

Submicroscopic Deletion in Patients with Williams-Beuren Syndrome Influences Expression Levels of the Nonhemizygous Flanking Genes

Giuseppe Merla, Cédric Howald, Charlotte N. Henrichsen, Robert Lyle, Carine Wyss, Marie-Thérèse Zobot, Stylianos E. Antonarakis, and Alexandre Reymond

Genomic imbalance is a common cause of phenotypic abnormalities. We measured the relative expression level of genes that map within the microdeletion that causes Williams-Beuren syndrome and within its flanking regions. We found, unexpectedly, that not only hemizygous genes but also normal-copy neighboring genes show decreased relative levels of expression. Our results suggest that not only the aneuploid genes but also the flanking genes that map several megabases away from a genomic rearrangement should be considered possible contributors to the phenotypic variation in genomic disorders.

Segmental aneuploidies (i.e., gain or loss of subchromosomal DNA fragments) are important contributors to human diseases¹ and, potentially, to phenotypic variation,^{2,3} as well as a major force of evolutionary changes.⁴⁻⁹ There is evidence that such genomic insertions and deletions contribute to phenotypic differences by modifying the expression levels of genes within the aneuploid segments.¹⁰⁻¹³ We hypothesize that these rearrangements also induce altered expression of the genes that lie near the breakpoints, although these do not vary in copy number; this effect could be mediated by disturbances of the copy number of long-range *cis* regulatory elements.¹⁴⁻¹⁷

To test this hypothesis, we assessed whether the human chromosome 7 (HSA7) recurrent DNA deletion causing Williams-Beuren syndrome (WBS [MIM 194050])¹⁸ influences the transcription levels of both the hemizygous genes within the deleted region and the nonhemizygous genes in the WBS flanking regions. WBS is a neurodevelopmental disorder characterized by numerous clinical aspects, including mental retardation with a unique cognitive and personality profile.¹⁹ Its incidence is estimated to be between 1:7,500 and 1:20,000, and sporadic *de novo* inheritance is usual.²⁰⁻²²

Material and Methods

Cell Culture, RNA, and cDNA Preparations

Human skin fibroblasts and lymphoblastoid cell lines were grown in HAM F-10 or RPMI 1640 media, respectively, supplemented with 10% fetal bovine serum and 1% antibiotics (Invitrogen). Total RNA was prepared from logarithmic growth-phase cells, with the use of RNeasy Mini Kit (Qiagen), in accordance with the manufacturer's instructions. After DNase treatment (Qiagen), the quality of all RNA samples were checked using an Agilent 2100

Bioanalyzer (Agilent Technologies). Total RNA was converted to cDNA with the use of Superscript II (Invitrogen) primed with poly d(T). For each cell line included in the study, 4.5 μ g of total RNA was converted to cDNA in three individual reactions; these were then pooled and were diluted 1:14.

Sample Population

Lymphoblastoid cell lines from 10 individuals with WBS and from 40 control individuals, as well as skin fibroblasts from 7 control individuals, were acquired from the cell culture collection of the Coriell Institute for Medical Research, and skin fibroblasts from 14 individuals with WBS and from 6 control individuals were obtained from the cell culture collections of the Centre de Biotechnologie Cellulaire, Hospices Civils de Lyon, Hôpital Debrousse, in Lyon, France. One more control was received from the Galliera Genetic Bank in Genova, Italy (table 1). Appropriate informed consent was obtained for each sample by the physicians in charge. DNA was extracted from each cell line of the sample population, with the use of PureGene (Gentra Systems), in accordance with the manufacturer's instructions. We assayed each DNA with a quantitative PCR approach, using SybrGreen dye and probes, mapping the region from the *BAZ1* locus to the *CYLN2* locus and the flanks of the commonly deleted region,²³ to determine the size of the deletions and to ensure (1) that none of the patients with WBS presented an atypical deletion²³⁻²⁹ or an inversion at 7q11.23^{18,30} and (2) that none of the controls were hemizygous for that same region. The results are presented in table 2.

To make sure that differences in expression levels measured in lymphoblastoid cells were not merely due to transformation, we established six lymphoblastoid cell lines from two blood samples collected at 1-wk intervals from the same individual, after informed consent. We measured expression levels of 25 HSA21 genes (*ITBG2*, *CBS*, *APP*, *PFKL*, *U2AF1*, *PRDM15*, *LSS*, *PDXK*, *SLC19A1*, *SLC37A1*, *PWP2H*, *MCM3AP*, *GART*, *CBR1*, *TMEM1*,

From the Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland (G.M.; C.H.; R.L.; C.W.; S.E.A.; A.R.); Servizio Genetica Medica, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy (G.M.); Centre de Biotechnologie Cellulaire, Hospices Civils de Lyon, Hôpital Debrousse (M.-T.Z.), and Interdisciplinary Group to Study the *ELN* Gene (M.-T.Z.), Lyon, France; and Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland (C.N.H.; A.R.)

Received April 26, 2006; accepted for publication May 31, 2006; electronically published June 23, 2006.

