with WS perform extremely well even at very young ages. *(B)* Individuals with DS showed essentially the same developmental trajectory across the three domains. In contrast, individuals with WS show three distinctly different trajectories. *(C)* Planned contrasts show that performance on the three tests differs significantly within the WS group, even when controlled for age. No between test differences are found in the DS group. (Reproduced from Jones et al., 1998.)

in most paradigms and that ERPs provide a powerful measure for showing differences in brain function in WS during facial recognition tasks. Thus, regardless of whether the neural circuitry is understood, the abnormal ERPs for facial recognition in WS illustrate how specific phenotypic tasks may be used to link human genetic variation with cognition or behavior.

With respect to social behavior in WS, volumetric and functional neural imaging has shown variations in orbitofrontal, dorsal cingulate, and dorsolateral prefrontal cortices. Meyer-Lindenberg and coworkers (**Reiss et al., 2004**; **Meyer-Lindenberg et al., 2005a**; **Mobbs et al., 2006**) have found reduced amygdala activation in individuals with WS for threatening faces but increased activation for threatening scenes, relative to matched normal controls. Taken together, these studies suggest neuroanatomic and functional variations that may contribute to the social behavior in WS. Of importance for the current review is that these studies may now be conducted in WS subjects with smaller deletions and may provide the basis for elucidating the neurogenetic developmental and functional origins of neural circuitry regulating human social behavior.

MOLECULAR GENETICS

What are the genetic and developmental origins of WS features? To address this, one must consider the evolutionary structure and expression of genes located in the WS region. Next, one must consider the mechanisms that may affect gene expression and therefore contribute to phenotypic variation in WS. We will briefly consider these and then address evidence for the contribution of single genes or clusters to WS physical and cognitive features from human and animal models.

Genomic Architecture of the WS Region on Chromosome Band 7q11.2

WS is associated with the deletion of an approximately 1.6 Mb region of chromosome band 7q11.23 that results in the loss of one copy of about 28 genes in most cases. A map of the WS region and the genes involved is shown below (Fig. 181–4), reported in 2003 (Hillier et al., 2003; Scherer et al., 2003), and available through genome sequence websites (http://genome.ucsc.edu and http://www.ensembl.org). The region commonly deleted in WS consists of a largely single copy stretch of DNA that is embedded in and directly flanked by a complex mosaic of repeated sequences, including transcribed genes and pseudogenes (**Korenberg et al., 1997**; **Perez Jurado, 1997**; **Osborne, 1999**). The deletion breakpoints fall within these duplications, and a subset of genomic variants appear to predispose to further meiotic instabilities or mispairing (**Robinson et al., 1996**). Therefore, it is not unexpected that most deletion breakpoints occur in common regions and most but not all individuals with WS have the same genes deleted (**Robinson et al., 1996**; **Nakayama et al., 1998**; **Wu et al., 1998**). This region of chromosome 7 is unstable during primate evolution and contains variable duplications and rearrangements in the normal human population. Some genomic variants may predispose to rearrangements, and inversions are more common in parents of WS subjects (Hobart et al., 2004). Duplications of the WS region have also been reported (**Somerville et al., 2005**). The diagnosis of WS is generally based on clinical criteria followed by molecular confirmation of a genomic deletion that includes ELN. This analysis usually employs fluorescence in situ hybridization (Korenberg, 2001; JCNS) and, more recently, genomic arrays. Insight into the cryptic duplications surrounding the WS region and their likely role in deletions arose from early studies that encountered duplicated regions during the establishment of large-fragment physical maps of the human genome (**Korenberg et al., 1998**). It is now appreciated that the WS region exemplifies a common architecture of genomic duplications that are associated with human genomic instability and increased risk of germline and somatic disease. It is an interesting question as to whether such regions may serve as sources of genetic variability that could contribute to human phenotypic variation or act as substrates for evolutionary selection. Understanding WS may provide clues to linking mechanisms of human evolution with human development and adult function.

