article

Williams syndrome deficits in visual spatial processing linked to GTF2IRD1 and GTF2I on Chromosome 7q11.23

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Purpose: To identify the relationship between specific genes and phenotypic features of Williams syndrome. **Methods:** Subjects were selected based on their deletion status determined by fluorescence in situ hybridization using a panel of 24 BACs and cosmids spanning the region commonly deleted and single gene analysis using Southern blotting. From the cohort of subjects, three had atypical deletions. Physical examinations and cognitive tests were administered to the three subjects and the results were compared to those from a cohort of typical WS subjects. **Results:** The molecular results indicate smaller deletions for each subject. In all three cases, typical Williams facies were absent and visual spatial abilities were above that of full deletion WS subjects, particularly in the qualitative aspects of visual spatial processing. **Conclusions:** Combining the molecular analysis with the cognitive results suggest that the genes GTF2IRD1 and GTF2I contribute to deficits on visual spatial functioning. ***Genet Med* 2003:5(4):311–321.**

Key Words: *Williams Syndrome, atypical deletion, chromosome 7q11.23, GTF2IRD1, GTF2I, visual-spatial cognition.*

We employ the rare disorder (1:20,000 live births), Williams syndrome (WS), to explore the genetic basis of human cognition and behavior. WS is a neurocognitive disorder commonly caused by a 1.5Mb deletion containing about 20 genes on chromosome band 7q11.23,[1,2] including the FK506 binding protein 6 (FKBP6),[3] human homolog of the *Drosophila* gene, *frizzled* (FZD9),[4] bromodomain adjacent to Zinc finger domain 1B (BAZ1B),[5–7] B-cell lymphoma 7 (BCL7B),[8] Transducin-beta like 2 (TBL2),[9] Williams syndrome basic helix-loop-helix (WS-bHLH),[10] syntaxin 1A (STX1A),[11] Claudin 3 (CLDN3),[12–14] Claudin 4 (CLDN4),[12,13] elastin (ELN),[15–17] LIM-kinase 1 (LIMK1),[18] eukaryotic initiation factor 4H (EIF4H),[19] heat-shock protein C046 (HSPC046),[20] replication factor C, subunit 2 (RFC2),[21,22] cytoplasmic linker protein (CYLN2),[23] GTF2I repeat domain containing protein 1 (GTF2IRD1),[24–27] and general transcription factor II-I (GTF2I).[28] The interest in WS is derived from the pattern of neurocognitive peaks and valleys, characterized by deficits in visual spatial processing and relative strengths in language and face recognition.[29] Other features of WS include facial features such as flat malar region, full cheeks, periorbital fullness, full nasal tip, prominent lips and wide mouth; cardiovascular disease which includes supravalvular aortic stenosis (SVAS) and/or peripheral pulmonary stenosis (PPS); transient neonatal hypercalcemia; a hoarse voice; gregarious personality; failure to thrive in infancy; and delayed language and motor milestones.[30]

Previous studies of individuals with smaller deletions have provided clear evidence that the gene for elastin is responsible for the congenital cardiovascular deficits of WS[15] but the contribution of these analyses to understanding the genes responsible for the cognitive deficits has been less clear. Determining the genes contributing to the distinct cognitive findings of WS has been challenging, in that the majority of subjects with WS physical features carry similar deletions[31–33] but express variable cognitive function.

In efforts to correlate genes to cognitive phenotypes, evaluations of cognition in subjects with smaller deletions have utilized similar standardized measures. However, such subjects are rare and detailed analyses of their cognitive domains are limited. The work in this paper provides the detailed molecular analyses of three subjects with smaller deletions and combines this with the results of their cognitive features as shown by performance on standard psychometric instruments. The results provide evidence to support the involvement of many regions in WS cognition but implicate the genes, GTF2I repeat domain-containing protein 1 (GTF2IRD1)[24] and general tran-

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scription factor II-I (GTF2I)[28] as disproportionately involved in the visual spatial deficits.

MATERIALS AND METHODS

Patients

We have previously shown the phenotypic spectrum of WS individuals from Japan. This study began with 60 Japanese subjects clinically diagnosed with WS.[34] The physical exams were conducted by clinicians with experience in genetic disorders. From the 60 individuals, further molecular studies identified three individuals with atypical deletions that were smaller than those commonly observed in any previous study.[31–33] These three cases became the focus of our study and a summary of their features is shown in Table 1.

Case 1 is a Japanese female who weighed 38 kg (−2SD) and measured 150 cm (−2SD) in height upon physical exam at 23 years of age. She had initially presented with cardiovascular anomalies including SVAS and PPS and because of a 110 mmHg pressure gradient from her SVAS, she underwent cardiac repair at age 18 years. She had no hoarse voice or facial features typically seen in Japanese individuals with WS. Magnetic Resonance Imaging (MRI) of the brain was read clinically as within normal limits, but no volumetric or statistical analyses were performed. She had no documented history of hypercalcemia, hyperacusis, or joint problems. After graduating from Universal Junior High School, she worked at a packaging company and was hoping to marry in the future.

Case 2 is a Japanese female who weighed 67 kg (+2SD) and measured 149 cm (−2SD) in height at 14 years of age. Upon physical exam at 17 years of age, she had cardiovascular anomalies including SVAS and PPS, and had a hoarse voice and dental anomalies, but did not have typical facial features. MRI of the brain was read as within normal limits, but no volumetric or statistical analyses were performed. She had no medical history of hypercalcemia, hyperacusis, or joint problems. She attended nursing school and acquired formal certification.

Case 3 is a Japanese female who weighed 34 kg (−1SD) and measured 131 cm (within normal limits) in height when she was seen at age 10. She did not have the typical facial features of WS or a hoarse voice observed in most WS patients. MRI of the brain was read as within normal limits, but no volumetric or statistical analyses were performed. Her medical history had no documented incidents of hypercalcemia, hyperacusis, or joint problems.

