Williams–Beuren syndrome: a challenge for genotype–phenotype correlations

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Many human chromosomal abnormality syndromes include specific cognitive and behavioural components. Children with Prader–Willi syndrome lack a paternally derived copy of the proximal long arm of chromosome 15, and eat uncontrollably; in Angelman syndrome lack of a maternal contribution of 15q11–q13 results in absence of speech, frequent smiling and episodes of paroxysmal laughter; deletions on 22q11 can be associated with obsessive behaviour and schizophrenia. The neurodevelopmental disorder Williams–Beuren syndrome (WBS), is caused by a microdeletion at 7q11.23 and provides us with one of the most convincing models of a relationship that links genes with human cognition and behaviour. The hypothesis is that deletion of one or a series of genes causes neurodevelopmental abnormalities that manifest as the fractionation of mental abilities typical of WBS. Detailed molecular characterization of the deletion alongside well-defined cognitive profiling in WBS provides a unique opportunity to investigate the neuromolecular basis of complex cognitive behaviour, and develop integrated approaches to study gene function and genotype–phenotype correlations.

INTRODUCTION

Williams–Beuren syndrome (WBS; MIM 194050) is a rare developmental disorder (1/20 000–1/50 000 live births) which usually occurs sporadically and has striking physical and behavioural features (1). WBS individuals have mild mental retardation, however, this camouflages an uneven cognitive profile. The Williams–Beuren Syndrome Cognitive Profile (WBSCP) includes relatively good verbal skills alongside very deficient visuo-spatial abilities (2,3). Children and adults with WBS also have characteristic personality traits, preferring the company of adults to peers and lacking shyness with strangers. Associated physical abnormalities include a characteristic dysmorphic face, short stature and heart abnormalities. WBS is caused by a chromosomal microdeletion at 7q11.23 thought to arise from recombination between misaligned repeat sequences flanking the critical region during meiosis (4,5). The breakpoints cluster within these repeat regions, resulting in a relatively homogeneous deletion (6). Twenty-four genes have been assigned to the WBS critical region (WBSCR) but only the elastin gene is associated with a phenotype (namely supra-valvular aortic stenosis; Fig. 1) (7). No credible cases without the deletion have been reported, so that haploinsufficiency for a single gene will not explain the spectrum of phenotypes associated with the disorder.

CLINICAL PHENOTYPE

Physically the WBS phenotype includes growth retardation, a dysmorphic face, heart abnormalities (typically supravalvular aortic stenosis, SVAS, and peripheral pulmonary stenosis, PPS), hyperacusis, infantile hypercalcaemia, and abnormal gait. The cardiovascular condition, SVAS, is a congenital narrowing of the ascending aorta, which also occurs as a sporadic or autosomal dominant condition (4,8). This obstructive vascular disease may be progressive and can lead to cardiac failure and early death without surgical intervention. SVAS is caused by deletion, mutation or disruption of the elastin (ELN) gene (4,7,9). As the main component of elastic fibres (~90% content), elastin provides strength and elasticity to extensible tissues that require resiliency, such as the heart, skin, lung and major blood vessels (10). Since elastin is an important structural component of the aorta and pulmonary arteries it is not surprising that haploinsufficiency causes vascular problems like SVAS and PPS, as well as some of the associated features seen in SVAS/WBS patients such as hernias and premature ageing. As usual with haploinsufficiency disorders, the severity of the cardiac abnormality is variable (ranging from heart murmurs to severe stenosis) both in individuals with isolated ELN mutations and those with classic WBS.

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BEHAVIOURAL AND COGNITIVE PHENOTYPE

The WBS neuropsychological profile is a striking one, characterized by strengths in certain complex faculties (language, music, face processing, and sociability) alongside marked and severe deficits in cognitive domains (2,3). Unlike many others with learning difficulties, WBS individuals have a 'gregarious' personality characterized by a lack of fear with strangers, over friendliness and charismatic speech rich in vocabulary (3). Approximately 70% also suffer from attention deficit disorder, and many experience anxiety and simple phobias (2,11,12). An interesting feature is the musical creativity observed in WBS individuals which is thought to be a means of expression, play, and improvisation rather than formal analytic skill in pitch and rhythm discrimination usually associated with musical ability (13).