Genes Deleted in the WS Region

The region commonly deleted in WS contains about 25 genes including the FK506-binding protein 6 (FKBP6), human homolog of the *Drosophila* gene, frizzled (FZD9), bromodomain adjacent to Zinc finger domain 1B (BAZ1B), B-cell lymphoma (BCL7B), Transducin betalike 2 (TBL2), WS basic helix–loop–helix (WS-bHLH), vacuolar protein sorting 37 homolog D (VPS37D), WBS chromosome region 18 protein (WBSCR18), WBS chromosome region 22 protein (WBSCR22), abhydrolase domain containing 11 (ABHD11), WBS chromosome region 27 protein (WBSCR27), WBS chromosome region 18 protein (WBSCR28), syntaxin1A (STX1A), Claudin3 (CLDN3), Claudin4 (CLDN4), ELN, LIM-kinase1(LIMK1), eukaryotic initiation factor4H (EIF4H), heat-shock protein C046 (HSPC046), replication factor C, subunit2 (RFC2), cytoplasmic linker protein (CYLN2), GTF2I repeat domain containing protein1(GTF2IRD1), WBS chromosome region 22 protein (WBSCR23), general transcription factorII-I (GTF2I), and neutrophil cytosolic factor 1 (NCF1). The flanking repeated regions contain variable numbers of pseudogenes or fragments for FKBP6, GTF2I, NCF1, and GTF2IRD2.

Evolution of the WS Region: A Model for Horizontal Variation and Potential Hotspots of Parent–Offspring Variation

Previously, we characterized the phylogenetic instability that gave rise to a novel gene in the WS region (Korenberg et al., 2001). The putative mechanism of this rearrangement was found to involve closely spaced alu family retrotransposon sequences that were capable of forming stem-loop structures that were then sensitive to exonucleolytic attack

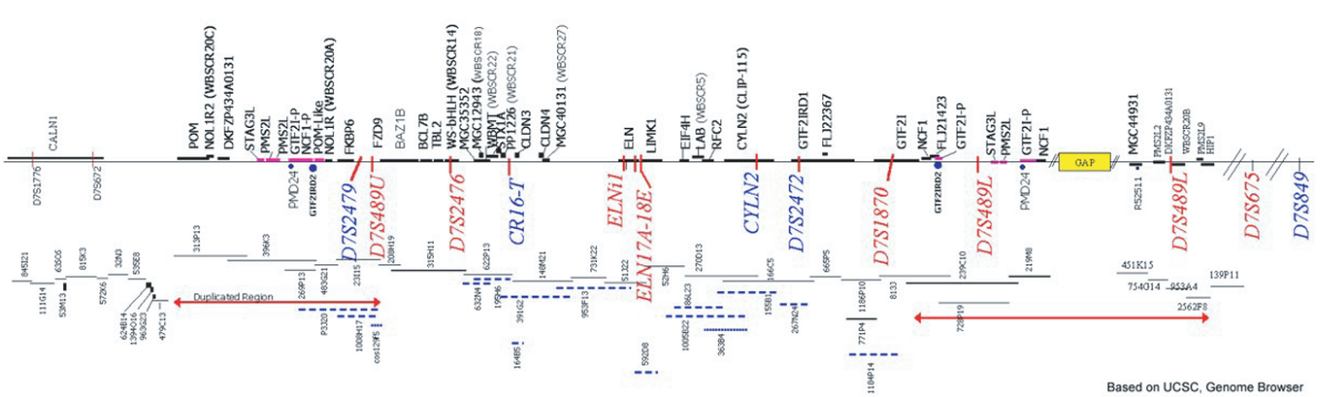

Figure 181–4. Physical map of Williams syndrome (WS) region of the genome.

(Korenberg, unpublished). This is diagramed at the left in Figure 181–8 below and we propose that similar target sites may also give rise to continuing genetic variation within the region including hot spots that may vary between parent and offspring. A similar mechanism may also lead to the instability and homogenization of DNA sequences located at the telomeric border of the deleted region. Figure 181–5 (**Antonell et al., 2005**) illustrates the relationship of repeated segments flanking and predisposing to WS in humans. This genomic structure may present challenges in generating genotype–phenotype maps of WS. The mechanism shown may also be at the heart of the genomic instability through primate evolution and within the human population, the consequence of which is a risk of WS.