Address for correspondence and reprints: Dr. Alexandre Reymond, Center for Integrative Genomics, Genopode Building, University of Lausanne, Lausanne, Switzerland. E-mail: alexandre.reymond@unil.ch

Am. J. Hum. Genet. 2006;79:332-341. © 2006 by The American Society of Human Genetics. All rights reserved. 0002-9297/2006/7902-0015\$15.00

Table 1. Cell Lines Employed

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

BTG3, *DSCR1*, *ETS2*, *IFNAR2*, *ANKRD3*, *WRB*, *GABPA*, *SON*, *IFNAR1*, and *CCT8*) that show a large variation in transcript levels in the normal population and found no significant differences in their expression levels in the assayed samples (Pearson $0.8 < r < 0.92$ [mean 0.87]; $P < .001$). The observed differences correspond to the experimental variation we measure between replicates.

Real-Time Quantitative PCR and Data Analysis

We opted for Taqman real-time quantitative PCR, to measure any small differences in gene expression levels. Primers and probes were designed using the PrimerExpress program (Applied Biosystems), with default parameters in every case for all the confirmed genes mapping on HSA7, from the centromere to the beginning of band 7q21.11. They can be divided into three groups of genes: 23 HSA7 test genes that are hemizygous in patients with WBS, 2 genes mapping in the low-copy repeats (LCRs) flanking the WBS deletion, and 24 HSA7 test genes that are nonhemizygous in patients with WBS. We also designed assays in 2 HSA7p genes, in 13 control genes, and in 3 normalization genes. The complete list of tested genes, their accession numbers and mapping positions, and the primers and probes used are indicated in table 3. Amplicon sequences were checked by both BLAST and BLAT against the human genome, to ensure specificity. Whenever possible (in 94% of cases), oligos were designed to span an intron. Non-intron-spanning assays were tested in standard \pm reverse transcriptase reactions of RNA samples for genomic contamination; in all cases, no amplification was observed in the absence of reverse transcriptase. High-performance liquid chromatography-purified, FAM-TAMRA-labeled, double-dye Taqman probes and qPCR mastermix (RT-QP2X-03) were obtained from Eurogentec.

The efficiency of each Taqman assay was measured using a dilution series of fibroblast cDNA and lymphoblastoid cells or a pool of cDNA samples of brain, liver, and testis, as described elsewhere³¹ (see table 3 for results). A working Taqman assay was obtained for 57 (85%) of the 67 assayed genes. We were unsuccessful for *RCP9*, *RABGEF1*, *FKBP6*, *FZD9*, *WBSCR14*, *CLDN4*, *MK-STYX*, *FGL2*, *AIP*, and *GRM3*. Six more genes (*TPST1*, *WBSCR17/GALNT9*, *CALN1*, *WBSCR27*, *WBSCR28*, and *WBSCR16*) were excluded because of a lack of expression in both fibroblasts and lymphoblastoid cell lines (see efficiencies in table 3). Note that *TRIM50/73/74*, *ATP50*, *SIM2*, and *UFD1L* are not expressed in skin fibroblasts, whereas *WBSCR24*, *WBSCR18*, *CLDN3*, *ELN*, *CACNA2D1*, *GABPA*, *IFNGR2*, and *DGCR8* are not expressed in lymphoblastoids. Thus, in at least one of the two studied cell lines, we were able to assess the relative expression level (REL) of 76% (51/76) of the selected genes, a proportion significantly above the one expected with genomewide technologies. Typically, microarrays hybridized with fibroblast or lymphoblastoid cell cDNA measure the expression of 30%–40% of human genes.

All RT-PCRs were performed in a 10- μ l final volume and in five

Table 2. Genotyping of Cell Lines

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Table 3. Assayed Genes, Primers, Probes, and Efficiencies

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replicates per sample and were set up in a 384-well plate format, with the use of a Biomek 2000 robot (Beckman). They were run in an ABI 7900 Sequence Detection System (Applied Biosystems) with the following amplification conditions: 50°C for 2 min, 95°C for 10 min, and 50 cycles at 95°C for 15 s and 60°C for 1 min. Each plate contained the appropriate normalization genes to control for any variability between the different plate runs.

Raw threshold-cycle (C_t) values were obtained using SDS2.2 (Applied Biosystems). To calculate the normalized relative expression ratio between individuals with WBS and controls, we followed methods described elsewhere.³¹ We exploited the ge-Norm method³² to select the three normalization genes: *AGPAT1*, *EEF1A1*, and *PSMAS*. They were used to normalize input cDNA for each sample, whereas mixes of 40 lymphoblastoid and 12 fibroblast control-cell-line cDNA samples (table 1) were used to define normal RELs.

Results

We used the high sensitivity of real-time quantitative PCR to accurately measure the expression of all the HSA7q genes mapping in the region 11.9 Mb downstream (band 7q21.11) to 8.4 Mb upstream (centromere position) of the WBS deleted region, in which we could design a working Taqman assay (i.e., efficiency ≥ 0.95 and ≤ 1.05 for expression in fibroblasts and/or lymphoblastoid cell lines) (see “Material and Methods” and table 3 for details). These genes can be divided into 17 HSA7q hemizygous genes that map within the WBS deletion, 14 HSA7q nonhemizygous genes, and 2 genes that map within the LCR flanking the deletion. This panel of genes was completed with 2 HSA7 nonhemizygous genes that map on the short arm of the chromosome (band 7p11.2) and 19 control, non-HSA7 genes. We compared the mRNA expression levels of these genes in nontransformed skin fibroblast cells obtained from 14 subjects with WBS and from 14 controls and in transformed lymphoblastoid cells obtained from 10 subjects with WBS and from 11 controls (see table 1 for cell lines; see table 2 for genotyping²³; and see table 3 for a complete list of assayed genes, primers, and probes).

