

Is It Williams Syndrome? *GTF2IRD1* Implicated in Visual–Spatial Construction and *GTF2I* in Sociability Revealed by High Resolution Arrays

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Genetic contributions to human cognition and behavior are clear but difficult to define. Williams syndrome (WS) provides a unique model for relating single genes to visual–spatial cognition and social behavior. We defined a ~1.5 Mb region of ~25 genes deleted in >98% of typical WS and then rare small deletions, showing that visual–spatial construction (VSC) in WS was associated with the genes *GTF2IRD1* and *GTF2I*. To distinguish the roles of *GTF2IRD1* and *GTF2I* in VSC and social behavior, we utilized multiple genomic methods (custom high resolution oligonucleotide microarray, multicolor FISH and somatic cell hybrids analyzed by PCR) to identify individuals deleted for either gene but not both. We analyzed genetic, cognitive and social behavior in a unique individual with WS features (heart defects, small size, facies), but with an atypical deletion of a set of genes that includes *GTF2IRD1*, but not *GTF2I*. The centromeric breakpoint localized to the region 72.32–72.38 Mb and the telomeric breakpoint to 72.66 Mb, 10 kb downstream of *GTF2IRD1*. Cognitive testing (WPPSI-R, K-BIT, and PLS-3) demonstrated striking deficits in VSC (Block Design, Object Assembly) but overall performance 1.5–3 SD above WS means. We have now integrated the genetic, clinical and cognitive data with previous reports of social behavior in this subject. These results combine with previous data from small deletions to suggest the gene *GTF2IRD1* is associated with WS facies and VSC, and that *GTF2I* may contribute to WS social behaviors including increased gaze and attention to strangers. © 2009 Wiley-Liss, Inc.

Key words: Williams syndrome; visual–spatial construction; social behavior; *GTF2IRD1*; *GTF2I*

INTRODUCTION

Williams syndrome (WS) is among the most compelling genetic disorders of human development and behavior and is due to a

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recurring deletion of about 1.5–1.8 megabases containing about 25–28 genes in chromosome band 7q11.23 [Ewart et al., 1993; Osborne, 1999], WS occurs once in approximately 7,500–20,000 live births [Greenberg, 1990; Stromme et al., 2002] and is associated with distinct physical, cognitive and behavioral features. Physical symptoms include small stature, a round face with small chin, flat malar region and thick lower lip, a hoarse voice, infantile transient hypercalcemia, hyperacusis, high sensitivity to music and cardio-

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vascular abnormalities, including supraaortic stenosis (SVAS) and peripheral pulmonary stenosis [Morris et al., 2006]. A subset of these features, which may change with age, is apparent in most persons with WS.

In the neurocognitive domain, performance is impaired, with a typical IQ of 60 [Bellugi et al., 2000]. Intriguingly, the hallmark difficulties in WS are highly specific and virtually universal deficits in visual–spatial function that are most clearly illustrated by tests of visual–spatial construction (VSC), especially those involving block construction and copying measured by the Wechsler and its derivatives [Wechsler, 1974], by the equivalent pattern construction test of the Differential Ability Scales (DAS) [Elliott, 1990] and by the Visual–Motor Integration (VMI) [Beery and Buktenica, 1989]. In WS, these striking defects in visual spatial construction stand in contrast to more subtle defects in processing stimuli that are more specifically social, such as language and faces [Bellugi et al., 2000]. The convergence of different methodologies including integrated analyses of genetics and behavioral performance, are needed to help clarify puzzling findings of impairment in some visual–spatial tasks (pattern construction, copying, drawing) and relative proficiency in others (face and object recognition) found in people with WS.

Of further interest for the current work is that persons with WS appear hypersociable from early in life [Doyle et al., 2004; Järvinen-Pasley et al., 2008]. In addition to general overfriendliness, individuals with WS seem highly engaging, affectionate, interpersonally sensitive, empathic, and socially disinhibited [Udwin and Yule, 1991; Gosch and Pankau, 1994; Dykens and Rosner, 1999; Sullivan and Tager-Flusberg, 1999; Tager-Flusberg and Sullivan, 2000; Plesa-Skwerer et al., 2004; Dykens et al., 2005]. This behavior may be called gregarious, with affected individuals showing eagerness to make eye contact with, and to approach strangers, even at ages when children are normally shy [Mervis and Klein-Tasman, 2000; Jones et al., 2000, 2001; Klein-Tasman and Mervis, 2003]. On the other hand, difficulties in peer interactions [Udwin and Yule, 1991; Gosch and Pankau, 1994, 1997] and in social judgment [Einfeld et al., 1997; Gosch and Pankau, 1997] are common, suggesting that the so-called “hypersociability” does not translate into more advanced social cognitive abilities in WS [Sullivan and Tager-Flusberg, 1999; Tager-Flusberg and Sullivan, 2000]. Structural [Reiss et al., 2004] and functional [Meyer-Lindenberg et al., 2005] magnetic resonance imaging (MRI) and cross-cultural data [Zitzer-Comfort et al., 2007] have suggested that the WS deletion contributes to social behavior. Taken together, evidence to date suggests that the sociability associated with WS is complex, in part genetically based, and carefully designed studies are needed to further elucidate its nature.

We have proposed that genes affecting measurable cognitive features may be implicated by their occurrence in individuals with small deletions [Botta et al., 1999; Korenberg et al., 2000; Bayes et al., 2003; Gagliardi et al., 2003; Heller et al., 2003; Hirota et al., 2003]. For example, deletion of *STX1A* through *GTF2I* [Botta et al., 1999] has been associated with a subset of cognitive as well as typical cardiovascular and facial features and with further cases, the deletion of the genes *GTF2IRD1* and *GTF2I*, located at the telomeric end of the deletion [Hirota et al., 2003; Tassabehji et al., 2005] has been implicated in WS defects of VSC. Further parsing of the effects of *GTF2IRD1* versus *GTF2I* on WS cognitive and social behavior has

been hampered by the small number of cases with small deletions and the limited cognitive data.

Previously, the variability of social behavior in children with WS has been documented using the Salk Institute Sociability Questionnaire (SISQ) [Doyle et al., 2004; Järvinen-Pasley et al., 2008] and direct observation according to a prespecified ethogram [Järvinen-Pasley et al., 2008]. These reports contrasted and quantified social behavior in toddlers with the typical WS deletion and compared these to a putatively atypical deletion case (5889), the molecular breakpoint analysis for which is reported here and was referred to incorrectly without data in the report of her social behavior in Järvinen-Pasley et al., 2008. Here we report on the clinical and developmental history, physical and cognitive features as well as high resolution molecular genetic analysis of the breakpoint in the atypical deletion Subject 5889 and integrate these data with the social behavior reported previously. We now report the specific WS features found in this subject from age 14 months to 7 years, including facies, cardiovascular (SVAS, PPS, renovascular stenoses, and hypertension), delayed growth, and deficits in VSC but also atypical WS features including social behavior and normal developmental milestones in combination with somewhat higher cognitive function. Further, to determine the breakpoint, we have developed a custom high density oligonucleotide array of the human genomic sequence spanning the 14 Mb region that includes the WS deletion and applied this to show that the deletion in 5889 results in the complete hemizygoty of all genes in the typical WS region except one, *GTF2I*. This provides the capability for molecular definition of breakpoints at the level of single exons, and, in combination with previous data on atypical subjects, allows the further parsing of genes with phenotypes in WS: the gene for *GTF2IRD1* with the visual–spatial constructive deficits, *GTF2I* with aspects of WS social behavior and both to abnormal cognitive development in WS.