COGNITIVE STRENGTHS

It has long been thought that brain function is modular, with the existence of independently functioning, innately specified modules, and supporters of that theory cite the fractionated pattern of cognitive phenotypes seen in WBS as evidence (14). However, current cross-syndrome/cross-domain studies suggest a different model, namely that early in life the brain is not specialized, and only becomes increasingly specialized and localized over developmental time (15–22). Recent studies on language and face recognition in younger individuals with WBS appear to support the latter model and challenge the general view that these skills are 'intact' in WBS by suggesting that all aspects of WBS language do indeed show delay and/or deviance throughout development (17–19). When infants and toddlers with WBS were tested alongside their Down syndrome (DS) counterparts they appeared equally delayed in vocabulary, despite outstripping them later in adulthood (19). This suggests that WBS language is not 'intact' and follows a different developmental trajectory to normal, which may turn out to be more like learning a second language (18). As with language, a different developmental trajectory was also apparent in face processing, where young children with WBS mainly rely on components or features. Normal controls use predominantly configural or holistic processes to recognize faces, but adults with WBS mainly rely on features and support these observations with measurements of brain activity in WBS individuals, where abnormal early waveforms are displayed showing less right lateralization and no difference in amplitude in response to human or monkey faces.
faces (22). On the whole, the evidence suggests that the superficially intact abilities in WBS depend on atypical cognitive and brain processes which are served by atypical neuronal processes. These results do not argue against modularity in the adult mature brain, but suggest that the link between modules, genes and phenotype is likely to be very complex and probably atypical in this clinical population.

COGNITIVE DEFICITS

WBS individuals have mild to severe mental retardation (mean IQ 51–70) and severe deficits in conceptual reasoning, problem solving, motor control, arithmetic and spatial cognition (14). Their specific deficits in spatial cognition are apparent when tested alongside age-matched DS individuals. Although both groups show abnormal spatial cognition, they fail in different ways. The drawing and block design tasks highlight these differences clearly, showing that WBS individuals focus on details at the expense of the whole picture, compared with DS where global configuration is maintained with poor internal detail (Fig. 2).

NEURAL DEVELOPMENT IN WBS

Neuroanatomical, structural and functional MRI (fMRI) studies are providing further converging evidence that the neural organization in WBS is abnormal, and that the roots of the mental and cognitive aspects of the disorder may lie in the disruption of normal neurodevelopment.

Auditory processing

WBS individuals suffer from hyperacusis and have a characteristic heightened emotional response (attraction or aversion) to music and noise (23,24). fMRI studies (24) show significant differences from normal in the neural processing of music and noise, with reduced activation in regions supporting music and noise processing (temporal lobes) coupled with increased activation in the cerebellum and amygdala, in accordance with the heightened emotional response (25). In addition, there is a more diffuse network of activation throughout the WBS brain during music processing. These studies suggest that auditory processing is carried out by different neural systems in WBS compared with normal, which could explain their atypical reactions to sound.

Brain morphology

In WBS the brain is reported to be significantly smaller (P < 0.05), due to decreased subcortical white-matter with increased cortical folding (26). The cerebellum is enlarged relative to a small cerebrum, but the limbic structures of the temporal lobe including the amygdala and Heschl’s gyrus in the primary auditory cortex are preserved (27). Neuronal abnormalities described in WBS (small neurones and an increased cell-packing density in the left hemisphere of the primal visual cortex) (28) may be related to the visuospatial deficits, although the location is unexpected since visuospatial functions mainly involve the right hemisphere while the left hemisphere is concerned with visual object recognition (29). Biochemical abnormalities in the left cerebellum have also been detected in WBS individuals during cognitive testing, which seem to be related to their cognitive deficits and provide more evidence that cerebellar neuronal integrity could be a requirement for some cognitive abilities (30).