The results of these analyses are summarized in table 4. We found extensive variability in gene RELs in humans (table 4, fig. 1, and data not shown), which is consistent with previous reports.^{16,17,33} In the population with WBS, all but two of the genes that map to the common deletion interval and that are hemizygous in patients with WBS show average relative expression levels (ARELs) that are approximately half of the normal ARELs (see table 4 and figs. 1C, 1D, and 2), which is consistent with partial results published elsewhere.³⁴ In contrast, the control genes show no significant variation in RELs between the patients and the controls (see table 4 and figs. 1A, 1B, and 2). Interestingly, one hemizygous gene per cell type deviates from

Table 4. ARELs

Gene	Category ^a	Controls		WBS		WBS/Controls			Pairwise <i>t</i> Test <i>P</i>
		AREL	SD	AREL	SD	<i>t</i> Test <i>P</i>	Mann-Whitney <i>P</i>	AREL Ratio ^b	
Lymphoblastoid cell lines:									
<i>GBAS</i>	2	1.38	.37	1.01	.20	.02	.04	.74	...
<i>PSPH</i>	2	1.19	.31	.99	.37	.23	.16	.83	...
<i>ZFD25</i>	2	1.15	.28	.98	.33	.24	.15	.85	...
<i>VKORC1L1</i>	2	1.31	.35	1.05	.42	.15	.15	.80	...
<i>GUSB</i>	2	.95	.18	.92	.36	.82	.35	.97	...
<i>ASL</i>	2	.81	.15	1.29	.39	.004	.004	1.59	...
<i>KCTD7</i>	2	.91	.42	.36	.08	.004	.005	.39	...
<i>NM_017994</i>	2	.99	.19	.89	.14	.18	.13	.89	...
<i>RSAFD1/NM_018264</i>	2	1.13	.23	1.20	.53	.71	.54	1.06	...
<i>AUTS2</i>	2	.57	.50	.20	.21	.06	.06	.35	...
<i>WBSCR20</i>	6	.63	.09	.30	.11	1.0 × 10⁻⁶	.0003	.48	...
<i>TRIM50/73/74</i>	6	1.07	.71	.71	.29	.22	.36	.66	...
<i>BAZ1B</i>	1	2.29	.92	.64	.22	.0006	.0004	.28	...
<i>BCL7B</i>	1	.74	.31	.26	.09	.002	.0004	.35	...
<i>TBL2</i>	1	.64	.14	.24	.08	5.0 × 10⁻⁶	.0003	.38	...
<i>WBSCR24</i>	1	NE	NE	NE	NE	NE	NE	NE	...
<i>WBSCR18</i>	1	NE	NE	NE	NE	NE	NE	NE	...
<i>WBSCR22</i>	1	.65	.20	.28	.09	.0003	.0003	.43	...
<i>STX1A</i>	1	.92	.33	.27	.10	.0003	.0003	.29	...
<i>WBSCR21</i>	1	1.18	.48	.44	.15	.001	.0009	.37	...
<i>CLDN3</i>	1	NE	NE	NE	NE	NE	NE	NE	...
<i>ELN</i>	1	NE	NE	NE	NE	NE	NE	NE	...
<i>LIMK1</i>	1	2.24	.80	.42	.14	.0001	.0004	.19	...
<i>WBSCR1/EIF4H</i>	1	.73	.15	.25	.09	1.1 × 10⁻⁶	.0003	.34	...
<i>WBSCR5</i>	1	.68	.21	.24	.07	.0001	.0003	.36	...
<i>RFC2</i>	1	.86	.28	.40	.12	.0009	.0003	.46	...
<i>CYLN2</i>	1	.81	.37	.23	.08	.001	.0003	.29	...
<i>GTF2IRD1</i>	1	.28	.21	.32	.19	.68	.65	1.14	...
<i>GTF2I</i>	1	.95	.19	.35	.11	2.0 × 10⁻⁶	.0003	.37	...
<i>HIP1</i>	2	.90	.46	.42	.17	.015	.02	.47	...
<i>RHBDL7/NPD007</i>	2	.62	.23	.50	.20	.23	.31	.80	...
<i>POR</i>	2	.56	.14	.50	.15	.37	.71	.89	...
<i>MDH2</i>	2	.86	.08	1.05	.14	.002	.005	1.23	...
<i>DTX2</i>	2	.78	.29	.83	.17	.62	.18	1.07	...
<i>CACNA2D1</i>	2	NE	NE	NE	NE	NE	NE	NE	...
<i>USP18</i>	C	1.19	.71	1.32	.62	.26	.66	1.11	...
<i>DGCR8</i>	C	NE	NE	NE	NE	NE	NE	NE	...
<i>ATP50</i>	C	.45	.11	.47	.16	.71	.21	1.05	...
<i>B2M</i>	C	.63	.12	.60	.28	.76	.54	.95	...
<i>DSCR2</i>	C	1.58	.59	1.99	.95	.29	.45	1.25	...
<i>IFNAR1</i>	C	1.08	.33	1.34	.66	.28	.54	1.25	...
<i>BTG3</i>	C	.79	.25	.56	.19	.04	.02	.71	...
<i>IFNGR2</i>	C	NE	NE	NE	NE	NE	NE	NE	...
<i>GABPA</i>	C	NE	NE	NE	NE	NE	NE	NE	...
<i>IL10RB</i>	C	.94	.11	1.19	.65	.24	.65	1.28	...
<i>SON</i>	C	1.00	.26	.86	.27	.26	.49	.86	...
<i>SIM2</i>	C	1.04	.47	.81	.55	.35	.31	.78	...
<i>UFD1L</i>	C	1.51	.49	1.84	.40	.04	.08	1.22	...
Nonhemizygous:									
All ^c	2	.89	.23	.78	.3588	.20
Centromere ^c	2	.98	.23	.86	.3988	.32
Telomere	2	.74	.14	.66	.2789	.51
Close ^d	2	.81	.23	.58	.3471	.04
LCRs	6	.85	.30	.51	.2960	.03
Hemizygous	1	1.00	.60	.33	.1233	.0005
Controls	C	1.00	.34	1.12	.51	1.12	.16
Skin fibroblasts:									
<i>GBAS</i>	2	.99	.35	.91	.23	.53	.89	.92	...
<i>PSPH</i>	2	.94	.34	.76	.32	.18	.31	.80	...
<i>ZFD25</i>	2	.90	.37	.78	.38	.45	.34	.87	...