MATERIALS AND METHODS

Subject

The family of Subject 5889 reported in this study was given informed consent by their geneticists, which was approved by the IRB of Cedars-Sinai Medical Center, Los Angeles. The clinical features were evaluated by a geneticist with experience in genetic disorders; a follow-up examination was performed when necessary.

Deletion Analysis

Fluorescence in situ hybridization (FISH). The chromosomes were prepared from peripheral blood by following protocols described by Korenberg and Chen [1995; supporting information (supporting information may be found in the online version of this article)]. The FISH study was performed in two-steps. First, three BAC probes that cover the entire common WS deleted and flanking duplicated regions were used to screen for the ends of the deletion (Fig. 2A). The probes included BACs 1008H17, 592D8 and 1184P14, of which, 1008H17 includes the genes FKBP6 and FZD9, 592D8 includes ELN, and 1184P14 includes *GTF2I*. The probe 815K3 was used for an internal experimental control. Next, a series

of eight additional BAC, PAC or cosmid probes were tested to define the breakpoint. These included BACs P195H06 (for *STX1A*), 1148G03 (for *ELN*), 54H15 and PACs 1186P10 (both overlapping *GTF2IRD1* and *GTF2I*), 239C10 (marking the flanking duplicated regions), and cosmids 182B11 (for *CYLN2*) and 183E1 (for *GTF2IRD1*).

Somatic cell hybrids and PCR. An immortalized lymphoblast cell line was generated from Subject 5889, grown under standard conditions [Korenberg and Chen, 1995] and somatic cell hybrids were constructed to isolate the deleted and the non-deleted chromosome 7 (GMP Genetics) [Yan et al., 2000]. Chromosome 7 colonies were selected using PCR of polymorphic markers located outside the WS region and DNA was extracted from colonies 9, 11, 12, and 13. To identify the telomeric breakpoint, we utilized PCR with primers within the deleted region (established by FISH) and 14 primer sets flanking the predicted breakpoint region from *GTF2IRD1* (73.661 Mb, NCBI build 36.2) through the 5'-end of *GTF2I* (73.671 Mb) as shown in Figure 2B and supporting information Table II (supporting information Table II may be found in the online version of this article).

Oligonucleotide isothermal high resolution DNA tiling microarray. A custom high resolution isothermal oligonucleotide DNA tiling array (Roche NimbleGen, Madison, WI) was developed and used to examine both breakpoints (Fig. 2C). All array CGH experiments were performed according to previously reported methods [Selzer et al., 2005] except that the copy number calling algorithm segMNT was used, which is available in NimbleScan software (version 2.4.27, www.nimblegen.com). DNA was isolated from both blood and cell lines generated from Subject 5889 (labeled with Cy3) and cohybridized to the array using the paternal DNA (labeled with Cy5) as a control as the deleted chromosome had been determined to be of paternal origin by using a panel of VNTR markers (raw data available upon request). The first step used DNA isolated from blood for screening on an initial array tiling all of chromosome 7. This analysis confirmed the 7q11.2 deletion, and showed that the centromeric breakpoint was located within the duplicated region, as found with BAC-FISH and as expected for typical WS deletions. To further define both breakpoints, a custom high density oligonucleotide array was generated that consisted of 385,000 probes spanning the region of 64–77 Mb of chromosome 7 (NCBI Build 36.1, HG18), which includes the putative WS duplicated region, located at 70–71.5 Mb and the flanking duplicated regions. Probes on the array were spaced at a median distance of 20 bp and contained 45–85 bp oligonucleotide probes which were adjusted in their C + G content to yield similar optimum hybridization temperatures (isothermal array, $T_m = 76^\circ\text{C}$) [Urban et al., 2006; Korbel et al., 2007]. In contrast to previous arrays and to optimize the ability to define a WS breakpoint within the centromeric duplicated region, oligonucleotide probes were included that occurred up to ninefold in the human genome. This would also provide the possibility to evaluate the contribution of pseudogenes and other copy number variation to WS phenotype. As the subject's deletion occurs on the chromosome of paternal origin, use of the father's DNA as the control provided the potential to precisely determine the subject's breakpoint with respect to single exons of the two related genes, *GTF2IRD1* and *GTF2I*, located at the telomeric end of the typical WS deletion. The breakpoints deter-

mined by array CGH analysis were validated by FISH and somatic cell hybrids with PCR.

Cognitive-Behavioral Analysis

Subject 5889 was diagnosed at 14 months and evaluated at 2½ to 7 years at Cedars-Sinai Medical Center, and seen at 2 years 6 months and at 4 years 4 months at the Salk Institute for Biological Studies. Participants reported here in the comparison groups for cognitive performance were individuals from a cohort of children initially recruited for a collaborative project with the Center for Neurodevelopmental studies at the University of California-San Diego, and included children with full WS deletions, children with Down syndrome (DS), as well as typically developing children (TD).

For the Wechsler Preschool and Primary Scale of Intelligence—Revised (WPPSI-R) [Wechsler, 1989], the cohort included seven WS aged 4–7 years, and 5889 was at age 4 years 4 months. For the Kaufman Brief Intelligence Test (K-BIT) [Kaufman and Kaufman, 1990], the cohort was 11 children with WS aged 4–10 years, and 5889 was at age 4 years 4 months. For the Preschool Language 3rd Edition (PLS-3) [Zimmerman, 1992], the cohort was 22 children with WS aged 1–5 years, and 5889 was at age 2 years 6 months and 4 years 4 months. No significant difference in ages was observed between the groups.

For the SISQ data used to map social features [Doyle et al., 2004; Järvinen-Pasley et al., 2008] versus gene deletion, the cohort included 34 WS aged 2–7 years, 18 DS aged 2–7 years, 18 TD aged 2–7 years and 5889 aged 2 years 6 months and 4 years 4 months. For the social observation study [Järvinen-Pasley et al., 2008], the cohort included 8 children with typical deletions for WS aged 3 years, mental age of approximately 16 months, and 8 chronological age- and mental age-matched children with DS, and 5889 aged 2 years 6 months.