ANALYSIS OF THE MOLECULAR LESION IN WBS

Mechanism of the deletion

WBS is thought to arise through unequal crossover between large duplicated regions that flank the WBS deletion (6). These low-copy repeat sequences (LCRs), spanning >320 kb, exhibit extraordinarily high nucleotide sequence identity (~98%) and are composed of smaller duplicons containing unique sequence elements from the internal border regions of the deletion, as well as genes and pseudogenes (Fig. 1). Similar repeats predispose other regions to a number of chromosomal rearrangements and microdeletion syndromes (e.g. 22q11.2 deletion syndrome; Smith–Magenis syndrome on 17p11.2) (31,32). The majority of WBS deletions (67%) result from interchromosomal rearrangements between chromosome 7 homologues, with 33% due to intrachromosomal rearrangements (33). Other rearrangements expected to be mediated by these flanking LCRs include duplications (not yet reported) and inversions arising from recombination between inverted repeats. An inversion polymorphism has recently been identified in families with WBS (34). Among parents of WBS children whose chromosome deleted during meiosis, 4/12 were heterozygous for the inversion, as were 3/11 atypical individuals who had chromosomal rearrangements in the region but no microdeletion. A similar situation has been reported on chromosome 8p, where a common non-pathogenic inversion polymorphism involving paired olfactory receptor gene clusters acts a predisposing factor for chromosome rearrangements (35).
GENOTYPE–PHENOTYPE CORRELATIONS

In classic WBS the deletion appears to be quite homogeneous and the main phenotypes (e.g. face, cognitive and behavioural profile, short stature) are highly penetrant. Twenty-four genes have been mapped to the WBS critical region with a variety of potential functions, some experimentally defined, some inferred by motifs (summarized in Table 1). To date only the elastin gene has been unambiguously associated with a phenotype (namely SV AS), and that came about following reports of an SVAS patient in whom a translocation disrupted the *ELN* gene (7). Since then a spectrum of mutations have been identified in individuals with isolated SVAS, 63% of which are truncating mutations and result in functional haploinsufficiency for elastin (9). In order to address the question of which of the remaining genes are dosage sensitive and contribute to the specific (9). In order to address the question of which of the remaining genes are dosage sensitive and contribute to the specific human cognition and behaviour, a twin-track approach has been adopted involving clinical, psychological and molecular analysis of patients with both typical and atypical WBS, alongside mouse models.

TYPICAL AND ATYPICAL WBS INDIVIDUALS

Molecular analysis of classic WBS individuals suggests that the majority of the deletions span a genomic region of ~1.5 Mb, with the common breakpoints residing between the direct repeats at duplicons B (Block B-cen and B-mid, see Fig. 1) (6,64). A few individuals with WBS have, however, been identified with smaller chromosomal deletions and, coupled with information from individuals with isolated SVAS and partial deletions, the critical region containing the main genetic determinants has been narrowed down to the telomeric end.

The characterized cases which have allowed the region harbouring the dosage-sensitive genes to be defined include three WBS individuals with atypical centromeric breakpoints and smaller deletions, excluding *STX1A* and the genes proximal to it (WBS 1–3, Fig. 1). Their telomeric breakpoints are more typical, interrupting the *GTF2I* gene (65) or just beyond (66). Other partial deletions were detected in individuals with isolated SVAS (SVAS 1–7; Fig. 1): (i) one with a small deletion (~30 kb) including *ELN* (SVAS 1) (67); (ii) two families with deletions encompassing both *ELN* and *LIMK1*, in which 11/13 deleted individuals also showed the impaired visuo-spatial constructive cognition associated with WBS (SVAS 2–3) (68); (iii) three SVAS individuals with *ELN–LIMK1* deletions who were not impaired on the cognitive tests (SVAS 4–6) (69); (iv) a large deletion, encompassing approximately 850 kb of the critical region including *ELN* and *LIMK1*, detected in an individual with SVAS and PPS but no aspects of the WBS cognitive profile (SVAS 7) (69,70). Lack of a WBS phenotype due to somatic mosaicism cannot be excluded in this latter case, but it seems unlikely because the deletion was detected in both lymphocytes and fibroblasts (70), and mosaicism is most likely to arise by mitotic recombination, which occurs at approximately $10^{-4}$ times the frequency of the meiotic recombination that produces the typical WBS deletion.