(continued)

Table 4. (continued)

Gene	Category ^a	Controls		WBS		WBS/Controls			Pairwise <i>t</i> Test <i>P</i>
		AREL	SD	AREL	SD	<i>t</i> Test <i>P</i>	Mann-Whitney <i>P</i>	AREL Ratio ^b	
<i>VKORC1L1</i>	2	1.10	.33	1.00	.41	.55	.67	.92	...
<i>GUSB</i>	2	.95	.31	1.06	.16	.29	.31	1.11	...
<i>ASL</i>	2	1.11	.32	.85	.23	.03	.08	.77	...
<i>KCTD7</i>	2	.87	.43	.59	.13	.06	.13	.68	...
<i>NM_017994</i>	2	1.09	.24	1.02	.17	.44	.69	.94	...
<i>RSAFD1/NM_018264</i>	2	.98	.19	.93	.16	.50	.47	.95	...
<i>AUTS2</i>	2	1.02	.46	.72	.43	.11	.13	.70	...
<i>WBSCR20</i>	6	.54	.21	.26	.04	.002	.0005	.48	...
<i>TRIM50/73/74</i>	6	NE	NE	NE	NE	NE	NE	NE	...
<i>BAZ1B</i>	1	1.02	.34	.31	.15	8.5 × 10⁻⁶	.00001	.31	...
<i>BCL7B</i>	1	.95	.37	.27	.06	4.6 × 10⁻⁵	.00001	.28	...
<i>TBL2</i>	1	.91	.30	.30	.08	1.7 × 10⁻⁵	.0001	.32	...
<i>WBSCR24</i>	1	.62	.45	.18	.04	.006	.0001	.29	...
<i>WBSCR18</i>	1	1.02	.33	.34	.13	9.9 × 10⁻⁶	.00001	.34	...
<i>WBSCR22</i>	1	.92	.22	.29	.07	3.9 × 10⁻⁷	.00001	.32	...
<i>STX1A</i>	1	.98	.59	.22	.06	.001	.0001	.23	...
<i>WBSCR21</i>	1	.92	.33	.27	.08	1.9 × 10⁻⁵	.0001	.29	...
<i>CLDN3</i>	1	.81	.56	.38	.17	.03	.04	.47	...
<i>ELN</i>	1	1.60	1.63	1.74	1.74	.85	.62	1.08	...
<i>LIMK1</i>	1	.94	.21	.26	.11	2.5 × 10⁻⁸	.00001	.28	...
<i>WBSCR1/EIF4H</i>	1	.91	.31	.28	.08	1.5 × 10⁻⁵	.00001	.30	...
<i>WBSCR5</i>	1	1.15	.48	.38	.12	.0001	.0001	.33	...
<i>RFC2</i>	1	.65	.24	.17	.06	2.4 × 10⁻⁵	.00001	.27	...
<i>CYLN2</i>	1	.61	.19	.18	.05	6.7 × 10⁻⁶	.00001	.29	...
<i>GTF2IRD1</i>	1	.69	.27	.16	.04	3.2 × 10⁻⁵	.00001	.24	...
<i>GTF2I</i>	1	1.36	.38	.72	.18	8.3 × 10⁻⁵	.0001	.53	...
<i>HIP1</i>	2	.51	.29	.29	.09	.03	.04	.56	...
<i>RHBDL7/NPD007</i>	2	.88	.38	.64	.17	.06	.10	.73	...
<i>POR</i>	2	.81	.23	.50	.10	.0006	.005	.62	...
<i>MDH2</i>	2	1.09	.18	.90	.16	.01	.01	.83	...
<i>DTX2</i>	2	1.06	.27	.95	.20	.29	.44	.90	...
<i>CACNA2D1</i>	2	1.01	.61	.94	.62	.80	.98	.94	...
<i>USP18</i>	C	1.05	.55	1.24	.47	.36	.21	1.18	...
<i>DGCR8</i>	C	.98	.63	.94	.32	.83	.84	.95	...
<i>ATP50</i>	C	NE	NE	NE	NE	NE	NE	NE	...
<i>B2M</i>	C	1.07	.57	1.15	.35	.67	.29	1.08	...
<i>DSCR2</i>	C	.87	.26	.80	.28	.53	.56	.92	...
<i>IFNAR1</i>	C	.85	.45	.82	.20	.42	.49	.97	...
<i>BTG3</i>	C	.86	.44	.60	.21	.08	.09	.69	...
<i>IFNGR2</i>	C	.93	.27	.85	.22	.41	.56	.91	...
<i>GABPA</i>	C	1.11	.57	1.37	.61	.30	.31	1.23	...
<i>IL10RB</i>	C	1.03	.40	1.25	.29	.15	.10	1.21	...
<i>SON</i>	C	.61	.24	.43	.10	.03	.03	.71	...
<i>SIM2</i>	C	NE	NE	NE	NE	NE	NE	NE	...
<i>UFD1L</i>	C	NE	NE	NE	NE	NE	NE	NE	...
Nonhemizygous:									
All ^c	2	.96	.16	.80	.2384	.0003
Centromere ^c	2	1.00	.09	.87	.1787	.03
Telomere	2	.89	.22	.71	.2879	.004
Close ^d	2	.94	.19	.74	.2478	.0001
LCRs	6	.54	.21	.26	.0448	.002
Hemizygous	1	.94	.26	.38	.3740	7.3 × 10⁻⁹
Controls	C	.94	.15	.95	.30	1.01	.87

