LETTER TO JMG

An atypical deletion of the Williams–Beuren syndrome interval implicates genes associated with defective visuospatial processing and autism

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Background: During a genetic study of autism, a female child who met diagnostic criteria for autism spectrum disorder, but also exhibited the cognitive–behavioural profile (CBP) associated with Williams–Beuren syndrome (WBS) was examined. The WBS CBP includes impaired visuospatial ability, an overly friendly personality, excessive non-social anxiety and language delay.

Methods: Using array-based comparative genomic hybridisation (aCGH), a deletion corresponding to BAC RP11-89A20 in the distal end of the WBS deletion interval was detected. Hemizygosity was confirmed using fluorescence in situ hybridisation and fine mapping was performed by measuring the copy number of genomic DNA using quantitative polymerase chain reaction.

Results: The proximal breakpoint was mapped to intron 1 of *GTF2IRD1* and the distal breakpoint lies 2.4–3.1 Mb towards the telomere. The subject was completely hemizygous for *GTF2I*, commonly deleted in carriers of the classic ~1.5 Mb WBS deletion, and *GTF2IRD2*, deleted in carriers of the rare ~1.84 Mb WBS deletion.

Conclusion: Hemizygosity of the GTF2 family of transcription factors is sufficient to produce many aspects of the WBS CBP, and particularly implicate the GTF2 transcription factors in the visuospatial construction deficit. Symptoms of autism in this case may be due to deletion of additional genes outside the typical WBS interval or remote effects on gene expression at other loci.

Key points

- Hemizygosity of GTF2I and GTF2IRD1 results in the WBS cognitive-behavioural profile including a severe visuospatial construction deficit and an overly friendly personality.
- GTF2I and GTF2IRD1 oppositely regulate Goosecoid, a gene involved in craniofacial development.
- A gene related to Goosecoid, called Goosecoid-like resides in the 22q11 DiGeorge/Velocardiofacial syndrome region also associated with autism and a visuospatial construction deficit.

Williams–Beuren syndrome (WBS) results from a ~1.5 Mb deletion in chromosome 7q11.23 that contains about 20 genes. The disorder occurs at a rate of 1:7500, as estimated in a recent study.[1] Three region-specific low-copy repeats (LCRs), referred to as centromeric, medial and telomeric blocks A, B and C, flank the WBS deletion site (fig 1). Most WBS deletions are 1.5 Mb in length (95%) and result from unequal crossing over between the centromeric and medial portions of the block B LCR containing *GTF2I* and *NCF1*, but sparing *GTF2IRD2*.[2] *GTF2IRD2* is deleted in the much rarer 1.84 Mb deletion cases mediated by portions of the block A LCR.

The cognitive–behavioural profile (CBP) of WBS includes severely impaired visuospatial ability, mental retardation, an overly friendly personality accompanied by excessive non-social anxiety, attention deficit, hyperacusis and/or love of music.[3] Language delay is usually present, but verbal IQ is generally, although not always, higher than performance IQ in WBS cases.[3–8] By contrast, although language delay is common to both disorders, subjects with autism tend to have higher performance IQs than verbal IQs and display excessive social versus non-social anxiety. However, difficulty reading emotions from the face and voice, an inability to get along with peers and poor social judgement are common features of both disorders.[9–11]

WBS cases with atypical CBPs and nested proximal deletions have helped generate genotype–phenotype correlations. For instance, there have been multiple reports of WBS cases with normal visuospatial ability that carry atypical deletions sparing the GTF2 transcription factors *GTF2IRD1* and *GTF2I*.[9–15] Furthermore, a recent report described a female child carrying a WBS deletion that extended through intron 1 of *GTF2IRD1*, but spared *GTF2I*, whose visuospatial abilities were less severely impaired than those of full-deletion carriers.[16] This finding suggests that hemizygosity of both *GTF2I* and *GTF2IRD1* is necessary to produce the severe visuospatial deficits seen in carriers of the classic WBS deletion, a hypothesis supported by our data.

Here, we describe a female child with many aspects of the WBS CBP, who also meets diagnostic criteria for autism spectrum disorder, as assessed by the Autism Diagnostic Interview–Revised[17] and the Autism Diagnostic Observation Schedule,[18] but lacks the classic medical and physical features of WBS. She carries a novel, de novo deletion between 2.4 and 3.1 Mb in length extending towards the telomere from intron 1 of *GTF2IRD1*, which results in hemizygosity of the genes at the distal end of the WBS deletion, including *GTF2IRD1*, *GTF2I* and

Abbreviations: aCGH, array-based comparative genomic hybridisation; BAC, bacterial artificial chromosome; CBP, cognitive–behavioural profile; FISH, fluorescence in situ hybridisation; HIP1, Huntingtin-interacting protein 1; LCR, low-copy repeat; PCR, polymerase chain reaction; UPL, Universal Probe Library; WBS, Williams–Beuren Syndrome; YWHAG, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein γ