This stem-loop model provides a way in which to understand and study how evolution of higher-order human behaviors, including sociability, may depend on genomic instability that is compatible with human high fitness levels and likely present as variation in the contemporary human population.

GENETIC BASIS OF WS FEATURES

WS provides the opportunity to link changes in single genes or clusters with the pathways underlying human development. In WS, we begin by asking how changes in gene copy number alter development and adult function. For WS, the success of this approach is ultimately dependent on two considerations; precise definition of genetic variation combined with detailed definition of physical, cognitive, and neuroanatomic phenotype. It is clear that WS features are ultimately due to the deletion of a common set of genes located in the 7q11.23 region, as well as to the effects of that deletion on the expression of genes in the region flanking the deletion.

Therefore, in order to identify genes that contribute to WS features, we and others have identified rare individuals with smaller deletions who show only parts of the features. In this case, we expect that their deleted genes contribute to their subset of WS features and that their nondeleted WS genes may shift some of their features more toward the normal. The significance of gene–phenotype associations inferred from individuals with small deletions must be understood in the context of the appropriate comparison groups for all physical or cognitive features.

There is a broad spectrum of mechanisms through which genetic variability can contribute to variation in phenotype. These include gender, the parental origin of the deletion, effects of the deletion rearrangement on neighboring transcriptional potential, the expression or sequence of genes and control regions on the nondeleted chromosome