NOTE.—NE indicates that the gene is not expressed in this cell line.

^a 1 = HSA7 hemizygous in WBS; 2 = HSA7 nonhemizygous in WBS; 6 = genes mapping in the LCR flanking the WBS commonly deleted region (i.e., present in six copies/genome); C = control genes.

^b Ratio of ARELs of patients with WBS compared with controls.

^c Does not take into account *GBAS* and *PSPH*, the two genes mapping to the HSA7 short arm.

^d Close nonhemizygous genes on both the centromeric and the telomeric side.

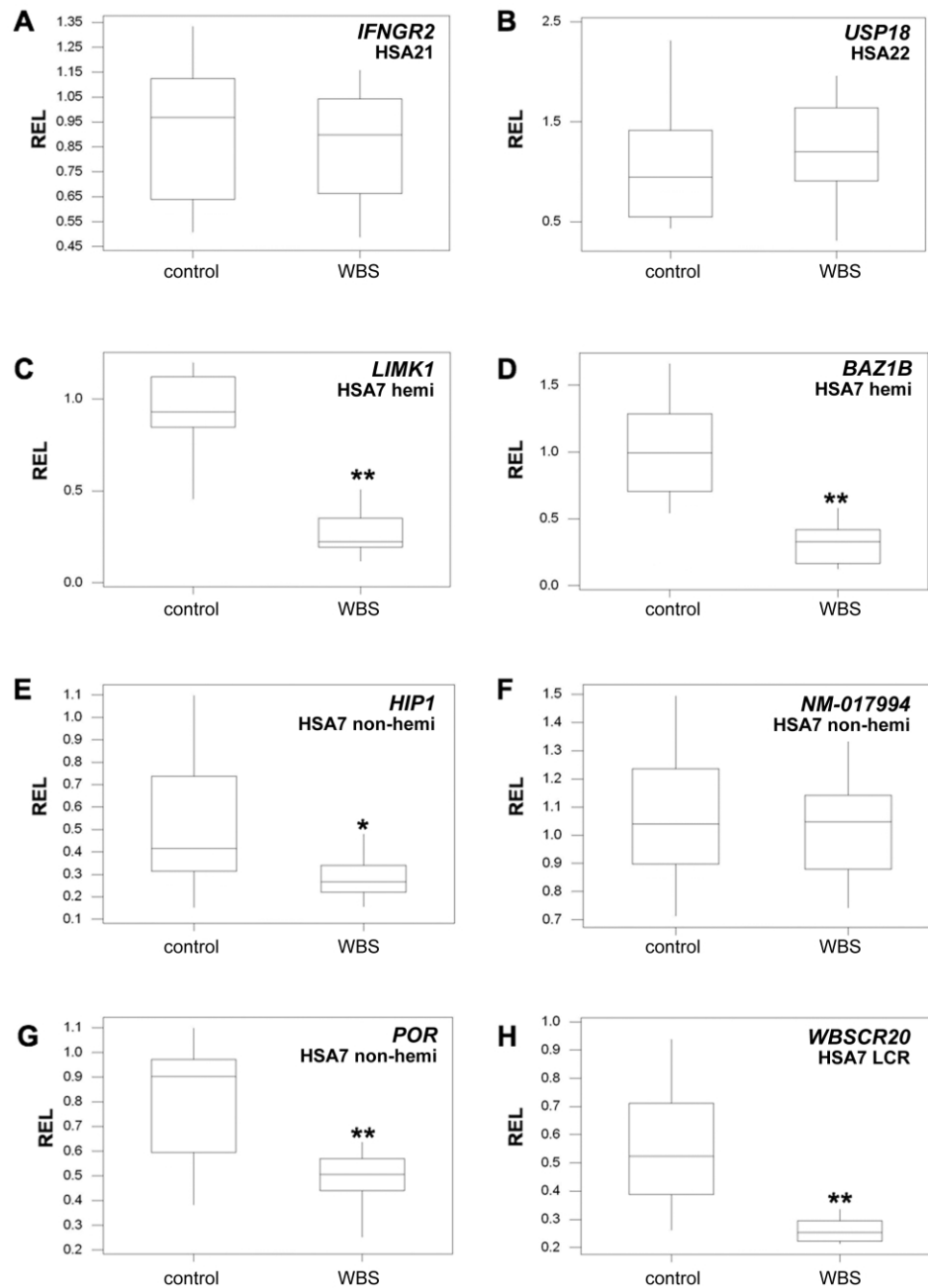


Figure 1. REL distributions measured in 14 patients with WBS and in 14 control skin fibroblasts. REL boxplots are shown for control genes (*IFNGR2* [A] and *USP18* [B]), for hemizygous genes that map to the commonly deleted WBS interval (*LIMK1* [C] and *BAZ1B* [D]), for nonhemizygous genes that map to the flank of the commonly deleted WBS interval (*HIP1* [E], *NM_017994* [F], and *POR* [G]), and for LCR genes that map to the repeats flanking the WBS deletion (*WBSR20* [H]). Asterisks indicate $P < .04$ and double asterisks indicate $P < .005$, at both t and Mann-Whitney tests.

this general pattern; the *GTF2IRD1* gene in lymphoblastoid cells and the *ELN* gene in fibroblasts show no significant changes in their ARELs between control and patient samples.

ELN haploinsufficiency has been linked to supravalvular aortic stenosis (SVAS) and to other stenoses.^{35–39} Here, we find that the REL of the *ELN* gene in skin fibroblasts is

not significantly different between the control population and the patients with WBS ($AREL_{\text{fibro}} = 1.08 \pm 0.31$; $P = .85$) (table 4). This result is in agreement with those obtained using microarray technology (A. Quattrone and G. Merla, unpublished data) but differs substantially from the one described elsewhere.⁴⁰ This discrepancy might be due to the very limited number (only one) of samples