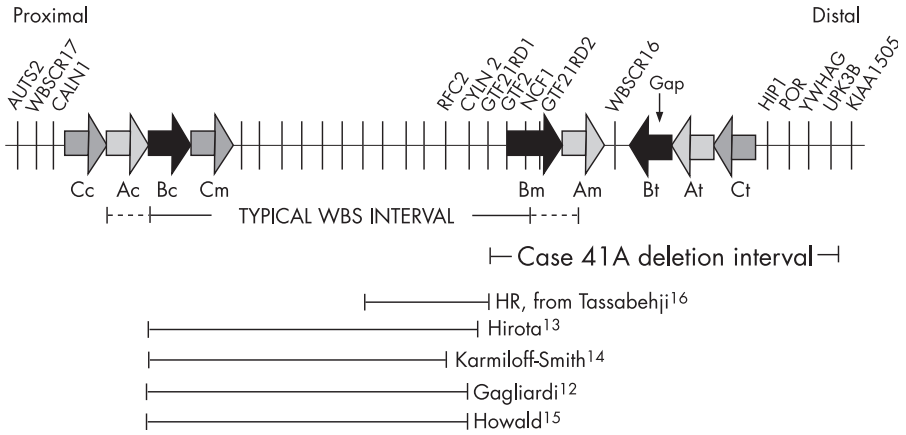

Figure 1 Schematic representation of the classic 7q11.23 William–Beuren syndrome (WBS) deletion region and the atypical deletion detected in patient 41A. The thick horizontal arrows represent the three large blocks of low-copy repeats (LCRs), labelled as blocks A, B and C, with centromeric (c), medial (m) and telomeric (t) sequence indicated. Single-copy regions flank the LCRs, between Cm and Bm, and between Am and Bt (which contains the gene *WBSCR16*). The common WBS deletion interval (approximately 1.5 Mb) is depicted as a solid line. The extended boundaries of the rare 1.84 Mb deletion are shown as dotted lines. Roughly 28 genes lie in the WBS deletion interval; only the distal genes that have been the focus of genotype–phenotype correlations are labelled for clarity. Atypical WBS deletions are displayed below the case 41A deletion interval.

GTF2IRD2 (figs 1, 2), as well as roughly 14 other genes which lie downstream of a gap in the genomic sequence that has yet to be resolved. Two of the 14 genes have previously been implicated in neuropsychiatric disorders, Huntingtin-interacting protein 1 (*HIP1*)[19] and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein γ (*YWHAG*).[20] Therefore, the symptoms of autism observed in our patient may be caused or exacerbated by hemizygosity of another gene or genes in the deletion interval or result from remote effects on gene expression at other loci.

MATERIALS AND METHODS

This project was approved under the guidelines of the Ministry of Health of Costa Rica, the ethical committee of the National Children's Hospital in San José, California, USA, and the institutional review board at Mount Sinai School of Medicine, New York, USA, in accordance with the Declaration of Helsinki. These approvals remain active. The parents of patient 41A provided written informed consent.

We are conducting an ongoing genetic study of autism in the isolated founder population of the Central Valley of Costa Rica.[21] Occasionally, we are referred atypical cases including patient 41A, a female child that smiled often and was overly friendly with strangers. She had a normal karyotype (G banding at 550), was negative for fragile X and lacked a constellation of physical features consistent with a known syndrome. However, we hypothesised that despite the normal karyotype data, mental retardation or atypical behavioural features could be indicative of an underlying submicroscopic chromosomal abnormality, so we decided to screen this patient using array-based comparative genomic hybridisation (aCGH).

Array-based comparative genomic hybridisation

Labelling of DNA samples, including a dye swap, and hybridisation to the Spectral Chip 2600 (Spectral Genomics, Houston, Texas, USA) whole-genome bacterial artificial chromosome (BAC) array, was carried out at Spectral Genomics, according to the manufacturer's protocol. Hybridised microarray slides were analysed with GenePix 4000B scanner (Axon Ins, Union City, California, USA), and data obtained were analysed using the Spectralware V.2.24 software (Spectral Genomics). Normalisation of the Spectral aCGH data was performed using the global linear regression and pin linear options provided in the Spectralware V.2.24 software. Clones were considered to be positive for a duplication or deletion if the $\log_2$ fluorescence intensities of the spots were >2 standard deviations (SDs) beyond the mean ratio of intensities for the autosomes.

Fluorescence in situ hybridisation confirmation of aCGH results

BAC DNAs for validation of aCGH results and LB agar stabs of individual BAC clones were obtained directly from Spectral Genomics and BAC PAC Resources Center (San Francisco,

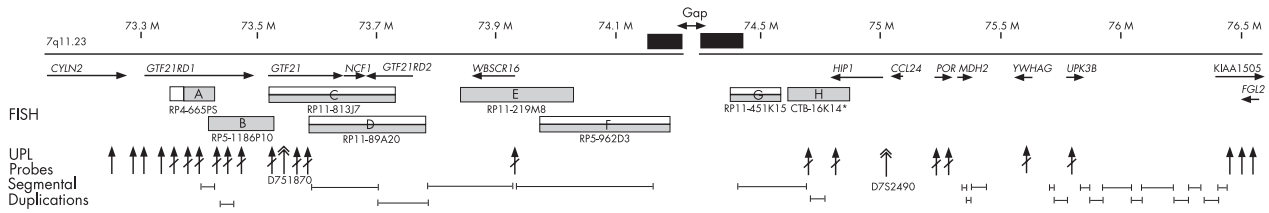

Figure 2 Atypical deletion of the distal William–Beuren syndrome (WBS) deletion interval of approximately 2.4–3.1 Mb. The proximal deletion breakpoint lies in intron 1 of *GTF2IRD1*, completely deleting *GTF2I*, *NCF1* and *GTF2IRD2*, the distal genes in the WBS deletion interval. Fluorescence in situ hybridisation (FISH) clones with hatch marks indicate that the subject is hemizygous for that sequence. Clones labelled with half-hatch marks gave diminished signals owing to cross hybridisation. All Universal Probe Library (UPL) probes listed in table 2 are displayed as upright arrows. A line crossing the arrow signifies that the subject is hemizygous for the sequence corresponding to the UPL probe. The double-headed upright arrow marks the location of microsatellites D7S1870 and D7S2490 used to determine the deletion that occurred de novo on a paternal chromosome. The location of segmental duplications, indicating highly repetitive sequence, has been approximated from the Database of Genomic Variants.