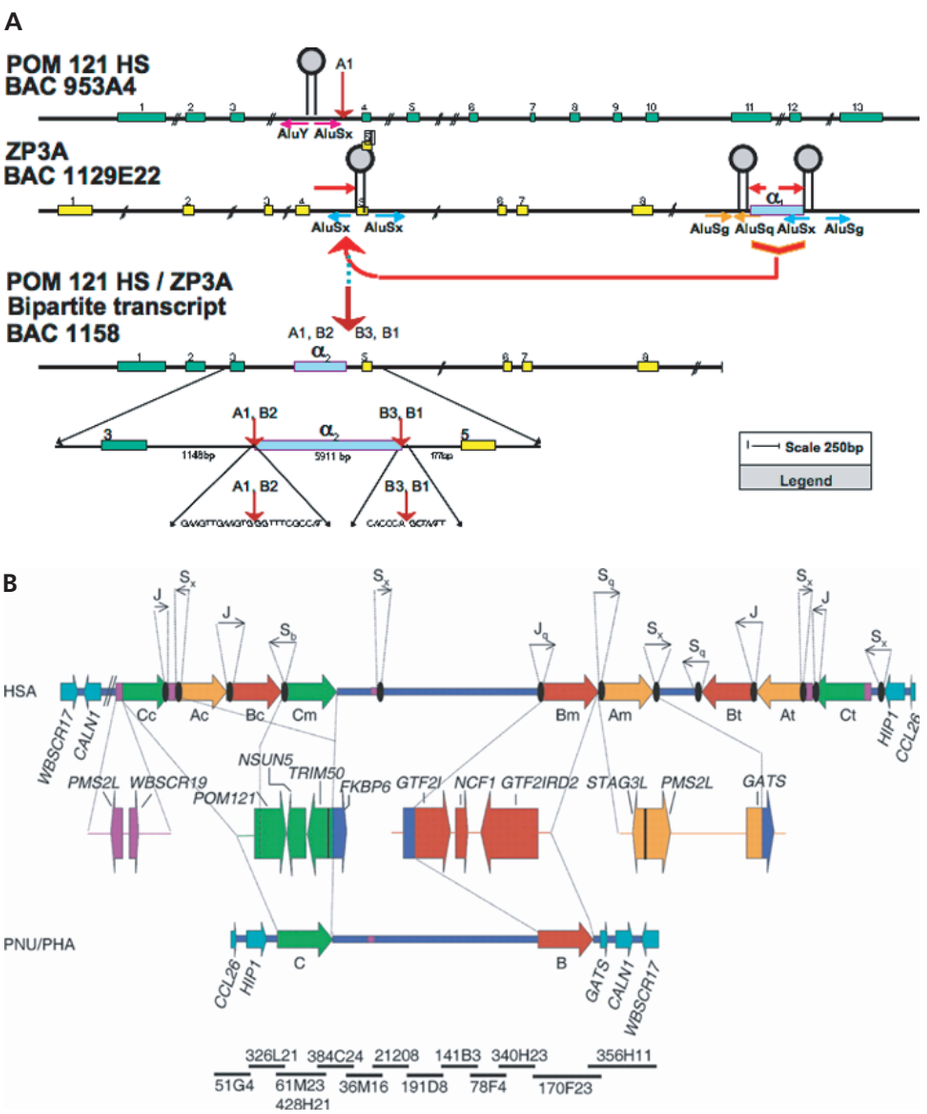

Figure 181–5. *(A)* The birth of a gene: the mechanism. *(B)* Duplicated region 7q11.23; unstable and predisposing to Williams syndrome (WS) (**Antonell et al., 2005**).

7, variation in the remaining 30,000 genes in the human genome, and finally stochastic and environmental effects. Because each of these mechanisms may affect gene expression, each of these sources of genetic variation must be established to evaluate the contribution of the deletion in any given individual for the phenotype. Ideally, gene expression in the brain during both fetal and adult life must be understood to assess this. Figure 181–6 illustrates the ideal situation in which a measured feature in the WS population differs almost entirely from that in the normal population and that, within each population, subsets of individuals exist whose phenotypes or gene expressions are not determined solely by gene copy number. It is clear that, in the normal population, a given trait may vary within four standard deviations (two above and two below the mean). This variation is largely due to genes that are not located in the WS region. Therefore, using atypical deletions to map genes for WS features is a powerful approach that will be realized through definition of genetic mechanisms and phenotypes with small variance but that clearly distinguish WS from normal. Finally, measuring parental or sibling features could provide an inexpensive way to increase the predictability of genetic contributions to WS behavior and cognition.

In WS, the deletion of ELN is associated with SVAS and PS, supported by similar phenotypes in humans with small ELN deletions and single base mutations (**Ewart et al., 1993**; **Hirota et al., 2003**; Tassabehji and Urban, 2006). The human ELN gene has 34 exons that span 45 kb of genomic DNA. The human ELN mRNA is about 3.5 kb and encodes a polypeptide of 70 kDa, which forms a structural protein that is a major component of elastic fibers and forms 50% of the dry weight of the aorta. It is of interest that the mouse model, heterozygous for the ELN deletion model, does not develop SVAS or PS but does show abnormalities of vessel wall elastin fibers and increased elastic lamellae that are similar to those found in SVAS and WS (**Li et al., 1998**; **Faury et al., 2003**). ELN hemizygosity in humans and mice induces a compensatory increase in the number of rings of elastic lamellae and smooth muscle during arterial development. However, it is not clear whether the deletion of other WS genes may modify the risk or expression of WS arteriopathy. Although the genetic origin of hypercalcemia in WS is unknown, it is of interest that animals exposed to hypervitaminosis D produce offspring with SVAS (**Friedman and Roberts, 1966**; **Chan et al., 1979**), and this may involve disruption of a common pathway. Data from familial SVAS do not support a significant role for elastin deletion to the typical facial features or hoarse voice of WS. Finally, the lack of significant cognitive defects in isolated familial SVAS augers against a prominent role for ELN deletion in the cognitive features of WS (Olson et al., 1995).

The gene LIMK1 has been implicated (**Frangiskakis et al., 1996**) but not substantiated (**Tassabehji et al., 1999**) as a cause of the visual–spatial features of WS, and the genes STX1A and FZD9 have been implicated simply by their brain-specific gene expression in the developing (FZD9) or adult (STX1A) central nervous system (CNS).

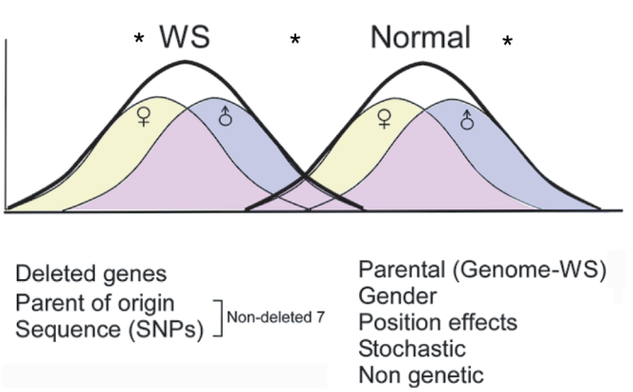

Figure 181–6. Cognitive mapping of Williams syndrome (WS): using sources of genetic variability.