Psychometric assessment. For the current study, we report on three measures of verbal and non-verbal intelligence (WPPSI-R, K-BIT, and PLS-3; SOM). For all measures, individual scores from Subject 5889 were compared to those of the full deletion WS group and those of the typically developed children group by conversion to z-scores with a mean of zero and standard deviation of one (see Fig. 4). Given the single-case contingency, we were forced to forgo inferential statistics and to rely on the base rates logic as a basis for comparison. Since z-scores reflect normal distributions, z-scores of 2.0 or higher were considered to be statistically significant (i.e., different from that population), as the probability of achieving such scores by chance is less than 0.05.

RESULTS

Physical and Developmental Features

A summary of the clinical features is shown in Table I and a complete clinical description of Subject 5889 is included in the SOM. Briefly, 5889 was born at 38 weeks gestation (weight at birth 10 centile, but at 4 4/12, 3 centile; height and head circumference <3 centile). She has the typical physical and developmental features of WS including growth delay through age 7, facial (Fig. 1 and Table I), ophthalmologic (strabismus), cardiac (SVAS, PPS), vascular stenoses (renal artery stenosis), and hypertension (Table I).

TABLE I. Summary of Frequencies of Clinical features of Subject 5889 Versus Typical WS

	Typical WS	Subject 5889
Craniofacial		
Bitemporal narrowing	+	+
Stellate iris	+	+
Epicanthal folds	+	+
Periorbital fullness	+	+
Medial eyebrow	+	+
Malar hypoplasia (abnormal cheekbones)	+	+
flare full cheeks	+	+
Short nose	+	+
Bulbous nasal tip	+	+
Long philtrum	+	+
Prominent lips	+	+
Small jaw	+	+
Dental Abn/malocclusion	85	+
Cardiovascular defects		
SVAS	75	+
PPS	50	+
VSD	10	-
Renal artery stenosis	+	+
Hypertension	50	No at infancy, yes at childhood
Genitourinary		
Structural anomaly		
Enuresis		
Nephrocalcinosis		-
Recurrent urinary tract infections		-
Musculoskeletal and supportive features		
Joint hypermobility	90	-
Joint contractures	50	-
Radioulnar synostosis		-
Long neck or sloped shoulders		+
Joint limitation or laxity		-
Rectal prolapse		-
Joint limitation		-
Long trunk		+
Hyperflexibility		+
Abnormal gait		-
Preoccupation, spinning, rhythmic movements		-
Hallux valgus		-
Sacral crease		-
Frequent urination or delayed continence		-
Strong attraction to music or perfect pitch		+
Inguinal hernia	40/50	-
Others		
Soft or velvety skin	+	+
Hypercalcemia	15	-
Hypothyroidism		-
Diabetes mellitus		-
Strabismus	50	+
Esotropia	50	+
Hypersensitivity to sound	+	-
Hoarse voice	+	+
Hyperacusis	80	-
Over friendly	+	-
Anxiety	+	-
Short attention span	+	-
Motor delay	+	-
Language delay	+	-



FIG. 1. Facial photographs of Subject 5889 at 14 months (A), 2.6 years (B), and 7 years (C) old. Reproduced with parental permission. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Motor developmental milestones are within the normal range (sitting at 6–7 months; cruising at 10 months; walking at 13 months) and neural exam normal (strength, tone and gait) from 4 months. However, in contrast to typical WS, language was not delayed and she was “highly talkative” at 29 months; results on the MacArthur Toddler form of the CDI (Child Development Inventory) were at the mean for typically developing children in sentence complexity, at the 50% for 24 Months (vocabulary production) and at 50% for 26 months (use of irregular nouns and verbs). In summary, although her physical development and milestones were typical for WS, she did not demonstrate the typical delay in language, or the overly social behavior, characteristic of young children with WS.

Deletion Analysis

Subject 5889 was diagnosed with WS by clinical presentation and FISH (Oncor, Gaithersburg, MD) at 14 months but was suspected to have an atypical deletion and was referred for molecular analysis in view of her normal developmental milestones.

The results of molecular cytogenetic analyses are shown in Figures 2 and 3. At the centromeric end, the probe BAC1008H17 was not present, suggesting that the breakpoint was located beyond FKBP6 and FZD9. At the telomeric end, however, the presence of a signal for probe BAC1184P14 but a decreased signal for BAC1186P10 suggested that the break occurred in the region of *GTF2IRD1*–*GTF2I* and further analyses were necessary to define the precise breakpoint with respect to these two genes (Fig. 2A). Therefore, somatic cell hybrids were generated and were analyzed with high density PCR probes spanning the region. PCR indicated that colonies 11 and 13 contained the deleted chromosomes 7 and colonies 9 and 12, the non-deleted. Further PCR probes narrowed the telomeric breakpoint to a 249 bp region located 10 kb downstream of the gene for *GTF2IRD1*, defined by probes RD 49 and RD50 (Fig. 2B and supporting information Table II (supporting information Table II may be found in the online version of this article)).

Chromosomal breakpoints were mapped by array CGH using a custom high resolution oligonucleotide isothermal DNA tiling array (Roche NimbleGen). While the custom array with a median probe spacing of 20 bp and more complete coverage of the duplicated regions can potentially map breakpoints to the

100–200 bp level, the centromeric breakpoint could not be further refined beyond the FISH analysis (Fig. 2A) due to the complexity of this region (Fig. 2C, lower left panel), which contains segmental duplications and other copy number variants. However, the telomeric breakpoint was localized to 73,665,076–73,666,056 bp, located 10 kb downstream of the 3'-UTR of the gene *GTF2IRD1*, which is consistent with the PCR analysis of the somatic cell hybrids. Therefore, the deleted region in Subject 5889 possibly includes genes *NSUN5* and *TRIM50*, most of *FKBP6* and all of the other genes in the interval through *GTF2IRD1*, but not the neighboring gene, *GTF2I* (Fig. 3).

Neurocognitive Performance

The upper panel of Figure 4 summarizes Subject 5889's z-scores on the WPPSI-R, K-BIT and PLS-3 subtests, as compared to WS children with typical deletions, while the lower panel of Figure 4 shows Subject 5889's scaled scores on these subtests relative to those of WS and the WPPSI-R, K-BIT and PLS3 typical norms. On the WPPSI-R, Subject 5889 obtained a Full Scale IQ (FSIQ) score of 78, with a Performance IQ (PIQ) score of 71 and Verbal IQ (VIQ) score of 88, which were 1–2 SD higher than typical WS children. Individuals with WS with classic deletions typically exhibit mean FSIQ of approximately 54–59 (SD = ~11) [Bellugi et al., 2000; Meyer-Lindenberg et al., 2006], with greater impairments seen in PIQ than in VIQ. Her verbal scores were significantly above that of other WS children on three out of five verbal subtests (Information, Arithmetic, Comprehension; z-scores = 2–6 above the WS means). Supporting her superior language skills relative to typical WS, Subject 5889's performance on the K-BIT and PLS-3 was also significantly higher than that of her typical WS peers. In contrast, her scores on VSC were striking, with high or normal performance on tests involving only recognition (Picture Completion SS 12; Geometric Design Recognition subcomponent raw score 6/7), but poor performance on all subtests that involve motor integration (Block Design 4, Object Assembly 3; Mazes 1, only 9/57 raw score on the drawing items of Geometric Design, and poor performance on forms 7–9 of the VMI).

