

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Somerville MJ, Mervis CB, Young EJ, et al. Severe expressive-language delay related to duplication of the Williams–Beuren locus. *N Engl J Med* 2005;353:1694-701.

SUPPLEMENTARY APPENDIX

Negative Medical History

An abdominal ultrasound, electrocardiogram, and chest radiograph performed at 13 months of age were normal and Patient 1 was successfully treated for iron deficiency anemia. An MRI at age six years, five months and an EEG at age seven years revealed no significant abnormalities. Assessment in the genetics clinic at age seven years showed a very sociable, hyperactive boy with mild dysmorphic features. Chromosome analysis at 550 band resolution was normal, as were the following investigations: quantitative amino acids, thyroid-stimulating hormone, routine electrolytes and hematology, urine mucopolysaccharide screen, oligosaccharide screen, specific molecular studies to detect the fragile X, X-linked alpha thalassemia-mental retardation and velocardiofacial syndromes.

Clinical Evaluation of Language Fundamentals – Preschool Version 2nd ed.

Patient 1's language abilities were too limited for him to complete the age-appropriate version of the Clinical Evaluation of Language Fundamentals (CELF). Therefore, the CELF-Preschool 2 [12] was administered. On the receptive subtests, Patient 1's responses indicated that he understood a variety of grammatical constructions, was able to categorize pictures of objects, and understood simple relational language. Overall, his performance was consistent with a moderate language impairment. In contrast, Patient 1 did not answer a single question correctly on the three primary expressive subtests: recall of sentences, expressive vocabulary, and word structure, indicating very severe expressive language impairment.

Table 1. Genotyping analysis of markers from the Williams-Beuren syndrome region

FAMILY MEMBER	MARKER	ALLELE 1	ALLELE 2	ALLELE 3	
		Size in base pairs			
Patient 1	D7S653	216	222		
Sister		222	222		
Father		216	222		
Mat GF		222	222		
Mat GM		222	222		
Patient 1		D7S672	140	150	
Sister	144		144		
Father	144		150		
Maternal Grandfather	140		140		
Maternal Grandmother	142		144		
Williams-Beuren syndrome region	Patient 1		D7S2476	156	162
	Sister	156		168	
	Father	156		156	
	Mother (reconstructed)	162		168	
	Maternal Grandfather	154		162	
	Maternal Grandmother	154		168	
	Patient 1	D7S3194	202	204	208
	Sister		204	204	
	Father		202	204	
	Mother (reconstructed)		204	208	
	Maternal Grandfather		208	208	
	Maternal Grandmother		196	204	
	Patient 1	D7S1870	134	132	136
	Sister		134	136	
	Father		134	134	
	Mother (reconstructed)		132	136	
	Maternal Grandfather		128	132	
	Maternal Grandmother		134	136	
Patient 1	D7S2455	210	210		
Sister		206	210		
Father		206	210		
Maternal Grandfather		202	202		
Maternal Grandmother		208	210		
Patient 1		D7S675	207	207	
Sister	207		211		
Father	207		211		
Maternal Grandfather	209		211		
Maternal Grandmother	207		209		

Markers were amplified using fluorescently labelled primers and the sizes of the resulting products determined using an Applied Biosystems 3730xl DNA analyzer and GeneMapper 3.5 software. Alleles not present in the father, but present in the maternal grandparents (and therefore inherited from the mother) are shaded. The mother's genotype at each marker has been reconstructed from the genotypes of her parents and offspring.

Table 2. Expression analysis of genes from the Williams-Beuren syndrome region

Gene	Comparative Expression Ratio (vs. control group n=9)	
	WBS Persons (n=9)	Patient 1 (duplication)
	Mean \pm SEM	Mean \pm SD
BAZ1B	0.42 (\pm 0.07)	1.47 (\pm 0.15)
LIMK1	0.50 (\pm 0.22)	1.57 (\pm 0.17)
WBSCR1	0.45 (\pm 0.05)	1.35 (\pm 0.10)
WBSCR5	0.38 (\pm 0.17)	1.05 (\pm 0.28)
RFC2	0.50 (\pm 0.06)	1.30 (\pm 0.02)
GTF2I	0.56 (\pm 0.08)	1.58 (\pm 0.13)
WBSCR16	0.99 (\pm 0.12)	0.94 (\pm 0.37)

All samples were run in triplicate and the experiment was repeated twice with consistent results. 5 μ g of total RNA extracted from transformed lymphoblast cell lines was DNase treated and reverse transcribed in a 20 μ l reaction with random hexamers using SuperScript™ First-Strand Synthesis System (Invitrogen). Real-time PCR was carried out using an ABI Prism7900HT sequence detection system with 10 μ l reactions containing 5 ng of template for 45 cycles of amplification. Primer sequences are available on our website (<http://www.utoronto.ca/osborne/>). *CYLN2* and *GTF2IRD1* expression was below the limit of reliable detection. Real-time PCR experiments were normalized using *ACTB*, *HPRT1* and *STARD7* as references. Each plate contained a water control and serially diluted concentrations of control genomic DNA (range 10-0.62 ng) from which a standard curve was generated for transcript quantification. Comparative expression ratios were calculated by dividing the averaged normalized values for each of the test genes by the normalized test gene values for the control group.

FIGURE LEGEND

Figure 1. Schematic of polymorphic marker analysis in Patient 1's family

The results of polymorphic marker analysis for the three informative markers within the WBS region are shown for Patient 1, his parents and maternal grandparents. The mothers' haplotype is inferred from that of her parents and of Patient 1. The markers used were *D7S2476*, *D7S3194* and *D7S1870*. Markers were amplified using fluorescently labelled primers and the sizes of the resulting products determined using an Applied Biosystems 3730xl DNA analyzer and GeneMapper 3.5 software.

Boxed haplotypes inherited by Patient 1 are shown in different colors for the grandmother and the grandfather. It can clearly be seen that the haplotypes corresponding to the duplicated 7q.23 region in Patient 1 were inherited from different grandparents, indicating that the unequal interchromosomal meiotic recombination occurred in the mother.