However, regardless of the theoretical attraction of these molecules as mediators of cognitive processes and their embryological substrata, it is important to test their significance in causing the phenotypes at hand when they are underexpressed by 50%, as is likely in the WS brain. When this is done, as illustrated in Figure 181–7, we see that deletion of these genes is not associated with the significant effects on overall cognition that are characteristic of WS. Therefore, it is important to ask which regions and their genes have been demonstrated in humans to be associated with changes in cognition when deleted.

The role of genes in the region from FZD9 through RFC2 appears unlikely to be largely responsible for the characteristic WS mental retardation, sociability, visual–spatial or memory deficits, language preservation, or facial features. This derives from cases RM1199 (Korenberg et al., 2001) and CS (**Tassabehji et al., 1999**), both of whom have heart disease with essentially normal range or mildly impaired cognitive and physical features.

In summary, the data from atypical deletions suggested that WS is due to the combined effects of a number of genes acting during development and determining adult function. The next section addresses whether a small number of genes are responsible for a diverse combination of WS cognitive features.

The significant challenge for the emerging field of human cognitive and behavioral genetics is to understand the genetic contributions to normal human cognitive variation. The striking deficits in WS visual–spatial constructive processing thus provided an opportunity to test whether the WS phenotype was a more focused phenotype that fulfilled the characteristics needed for genetic mapping in partial deletions: large differences between the normal and WS populations with narrowed variation within WS. Combining data from subjects with atypical deletions suggested that visual–spatial deficits are associated with an atypical but still large deletion (STX1A through GTF2I) (Botta et al., 1999). The visual–spatial constructive deficit was more precisely proposed to be due to a narrowed region including two genes, GTF2IRD1 and GTF2I, by Korenberg and coworkers (**Korenberg et al., 2000**; **Hirota et al., 2003**). Further, it was suggested that GTF2IRD1 and GTF2I along with RFC2 and CYLN2 contribute to a significant part of the mental retardation (Korenberg et al., 2001).

The data linking WS cognitive dysfunction to deletion of GTF2I and GTF2IRD1 heightened interest in their possible role in mammalian brain development and cognitive function. GTFII-I encodes a 957-amino acid polypeptide with an N-terminal hydrophobic zipper-like region; 6 directly repeated 90-residue stretches, each with a potential helix–loop–span–helix motif; a consensus site for mitogen-activated protein (MAP) kinase; and several Src autophosphorylation sites (Roy et al., 1997). Although it was first isolated as a downstream target of BTK (Yang and Desiderio, 1997), it is now known to have a number of splice forms (Cheriyath and Roy, 2000) and to be broadly expressed in the developing and adult brain. Further, it is an attractive candidate as a mediator of dosage-dependent developmental variation in that its functions include transcriptional regulation through direct DNA binding (Roy et al., 1997; 2001; 2002) and, more recently, a cytoplasmic role as a negative regulator of agonist-induced calcium entry that suppresses surface accumulation of TRPC3 channels (Caraveo et al., 2006). The gene encoding GTF2IRD1 was independently described for its regulatory role in muscle development (O'Mahoney et al., 1998), as a DNA-binding protein and interactor with FOXH1 (Ring et al., 2002), and as a binding factor to the HoxC8 promoter (Bayarsaihan and Ruddle, 2003). Related to its downstream genomic neighbor, GTF2I, GTF2IRD1 contains 944 amino acids with 5–6 direct repeat domains, each containing bHLH motifs related to those in GTF2I, as well as a leucine zipper, nuclear localization domains, and putative phosphorylation sites. Highly spliced, GTF2IRD1 isoforms are broadly expressed and known to regulate the activity of GTF2I. The genetic and developmental promise of GTF2I and GTF2IRD1 suggests that understanding their role in WS may provide clues to elucidating human brain development and adult function.