Relationship Between Neurocognitive Performance and Social Behavior

In order to investigate whether the behavioral dimensions of sociability may be independent of, and/or not easily explained, by the level of cognitive functioning in WS, we carried out correlations between the SISQ Approach Strangers subscale and IQ on data from individuals with WS using the cohort previously reported [Doyle et al., 2004]. Twenty children and adolescents with WS (11 M, 9 F; M age = 13.47, SD = 2.31; range 7.9–16.7 years) were administered the SISQ and either the WISC-III (Wechsler Intelligence Scale for Children, 3rd edition 1991) or WISC-R [Wechsler, 1974]. Mean FSIQ = 52.0, SD = 7.7, range 42–66. SISQ Approach Strangers scores were not correlated with Full Scale IQ ($r = 0.031$, $P = 0.90$). Further, there were no differences between males and females (FSIQ; $t(18) = 3.43$, $P = 0.74$; Approach Strangers; $t(18) = -0.070$, $P = 0.95$).

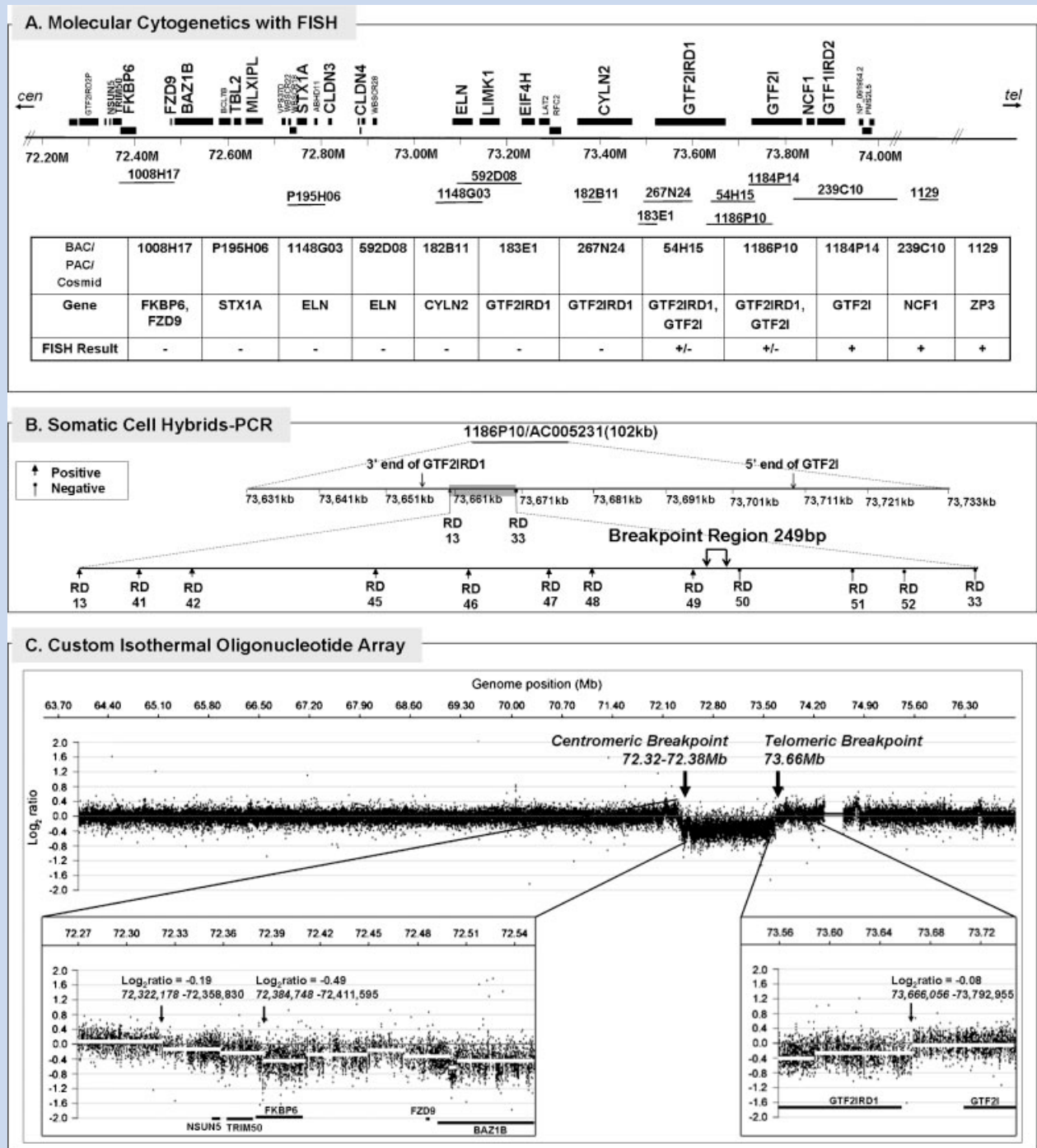


FIG. 2. Deletion breakpoint analysis of Subject 5889. **A:** Physical map of BAC/PAC/cosmid probes used for molecular cytogenetics with fluorescence in situ hybridization (FISH) and summary of results defining deletion in 5889. **B:** Polymerase chain reaction (PCR) with somatic cell hybrids of the distal breakpoint region identified by FISH. **C:** High resolution array CGH analysis of a 13 megabase region on chromosome 7 (64–77 Mb) using a custom isothermal oligonucleotide tiling array. The top panel shows the complete tiled region in a 10X window averaged view (200 bp probe spacing). Bottom left and right panels show zoomed in views of the proximal and distal breakpoints for the deletion in which all data points (unaveraged data set with 385,013 probes and 20 bp median spacing) were analyzed. Red line segments in both bottom panels denote copy number calls by the analysis algorithm (see Materials and Methods Section).