FISH analysis

Human metaphase and/or interphase chromosome preparations were made from Epstein-Barr virus transformed lymphoblastoid cell lines and/or peripheral blood by standard methods.[35] Fluorescence in-situ hybridization (FISH) analysis was performed with multi-color FISH using bacterial artificial chromosomes (BAC), P1-derived artificial chromosomes (PAC), or cosmid DNAs as described previously[35] and are indicated in Table 2 and in Fig. 1. To determine whether a given probe was deleted for each individual, more than 20 metaphase cells were evaluated and scored for the presence, absence or intermediate signal from a test probe hybridized simultaneously with a control probe located outside the common deleted region or whose deletion status was established by independent experiments. Deletions were defined in cells with two nonoverlapped chromosomes, with probes that generated signals on one chromosome but control signals were generated on both.

Construction of a physical map of the WS region

A physical map (Fig. 2) of the largely single copy region deleted in WS was generated using BACs, PACs, and cosmid clones as described previously.[35] PAC clone 632H11 and 391G2 were described in Meng et al., 1998.[3] All cosmid clones were obtained as gifts from the L.C. Tsui group at the Department of Genetics, Hospital for Sick Children, Toronto, Canada.[36] The position of each clone used for the analysis of subjects' breakpoints was confirmed by the polymerase chain reaction (PCR) using published sequence data for gene sequences, as described in Korenberg et al., 2000.[35] Clones shown on the map in Figure 2 were used for FISH analysis.

DNA extraction and dosage blotting

Genomic DNA from Epstein-Barr virus transformed lymphoblastoid cell lines was prepared using Puregene DNA Isolation Kit (Gentra, Mineapolis, Minnesota). DNA samples isolated from subjects 1 to 3, from normal controls, and from WS subjects with typical deletions were then digested with PSTI, size fractionated by agarose gel electrophoresis, and blotted onto nylon membranes (Amersham Hybond N). For the FZD9 gene, a 283 bp probe for the cDNA encoding FZD9 was generated by PCR using the following primers, (FZD9 forward: 5'-tgtcaaggtcaggcaagtgag-3'; FZD9 reverse, 5'-ctcacctcctaccttc-ccccttcccagcca-3'). For the FKBP6 gene, a 523-bp probe from exon 9 was generated as described in Meng et al., 1998.[3] These cDNA fragments on a control plasmid (D17S HHH202) mapping to chromosome 17 were labeled by random priming and hybridized as described.[37] To determine sequence copy number, the ratio between the two chromosome fragments and control bands was determined within each DNA sample and compared to normal control DNAs by quantitative densiometry.[37] A representative autoradiogram is shown in Figure 3.

Psychometric testing

For this study, the Wechsler Adult Intelligence Scale-Revised (WAIS-R)[38] and Wechsler Intelligence Scale for Children-Third edition (WISC-III)[39] were used to compare the cognitive profiles of our subjects to individuals with typical WS[40] (Searcy et al., unpublished data, 2003). The sample size of our full deletion group includes 91 North American WS adolescents and adults with detailed genetic analyses. The WAIS-R and WISC-III consist of 11 and 10 subtests, respectively, grouped into measures of verbal intellectual ability (Verbal IQ; VIQ) and visual spatial intellectual ability (Performance IQ; PIQ) that together yield a Full Scale IQ (FSIQ). All subjects were administered the WAIS-R or WISC-III according to standardized instructions. Case 1 and Case 2 were administered the Japanese version of the WAIS-R at the Psychological Corpora-

Table 1
Physical Features of atypical Japanese cases compared to North American WS and Japanese WS cases

	North American cases[a] 315 subjects (% incidence)	Japanese cases[b] 60 subjects (% incidence)	Case 1 Japanese	Case 2 Japanese	Case 3 Japanese
GA, > 41W	(50)		38W	40W	40W
Birth weight, SGA, mean = 2600 g	(25–70)	28/60 (47)	2960g	2500g	2600g
hypercalcemia	(15)		U	U	U
Craniofacial		56/60 (93)			
Dolichocephaly	+	+	−	−	−
Bitemporal narrowing	+	+	−	−	−
Medial eyebrow flare	+	+	−	−	−
Periorbital fullness	+	+	−	−	−
Epicanthal folds	+	+	−	−	−
Stellate irides	+	+	−	−	−
Full nasal tip	+	+	−	−	+
Short, upturned nose	+	+	−	−	+/−
Flat malar region, full cheeks	+	+	−	−	+
Long smooth philtrum	+	+	−	+/−	−
Full lips	+	+	−	+/−	+/−
Dental abn/malocclusion	(85)	+	+	+	+
Cardiovascular disease, any abn	(80)	59/60 (98)			
SVAS	(75)	51/60 (85)	+	+	+
PPS	(50)	20/51 (40)	+	+	
Mitral valve prolapse/VSD	(10)	11/51 (21.5)	−	−	−
High blood pressure	(50)		−	−	−
Skeletal and connective tissue					
Joint hyperelasticity	(90)		−	−	−
Joint contractures	(50)		−	−	−
Kyphosis	(20)		−	−	−
Lordosis	(40)		−	−	−
Radioulnar synostosis	(20)		−	−	−
Inguinal/umbilical hernia	(40/50)	12/60 (20)	−	−	−
Genitourinary tract					
Congenital malformation	(20)		−	−	−
Enuresis/bladder dysfunction	(50)		−	−	−
Nephrocalcinosis	(<5)		−	−	−
Others					
Strabismus	(50)		+	−	−
Esotropia	(50)		−	−	−
Hoarse voice	+		−	+	−
Hyperacusis	(80)		−	−	−

[a]Morris, et al. 1988.
[b]Kimura, et al. 2000.
GA, Gestational age; SGA, Small for gestational age; SVAS, Supravalvular aortic stenosis; +, Present; −, Not Observed; U, Unknown.

Table 2
FISH analysis showing approximate deletion spans in the atypical Japanese cases and in typical subjects with WS

	D7S489U																					D7S489L		
	B1008H17	C12915	B315H11	P632N4	P391G2	P195h6	C82c2	B592D8	C34b3	C152a8	C128d2	C102f12	C135f3	B155B1	B363B4	C82b11	C209c11	C47d1	C160g4	C183e1	P267N24	C15e11	B1184P14	B239C10
Typical WMS	+/−	+/−	−					−															−	+/−
Case 1	+/−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+/−	+	+	+	+
Case 2	+/−	−	−	−	−	−	−	−	−	−	−	+/−	+	+	+	+	+	+	+	+	+	+	+	+
Case 3	+/−	+/−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+

−, Deletion; +, No deletion; +/−, Partial Deletion; B, BAC clone; P, PAC clone; C, Cosmid clone.