There is obvious ambiguity regarding the role of *LIMK1* haploinsufficiency in cognitive spatial impairment (68–70). The WBS cognitive profile is highly penetrant, so reduced penetrance does not explain its absence in four independent *LIMK1*-deleted SVAS individuals (SVAS 4–7) (69). Perhaps there is some additional undetected lesion within the WBS critical region in the two families described with relatively small *ELN–LIMK* deletions and visuospatial problems (SVAS 2–3) (68). In contrast, haploinsufficiency for *STX1A* and the other proximal genes deleted in the SVAS individual (SVAS 7), but not in some WBS cases (WBS 1–3), do not appear responsible for any of the main physical, mental and cognitive aspects of WBS. They should not, however, be completely excluded from playing a role in the variably penetrant components like infantile hypercalcaemia.

Further insights into genotype–phenotype correlations comes from an intriguing report of an individual with many clinical features of WBS (milder facial features, SVAS, short stature) and a peculiar cognitive profile, but without the specific spatial and constructive impairment typically seen in WBS (71). The telomeric deletion breakpoint in this individual is atypical, mapping around the *CYLN2* gene (deletion status of the *GTF2IRD1* gene is not clear), but does not include the *GTF2I* gene (WBS 4; Fig. 1). The authors conclude that hemizygosity for *LIMK1* and *CYLN2*, while not sufficient to generate the WBSCP, may cause alterations in the cognitive profile, resulting in the milder phenotype with some cognitive impairment (borderline IQ) and a variable loss of visuo-spatial and constructive abilities. These observations suggest that, while the WBS phenotype is the result of haploinsufficiency for a number of genes, the deletion of the *GTF2IRD1* and/or *GTF2I* genes located on the telomeric side of the critical region appears necessary for the unique WBS cognitive profile. It may be significant to note that Osborne *et al.* (34) detected an individual with atypical WBS features (WBS face, malocclusion, some aspects of the behavioural profile and developmental delay), who was heterozygous for the WBSCR inversion polymorphism, and hypothesised that *GTF2I* may have been involved in the rearrangement, thereby contributing to the observed phenotype. More patients with similar deletions/rearrangements in the vicinity of these telomeric genes are required to allow more definite conclusions to be drawn regarding their role in the pathology of WBS.

Cytogenetically visible deletions involving 7q11.23, but extending past the critical region, have also been identified in WBS individuals who consequently exhibit more severe phenotypes (including infantile spasms) (72–74), however, no credible cases of WBS without the deletion have been reported.

MOUSE MODELS

A high degree of synteny and linkage conservation exists between human chromosome band 7q11.23 and mouse chromosome 5G1–G2 (6). The orthologous region in the mouse lacks the characteristic duplicated blocks but has the full complement of genes which is inverted with respect to the human map, possibly because the chromosome 7 LCRs in humans correspond to sites of evolutionary breakpoints. Mice harbouring targeted deletions of three individual genes (*Eln*, *Limk1* and *Cyln2*) have provided an insight into their contribution to the pathology of WBS. The *Eln* mouse model uncovered a regulatory function for elastin in arterial development and confirmed that the pathophysiological mechanism for
SVAS is haploinsufficiency through lesions in elastic arteries that arise from increased cellularity and narrowing of the lumen (50). \( E_{ln}^{+/-} \) mice die after birth due to subendothelial accumulation of proliferating smooth muscle cells, in both systemic and pulmonary arteries, that eventually obliterate the vascular lumen. \( E_{ln}^{+/-} \) mice do not have SVAS (normal gross appearance and life expectancy), despite having abnormal elastic lamellae in the aorta at birth, but they are hypertensive and have altered arterial compliance (75). Hypertension is often seen in WBS, indicating that a similar mechanism exists in humans. In this context, hypertension may be an important adaptive response to maintain cardiovascular function and the compensatory increase in the lamellar units results in an increased risk of obstructive vascular disease in man.