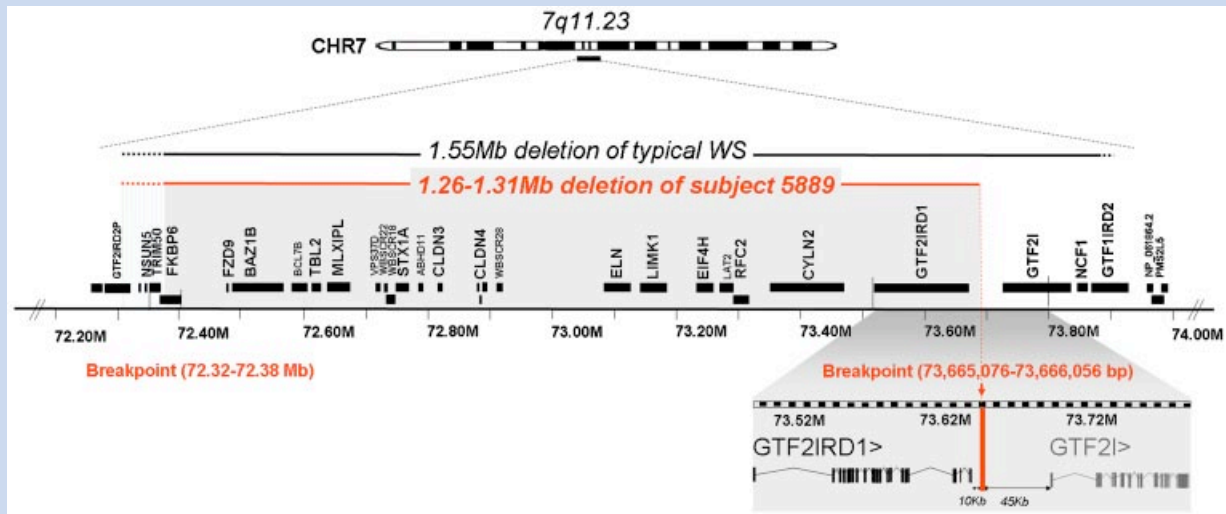


FIG. 3. Molecular map of the Subject 5889. High resolution molecular determination of deletion breakpoints in Subject 5889 occur within 72.32–72.38 Mb at centromeric end and 10 kb downstream of *GTF2IRD1* at telomeric end.

DISCUSSION

WS provides a unique model in which to link human behavior with human genetics. The role of specific genes in WS is emerging from the analysis of atypical deletions but at present, these are limited to a role for *GTF2IRD1* and *GTF2I* in visual–spatial processing [Hirota et al., 2003] and suggested facial features [Hirota et al., 2003; Tassabehji et al., 2005] and further progress calls for high resolution genomic analyses combined with paradigms that differentiate cognitive and social features. Moreover, there are no previous reports that clearly distinguish the roles of *GTF2IRD1* versus *GTF2I*. The current report provides the evidence to dissect genes for WS, particularly for *GTF2IRD1* in a subset of facial features (ages 14 months–7 years) and VSC, and for *GTF2I* in aspects of the social behavior of WS (Fig. 6). Moreover, Subject 5889 is a child with many physical characteristics of WS including short stature, SVAS, PPS and other vascular stenoses. Taken together with her physical features, the deletion in 5889 shows that *GTF2I* may not play a necessary role in the short stature, facial features, SVAS, PPS and renal-vascular stenoses, but may play an important role in some aspects of cognition and social behavior seen in WS.

At the beginning of study, it was a challenge to determine the breakpoint in the duplicated region as the assembled human genome was not available. In this report, we further refine the molecular breakpoints of Subject 5889 by combining high resolution oligonucleotide array CGH analysis with multicolor fluorescence hybridization and PCR with somatic cell hybrids. The centromeric breakpoint maps to the region 72.32–72.38 Mb, which includes most of *FKBP6* and possibly includes *NSUN5* and *TRIM50*. Due to the presence of segmental duplications and copy number variants in this region, further molecular analysis (e.g., CGH with alternate reference DNAs) will be required to more precisely map this breakpoint. The telomeric breakpoint maps to a 249 bp region located between *GTF2IRD1* and *GTF2I*, a

region with no homology to the centromeric breakpoint. The gene for *GTF2I* is left intact, along with 45 kb of the upstream putative control region, which may contribute to more normal function of this non-deleted gene. The location of the breakpoints in non-homologous DNA sequences suggests that the break in 5889 may have occurred by non-homologous end joining (NHEJ) or other processes that do not require homology at the breakpoint, and support the proposal that other mechanisms predispose to genomic instability of the WS region [Korenberg et al., 2000; Korbel et al., 2007].

WS facial features are associated with atypical deletions but the genes have not been completely defined. The classic WS facial appearance has been associated with deletions that include *CYLN2*, *GTF2IRD1*, and *GTF2I* [Botta et al., 1999; Heller et al., 2003] but not with those that preserve this region [Tassabehji et al., 1999; Hirota et al., 2003; Karmiloff-Smith et al., 2003; Tassabehji et al., 2005; Howald et al., 2006; van Hagen et al., 2007]. Deletion of *CYLN2* in combination with *GTF2IRD1* and *GTF2I* has been associated with typical WS facial dysmorphism; for example, Patients A and B [Botta et al., 1999] and Cases 2 and 3 [Heller et al., 2003]. However, deletion of *CYLN2* combined only with partial deletion of *GTF2IRD1* (and preservation of *GTF2I*) has shown “mild” facial features. For example, family K3321 with four members [Morris et al., 2003] had long philtrum in all and in one of four, flat malar region, bitemporal narrowing or full cheeks reported; HR [Tassabehji et al., 2005] had “facial features . . . suggestive of, but not classical for, WBS.” A contribution of *CYLN2* is suggested by the association of its partial deletion (with preservation of both *GTF2IRD1* and *GTF2I*) with “mild” facial features (e.g., a case in Gagliardi et al., 2003 and IM3 in Howald et al., 2006). In contrast, WS deletions that do not affect *CYLN2*, *GTF2IRD1*, or *GTF2I*, are not reported to have WS facial features (e.g., Cases 2 and 3 [Hirota et al., 2003], CS [Tassabehji et al., 1999, 2005; Karmiloff-Smith et al.,