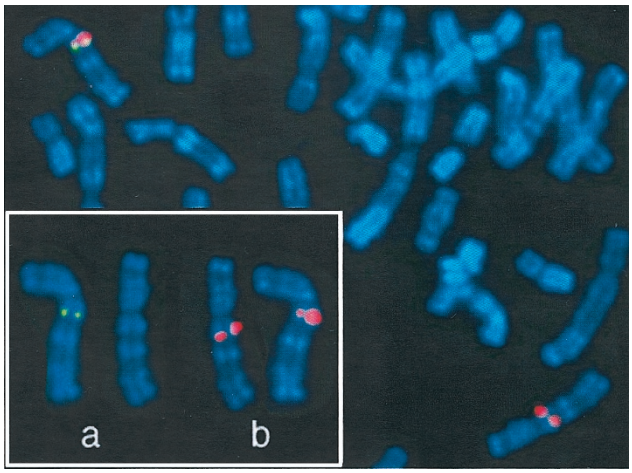

Fig. 1 Cytogenetic analysis of WS with dual color-FISH (Fluorescence in situ hybridization). Two differently labeled BAC DNA probes, (a) BAC 592D8 for ELN (FITC, green) and (b) BAC 1184P14 for GTF2I (Cy3, red), were cohybridized to reverse-banded metaphase chromosomes derived from Case 1 lymphoblastoid cell line and reverse-banded with Chromomycin and Distamycin A3. The gene ELN was seen deleted on chromosome band 7q11.23, whereas GTF2I showed clearly no deletion.

tion, Japan. Case 3 was administered the Japanese version of the WISC-III at Tokyo Women's University, Japan. Experienced professionals conducted all tests.

Factor analytic studies of the WAIS-R standardization group[41,42] have most consistently identified a three-factor structure for the WAIS-R: Verbal Comprehension (VC), Perceptual Organization (PO), and the Freedom from Distractibility(FD). The WISC-III has a similar factor structure, but also includes a fourth, Processing Speed factor, that was not analyzed because the subtests necessary to calculate this index were not administered to all subjects. These indices measure verbal knowledge and understanding obtained by formal and informal education (VC), the interpretation and organization of visually presented material (PO), and the ability to attend and concentrate (FD). In addition to individual subtests, these factor scores were used for further analysis when comparing the small deletion WS cases to our North American full deletion WS group. We next determined individual strengths and weaknesses in the Verbal and Performance subtests by comparing each subtest's scaled score to the subject's scaled scores averaged across all subtests. For a given individual, scaled scores that were significantly above or below the mean scaled score were interpreted as specific strengths or weaknesses.[42]

The above analyses of performance on the Wechsler scales utilized quantitative methodology, looking for statistical differences between the special deletion cases and our sample of full deletion WS. However, given the small number of special cases, statistical comparisons of performance may be less informative than qualitative evaluations. Qualitative analysis was therefore undertaken to examine types of error and strategies used for individual problem solving. We examined performance on the subtests of the Wechsler scales, as well as performance on a drawing copy task of an elephant, from the Boston Diagnostic Aphasia Examination (BDAE).[43,44] We were particularly interested in the integration of global and local aspects of visual spatial processing due to reports of a local processing bias in full deletion WS.[45] Our qualitative analysis followed the principles of the Boston Process Approach that is commonly employed in the clinical neuropsychological examination.[46,47]

RESULTS

Clinical findings

All three subjects in our study were ascertained initially by cardiovascular anomalies. The features of Williams syndrome including the facial features are quite distinct to Japanese physicians. However, the typical physical features of individuals with WS were not observed in our three subjects. This was considered in the construction of Table 1, which shows the subset of features observed in each subject in comparison to features previously defined in Japanese and North American subjects with WS. It is of interest that the deletion appears to cause facial features that are comparable within ethnic groups. Permission for photographs was not obtained and no further data were permitted to be shown.

FISH analysis

Typical WS subjects with the common deletion

To estimate the size of the deletions, FISH analysis was performed on 31 cases using 592D8 (containing the ELN and