\( \text{Limk1} \) encodes a serine/threonine kinase, specifically expressed in neuronal tissue, which regulates actin reorganization through phosphorylation and inactivation of ADF/cofilin, a stimulus-responsive mediator of actin dynamics (53). Actin cytoskeletal reorganization is essential for various cell activities including the directional movement of neurones and neurite outgrowth. Actin remodelling may, therefore, be crucial to the establishment and modification of dendritic spines that make up the majority of the synaptic connections within the hippocampus and are associated with the formation and maintenance of memory and learning (76). \( \text{Limk1}^{+/-} \) mice show significant abnormalities in dendritic spine morphology and synaptic function, including long-term hippocampal potentiation (a form of synaptic plasticity considered critical to learning and memory) (77). Surprisingly, despite these abnormalities they are viable with a grossly normal nervous system. This may be a consequence of \( \text{Limk2} \) or \( \text{Tesk} \) activity (both able to regulate cofilin) providing some of the missing function (78). In fact, it does appear that there was some kinase activity regulating actin turnover levels in the knockout mice and Meng et al. (77) detected significant levels (>50% of wild-type) of phosphorylated actin in \( \text{Limk1}^{-/-} \) brain samples. In behavioural tests

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( POM121 )</td>
<td>Component of nuclear pore complex that mediates bi-directional macromolecular traffic in cells</td>
<td>(36)</td>
</tr>
<tr>
<td>( NOL1R )</td>
<td>Protein with a NOL1/NOP2/sun domain. May play a role in the regulation of the cell cycle</td>
<td>(37)</td>
</tr>
<tr>
<td>( FKBP6 )</td>
<td>Immunophilin protein. Role in male fertility and homologous chromosome pairing in meiosis</td>
<td>(38,39)</td>
</tr>
<tr>
<td>( FZD9 )</td>
<td>‘Frizzled’ proteins act as receptors for Wnt signalling proteins. May be involved in tissue polarity and development</td>
<td>(40)</td>
</tr>
<tr>
<td>( BA21B )</td>
<td>Protein with a bromodomain. May be involved in chromatin-dependent regulation of transcription</td>
<td>(41)</td>
</tr>
<tr>
<td>( BCL7B )</td>
<td>Member of BCL7 protein family. Unknown function</td>
<td>(42)</td>
</tr>
<tr>
<td>( TBL2 )</td>
<td>( \beta )-transducin protein with four putative WD40 repeats. May play a role in intracellular signalling pathways or cytoskeletal organization</td>
<td>(43)</td>
</tr>
<tr>
<td>( WBSCR18 )</td>
<td>Protein has a DNAJ domain involved in protein folding</td>
<td>(44)</td>
</tr>
<tr>
<td>( WBSCR14 )</td>
<td>bHLH-LZ transcription factor. May play a role in cell proliferation and/or differentiation</td>
<td>(45,46)</td>
</tr>
<tr>
<td>( WBSCR22 )</td>
<td>Protein with 5-adenosyl-L-methionine binding motif. May be involved in DNA methylation</td>
<td>(47)</td>
</tr>
<tr>
<td>( STX1A )</td>
<td>Syntaxin 1A protein plays key role in intracellular transport and neurotransmitter release</td>
<td>(48)</td>
</tr>
<tr>
<td>( WBSCR21 )</td>
<td>Protein has a ( \alpha/\beta )-hydrolase fold domain. Unknown function</td>
<td>(49)</td>
</tr>
<tr>
<td>( CLDN3 )</td>
<td>Protein component of tight junction strands in liver epithelial cells. Role in maintaining cellular polarity</td>
<td></td>
</tr>
<tr>
<td>( CLDN4 )</td>
<td>protein component of tight junction strands in kidney epithelial cells. Role in maintaining cellular polarity</td>