California, USA), respectively, and are listed in supplementary table A (details available online at http://jmg.bmj.com/supplemental). BAC DNA was prepared according to the product manual of the Nucleobond Plasmid Maxikit (BD Biosciences Clontech, San Jose, California, USA). Each BAC was labelled using the Nick Translation Reaction Kit (Vysis, Downer's Grove, Illinois, USA) and Spectrum Green-11-dUTP (Vysis) or Spectrum Red-11-dUTP (Vysis). Single or dual-coloured fluorescence in situ hybridisation (FISH) was performed on metaphase spreads from the peripheral blood of the participant or established lymphoblastoid lines dropped on to precleaned glass slides using a standard protocol. Images were captured with an ImagePoint cooled CCD video camera (Photometrics, Tuscon, Arizona, USA) through a Labophot-2A fluorescence microscope (Nikon, Melville, New York, USA) using Cytovision FISH software (Applied Imaging, San Jose, California, USA).

Genomic quantitative polymerase chain reaction for deletion mapping

We adapted the Universal Probe Library (UPL) system from Roche (Roche, Nutley, New Jersey, USA) to perform genomic quantitative polymerase chain reaction (PCR). The Roche system offers a library of eight-nucleotide fluorescence-labelled probes containing locked nucleic acids to ensure tight binding with the target sequence. Probes are chosen according to the primer sequences specified using ProbeFinder V.2.04 software (Roche, http://www.universalprobelibrary.com). This system is a precise and much cheaper alternative to traditional TaqMan assays, as the complete UPL covers >99% of the transcriptome using only 90 probes paired with transcript-specific primers.

Primers to be used with the UPL probes were designed using gene sequences from the UCSC Genome Browser (NCBI Build 35, http://genome.ucsc.edu/) and the ProbeFinder application. Supplementary table B includes all primers and probes used for the genomic deletion analysis (available online at www.jmg.bmjjournals.com/supplemental). Reactions were performed with a volume of 10 ml containing 25 ng DNA, 400 nM of each primer, 100 nM UPL probe (Roche) and 1× Platinum Quantitative PCR SuperMix-uracil-N-glycosylase, with Rox (Invitrogen, Carlsbad, California, USA). All reactions were performed in quadruplicate with an ABI Prism 7900HT sequence detection system (Applied Biosystems, California, USA). In addition to the case and parental DNA (case 41A, 41B father and 41C mother) and the genes to be assayed for copy number, we included a trio (child and two parents) from the CVCR as control genomic DNA samples (C1, C2 and C3) and four reference genes (*COBL*, *GUSB*, *PPIA* and *SNCA*) on each plate. PCR conditions were as follows: 2 min uracil-N-glycosylase activation at 50℃, then 2 min denaturation at 95℃, followed by 40 cycles of 95℃ for 15 s and 60℃ for 1 min.

Quantitative PCR to evaluate cDNA expression

cDNA was generated with SuperScript III First Strand kit according to the manufacturer's instructions. The products were diluted either 1:250 (for reference probes) or 1:25 (for all other probes), and 4.5 μl of the diluted reaction was used in the PCR reactions.

Four genes within the deletion interval and three reference genes were assayed by TaqMan gene expression probes (Applied Biosystems) according to standard protocols. Expression probes are listed in supplementary table C (available online at http://jmg.bmj.com/supplemental).

Quantitative PCR data analysis

The data were first evaluated using the Sequence Detection Software V.2.1 (Applied Biosystems). The baselines and thresholds for each gene were adjusted to measure Ct values at the most linear range possible. Quadruplicates having a SD >0.2 were evaluated and outlying Ct values >2 SDs from the mean of the quadruplicate were excluded from further analysis. The remaining Ct values were then fed into the qBase program[22] and analysed using the default options and settings. The reference genes were excluded from the analysis one at a time on the basis of the coefficient of variation, until two remained.[23] For genomic quantitative PCR experiments, relative quantification was performed and then calibrated by setting control DNA sample C2 to a value of 1. Single-copy deletions were detected when relative copy number values were <0.75. In the quantitative PCR expression assays, relative quantification was performed, calibrated by setting 41C to a value of 1, then all values were normalised to the mean of 41B and 41C as 1. (For raw data from both the genomic quantitative PCR