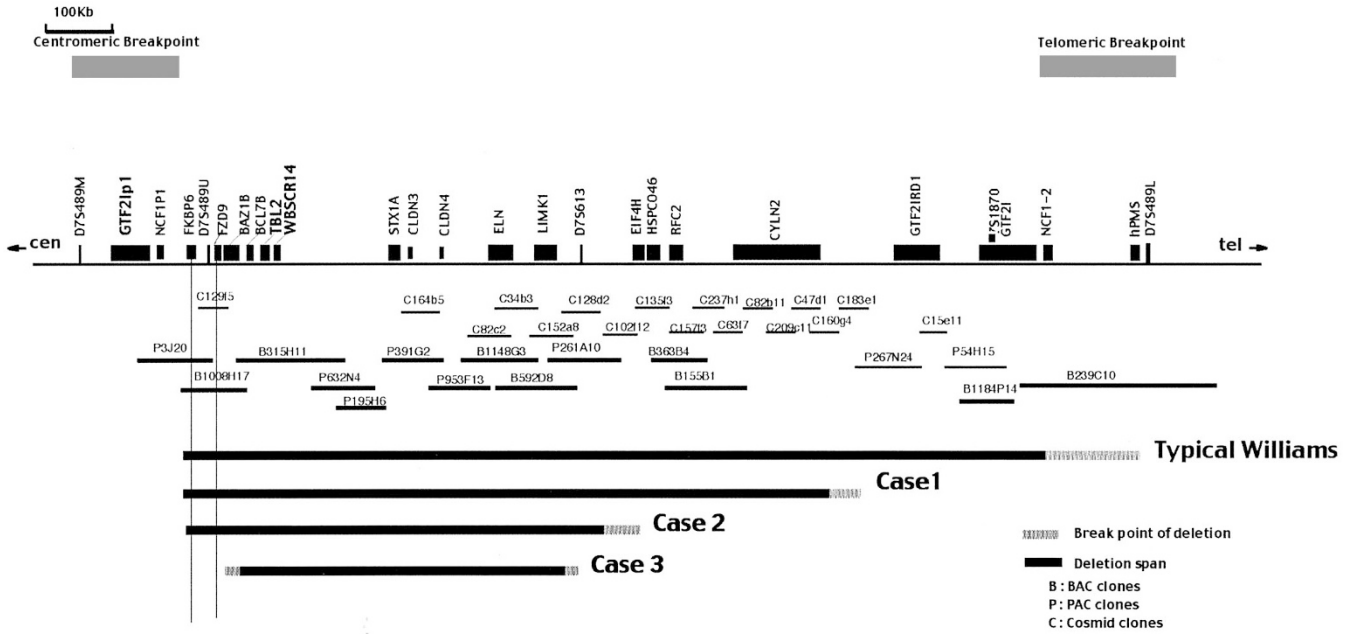

Fig. 2 Physical map of the common WS deletion. Genes mapping in this region are represented by black boxes (names reading vertically). BAC, PAC, and cosmid clones spanning this region are indicated below the genes and are described in the Methods. The black horizontal lines depict the approximate size and extent of deletions in the 3 cases with atypical deletions and in typical subjects with WS.

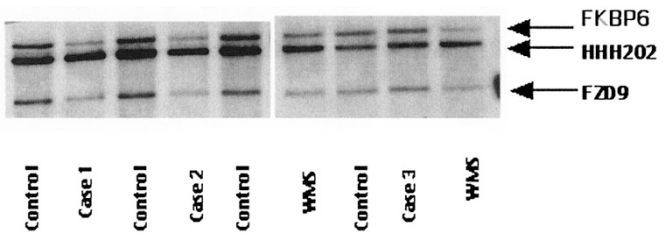

Fig. 3 Representative autoradiograms from Southern blot dosage analysis of FZD9 and FKBP6. Alternating lanes containing DNAs from diploid control and Cases 1, 2, and 3 are indicated. Autoradiographic bands corresponding to each DNA sequence are noted at the right of the figure.

LIMK1 genes) and 1184P14 (containing the 5' end of GTF2I gene and marker D7S1870). The two clones showed no signal in 11 typical WS cases. Further analysis was performed using 1008H17 (containing the genes for FZD9 and FKBP6 genes) in 11 cases. The results indicated that BAC 1008H17 generated intermediate signals in 8 cases. Preliminary analyses on a subset of these were referred to in Kimura et al., 2000.[34] The table of results is shown on our website.[48]

Three atypical subjects with smaller deletions

The results of the three cases are summarized in Table 2. The FISH analysis performed in the three atypical cases utilized 24 clones spanning the common deletion indicated in Fig. 2 including BACs (1008H17, 315H11, 592D8, 363B4, 155B1, 1184P14, and 239C10), PACs (632N4, 391G2, 195H6, and 267N24), and cosmids (129f5, 82c2, 34b2, 152a8, 128d2, 102f12, 135f5, 82b11, 209c11, 47d1, 183e1, 160g4, and 15e11). These cover the region from D7S489U to D7S1870 on the physical map. The results for Case 1 indicate that no signals were observed for cosmids 129f5 through 183el and intermediate signals were observed for BAC 1008H17 and cosmid 160g4. Case 2 had no signals detected from cosmids 129f9 through 128d2 and intermediate signals were seen for BAC 1008H17 and cosmid 102f12. The FISH analysis for Case 3 exhibited no signal for BAC 315H11 to cosmid 152a8, with signals from BAC 1008H17 partially detected.

Gene dosage analysis

Because cosmids and BACs containing the region of FKBP6 and FZD9 appear nondeleted in many subjects with WS, gene dosage analysis was performed in 10 typical WS cases; in Case 1, Case 2, and Case 3, and in normal control subjects using the Southern blotting technique to evaluate the deletion of FKBP6 and FZD9. The ratio of intensities of these fragments versus the control fragments was approximately 0.5 in typical WS cases and Case 1 and Case 2. For Case 3, the band intensities corresponding to FKBP6 and FZD9 were not significantly different from 1.0.

In summary, the genes deleted in the three atypical cases were inferred from the FISH analysis and dosage analysis. In Case 1, the deletion spans from the FKBP6 through CYLN2. The deleted region did not include GTF2IRD1 and GTF2I. The deletion in Case 2 extends from FKBP6 through LIMK1 and did not include EIF4H, RFC2, CYLN2, GTF2IRD1, and GTF2I. In Case 3, the deletion is defined from approximately BAZ1B through LIMK1 and did not include FKBP6 and FZD9 or EIF4H, RFC2, CYLN2, GTF2IRD1, and GTF2I.

Table 3
Summary of WAIS-R and WISC-III results of Japanese cases

WAIS-R results			
Verbal subtests	*Typical WS (SD)	Case 1, 28 y/o	Case 2 21 y/o
Vocabulary	5.21 (2.11)	3	2
Similarities	6.46 (1.8)	4	7
Information	4.68 (2.14)	3	2
Arithmetic	3.79 (1.58)	4	3
Digit span	5.01 (1.95)	8	5
Comprehension	4.66 (2.05)	4	8
Performance subtests			
Picture completion	5.04 (1.72)	6	3
Block design	4.0 (1.71)	5	2
Picture arrangement	5.28 (2.05)	5	11
Object assembly	3.63 (2.27)	9	3
Coding/digit symbol	3.91 (1.4)	7	4
VIQ	71.79 (8.07)	64	65
PIQ	66.18 (8.09)	72	56
FSIQ	67.67 (8.25)	64	55
VCI	73.42 (9.29)	64	71
POI	66.34 (10.05)	81	57
FDI	67.65 (8.99)	77	65
WISC-III results			
Verbal subtests	WISC-R **Typical WS	Case 3, 12 y/o	
Vocabulary	3.47 (2.3)	4	
Similarities	5.33 (3.4)	8	
Information	3.13 (2)	4	
Digit Span	3.69 (1.5)	6	
Comprehension	4.6 (1.9)	5	
Arithmetic	2.07 (1.7)	1	
Performance subtests			
Picture completion	4.67 (2.7)	1	
Block design	2.2 (1.5)	4	
Picture arrangement	3.47 (2.6)	2	
Object assembly	2.36 (2.1)	5	
Coding/digit symbol	2.33 (2.4)	3	
VIQ	61 (10.9)	65	
PIQ	54.3 (10.2)	51	
FSIQ	54 (10)	54	
VCI	65.50 (11.47)	72	
POI	56.05 (10.44)	55	
FDI	50.72 (8.27)	56	

*$n = 76$.
**$n = 15$.
VIQ, Verbal IQ; PIQ, Performance IQ; FSIQ, Full Scale IQ.
With index scores: VC, Verbal Comprehension; PO, Perceptual Organization; FD, Freedom from Distractibility.