<td>(50)</td>
</tr>
<tr>
<td>( ELN )</td>
<td>Structural protein, component of elastic fibres. Role in arterial morphogenesis</td>
<td>(51,52)</td>
</tr>
<tr>
<td>( LIMK1 )</td>
<td>Serine/threonine kinase with LIM domains. Role in actin cytoskeletal reorganization essential for directional movement of neurons</td>
<td>(53)</td>
</tr>
<tr>
<td>( EIF4H )</td>
<td>Protein contains an RNA recognition motif. Stimulates initiation of protein synthesis at the level of mRNA utilization</td>
<td>(54)</td>
</tr>
<tr>
<td>( WBSCR5 )</td>
<td>LAB (linker for activation of B cells proteins. Role in normal B-cell development</td>
<td>(55,56)</td>
</tr>
<tr>
<td>( RFC2 )</td>
<td>Component of replication factor C complex which is an activator of DNA polymerases during replication</td>
<td>(57)</td>
</tr>
<tr>
<td>( CYL2 )</td>
<td>Cytoplasmic linker protein. Role in regulating microtubule dynamics</td>
<td>(58)</td>
</tr>
<tr>
<td>( GTF2IRD1 )</td>
<td>Member of GTF2I transcription factor family with leucine-zipper and five I-repeats. May play a role in activating/repressing gene transcription</td>
<td>(59,60)</td>
</tr>
<tr>
<td>( GTF2I )</td>
<td>Multifunctional transcription factor. Contains a leucine-zipper and six HLH-like domains (I-repeats). Functions both as a basal factor and as an activator of gene transcription</td>
<td>(61,62)</td>
</tr>
<tr>
<td>( NCF1 )</td>
<td>Component of phagocyte NADPH-oxidase system. Role in immunity</td>
<td>(63)</td>
</tr>
<tr>
<td>( GTF2IRD2 )</td>
<td>Gene containing both I-repeats and a Charlie8-like transposase motif. Function unknown</td>
<td>Our data</td>
</tr>
</tbody>
</table>
Limk\(^{-/-}\) mice displayed altered fear responses and deficiencies in spatial learning, but their relevance to the cognitive deficits in humans with WBS is not yet clear.

Cyln2 is abundantly expressed in dendrites and cell bodies of many neurones in the brain and encodes a highly conserved protein, CLIP-115 (79). CLIP-115 is a member of a family of cytoplasmic linker proteins (CLIPs) that specifically associate with the ends of growing microtubules. They are involved in regulating microtubule dynamics and establishing interactions between microtubule tips and various cellular structures, including cargoes destined for transport by dynein. CLIP115 is thought to play a role in the formation and/or turnover of gap junctions (80). Using a gene targeting approach, Hoogenraad et al. (81) provided evidence that mice haploinsufficient for Cyln2 had features reminiscent of WBS, that included mild growth deficiency, brain abnormalities, hippocampal dysfunction and particular deficits in motor coordination. Although the encoded CLIP-115 protein is not essential for life (Cyln2\(^{-/-}\) mice are viable), it does appear to be necessary for normal development. For example, hetero- and homozygous Cyln2 knockout mice displayed mildly abnormal brain morphology (enlarged brain-ventricle size, a smaller corpus callosum but no evidence of smaller brains). Microtubule dynamics in Cyln2\(^{-/-}\) mice were largely unaffected apart from an increased accumulation of CLIP-170 and dynactin at microtubule tips. This protein redistribution may be significant in disrupting dynein motor regulation and, together with the loss of CLIP115-specific functions, could underlie the neurodevelopmental abnormalities seen in the mice. Perhaps this is also the case in WBS since there is some overlap in the neurological phenotypes.