AQ6

Imprinting as a Source of Neurocognitive Variation in WS

An important question in understanding WS is the origin of the variation in neurocognitive phenotype seen in WS with typical deletions. One

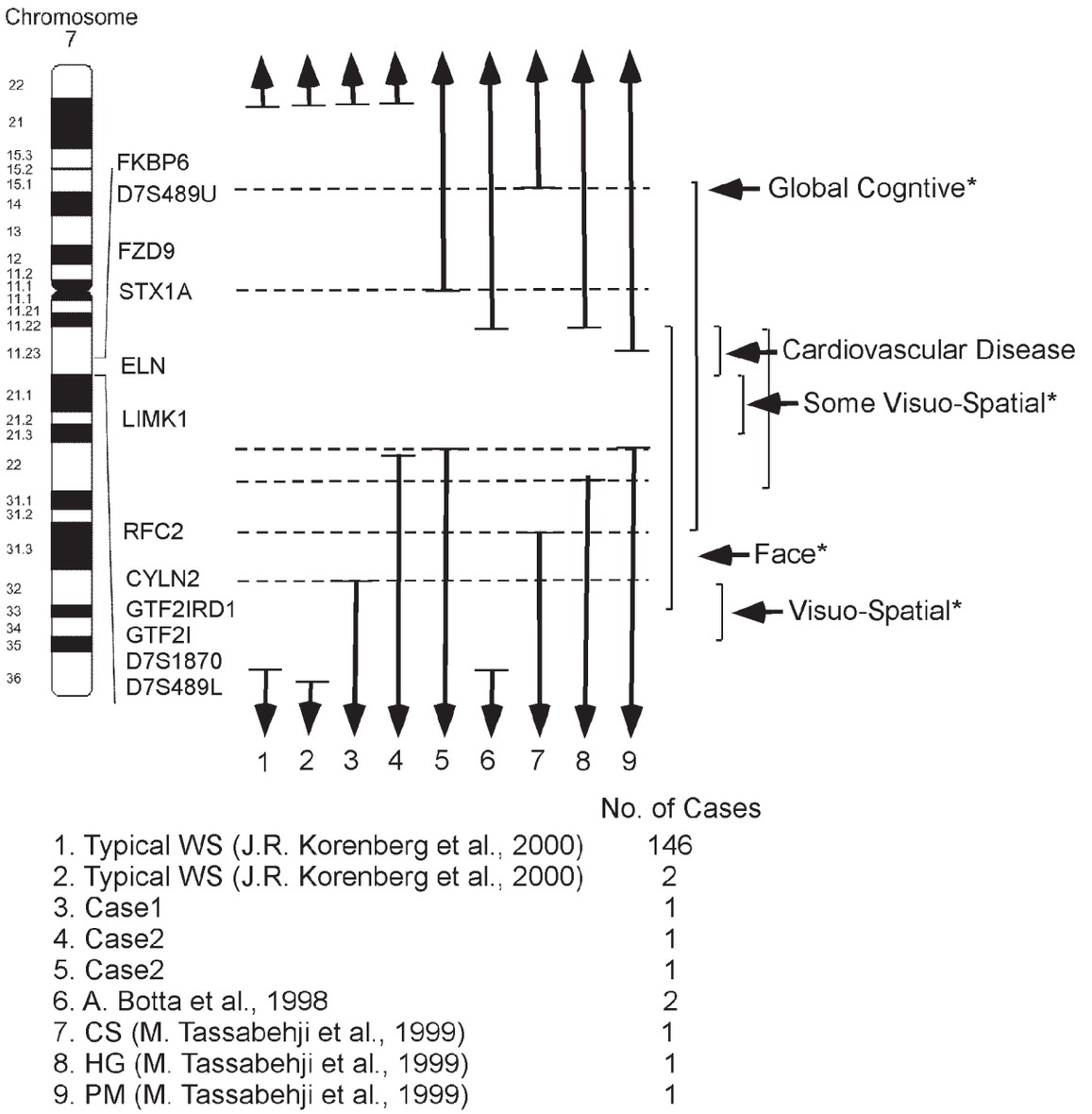

Figure 181–7. Mapping of phenotype to minimal genotype in Williams syndrome (WS). Deleted regions are demarcated by vertical lines. *Position effects notwithstanding. Vertical lines on both figures indicate the regions deleted, and the number of subjects carrying the common WS deletion are associated with some of the typical facial features, mental retardation, and heart disease; the larger deletion associated with similar features; or the smaller deletions, which include subregions of *Synaxin1A* through *RFC2*, associated with only the typical heart disease supravalvar aortic stenosis (SVAS) and subtle cognitive deficits that fall within the normal range. Gene symbols are noted in the corresponding regions. Subject VIII has a subtle defect in visual ± spatial processing. *Indicates individuals with deletion or single base pair mutation of *elastin*, all associated only with SVAS and normal cognition. Small vertical brackets indicate deleted regions that differ among subjects and therefore provide the potential to assign specific WS features to single regions or genes. Some brackets indicate regions that, from the current data, are likely to contain a gene or genes that when deleted contribute in some measure to the WS features denoted. The significance of these data is that deletion of *STXIA*, *ELN*, *LIMK1*, *WSCR1*, and *RFC2* does not appear to be strongly associated with the characteristic facial or cognitive features seen in WS, although they may contribute. In contrast, deletion of the region telomeric to *WSCR1* is associated with characteristic features of WS cognition.

possible contributing factor is imprinting, the differential expression of a gene determined by its passage through the maternal versus the paternal germline. Although previously thought to be an invariant property of a given gene, tissue-specific imprinted expression limited to the brain has been demonstrated in the 15q-Angelman syndrome (Rougeulle et al., 1997). It is not unreasonable to consider that such partially imprinted expression may also result in neurocognitive variation in WS. Subsequent studies (Wang et al., 1999) did not support earlier work (Perez-Jurado et al., 1996), suggesting that small head circumference and postnatal growth were related to maternal inheritance. Although preliminary results (Korenberg et al., 1999) indicated no significant difference, larger numbers of subjects, other cognitive variables, and the development of more sensitive and specific measures may be required to elucidate more subtle effects of genetic imprinting on gene expression in WS.

The initial map of cognition is important in setting out the approach for defining the genetic origins of other domains of cognitive function and neuroanatomic structure. Moreover, it emphasizes that, with a small number of subjects, however rare, significant understanding can be gained. It is the development of sensitive measures and their study in rare individuals that will ultimately provide clues to the critical steps in the pathways of human cognition.