Neurocognitive testing

The results of the WAIS-R and WISC-III testing are shown in Table 3 and Fig. 4. Case 1 performed in the "Extremely Low range" of intelligence as defined by the WAIS-R Manual,[38] earning a FSIQ of 64, a VIQ of 64, and a PIQ of 72. Similarly, she earned a VC standard score of 64, and a PO score of 81. Her FD score was 77. Note that the PIQ score is 8 points higher than her VIQ score, a highly atypical pattern that occurred in only 11% of our full deletion WMS group, and only 4% had a difference of 8 points or greater. Furthermore, Case 1's Perceptual Organization score is 17 points higher than the Verbal Comprehension score. A PO>VC pattern occurred in only 22% of our full deletion WS sample, and the largest of those differences was 3.25 points. Within the Verbal scale, she demonstrated strengths on the Object Assembly (nonverbal reasoning and synthesis of meaningful information) and Digit Span (immediate auditory memory) subtests. She exhibited weaknesses on the Vocabulary (word knowledge) and Similarities subtests (verbal abstraction). Of key interest was her performance on the Object Assembly subtest of the Performance Scale, which measures the ability of a subject to put together parts and assesses thought processes that involve visualizing at global and local levels. Case 1 achieved a scaled score of 9 (average range) on this subtest by completing 3 of the 4 puzzles and even received bonus points for the speed of her solutions on two items. Additional qualitative analysis of her Block Design performance also reveals interesting information about the nature of her visual spatial processing. This subtests requires the subject to arrange blocks consisting of solid red, solid white, and half red/half white faces, and to match designs of increasing complexity. Case 1 correctly constructed eight out of ten items, including both 2×2 and 3×3 block configurations, receiving bonus points for speed of completion on 4 items. This level of performance is quite rare among our large sample of WS. Furthermore, though incorrect, her production on items 8 and 10 were similar to the correct designs (Fig. 5). In particular, we note that the outer configuration for all solutions, even when incorrect, was maintained. Errors, rather, involved rotations of inner detail. These errors suggest relatively better global integrative processing, a finding that contrasts sharply with that of full deletion WS. Furthermore, a video recording of her performance of the Block Design, Picture Completion, and Digit Symbol subtests was viewed and significant speed and agility were noted during her successful completion of the above tasks. For example, in the Block Design subtest, she utilized a bimanual approach to manipulate the blocks and a block by block strategy to compare the test design to her working model, suggesting a methodical and organized problem solving strategy that again is atypical of full deletion WS. Finally, her approach to the Digit-Symbol subtest (measuring graphomotor integration, processing speed, attention, and working memory) was striking in speed and accuracy, scoring over 1.5 standard deviations above the mean of our WS sample. The quality of her symbol reproduction was also markedly accurate, failing to demonstrate the fine manual-motor control deficits prevalent in our full deletion WS sample.

Case 2 also performed in the Extremely Low range of intelligence on the WAIS-R, earning an FSIQ of 55, a VIQ of 65, and a PIQ of 56. The values for the VC, FD, and PO factor scores were 71, 65, and 57 respectively. Case 2 demonstrated strengths in Comprehension (measuring social judgment and reason-

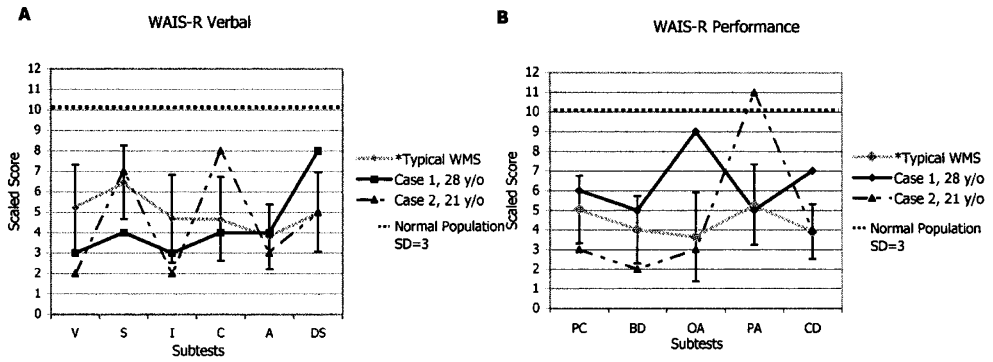

Fig. 4 Graphical Comparison of Neurocognitive results (WAIS-R and WISC-III) of each case to individuals with typical WS. Vertical lines at each subtest denote standard deviations (listed in Table 3). Dashed lines indicate scores the normal population receive on these standardized tests.