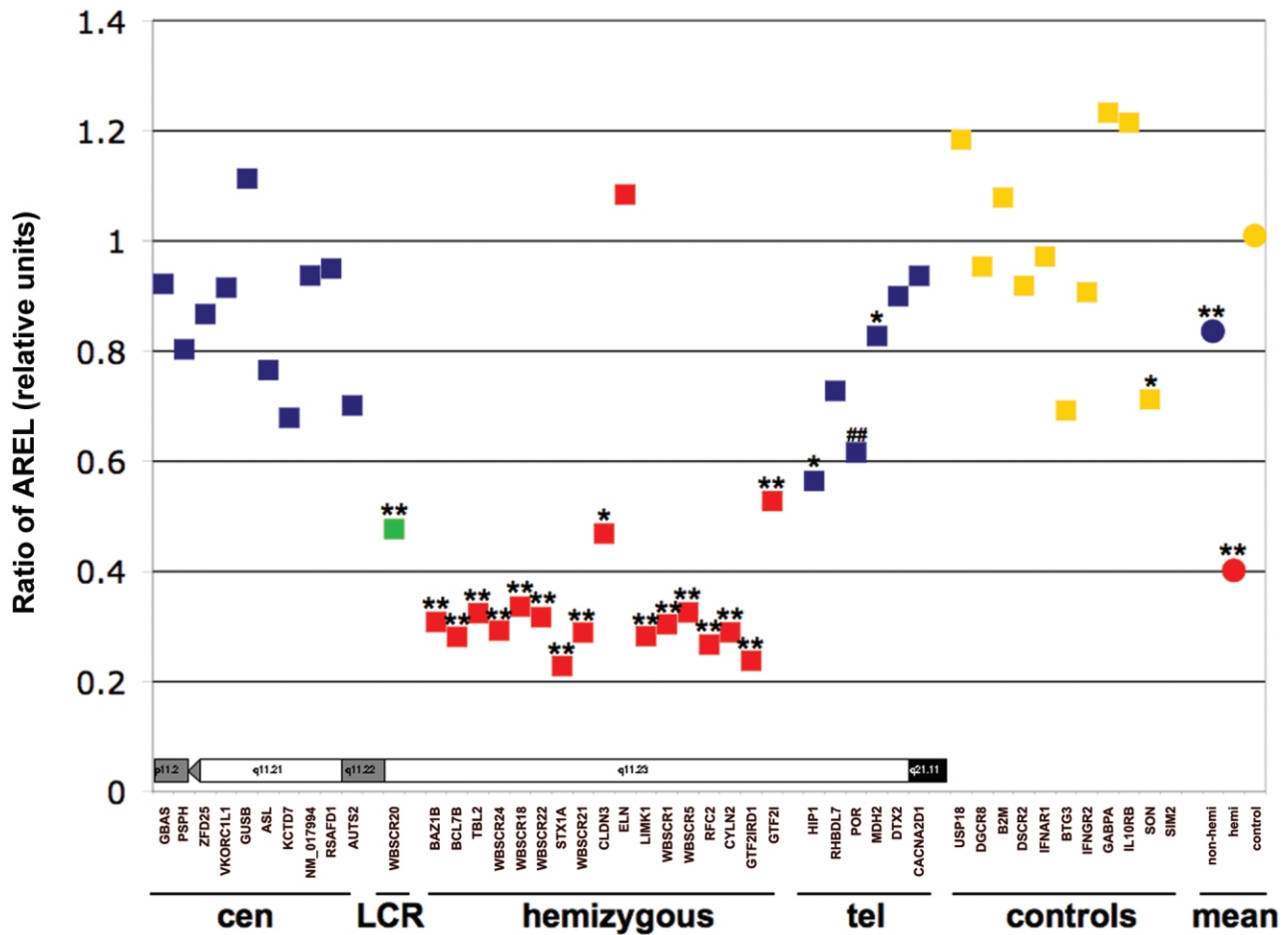


Figure 2. Differences of expression levels in patients with WBS and in controls. Ratio of ARELs from 14 patients with WBS and from 14 controls, measured in skin fibroblasts. *Left to right*, Two HSA7 short-arm genes, HSA7 long-arm genes from the centromere to the telomere, followed by the control genes (*squares*) and the mean (*disks*) of each tested gene category. Nonhemizygous WBS HSA7 genes (*blue*) map centromerically (*cen*) or telomerically (*tel*) of the deletion. LCR = Gene mapping to the repeats flanking the WBS deletion (*green*). hemizygous = Hemizygous WBS HSA7 genes (*red*). controls = Control genes mapping outside HSA7 (*yellow*). A schematic representation (not to scale) of the HSA7 cytogenetic bands in which the assessed genes map is presented in the lower part of the graph. Asterisks indicate $P < .05$ and double asterisks indicate $P < .001$, at both t and Mann-Whitney tests for individual genes and also at pairwise t test for categories. A double number sign (##) indicates that the t and Mann-Whitney tests are significant at $P < .001$ and $P < .005$, respectively (see table 4 for details).

studied and/or to the less sensitive method used in the latter study.⁴⁰ As demonstrated by the large SD (table 4), we observe a large variation in relative expression of *ELN* in the patients with WBS. It is, therefore, possible that the incomplete penetrance of the SVAS phenotype is correlated with the REL of *ELN*^{41,42}—that is, that patients who are under a compensatory mechanism of expression are less likely to present the phenotype. Consistently, the AREL of the *ELN* gene in patients with WBS with SVAS (AREL = 1.15 ± 1.08) is lower than it is in patients with WBS without this phenotype (AREL = 2.56 ± 1.97); however, this difference is not significant. To confirm this hypothesis, we will need to measure the relative expression of this gene in a large number of patients.

A mouse model and recent functional data suggest that