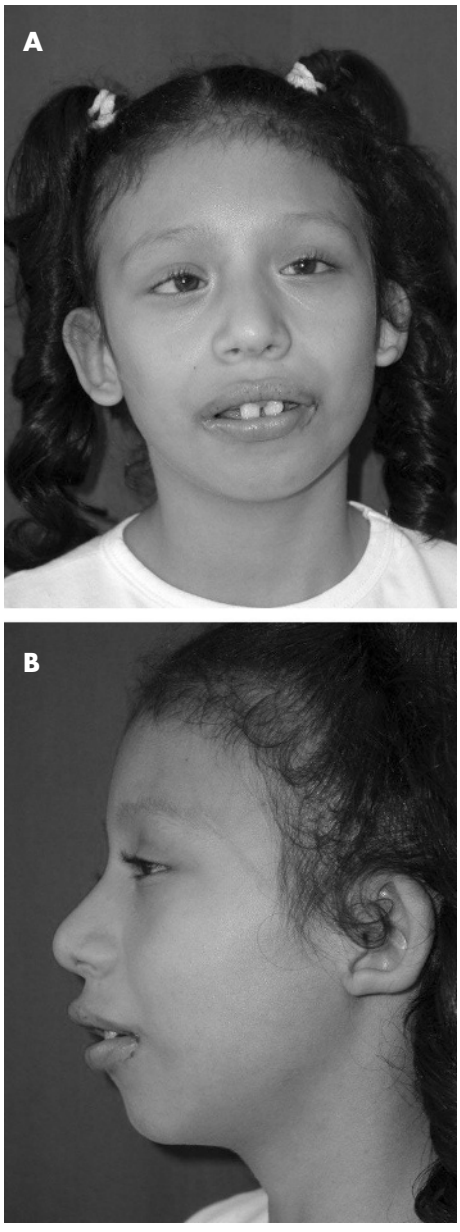

Figure 3 Anteroposterior (A) and lateral (B) photographs of patient 41A. Note the abnormal external rotation of the ear on the lateral photograph. Parental consent was obtained for publication of this figure.

mapping assays and quantitative PCR expression assays, refer to supplementary tables D and E, respectively, available online at http://jmg.bmj.com/supplemental.)

Microsatellite genotyping

The microsatellite primer pairs D7S1870 in *GTF2I* and D7S2490 at the distal end of *HIP1* were synthesised by Integrated DNA Technologies (Coralville, Iowa, USA). PCR conditions are available on request. The microsatellites were genotyped using the ABI 31-30 XL (Applied Biosystems) and genotype calls were performed using Genemapper V.3.7 (Applied Biosystems).

RESULTS

Patient 41A (age 6 years and 6 months at the time of interview)

The mother of patient 41A (41C, 18½ years old at the time of the child's birth) experienced bleeding in both the first and third trimesters and gained <7 kg during the pregnancy. The child was delivered at term without complications, although she was born with an orbital hemangioma that required surgery at 2 years and 8 months of age, resulting in strabismus. Her birth weight, height and head circumference were 2930 g (20th centile), 47 cm (10th centile) and 32 cm (<5th centile), respectively. (These norms are from the National Center for Health Statistics, as none are available for Costa Rica.) She has one younger male sibling who is in good health and had no perinatal complications. She did not have a social smile until she was 1 year old and had delayed acquisition of phrase speech (3½ years of age). She developed sphincter control at 42 months; other motor milestones were generally normal, or only mildly delayed. Her parents placed her in first grade in a regular school, but she had to be removed because of aggression against her peers and other disruptive behaviour. She is very interested in music, and certain sounds, but does not have hyperacusis according to parental reports, and clinical observation, although hyperacusis was not formally tested.

The patient met the Autism Diagnostic Interview–Revised criteria for autism in all three domains. Her most severe symptoms included deficits in the comprehension of simple language, articulation, reciprocal conversation, attention to voice, empathy and socialising with peers. She met the Autism Diagnostic Observation Schedule criteria for autism in the social, play and restricted behaviour domains, but was 2 points below the cut-off score in the communication domain. She smiles easily and is superficially friendly with strangers, although she exhibits minimal reciprocal social interaction. She also displays excessive non-social anxiety and moderately severe attention deficits.

Her cognitive profile on the Wechsler Preschool and Primary Scale of Intelligence—third edition[24] showed a verbal IQ of 53, a performance IQ of 57 and a Full-Scale composite IQ of 49, which places her in the extremely low range when compared with her peers. She had an IQ of 67 using the Leiter-R,[25] a non-verbal IQ test. Her verbal and performance abilities were also both in the extremely low range. We noted no significant differences in performance on the verbal subtests, suggesting that her verbal cognitive abilities are similarly developed. Notably, she showed an extreme weakness in visuospatial construction (block design score of 1; see supplementary tables Fa and Fb for full details of the IQ results, available online at http://jmg.bmj.com/supplemental). Developmental testing showed an adaptive behaviour composite score of 50, a communication skills summary score of 44, a daily living skills domain summary score of 64 and a social skills summary score of 54 (Vineland Adaptive Behavioral Scales).

At the time of the evaluation, the patient weighed 21 kg (50th centile), measured 120 cm in height (50th centile), and had a head circumference of 51 cm (50th centile). The results of our medical evaluation did not show any cardinal medical features of WBS, such as heart defects (negative echocardiogram and no heart murmur), joint laxity, loose skin or genitourinary dysfunction (negative renal ultrasound), although more extensive diagnostic testing might uncover such abnormalities. She also had no endocrine or serum calcium abnormalities and did not have feeding or sleeping difficulties as an infant. Her blood pressure was normal. Her head and facial features were remarkable for asymmetrical, abnormally shaped external ears, most prominent on the left side and iatrogenic strabismus due to the orbital hemangioma (fig 3). The only facial features consistent with WBS that she displayed include widely spaced teeth, a wide mouth and prominent lips. Specifically, she did not display dolichocephaly, bifrontal narrowing, mid-face hypoplasia, periorbital fullness, an upturned nose, a bulbous nasal tip or a long philtrum.