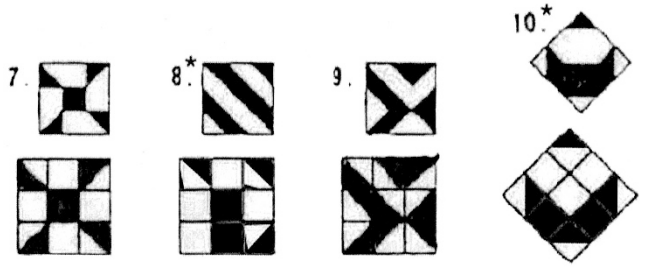

Fig. 5 Block Design Results of Case 1. Note designs 1 to 7 and 9 were successfully completed. *Case 1 was not able to complete designs 8 and 10.

ing) and Picture Arrangement (visual sequencing and anticipation of antecedents and consequences in social situations), with relative weaknesses on the Vocabulary, Information (measuring her fund of general information), and Block Design subtests. As with Case 1, however, the qualitative analysis of Case 2's performance on the Block Design subtest is more informative than the score itself. Case 2 correctly completed four of ten items (attempting only seven), though all of Case 2's Block Design constructions maintained the 2×2 or 3×3 configuration with all errors consisting of internal block rotation. This pattern again suggests improved global integrative processing relative to that of the full deletion WS group. Also, the quality of the symbol reproductions from the Digit Symbol subtest were highly accurate compared to full deletion WS.

Case 3 also performed in the Extremely Low range of intelligence on the WISC-III, with a FSIQ of 54, a VIQ of 65, and a PIQ of 51. We note that these scores are the lowest of the three small deletion cases. Her VC (72), FD (56), and PO (55) factor scores followed a similar pattern. Case 3 demonstrated a relative strength in Similarities (verbal abstraction) and a weakness in Arithmetic (working memory, numerical reasoning) subtests. Like cases 1 and 2, her symbol reproductions were more accurate than seen in full deletion WS. Also, Case 3 demonstrated Low Average range performance (scaled score of 7) on the Mazes subtest of the WISC-III, one which is not used in the calculation of the IQ scores. This subtest requires the subject to draw a line from the center to the outside of each of 9 mazes without crossing any of the lines representing walls. It requires planning ability, perceptual organization, visuomotor control, and speed. In summary, Case 3 demonstrated relative strengths on measures of visuoconstruction and manual motor control despite her extremely low omnibus IQ scores.

Finally, Figure 6 provides the results of the elephant copy task from the Boston Diagnostic Aphasia Examination (BDAE)[44] for each of the three cases along with drawings from age matched individuals with WS from Japan and North America. It is apparent from the figure that the drawings of each small deletion case (including Case 3, whose IQ scores are quite low) were superior to those of our full deletion WS cases. In particular, and consistent with performance on the Block Design subtest of the Wechsler Scales, the special deletion cases demonstrated global integration of individual details, reflected in the accurate reproduction of the outer configuration and three-dimensional representation of the elephant compared to the simplistic and highly fragmented drawings of the full deletion WS group. Because of the pictorial nature of Japanese writing, it might be argued that the superior reproductions are the result of cultural differences between Japanese and North American samples. However even when compared to WS drawings to elephant drawings from a sample of Japanese persons with WS and full deletion (see Fig. 6), the small deletion cases demonstrate a qualitatively better performance.

DISCUSSION

The summary of molecular studies seen in the phenotypic map (Fig. 7) along with clinical and cognitive data collected, provide the basis for a WS phenotypic map. By "phenotype" we mean any measured physical or cognitive parameter. The purpose of constructing a "phenotypic map" is to define molecularly the chromosomal regions and ultimately the genes, which are responsible for the variation in particular features. Although there are approximately 20 genes[1] known on the common deleted region for WS on 7q11.23, underexpression of a portion of them mapping telo-

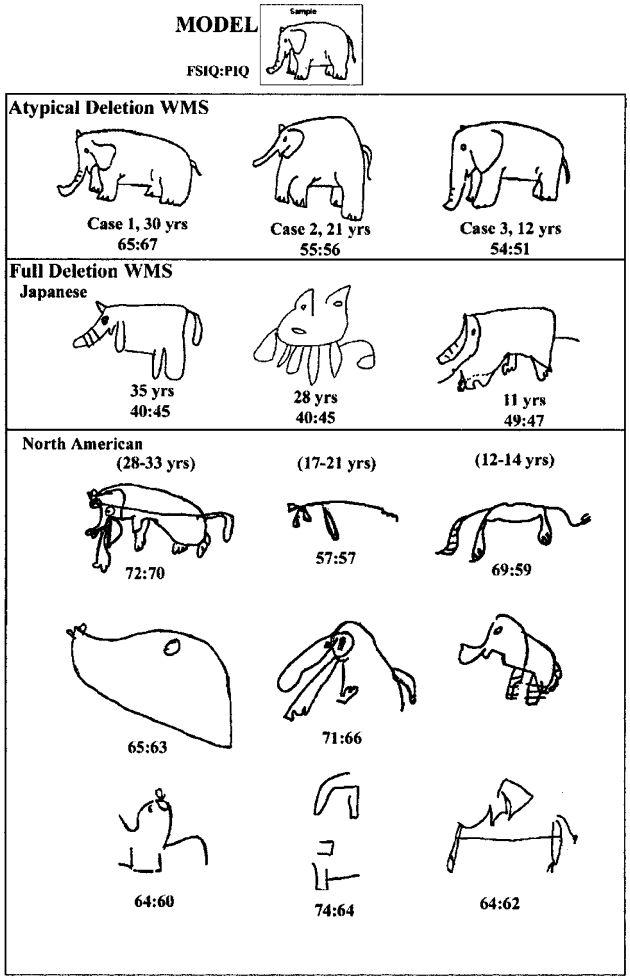

Fig. 6 Drawing copy task with of elephant by smaller deletion cases (Cases 1, 2, and 3) and age and IQ matched full deletion WS cases.

meric to RFC2 may produce the characteristic visual spatial deficits seen in persons with WS.[49,50]

Thus, it is the studies that identify rare individuals with atypical deletions for WS that provide the opportunity to correlate clinical and molecular levels of WS by relating specific features such as visual spatial cognition to explicit subsets of chromosome 7q11.23 genes in the common WS deletion. It was reported that deletion of only elastin through GTF2I,[49] was associated with the facial features of WS as well as with global intellectual deficits (Full Scale IQ = 48) with particular difficulties noted in visual perceptual and visual motor abilities (determined by the WISC-R[49a] with subtest data not reported). However, the second child from that study who carried the same apparent deletion revealed global deficits but no significant pattern (subtests not reported). In addition, Tassabehji et al., 1999[50] reported two individuals carrying a deletion for elastin and LIM kinase-1 with normal or low normal range function and a mixed pattern showing relative strengths in pattern construction and word definition uncharacteristic of WS.[50] In the same study, one seven-year-old subject (CS) carried a deletion of most of the WS region with the exception of the genes telomeric to RFC2, but nonetheless demonstrated Verbal and Performance scores slightly above the mean of their full deletion WS group.[50] The results of these studies suggested that, although many genes may contribute to global functioning deficits, the genes distal to RFC2 might contribute more to visual spatial cognitive processes.