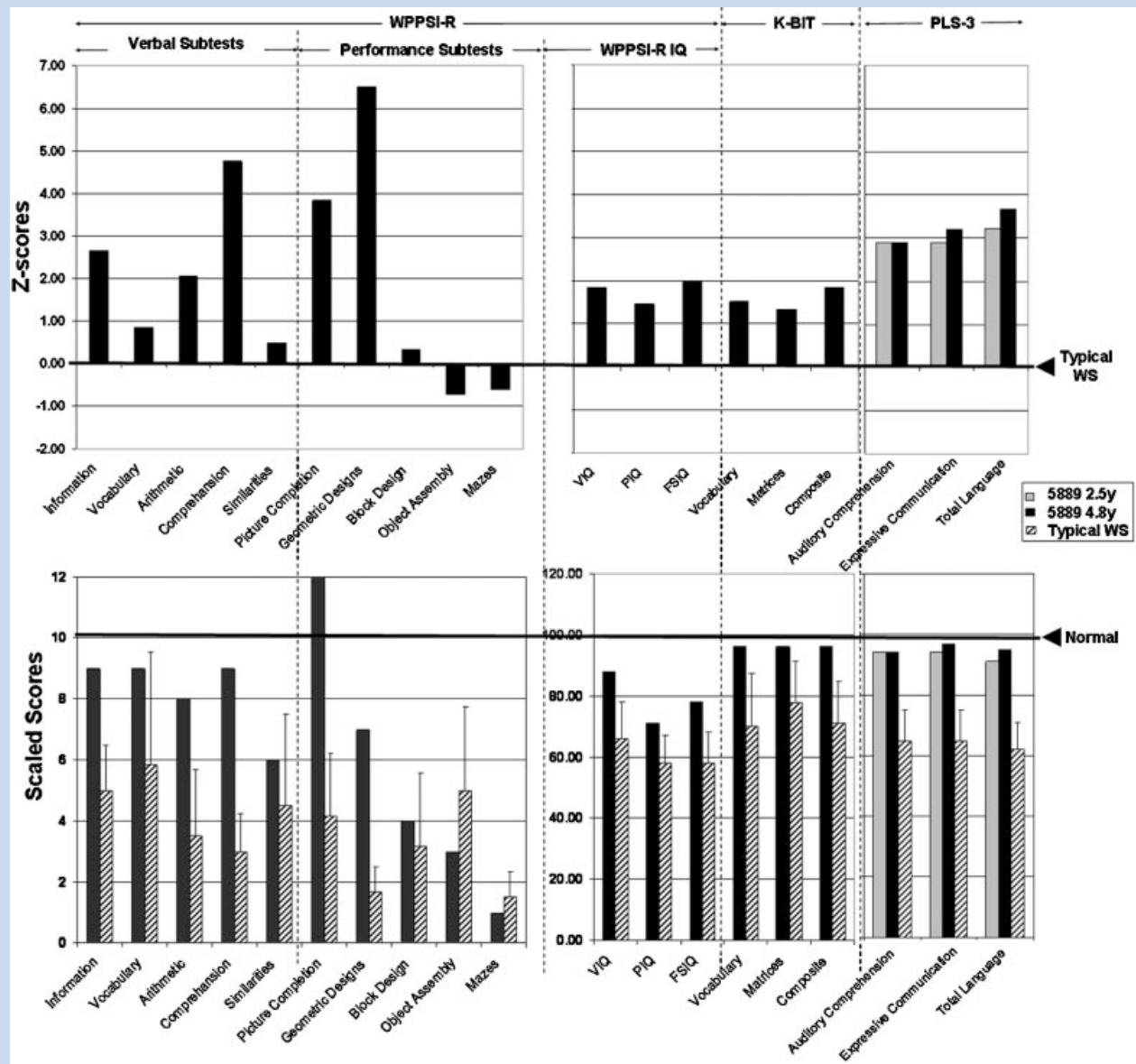


FIG. 4. Subject 5889's scores on cognitive measures (WPPSI-R, K-BIT and PLS-3) as compared to mean WS performance (upper panel, Typical WS score denoted by right arrow) and compared to WS children with typical deletion (hatched bars) and typically developed children (lower panel, Normal score denoted by right arrow).

2003], CO3 [Howald et al., 2006], and WS75 [van Hagen et al., 2007]). Thus, the evidence from small deletions implicates a role for *CYLN2* and *GTF2IRD1* in mild facial features and the case reported here illustrates that full deletion of *GTF2IRD1* is associated with typical WS facies (Fig. 6). The significance of case HR [Tassabehji et al., 2005] (who quantitatively showed "mild" features) for determining the genetics of WS facial features is unclear as the deletion included only the first of 26 exons for *GTF2IRD1*. It is of interest that although decreased expression was detected, the gene region probed was not specified and may represent a transcript not decreased in full deletion as this decrease has not been confirmed by

other groups [Merla et al., 2006; Edelmann et al., 2007]. The current Subject 5889 has the typical facial features of WS (shown in Table I and Figure 1) and these results allow the conclusion that deletion of the gene for *GTF2IRD1* is associated with the WS facial features seen in 5889. Recent results showed that craniofacial tissues are one of the distributions of *Gtf2ird1* in mouse [Palmer et al., 2007]. However, the null mutation of *Gtf2ird1* produced by the targeted insertion of a nls-LacZ cassette into exon 2 of the gene does not exhibit craniofacial dysmorphism or dental abnormalities [Palmer et al., 2007]. This is different from the previous report by another group, in which the null mutant mice contain a deletion of