Array-based comparative genomic hybridisation

Analysing the aCGH data, clone RP11-89A20 showed a signal consistent with a deletion of this region (table 1). The two flanking clones, RP11-137M14 (proximal) and RP11-451M14 (distal), located approximately 3.3 Mb apart, were not deleted. Only the clones in the WBS deletion interval were considered for further follow-up. Refinement of the deletion interval was conducted with a combination of FISH and genomic qualitative PCR with UPL probes.

Deletion mapping by FISH and genomic quantitative PCR

Given the genomic location of the deleted BAC within the classic WBS deletion interval, we first tested the ~180 kb probe LSI *ELN* (Vysis) used to screen for the WBS deletion. The LSI *ELN* probe gave two clear signals on both metaphase

Table 1 Patient 41A: results of an array-based comparative genomic hybridisation from Spectral Genomics

Clone name	Cyto band	Cy5 test log ratios	Cy3 test log ratios	Clone gain or loss	Known genes in the BAC clones
CTC-232B23	1pter	1.542	0.803	G	CENTB5, SCNN1D, B3GALT6, DVL1, PUSL1, TNFRSF18, UBE2J2, TNFRSF4
RP4-703E10	1p36.32–1p36.33	1.399	0.647	G	Observed in 14/20 Costa Rican cases
RP1-45P21	6p22.2	1.36	0.733	G	Cluster of BTN genes in the extended MHC
B39H04	7q11.23	0.799	1.236	L	GTF2IRD1
RP11-89A20	7q11.23–7q11.23	0.774	1.244	L	GTF2I
RP11-89A20(E)	7q11.23	0.755	1.321	L	GTF2IRD2
RP11-65C22	17q24–17q24	1.487	0.797	G	Solute carrier family 39 (metal ion transporter)

BAC, bacterial artificial chromosome; G, gain (duplication); L, loss (deletion).
The data were analysed using the global linear and pin lowess options of the Spectral V.2.24 software. The known CNV column indicates whether this particular clone was duplicated or deleted in normal people. We indicate a new CNV because this clone was duplicated in 14/20 Costa Rican cases, with autism hybridised in the original study (results to be published elsewhere). Importantly, we tested many of the putative CNVs indicated by the array data and found them to be false-positive results, therefore we only followed up the most convincing findings in our patients.

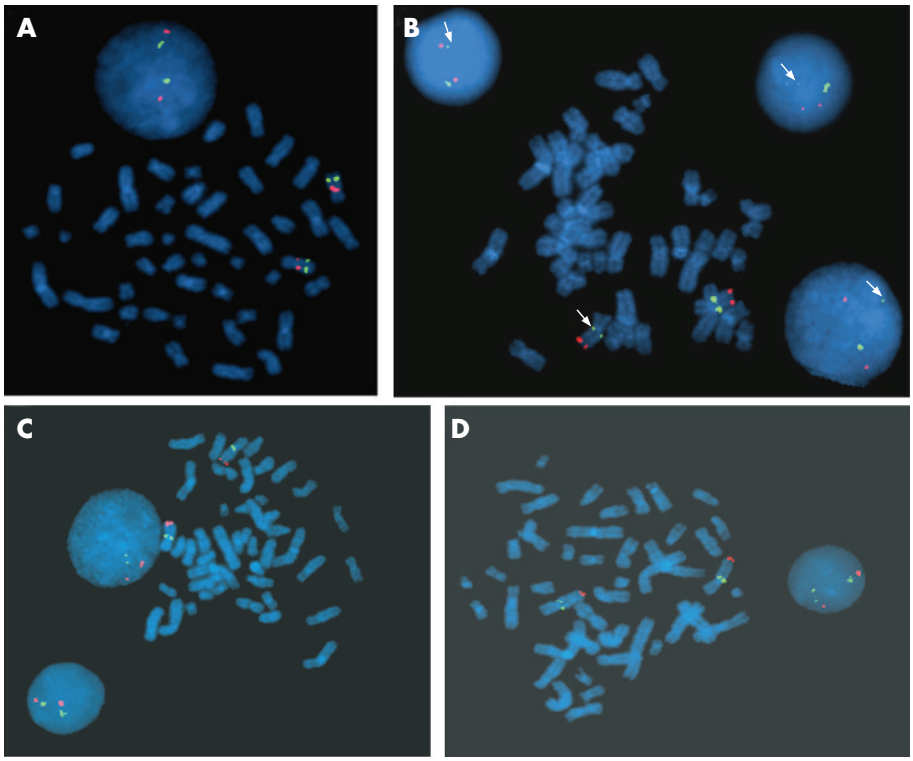

Figure 4 (A) Fluorescence in situ hybridisation (FISH) results for patient 41A performed with the William–Beuren syndrome (WBS) probe LSI *ELN* (Vysis). The *ELN* probe (orange signal) is labelled with Spectrum Orange dUTP and LSI D7S486, D7S522 (green signal) is labelled with spectrum green dUTP. (B) FISH results for patient 41A performed with the SGI BAC RP11-89A20 (green) labelled with spectrum green dUTP, and the control probe for the 7q subtelomere (orange, TelVysion 7q (Vysis)) labelled with spectrum orange dUTP. A normal green signal and a second weaker green signal (indicated with arrowheads) of varying intensity are observed on both metaphase chromosomes and interphase nuclei. Control probe signals appear equal in intensity on both chromosomes 7. (C, D). FISH results for the father and mother, respectively, of patient 41A, performed with the SGI BAC RP11-89A20 (green) labelled with spectrum green dUTP, and the control probe for the 7q subtelomere (orange, TelVysion 7q (Vysis)) labelled with spectrum orange dUTP. Two normal green signals are observed, and control probe signals are also of equal intensity on both chromosomes 7.