hemizygosity of *CYLN2* and *WBSR14* might contribute to the cognitive profile and to impaired glucose tolerance or silent diabetes, respectively, in patients with WBS.^{43–46} Whereas the study of patients with WBS with atypical deletion suggests that hemizygosity of *GTF2IRD1* and *GTF2I* is linked to their visual spatial processing deficits,^{23–29,47,48} *Gtf2ird1*-null mice display craniofacial abnormalities, thus suggesting a possible link between hemizygosity of *GTF2IRD1* and craniofacial abnormalities displayed in

Table 5. Numbers of UniGene ESTs for TRIM50 and WBSR20

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

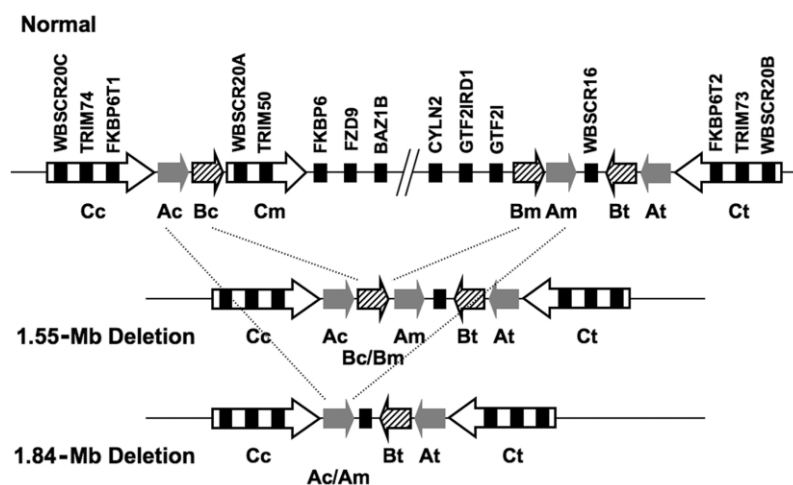


Figure 3. Schematic partial transcript map of the 7q11.23 region in normal chromosome (*top*) and in chromosomes bearing the classical WBS 1.55-Mb (*center*) and 1.84-Mb (*bottom*) deletions. The different centromeric (c), middle (m), and telomeric (t) duplicons within the LCRs are represented by specific arrows that specify their relative orientation and type. Gray arrow indicates BLOCK-A (A); striped arrow indicates BLOCK-B (B); and white arrow indicates BLOCK-C (C). Genes are depicted by black rectangles, with their names given above.

patients with WBS.⁴⁹ In this article, we find that the relative expression of *GTF2IRD1* is significantly decreased in the fibroblasts of patients with WBS ($AREL^{fibro} = 0.24 \pm 0.02$; $P = 3.2 \times 10^{-5}$) but is not affected in lymphoblastoid cell lines ($AREL^{lympho} = 1.14 \pm 0.21$; $P = .68$) (table 4). Thus, we cannot assess the contribution of the *GTF2IRD1* gene to the WBS cognitive phenotype, because its expression might be under special control in the CNS of patients with WBS.

Remarkably, a significant decrease in relative expression was observed for the nonhemizygous genes *ASL*, *KCTD7*, *HIP1*, *POR*, and *MDH2*, which map outside the common deletion region (see table 4 and figs. 1E–1G and 2), although the decrease was not as large as that observed for hemizygous genes. This decrease is significant even if we consider all the tested nonhemizygous genes in fibroblasts mapping to HSA7q or the subset of closest-tested hemizygous genes in lymphoblastoid cells (table 4 and fig. 2).