Phenotypic Mapping

Social Behavior

Volumetric and functional neural imaging has shown variations in brain regions associated with human social behavior. These include volumetric measures of orbitofrontal, dorsal cingulate, and dorsolateral prefrontal cortices (**Reiss et al., 2004**; Meyer-Lindenberg et al., 2005; **Mobbs et al., 2006**) and reduced amygdala activation for threatening faces relative to matched normal controls (**Meyer-indenberg et al., 2004**). Taken together, these studies suggest neuroanatomic and functional variations that may contribute to the social behavior in WS. Of importance for the current review is that these studies may now be conducted in WS subjects with small deletions and may provide the basis for elucidating the neurogenetic developmental and functional origins of neural circuitry regulating human social behavior.

Links to Mammalian Developmental Models

Human brain mapping (Gaser et al., 2006; Van Essen et al., 2006) has revealed quantitative variations in cortical thickness, folding, and gyrification. Such quantitative measures may provide the opportunity to compare single subjects' brain maps with those of a population of normal or WS brains. Where a rare subject carries a smaller deletion, this approach may provide the basis for correlating cortical variations with smaller numbers of WS genes and, thereby, clues to the disturbances in brain developmental pathways underlying human cortical formation. Such data also provide a more direct potential link between overall brain structure in WS and in mouse models. Findings (**Thompson et al., 2005**; Gaser et al., 2006; Van Essen et al., 2006) suggest that WS cortical folding abnormalities extend across a region from dorsoposterior to ventroanterior regions of each hemisphere. It is of interest that regional cortical area sizes are disturbed in mouse mutants for genes that affect early patterning of the telencephalon (Leingartner et al., 2007) and that the variations in cortical size are related to performance on functional tests of the region. In WS, although there is a tentative correlation of a subset of regional volumes with preserved versus reduced cognitive functions, evidence for correlation with disturbed CNS gradients is unclear. Approaches to brain mapping may provide gene candidates in the WS region with dosage-sensitive effects on early regional cortical development in humans. Genetic links to brain function are beginning to emerge. The finding that deletion of GTF2I/GTF2IRD1 is associated with abnormal visual–constructive processing (**Korenberg et al., 2000**; **Hirota et al., 2003**) which is independently associated with abnormal posterior parietal structure (**Meyer-Lindenberg et al., 2004**; **Reiss et al., 2004**), suggests that variations in these two genes may contribute to posterior-cortical development and adult visual–spatial function (Korenberg, unpublished). Such genetic links may be tested in mouse models (Table 181–1) and ultimately help to elucidate the developmental mechanisms that alter human and rodent neural circuitry affected in WS (VanEssen et al., 2006).