chromosomes and interphase nuclei, indicating that patient 41A does not carry a deletion of the *ELN* gene (fig 4A). We then selected a tiling path of FISH clones across the immediate interval surrounding RP11-89A20 to confirm the Spectral Genomics array data. The deletion was confirmed using the clones in supplementary table A (available online at http://jmg.bmj.com/supplemental), and the results are depicted schematically in fig 2. Figure 4B shows FISH results for RP11-89A20 (clone D in fig 2) that gave one normal signal and a second weak signal of varying intensity, although this clone was clearly deleted according to the Spectral Genomics software. A diminished signal could indicate that this segment of DNA was only partially deleted or that the BAC was partially hybridising to non-allelic homologous sequence elsewhere. The second alternative is most likely, given that others have observed a similar hybridisation pattern using FISH clones in the block B LCR[26]; the results obtained with the adjacent proximal and distal overlapping clones, the UPL probe data and the original Spectral Genomics array data all indicate a deletion. Hybridisation of RP11-89A20 (clone D) to paternal and maternal metaphase chromosomes and interphase nuclei showed two clear signals, indicating that this deletion is de novo in patient 41A (fig 5C,D). In addition, parental nuclei often displayed a second weak signal adjacent to a strong signal in many of the nuclei, as did the normal chromosome 7 from 41A. However, this second signal was often too close to resolve from the stronger signal hybridising to its identical sequence in block B and was only clearly visible as a discrete albeit weak signal on the deleted chromosome 7 of 41A.

BACs RP11-813J7 (clone C) and RP11-219M8 (clone E) also gave a normal signal and a second weaker signal when hybridised to patient 41A (fig 5A, B, respectively). BAC RP11-813J7 shares substantial overlap with RP11-89A20 and therefore displays a similar hybridisation pattern. Dual-colour FISH with PAC RP5-1186P10 (clone B) gave one signal indicating that this segment of DNA is deleted. BAC RP11-451K15 (clone G) gave a strong signal and a slightly diminished signal (fig 5C), consistent with partial cross hybridisation to other repetitive sequences as has been reported by Antonell *et al*.[26] Dual-colour FISH results for PACs RP4-665P5 (clone A) and RP5-962D3 (clone F) also showed two signals for each PAC, but again, the signals appear diminished on one chromosome 7 (fig 5D). Clone A is not completely deleted, as indicated by the UPL probe data (fig 6). Figure 5 (E, F, G) shows the results of interphase FISH with BAC CTB-16K14[27] (clone H) for patient 41A, her mother, and her father, respectively. CTB-16K14 is an unsequenced BAC from the Caltech BAC library. Nuclei of patient 41A exhibit a single hybridisation signal, indicating a deletion of the sequence containing *HIP1*, whereas both parents have normal hybridisation patterns, with two signals of equal intensity.

These data are also consistent with the fine-mapping UPL probe data (fig 6, raw data in supplementary data table D, available online at http://jmg.bmj.com/supplemental) that place the centromeric breakpoint of the deletion within the first intron of *GTF2IRD1* in clone A, and the telomeric breakpoint somewhere between gene *UPK3B* and the putative gene KIAA1505. Note that a gap in the genomic sequence exists