The results of the current study provide evidence to support the association of two genes with visual spatial performance in WS. All three subjects had smaller overlapping deletions, and that of Case 1 differed least from the common deletion, including only cosmids 129f5 through 183e1, with a partial deletion of cosmid 160g4. These analyses imply that Case 1 had two genes remaining, GTF2IRD1 and GTF2I. GTF2IRD1 (BEN) is differentially expressed in brain and heart during development and in the adult. It encodes helix-loop-helix domains similar to those found in GTF2I,[28] a transcription factor that is a target of the Bruton's tyrosine kinase (BTK).[51]

With these molecular data, we then considered the cognitive aspects of each subject. We have compared the results from the WAIS-R and WISC-III to those from a cohort of subjects with WS[40](Searcy et al., unpublished data, 2003) as shown in Figure 4 and Table 3. All three subjects had Full Scale Intelligence Quotients (FSIQ) within the range of our full deletion WS ($M = 67.67$, $SD = 8.25$). Early publications[45,52] reported IQs of typical WS in the 55 to 60 range, however, as discussed in the Searcy et al.,[40] there has been a steady increase in mean IQ. Although there exists considerable within-group variability, the mean IQ of our sample has remained quite stable.

The results for Case 1 were of interest because her PIQ was 8 points greater than her VIQ. This differed in both direction and magnitude from our WS sample in which 89% show the reverse, a VIQ>PIQ on the WAIS-R. Furthermore, a PIQ>VIQ difference > 6 points occurred in only 4% of the typical WS group. Case 2 and Case 3 achieved VIQ scores greater than PIQ scores, characteristic of subjects with WS, although only for Case 3 was the difference statistically significant.

For a more focused evaluation of cognitive abilities, three factor-analytically derived indices were calculated and compared to our full deletion WS group: Verbal Comprehension (VC), Perception Organizational (PO), and Freedom of Distractibility (FD). These indices combine subtests based on intercorrelations among those subtests and reflect the particular abilities (i.e., verbal comprehension or visual spatial organization) required to successfully complete the task. A typical WS pattern is a VC factor greater than PO and FD, which occurred in nearly 80% of our full deletion sample. However, Case 1 did not show this pattern, as her VC factor score was lower than both her PO and FD factor scores. Her high PO score reflects the ability to integrate visual stimuli to solve problems involving visual spatial and visual-motor skills, suggesting a relative strength in her perceptual organization skills compared to her verbal abilities.

The cognitive profiles of our atypical WS cases were then further parsed by an analysis of the relative strengths and weaknesses seen in subtests. Typical WS patients show relative strengths in verbal abstract reasoning, visual attention to detail, visual sequencing in social situations and weaknesses in numerical reason-

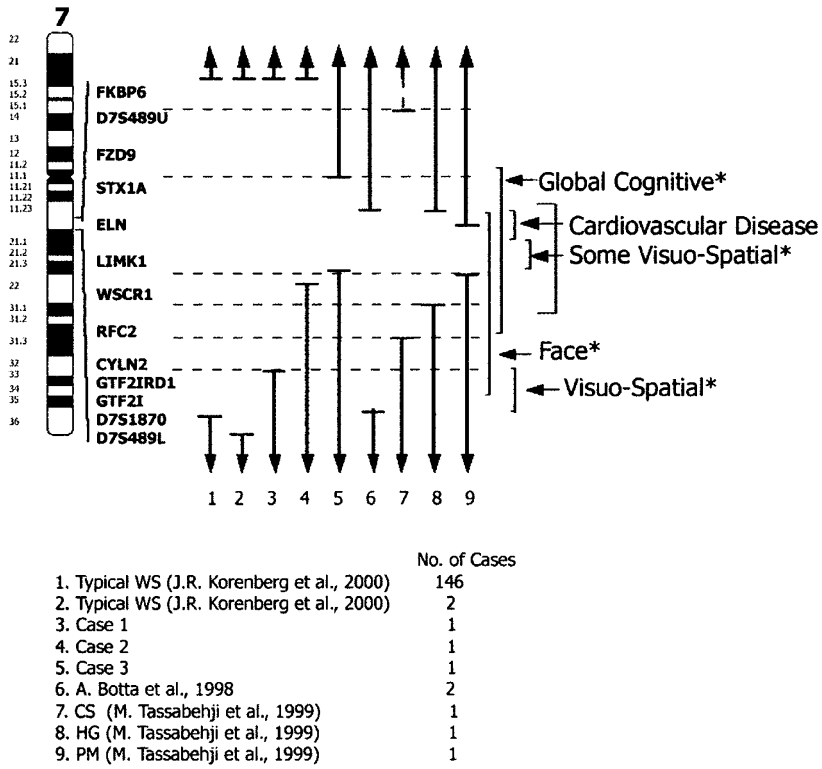

Fig. 7 Mapping of Phenotype to Minimal Genotype in Williams Syndrome. Deleted regions are demarcated by vertical lines. *Position effects not withstanding.

ing, visuoconstruction, and working memory[40,52] (Searcy et al., unpublished data, 2003). The strength in Object Assembly of Case 1 (scaled score = 9) demonstrated her abilities to visualize at global and spatial levels and to visually process and plan solutions. Her performance on this subtest was higher than her own mean Performance scale score, and higher than the mean Object Assembly score of our typical WS group (M = 3.63; SD = 2.27). Further, the video record of Case 1's performance on the WAIS-R Block Design subtest, indicated methodical and ordered strategies for solving visual spatial problems, especially with respect to global (i.e., "gestalt") processing. This is in striking contrast to typical WS subjects. For Case 1, age was not a mediating factor, as the Searcy et al. data[40] show, the correlation between Block Design scaled scores and age is minimal, accounting for only 5% of the total variance. Our intelligence test data are consistent with the findings of Mervis et al.[53] in demonstrating a leveling off of such improvement by late adolescence. We interpret these data as an indication of a qualitative strength in Case 1's visual integration and visuoconstruction abilities.