c-myc transgenic mice have recently been reported that contain a transgene-induced deletion of \(~40\) kb of the Gtf2ird1 gene on both chromosome 5G homologues (82). Despite the deletion Gtf2ird1 expression was detected, albeit at significantly reduced levels, and mice homozygous for the deletion were viable and fertile with a normal lifespan. No obvious behavioural or morphological abnormalities were observed apart from the increased liver cell proliferation and hepatocellular carcinomas, expected due to c-myc overexpression. It remains to be seen whether any subtle neurological or behavioural abnormalities do exist in these mice since these analyses were not carried out.

**CANDIDATE GENES FROM THE CRITICAL REGION**

Of the genes in the WBS critical region distal to STX1A only the role of ELN in SVAS has been unambiguously established. LIMKI haploinsufficiency alone is unlikely to cause the cognitive deficits specific to the WBS profile, but the mouse studies suggest that it could contribute to the phenotype, and a half dose of CYLN2 may lead to hippocampal dysfunction and particular deficits in motor coordination, analogous to the mouse models. Transcription factors are often dosage sensitive and manifest in hemizygotes as dominant developmental disorders so, of the remaining genes, the unique transcription factors GTF2IRD1 and GTF2I stand out as strong candidates for the mental and cognitive aspects of the phenotype. Both genes are ubiquitously expressed and belong to a novel family of transcription factors that appear to have the capacity for multiple protein–protein and protein–DNA interactions. Their distinct structure, comprising an amino-terminal hydrophobic leucine-zipper and multiple helix–loop–helix (HLH)-like domains [now called I-repeats (PF02946)], is compatible with multifunctional capabilities. Functional studies have uncovered unique properties of GTF2I (or TFII-I) that enable it simultaneously to function both as a basal factor and as an activator, linking signal transduction pathways to transcription by its activation in response to certain extracellular signals (83). Unlike other HLH proteins, GTF2I is a multifunctional transcription factor that can bind both enhancer and core promoter elements and has been shown to interact with both E-box and Inr elements (62). GTF2I can also bind proteins, for example, transcription factors such as SRF, Phox, UBF, NF-kB, STAT1, STAT3 and c-myc (83), as well as signalling molecules like Bruton’s tyrosine kinase (84). It, therefore, appears to play an important role as a coordinator of the basal transcription machinery as well as of diverse cell signalling responses.

GTF2IRD1 also seems to be promiscuous in its function, binding and interacting with both DNA and protein, and has been shown to play a role in either activating or repressing gene transcription (85,86). For example, it can bind to the EFG site within the early enhancer of \(Hoxc8\), and may behave as a repressor of \(Hoxc8\) (85). The mouse Gtf2ird1 protein has been reported to bind to the retinoblastoma protein through its C-terminal end and may serve as a positive regulator of the cell cycle and development (87). GTF2IRD1 also specifically binds the bicoid-like motif of the Troponin I slow (\(Tnls\)) upstream enhancer and is involved in the confinement of \(Tnls\) expression to slow-twitch fibres during early stages of muscle development (60,86). Lack of stamina and myopathies associated with WBS could be due to altered expression of proteins that regulate the contractile or metabolic properties of skeletal muscles (59).