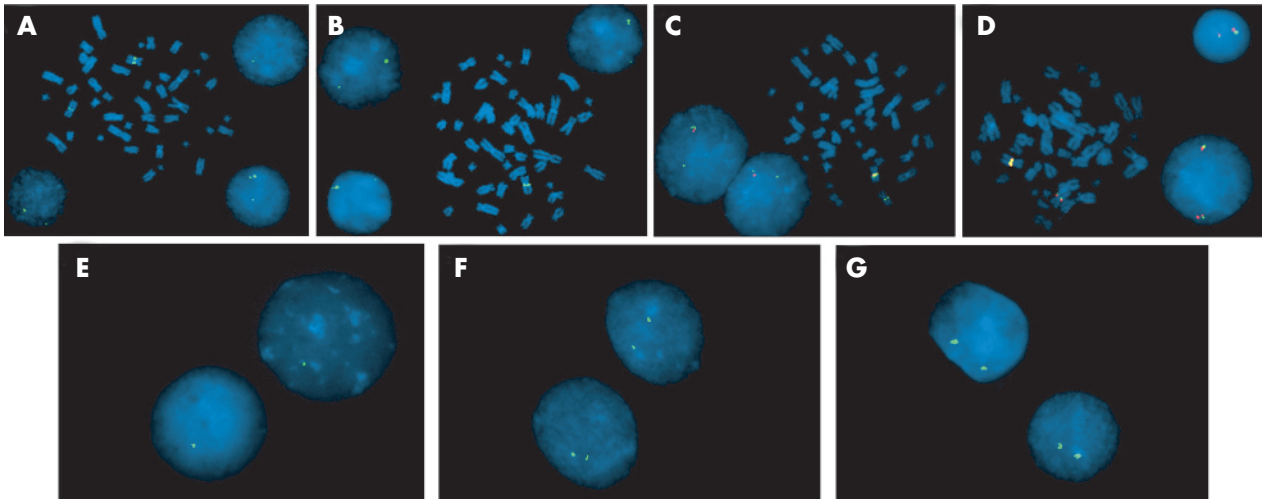

Figure 5 Fluorescence in situ hybridisation (FISH) results for patient 41A performed with BAC and PAC clones that span the distal end of the William–Beuren syndrome (WBS) region (fig 2). (A) BAC RP11-813J7 labelled with spectrum green dUTP. A normal green signal and a second weaker signal are observed on both metaphase chromosomes and interphase nuclei. (B) BAC RP11-219M8 labelled with spectrum green dUTP. A normal green signal and a second faint signal are consistently observed on both metaphase chromosomes and interphase nuclei. (C) Dual-colour FISH results with PAC RP5-1186P10 (spectrum red dUTP) and BAC RP11-451K15 (spectrum green dUTP). A single red signal and two green signals of equal intensity are present. (D) Dual-colour FISH results for PACs RP4-665P5 (spectrum green dUTP) and RP5-962D3 (spectrum red dUTP). (E, F, G) Interphase FISH results with BAC CTB-16K14[27] (spectrum green dUTP) for patient 41A, her mother and her father, respectively. Nuclei of patient 41A exhibit a single green hybridisation signal, whereas both parents have normal hybridisation patterns, with two signals of equal intensity.

between *WBSCR16* and *HIP1*; therefore, placement of additional UPL probes in this region was not possible. Supplementary table B contains a list of the genes in the proximal portion of the deleted interval, upstream of the gap in the genomic sequence and downstream of the gap, respectively (http://jmg.bmj.com/supplemental).

Analyses of gene expression

Quantitative reverse transcriptase-PCR analyses of *GTF2IRD1*, *GTF2I*, *HIP1* and *YWHAG* were performed on cDNA generated from RNA extracted from transformed lymphocytes derived from patient 41A, her father (41B) and her mother (41C). Expression of *GTF2I* was decreased to about 60%, and expression of *YWHAG* was decreased to about 50%, in patient 41A compared with both parents (supplementary data fig A, supplementary table C, raw data in supplementary data table E, available online at http://jmg.bmj.com/supplemental). Expression of *HIP1* was unchanged in either patient 41A or in the WBS control. However, expression of *GTF2IRD1*, using probes targeting exons 2 and 11, deleted in patient 41A, was increased in both patient 41A and the WBS control (NIGMS cell line GM13460, Coriell) and was at least 2.5 times the expression observed in 41C. The father, 41B, exhibited an intermediate increase in expression for both *GTF2IRD1* probes that fell between the levels of the deletion carriers and 41C. The abnormal expression values for *GTF2IRD1* in the father may be

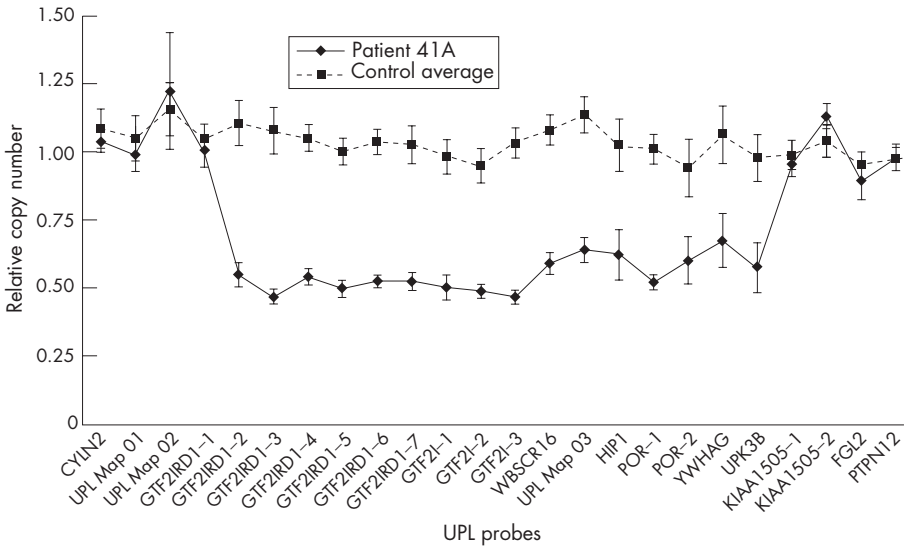

Figure 6 Graph of the Universal Probe Library (UPL) probe data used to fine map the deletion carried by patient 41A, displaying relative genomic copy numbers and standard deviations (SDs) from the qBase program. The control average series is the mean of the values and SDs of the three control DNA samples (C1, C3 and the calibrator C2) as well as the parents of patient 41A (41B and 41C). Single-copy number deletions were detected in the patient when relative quantification values dropped to <0.75. The deletion spans from probe *GTF2IRD1*-2 to *UPK3B*.

indicative of some kind of genomic rearrangement; however, we were unable to detect the parental inversion that has been described previously in the father.[28]

Microsatellite data

Data from microsatellites upstream, D7S1870 and downstream of the gap, D7S2490 (fig 2), indicate that the deletion in our patient occurred de novo on a paternal chromosome (data not shown).