AQ7

The current view of developmental brain processes in humans has rested on inferences from grossly abnormal postnatal or adult structures whose origins lie in early development. However, it is important to test these hypotheses in humans. We have asked whether classical neuroanatomic analyses of gyrification or sulcal development in this region may identify unique or transitory target structures, as landmarks relating to the expression of genes linked by independent lines of evidence, to development. Figure 181–8 illustrates such a target. Identified by Floyyd Gilles, a striking cell layer appears precisely during the formation of the parieto-occipital sulcus, sufficiently close to the IPS (intraparietal sulcus) to be of significant suspicion for a role relating abnormal GTF2IRD1 or GTF2I expression to adult parietal circuit structure and function underlying the sophisticated human performance underlying visual–spatial integration.

Through integration with data from the normal and WS populations, we have proposed that deletion of GTF2IRD1 and GTF2I appear to contribute disproportionately to the visuospatial constructive deficits and linked posterior parietal variants in WS (Botta et al., 1998; **Hirota et al., 2003**), and we have here proposed a complete developmental and phylogenetic approach to test this hypothesis in humans and to evaluate its mechanisms in mice (Korenberg, unpublished).

Figure 181–8. A striking cell layer appears precisely during the formation of the parieto-occipital sulcus, sufficiently close to the IPS (intraparietal sulcus). (Floyyd Gilles, 1983.)

THE PATHOGENESIS PUZZLE: PUTTING TOGETHER THE PIECES

The pathogenesis of WS is emerging with our understanding of mammalian brain development but, unlike less uniquely human cognitive syndromes, studies of WS are leading the way to elucidate the genetic and neural basis of human neurobiology. With the emergence of human genomic sequence and cDNA isoforms, the more obvious associations of WS deletion of GTF2I and GTF2IRD1 appear to be associated with visual–spatial constructive defects, linked to the superior parietal region, and exemplified in part by copy tasks. The association of an elastin deletion with SVAS is fundamental, but it is not at all clear whether other genes in the region modify the risk or the expressivity. Craniofacial features appear to be more affected by genes from CYLN2 through GTF2I. The early association of LIMK1 deletion with subsets of WS features remains obscure, as does the association of such appealing genes at the synapse as STX1A. There is a suggestion from both human WS deletion variants and emerging understanding of HDAC (histone deacetylase) that genes centromeric to STX1A may act synergistically with those telomeric to RFC2 in regulating transcription. Finally, two fundamental realizations are growing in acceptance. The solution to human cognitive neurobiology may need to come in part from human studies such as provided by functional neural imaging, and from studies of brain development and function in nonhuman primates. Techniques are needed to integrate across experimental domains currently without common language or statistics. The relationship of music to language and to morality requires new vocabulary and acceptance among hard-core scientists. The absence of gyrification and other basic developmental and cognitive processes in mice may be limiting until questions are better focused. Mice modified with human regulatory regions may not be enough. Second, in order to map adult structures back to their prenatal roots, more attention must be paid to integrating the deep insights of the past and present masters of human brain development and structure with the sharp tools of the common genomic era.

ACKNOWLEDGMENTS

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AQ3: Inserted expansion of “TSH”—thyroid stimulating hormone—OK?
AQ4: Please provide year of publication for “Reiss et al.”
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AQ6: Inserted expansion of “MAP”—mitogen-activated protein—OK?
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