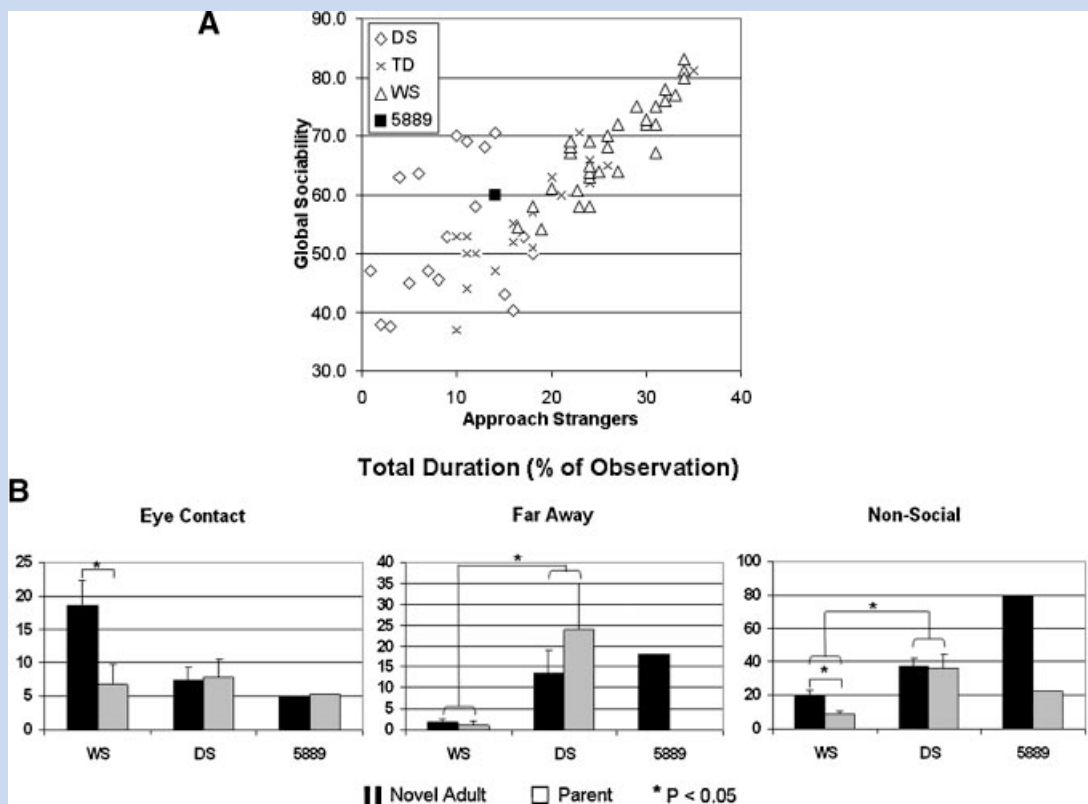


FIG. 5. Quantification of social behavior in Subject 5889 versus WS, Down syndrome (DS) and controls (typically developed, TD). Data replotted from Doyle et al. (2004) and Järvinen-Pasley et al. (2008). **A:** The atypical social behavior of Subject 5889 was reflected in parental ratings of social behavior using the SISQ at age 2.55 [Doyle et al., 2004, vs. WS only] and were repeated at age 4.6 years [Järvinen-Pasley et al., 2008, vs. a single typical WS case only]. In contrast with the age-matched typical WS group, as well as children with DS and TD, the parental ratings (converted to z-scores for comparison) revealed that 5889's Global Sociability and Approach Strangers scores both at 2.55 years ($z = -2.59$ and -2.39 , respectively) and at 4.86 years ($z = -2.6$ and -3.2), were significantly different from her peers in the typical WS group. **B:** A number of key differences in direct social behavior were noted between the Subject 5889 and children with typical WS deletions [shown in Järvinen-Pasley et al., 2008], relative to control children with DS for the mean total durations of three ethogram behaviors. Overall, WS children with full deletions were significantly less likely than children with DS to spend time far away ($P = 0.007$), and to engage in non-social activities with ($P = 0.01$), with adults, whether familiars (mothers) or novel (the experimenter), highlighting their tendency toward social over non-social activities. Furthermore, WS children with full deletions were significantly more likely to maintain eye contact with the novel adult than did children with DS ($P = 0.038$). Compared to the typical WS group, Subject 5889 (tested at age 2.5 years) spent a greater amount of time far away the novel adult ($z = 7.96$), and engaged in non-social activities for a greater amount of time with both the parent ($z = 2.39$) and the novel adult ($z = 6.00$). The difference in the amount of eye contact toward the unfamiliar adult failed to reach significance ($z = 1.24$), due to a large SD within the WS group. These results provide converging evidence for the SISQ data presented above, showing that Subject 5889 did not display the hypersociability characteristic of individuals with WS full deletions.

~40 kb from downstream of *Cyln2* to exon 1 of *Gtf2ird1*, and display craniofacial dysmorphism and dental abnormalities [Tassabehji et al., 2005]. A more complete and specific *Gtf2ird1* null mutant mouse model may be required to explain the discrepancy.

The cognitive performance of Subject 5889 differed significantly from that of age-matched WS children with typical deletion (Fig. 4). Specifically, her performance on many language tasks (except

Similarities) and some performance tasks (Picture Completion and the Spatial Recognition part of Geometric Designs) were well above the typical range for WS, suggesting that her normal copy number of GTF2I may have contributed to her increased function in both verbal and performance domains. However, her poor performance on some of the spatial tests (Block Design, Object Assembly, Mazes, Drawing part of Geometric Designs) is similar to

FIG. 6. WS Cognition: Gene map of visuospatial construction and social behavior. **A:** Summary of facial features, cognition test scores, and gene copy number analysis of GTF2IRD1 for WS patients with typical and atypical AT deletions. **B:** WS region gene map and deletion summary of typical and atypical WS patients and corresponding mapping of reported phenotypes.

WS subject	WS facial features	Cognition						Copy number of GTF2IRD1 in genome			
		IQ (SD)	Verbal performance (SD)	Spatial performance (SD)	Visual-spatial construction						
					Block design (SD)	Copying /VMI	Object assembly (SD)		Picture completion (SD)		
WS with typical deletion	Children (4-7 y) ¹	61 (11.2) ^F	66 (12.8) ¹	60 (10.4) ⁴	3.29 (2.21) ⁵	poor	4.71 (2.83) ⁶	6 (2.89) ⁷	1		
	Adolescence ⁸	64 (10) ¹	61 (10.8) ⁴	64.3 (10.2) ³	2.2 (1.5) ¹⁸	poor	2.36 (2.1) ¹⁸	504 (1.72) ¹⁹			
	Adult (40-90y) ¹¹	67.67 (9.26) ¹¹	71.79 (9.07) ¹¹	66.18 (9.06) ¹⁴	4.0 (1.71) ¹⁵	poor	3.62 (2.27) ¹¹	4.67 (2.7) ¹⁵			
WS with atypical deletion	AT1	yes	48 ¹⁰							1	
	AT2	yes	69 ¹⁰								
	AT4	yes	78 ²	88 ²	71 ⁴	4 ⁵	poor	3 ⁶	12 ⁷	1	
	AT5	mild	101 ¹⁸	106 ¹⁸	T=31 ¹⁸ , T=41 ²¹						1 + partial
	AT6	mild	94 ¹⁸	98 ¹⁸	T=38 ¹⁸ , T=58 ²¹						1 + partial
	AT7	mild	91 ¹⁸	95 ¹⁸	T=36 ¹⁸ , T=46 ²¹						1 + partial
	AT8	mild	74 ¹⁸	75 ¹⁸	T=24 ¹⁸ , T=31 ²¹						1 + partial
	AT9	mild		delay	serious deficits						1 + partial
	AT12	mild	83 (WS 59, SD 16.22) ²²	mildly restricted		2.00 (WS 1.40, SD 1.20) ²³	VMI 4.30 (WS 3.20) ²⁴				2
	AT13	no		110 (WS 58, SD 15.22) ²²	115 (WS 48, SD 2.18) ²²						2
AT15	no	64 ¹⁰	64 ¹⁰	72 ¹⁴	6 ¹⁵		9 ¹¹	9 ¹¹		2	
AT17	no				26 (WS 14) ²⁷	Beery-VMI 37 (WS 7) ²⁸	34 (WS 25) ²⁸			2	

¹ n=7; ² WPPSI-R IQ; ³ WPPSI-R Verbal IQ; ⁴ WPPSI-R Performance IQ; ⁵ WPPSI-R score; ⁶ n=15; ⁷ WAIS-R IQ; ⁸ WAIS-R Verbal IQ; ⁹ WAIS-R Performance IQ; ¹⁰ WAIS-R score; ¹¹ n=76; ¹² WISC-III IQ; ¹³ WISC-III Verbal IQ; ¹⁴ WISC-III Performance IQ; ¹⁵ WISC-III score; ¹⁶ WISC-R IQ; ¹⁷ BLS IQ; ¹⁸ KBIT IQ; ¹⁹ PPVT-R score; ²⁰ T scores of DAS pattern construction; ²¹ T scores of DAS recall of digits; ²² IQ compared to age matched typical WS (mean 89.67, SD 16.29, n=8); ²³ Blocks-WISC-R score compared to age matched typical WS (mean score 1.40, SD 1.20, n=9); ²⁴ VMI score compared to age matched typical WS (mean score 3.20, SD 0.38, n=9); ²⁵ BAS II Verbal score compared to typical WS (mean 59, n=31, SD 10.29), with normal score=100 (SD=15); ²⁶ BAS II spatial score compared to typical WS (mean 48, n=31, SD 2.18), with normal score 100 (SD 15); ²⁷ WISC-RN score compared to typical WS (mean score 18, SD 7, n=10) and age matched typical WS (mean score 14, n=1); ²⁸ Beery-VMI score compared to typical WS (mean score 4.8, n=53) and age matched typical WS (mean score 14, n=1); ²⁹ WISC-RN score compared to typical WS (means score 30, n=1) and age matched typical WS (mean score 25, n=2).