DISCUSSION

Our results show that hemizygosity of the GTF2 transcription factors is sufficient to produce multiple aspects of the WBS CBP, including impaired visuospatial construction abilities, an overly friendly personality accompanied by excessive non-social anxiety and language delay. Although mental retardation and autistic traits in patient 41A might well be a consequence of a reduced dosage of the GTF2 transcription factors, these symptoms might also be caused or exacerbated by other genes deleted in patient 41A, the prime candidates being *HIP1* and *YWHAG*. Expression of *GTF2I* and *YWHAG* in transformed lymphocytes from patient 41A was reduced to 60% and 50% of the parental values, respectively. Expression of *GTF2IRD2* was not evaluated, as this gene has an extra copy in humans that is transcribed, although it is not clear if it is functional. The expression data for *GTF2I* in patient 41A falls within the range described in a previous analysis of gene expression of transformed lymphocytes from 10 full WBS deletion carriers and their parents using semiquantitative reverse transcriptase-PCR (range 42–70%).[29] We found increased expression of *GTF2IRD1* in both our cases and in the WBS control cell line. These data are consistent with a recent report documenting decreased expression of *GTF2IRD1* in cultured skin fibroblasts from cases with typical WBS deletions, but normal or even increased expression of *GTF2IRD1* in transformed lymphocytes.[30] Only one group has reported decreased expression of *GTF2IRD1* in a typical WBS deletion carrier[16]; the same group also found decreased expression of *GTF2IRD1* in the atypical deletion carrier HR using semiquantitative reverse transcriptase-PCR. The reason for this discrepancy is unclear; however, expression of *GTF2IRD1* in transformed lymphocytes is not representative of gene expression in fibroblasts, and different regulatory elements are probably important for region-specific expression. Finally, expression of *HIP1* was reduced in both transformed lymphocytes and skin fibroblasts from typical WBS deletion carriers who have two copies of *HIP1*[30]; however, we did not observe decreased expression of *HIP1* in our patient or in the WBS typical deletion carrier used as a control. This discrepancy might have arisen because our Taqman probe was designed to the 5′ untranslated region of *HIP1*, whereas the probe used by Merla *et al*[30] targeted exon 31 of *HIP1*. Thus, it is possible that we have measured different splice forms and that expression of only one of the splice forms is altered.

GTF2 transcription factors, visuospatial ability and symptoms of autism

In addition to evidence from atypical WBS deletion carriers, other data also implicate *GTF2I* in visuospatial ability. Holinger *et al*[31] examined neurons in the brain of five patients with WBS and five age-matched controls and observed a lack of staining for GTF2I in neurons from the posterior parietal lobe, Brodmann area 7. These neuropathological data are consistent with a functional MRI study showing isolated hypoactivation of the parietal portion of the dorsal visual stream in 13 patients with WBS (and normal IQ) on a task designed to test the integrity of both the dorsal and ventral visual streams.[32] Additionally, a recent study showed down regulation of *GTF2I* in association with age-dependent deficits of hippocampal-dependent spatial memory in mice.[33] Furthermore, defects in the anterior hippocampus have been noted in a structural and functional imaging study of patients with WBS.[34] Therefore, reduced expression of *GTF2I* in the hippocampus may contribute to impaired spatial cognition and navigation observed in patients with WBS.[35]

Other evidence suggests that the GTF2 family of transcription factors might contribute to abnormalities in speech and language observed in patients with autism and WBS. For example, Laws and Bishop[5] studied language impairment and social deficits in traditional WBS deletion carriers and found many similarities between autism spectrum disorders and WBS, especially in the realm of pragmatic language. In fact, patients with WBS resemble patients with Asperger's disorder in that they often fail to discriminate relevant from irrelevant information in conversation, and their conversational content is often incongruent with the conversational intention of others. They may also convey a paucity of information despite apparent volubility and an extensive vocabulary. Another group found that children and adults with WBS did poorly on a basic test of emotional recognition from facial and vocal cues, especially negative emotions, despite the fact that people with WBS are drawn to faces and seek eye contact.[8] Finally, a duplication of the WBS deletion interval has been associated with abnormalities in language,[29] and delayed acquisition of language is characteristic of both WBS and autism; therefore the GTF2 transcription factors could also have a role in normal speech and language development.

Other candidate genes for autism outside the WBS deletion interval

Of the roughly 14 genes deleted downstream of the WBS deletion interval, *HIP1* and *YWHAG* are the most compelling candidates for susceptibility to autistic traits. HIP1 is a multifunctional nucleocytoplasmic protein that was first identified in screens for proteins that interact with Huntingtin.[19] It is a proapoptotic mediator involved in the differentiation and growth of hippocampal neurons[36] and also mediates the endocytosis of GluR1-containing AMPA receptors in primary hippocampal neurons.[37] *Hip1*-null mice show no gross changes in brain morphology, or hippocampal or striatal cell densities and appear normal during embryogenesis, yet develop a neurological phenotype by 3 months of age, characterised by wasting, tremor and gait ataxia secondary to a rigid thoracolumbar kyphosis. (Patient 41A does not have a history of spinal abnormalities.) *HIP1* exhibits dosage sensitivity in mice.

The other gene in the deletion interval that might confer aspects of the autism phenotype is *YHWAG*, a member of a conserved multigene family of phosphopeptide-binding proteins involved in signal transduction, protein localisation, cell-cycle checkpoint control and apoptosis.

CONCLUSION

In summary, our most important finding is that, in addition to *GTF2IRD1*, hemizygosity of *GTF2I* is necessary for full manifestation of the visuospatial construction deficit that is a core component of the WBS CBP. *GTF2I* may also contribute to impaired hippocampal-dependent spatial navigation, and other roles for this gene related to language or the overly friendly personality are also possible. Finally, several genes both flanking and within the WBS deletion interval could be candidate genes for autism susceptibility owing to their role in neurodevelopment. Association studies and mutation screens targeting the genes discussed in this paper are warranted

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The supplementary tables are available at http://jmg.bmj.com/supplemental

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