**CONCLUSION**

Williams–Beuren syndrome provides a good example of the approaches, progress and problems in using microdeletion phenotypes to define genes affecting human cognition and behaviour. The strategy for genotype–phenotype correlations faces a number of difficulties. First, it is not sufficient simply to enumerate the deleted genes because few genes show haploinsufficiency and often cells function normally with a 50% level of gene action. Second, the mouse repertoire of measurable behaviour is limited, so we may not be able to detect equivalent cognitive deficits in mice even if they exist. Therefore, mouse knockouts are of limited use for confirming correlations. Third, the gene dosage effects that cause these phenotypes are often dependent on genetic background, reducing the value of single clinical cases and making cross-species comparisons particularly unreliable. Finally, detailed cognitive profiling has illustrated the indirect route through which genes can contribute to impairments. For example, the developmental trajectory in the language of WBS children is affected by an alteration in the relative quality or accessibility of information required for successful development, a process that seems to be mediated by genetic influences (16).
As a consequence, the relationship between genetic abnormalities and cognitive processes is still poorly understood, and clues about pathways that might be involved in cognition could come from studies of mental retardation. Mental retardation pathogenesis affects pathways involved in synapses, axon maintenance, and trafficking (88–90). In fragile X syndrome, protein synthesis in dendrites may be a critical step in learning and memory and the gene product (FMRP) is involved in the synaptic regulation of protein synthesis (90). It is interesting that LIMK1 is involved in dendritic spine morphogenesis and synapse formation and CLIP115 is thought to play a role in the formation and/or turnover of gap junctions of neurons, and both mouse models present with abnormal brain morphology and show some behavioural phenotypes. Whether and how these genes contribute to the neuropathology seen in WBS remains to be seen, but it is becoming clear from patient studies that the transcription factors GTF2I and GTF2IRD1 probably play a crucial role in the development of the typical WBS cognitive and behavioural profile.

A key question is whether the genetic effects are additive. That is, does the WBS phenotype consist of a superimposed set of independent single-gene haploinsufficiency effects, or is something more complex going on? It is intriguing that, apart from ELN and the cardiovascular aspects of WBS, no other part of the phenotype has been recognized as an isolated Mendelian dominant character in families with a point mutation in one of the critical genes. Is this just because these are relatively soft signs, hard to score reliably in otherwise unaffected individuals? The very high penetrance of the visuospatial cognitive deficit is also unexpected for a feature dependent on simple haploinsufficiency, and biased ascertainment cannot explain this. Could the overall WBS phenotype depend in part on functional relationships between genes within the region, or perhaps even on the presence of a large deletion itself? The role of position effects silencing genes flanking the deletion breakpoints must also be considered.

The situation in WBS is mirrored to some extent in 22q11.2 deletion syndrome [encompassing DiGeorge syndrome (DGS), velo-cardio-facial syndrome (VCFS) and conotruncal anomaly face syndrome], with clinical features that include cardiac defects, characteristic face, cleft palate, hypoparathyroidism and psychiatric disorders. Although extensive work on mouse models has provided a candidate gene, TBX1, implicated in the main clinical features for VCFS/DGS (91), proof that haploinsufficiency of this gene alone is responsible for the syndrome in humans is lacking. No $TBX1$ mutations have been detected in non-deleted individuals, and no cases with a de novo mutation inactivating the gene have been identified (92). In addition the molecular basis for the psychiatric disorders associated with VCFS/DGS is not clear since $TBX1$ is not expressed in the brain. In humans, haploinsufficiency of additional genes may contribute to the aetiology of this disorder too. It is interesting to speculate that chromosomal microdeletion syndromes like WBS may sometimes be more than the sum of their parts. Further progress requires a mouse model of WBS that will complement but not replace human studies at the molecular, clinical and psychological levels.

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Note Added in Proof

Kitagawa et al. (41) have recently described a novel multifunctional ATP-dependent chromatin remodeling complex (WINAC), one component of which is BAZ1B (or WSTF). WINAC promotes both the assembly and disruption of nucleosome arrays and can also reconstitute chromatin upon newly replicated DNA. BAZ1B appears to function as a platform protein for the assembly of components in WINAC, and is also capable of interacting directly with the vitamin D receptor (VDR). Normal levels of BAZ1B were shown to be necessary to support normal activities of VDR but it is not obvious what the phenotypic consequences of impaired VDR signaling are. Since BAZ1B appears to be necessary for expression of enzymes involved in both synthesis and catabolism of vitamin D, haploinsufficiency for this gene could have a potential role in the infantile hypercalcaemia seen in many WBS patients.

References