WS subject	Social behavior description						Copy number of GTF2I in genome	
	Increased eye contact	Overfriendly towards strangers	Anxious	Hyperactive	Friendly	shy		
WS with typical deletion	yes	yes	yes	yes	yes	no	1	
WS with atypical deletion	AT1		yes	yes			1	
	AT2				mild	yes	1	
	AT3					yes	1	
	AT4	no	no			yes	yes	2
	AT9		no					2
	AT10		no				yes	2
	AT12		no	no		yes		2
	AT13		no		no			2
AT17						yes	2	

Quotes:
 AT1: "The girl was hyperactive with overfriendly behavior towards unfamiliar adults and children. Anxiety was a characteristic feature."
 AT2: "His behavior was friendly, cheerful, and slightly hyperactive."
 AT3: "very friendly nature"
 AT9: "She does not exhibit the overly friendly personality."
 AT10: "She didn't rush up to people. Her interaction with nurse was normal. She wanted to get married. She was reserved."
 AT12: "His social behavior was friendly, and no anxiety trait or overfriendly manner could be detected."
 AT13: "Her parents did not report problems with hyperactivity or concentration." "She displays normal interaction with adults and peers".
 AT17: "in contrast to typical WS patients he had a shy appearance."

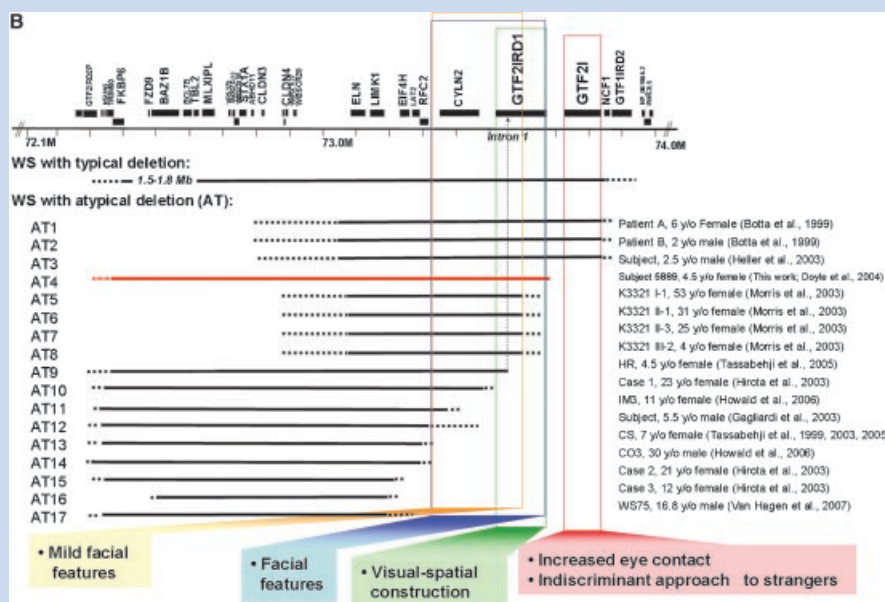


FIG. 6.

that of typical WS, suggesting that *GTF2IRD1* may be important for specific aspects of visual–spatial cognition, such as visual–motor integration. Previously, all atypical deletion subjects were deleted for only part of *GTF2IRD1* or both *GTF2IRD1* and *GTF2I*, preventing the dissection of their individual contribution to visual–spatial features. This is the first report parsing the parts of visual–spatial performance that are determined by the deletion of *GTF2I* versus *GTF2IRD1*. Taken together with data from the literature, we suggest that the deletion of *GTF2IRD1* is not sufficient to disturb all visual–spatial performance but is more important for the typical motor deficits in VSC, a hallmark of WS cognitive dysfunction (Fig. 6).

In this report, atypical WS social behavior was associated with atypical deletion preserving *GTF2I*. The observational results (Fig. 5B) previously reported [Järvinen-Pasley et al., 2008] closely mirrored the parental ratings (Fig. 5A) [Doyle et al., 2004] in that 5889 did not exhibit the highly pronounced social drive that is characteristic of children with WS full deletion of her age. Her reduced indiscriminant approach to strangers relative to typical WS children, combined with her decreased tendency to maintain eye contact and her preference for non-social over social activities, differentiate her social behavior from that seen in age-matched typical WS. It is possible that Subject 5889's higher overall functioning may contribute to her social behavior, although the results reported here in the cohort of typical WS did not show a correlation between SISQ and IQ. Similar findings indicating a lack of association between sociability and intelligence in individuals with WS have been reported in the literature [Frigerio et al., 2006; Porter et al., 2007]. Combined with the genetic analyses reported here, the social data support the involvement of the gene for *GTF2I* in aspects of hypersociability, particularly the lack of indiscriminant approach seen in WS, and moreover, suggest that with additional atypical deletion cases, it may be possible to further parse social behavior in WS (Fig. 6). However, other genes and interactions with *GTF2I* may clearly contribute and the roles for *GTF2I* and *GTF2IRD1* will be better distinguished when patients with just one of these genes deleted are found. The individual roles and interactions of *GTF2IRD1* and *GTF2I* in WS need to be clarified.

Our results suggest a possible role for *GTF2I* and *GTF2IRD1* in WS social and cognitive behavior. *GTF2IRD1* [Bayarsaihan et al., 2003] and *GTF2I* [Roy, 2001] may jointly contribute to WS behavior in that they are distantly related genes that encode transcriptional regulators and may each be related to different features or may act synergistically to determine distinct WS phenotypes. Further, previous studies have associated neuropsychiatric disorders with variations in neurohormones, including oxytocin and Arg-vasopressin, and their receptors [Witt et al., 1990; Witt and Insel, 1994; Modahl et al., 1998; Goldman et al., 2008]. It may be of interest to investigate *GTF2I* and *GTF2IRD1* in their regulatory role in neurohormones and their receptors. Finally, recent reports of *Gtf2ird1*-targeted mice revealed reduced fear and aggression and altered serotonin metabolism [Young et al., 2008], suggesting that it may be of interest to examine these pathways in typical WS subjects and Subject 5889.

So, is it WS? With respect to facial features, small stature and aspects of VSC, deletion sparing *GTF2I* resembles typical WS. In contrast, the typical tendency to indiscriminately approach strang-

ers is not seen in this child and suggests an association of *GTF2I* with a part of the social behavior in WS. Finally, the involvement of *GTF2I* and *GTF2IRD1* in regulation and development suggests that understanding their role in WS may provide insight into human brain development and adult function in the normal population.

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