Case 2 showed strengths in Comprehension and Picture Arrangement subtests and weaknesses in Vocabulary, Information and Block Design subtests. These results indicate relative strengths in social judgment, verbal expression (Comprehension), and the ability to perceive details of pictures and to detect sequences. Moreover, despite her low score on the Block Design subtest, her solutions consistently reflected preserved configural processing, atypical for the full deletion WS group.

Case 3 was administered the WISC-III and demonstrated strength in the Similarities subtest and a weakness in the Arithmetic subtest, typical of WS subjects. Strength in the Similarities subtest reflects relative skills in verbal conceptualization and abstract reasoning in categorically organizing objects.[54] Her low Arithmetic scores indicate deficient attention and working memory in addition to poor facility with numbers, all common in full deletion WS subjects. One caveat that limits the interpretation of scores from Case 3 is that our typical WS subjects were administered the WISC-R, whereas Case 3 was administered the WISC-III which yields lower IQs than the WISC-R (data not shown) and differs in several subtests. Regardless, WISC-R results reflected relative strengths and weaknesses in full deletion WS and a qualitative analysis of performance by Case 3 revealed relative strength in visuomotor control, consistent with the pattern observed in the remaining two cases but not typical of WS with full deletion.

Finally, in contrast to both the Japanese and our North American cohort with full deletion WS, the elephant drawings for all three subjects with atypical deletions are good copies with all of their global elements in place. When interpreted in the context of the qualitative Block Design data, all 3 subjects demonstrate a dramatic reduction in the degree of constructional dyspraxia that characterizes full deletion WS.

Cultural differences between the Japanese cases and the North American full deletion WS groups are not likely to explain the observed differences in functioning, in that the Japanese WAIS-R standardization sample is similar in pattern to that observed in the North American version.[55] Specifically, there is no relative advantage on visuospatial tasks (e.g., Block Design) in the Japanese group relative to North American samples. Similarly, the fact that

the three special deletion cases from Japan all produced better copies of the elephant picture than the full-deletion Japanese WS cases (Fig. 6) suggests that differences in written language (complex character vs. letter based) cannot entirely account for their relative strengths on these tasks.

Although this study must be expanded by further testing and with greater numbers of WS subjects with atypical sized deletions, these data support our proposal that the lack of deletion of GTF2IRD1 and GTF2I is associated with some preserved aspects of visual spatial function and conversely, that deficits in these functions are in part due to the deletion of these genes in typical WS. We have previously suggested[56] that clusters or single genes can be implicated as responsible for a large part of the variability of a trait when the penetrance and expressivity of the trait is similar in full aneuploidy and in the subset of individuals who are aneuploid for the candidate region. Moreover, even single cases can be highly informative for assigning genes to phenotypes. However, for this type of analysis to be valid, the phenotypes and their variation must be well defined in the partially aneuploid individuals as well as in the normal and WS control groups. It is particularly important to note that control groups with "typical" or "full deletion" WS must be characterized in detail genetically but none of the current published studies of atypical deletions has done so. Therefore, the current report represents an intermediate qualitative step and although valid for hypothesis generation, future quantitative studies are essential. Such studies must identify further WS individuals with atypical deletions or rearrangements, present evidence for other possible sources of genetic and nongenetic instability, focus on well-defined and novel cognitive features in large normal and WS control groups, dissect subdomains of visual-spatial function, and finally, relate these to the results of quantitative functional and structural brain imaging. These studies are in progress.

Nonetheless, integrating previous and current molecular studies with those of the physical features and cognitive profiles of our three subjects and individuals with typical WS provides an opportunity to narrow the genes potentially involved in additional WS phenotypes. Some of the facial features must clearly be due to the deletion of GTF2I and GTF2IRD1, from their lack in the three atypical deletions. For other physical features, more detailed examination is necessary which was not possible in the current cases. The observation that all three subjects exhibited global cognitive deficits and none showed the facial features characteristic of WS suggests that, in contrast to previous reports, the deletion of genes within the region BAZ1B through elastin may be responsible for significant global deficits in cognitive function. Further, these results provide evidence that helps to define the region associated with characteristic WS weakness in spatial construction to the region telomeric to CYLN2, although this and other genes may also contribute. Because the two smaller deletions appeared to have more severe cognitive impairments, it is difficult to assess the role of CYLN2 or FZD9 although this will be approached in future studies. Some aspects of WS neural phenotypes may be approached in the mouse as suggested by the subtle decreases in adult weight, muscular dyscoordination, and abnormal fear conditioning reported for the CYLN2 conditional knock-out model.[23] However, neither the heterozygous nor homozygous knock-out for CYLN2 were reported to have significant defects in visual-spatial function. Further, narrowing the genes responsible for the subtle anomalies in corpus callosum and ventricular volume observed in the mouse or more importantly, for the significant behavioral, linguistic, and volumetric regional abnormalities seen in humans,[57] will depend on the definition of further human subjects with atypical deletions in whom detailed cognitive and physical information can be combined with functional brain imaging, that are not yet available in the current cases. Moreover, the overlapping variation seen in the cognitive subtests of the three subjects emphasizes the need to apply finer tools and additional subjects with atypical sized deletions to explore the genetic origins of WS cognition. Finally, our evidence suggests that some of the characteristic facial features are associated with deletion of the region telomeric to elastin but with variable expressivity and possible interaction with other genes in this region. Explicit molecular and cognitive data are also needed to explain the effects of breakpoint position, on neighboring genes as well as the effect of parent of origin on genes in the WS region. However, this study reflects the importance of the role GTF2IRD1 and GTF2I may play in the development of neural pathways involved in visual spatial cognition. The current report therefore provides a beginning and future studies of these and other individuals with partial deletions are required to understand the role of GTF2IRD1 and GTF2I or of CYLN2, and the genetic mechanisms linking variation in their expression with variation in human neurodevelopment and cognition.

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