Two of the tested genes, *WBSCR20* and *TRIM50*, map in the LCRs. Three highly similar copies (*WBSCR20A*, *WBSCR20B*, and *WBSCR20C* and *TRIM50*, *TRIM73*, and *TRIM74*; >98% identity each) of each of these genes are present within the studied region (see table 3 and fig. 3).⁵⁰ *TRIM50* (also known as “*TRIM50A*”) and *WBSCR20A* map centromerically to *FKBP6* within repeat BLOCK-C-mid; *TRIM73* (also known as “*TRIM50B*”) and *WBSCR20B* map telomerically to *FKBP6T2* in BLOCK-C-tel; whereas *TRIM74* (also known as “*TRIM50C*”) and *WBSCR20C* map to the BLOCK-C-cen interval centromeric to *FKBP6T1* (see schematic representation in fig. 3).^{50–52} *WBSCR20A* and *TRIM50*, only one copy of each gene, are hemizygous in patients with WBS (fig. 3).¹⁸ We designed a Taqman assay able to simultaneously recognize all three copies and col-

lectively measure the RELs of all copies. Both *WBSCR20A/B/C* and *TRIM50/73/74* exhibit RELs decreased by about one-half and one-third, respectively, in patients with WBS (see table 4 and figs. 1H and 2), a result that deviates noticeably from the theoretically predicted decrease of 17%. It is possible that different levels of expression of the three copies account for this discrepancy. Consistently, the study of UniGene clusters suggests that *TRIM50* is expressed more than *TRIM73* and *TRIM74* together. Conversely, *WBSCR20A* is not expressed at a higher level than the B and C copies (table 5). These observations suggest that the decrease in relative expression cannot be explained solely by copy-specific expression-level differences. A possible explanation would be that the number of BLOCK-C repeats is polymorphic in the population; however, published results suggest only that the number of BLOCK-A and BLOCK-B copies are polymorphic (see fig. 3).^{52,53} Thus, our results are consistent with the hypothesis that the nondeleted copies in *cis* with the deletion are possibly affected in their expression.

Discussion

Our results suggest that, in genomic disorders, not only the aneuploid genes but also the normal-copy genes that map close to a deletion should be considered as candidate genes for features of these abnormal phenotypes, although we cannot exclude the possibility that what we observe here is only a 7q11.23 region-specific phenomenon. For example, the *HIP1*, *POR*, and *KCTD7* genes, located at distances of 0.7, 1.2, and 6.5 Mb from the WBS region, respectively (table 1), show significantly dysregulated patterns (*t* test $P = .015$ and $P = .025$, $P = 6.3 \times 10^{-4}$, and

$P = 3.8 \times 10^{-3}$, respectively; Mann-Whitney test $P = .025$ and $P = .04$, $P = 5.1 \times 10^{-3}$, and $P = 4.7 \times 10^{-3}$, respectively) (table 4) and are thus good candidates for involvement in certain WBS phenotypic features. Remarkably, it appears that this deregulation is more pronounced for genes mapping closer to the breakpoint (fig. 2). This finding also suggests the presence of very distant long-range *cis*-regulatory elements—to an extent, undescribed elsewhere—and substantiates the notion that functional gene domains extend way beyond their transcription units.⁵⁴ Although this phenomenon was observed in both a transformed and an untransformed cell line, we cannot be certain that the relative expression pattern is the same in the tissues affected with the different phenotypes. However, data obtained elsewhere, from partial Down syndrome mouse models, have shown that relative expression from aneuploid genes is significantly similar across different tissues and developmental stages.^{10,11}

Even though deletions or duplications of large genomic regions result in significant gene expression changes, our results show that the changes are not always directly correlated to copy number, which suggests an underlying complexity that might involve the size of the deletion, the altered structure of chromatin, a dosage-compensation mechanism, or a combination of these factors. In particular, we identified two transcripts within the commonly deleted WBS region for which there were no significant expression differences. Our observations also suggest that changes in the expression levels of genes neighboring large-scale copy-number polymorphisms^{2,3,55–57} might play an important role in phenotypic variation in normal populations and, possibly, in evolution.

Acknowledgments

We thank B. Conrad, E. T. Dermitzakis, S. Deutsch, P. Descombes, M. Docquier, M. Gagnebin, C. Gehrig, H. Kaessmann, C. UCLA, and the members of the GRIEG (Group Interdisciplinaire pour l'étude de l'élastine et son gene) group, for assistance and/or critical reading of the manuscript, and A. Quattrone, for sharing unpublished data. This work was supported by grants from Telathon Action Suisse, the Jérôme Lejeune Foundation, the Désirée and Niels Yde Foundation, the Novartis Foundation, the Swiss National Science Foundation (to A.R.), the European Commission (grant number 037627) (to S.E.A. and A.R.), and the Italian Ministry of Health (to G.M.).

Web Resources

Accession numbers and URLs for data presented herein are as follows:

Coriell Institute for Medical Research, <http://www.coriell.org/index.php/content/view/31/78/> (for cell lines)
 Galliera Genetic Bank, <http://ggb.galliera.it> (for cell lines)
 geNorm, <http://medgen.ugent.be/~jvdesomp/genorm/> (for selection of normalization genes)
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for WBS)
 UniGene, <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=>

unigene (for sets of transcript sequences that appear to come from the same transcription locus)

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