ORIGINAL ARTICLE

A cryptic deletion of 2q35 including part of the *PAX3* gene detected by breakpoint mapping in a child with autism and a de novo 2;8 translocation

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We report a de novo, apparently balanced (2;8)(q35;q21.2) translocation in a boy with developmental delay and autism. Cross species (colour) paint (Rx) and SKY FISH, forward and reverse chromosome painting, and FISH with subtelomeric probes were used to examine the patient's karyotype, but further rearrangements were not detected. FISH with region specific clones mapping near 2q35 and 8q21.2 breakpoints and STS mapping performed on the isolated derivative chromosomes were used to refine the location of the breakpoints further. A cryptic deletion of between 4.23 and 4.41 Mb in extent and involving at least 13 complete genes or transcription units was found at the breakpoint on 2q35. The deletion includes the promoter and 5′ untranslated region of the paired box 3 (*PAX3*) gene. The child has very mild dystopia canthorum which may be associated with the *PAX3* haploinsufficiency. The 8q21.2 breakpoint is within *MMP16* which encodes matrix metalloproteinase 16. We postulate that the cryptic deletion and rearrangement are responsible for the patient's phenotype and that a gene (or genes) responsible for autism lies at 2q35 or 8q21.2. The results present a step towards identifying genes predisposing to autism.

A utism is a severe neurodevelopmental disorder of childhood. Diagnosis is based on the presence of a combination of abnormal social, communicative, and behavioural functioning presenting before the third year of life and persisting into adulthood. It is a relatively common condition with an incidence of about 5 in 10 000 and an average male to female ratio of 3.8:1, the latter varying according to the absence or presence of mental retardation.[1] A major genetic component for autism has been strongly suggested by twin studies which show a much higher concordance for monozygotic (31-90%) than dizygotic (0-3%) twins.[2–4]

Autism is genetically heterogeneous. Nearly all chromosomes have been implicated in one study or another, with the exclusion of chromosomes 12, 14, 19, and 20.[5] Genome wide screens by linkage analysis have suggested that loci at 1p, 2q, 4q, 5p, 6q, 7q, 10q, 15q, 16p, 18q, 19p, 22q, and Xp are implicated in the aetiology of autism. The International Molecular Genetic Study of Autism Consortium (IMGSAC)[6 7] and the Paris Autism Research International Sibpair Study (PARISS),[8] and a recent sib pair based study restricted to cases of autism with phrased speech delay[9] all identified susceptible regions on chromosome 2q at around 2q32. The IMGSAC excluded the entire X chromosome in stage 2 of their study,[6] although one marker (DXS996) reached a maximum lod score of 0.5 in the stage 1 data set. On the other hand, the PARISS implicated Xp as a susceptibility region potentially involved in autism. Particular interest has focused on chromosome arms 2q, 7q, and 15q. Ghaziuddin and Burmeister[10] reported four cases (two unpublished) of 2q37 deletions associated with autism and also reported on other published cases, with deletions ranging from 2q31 to 2qter. Conrad *et al*[11] also reported three cases of 2q37 deletions associated with features that are highly suggestive of autism. Here we have studied an autistic patient with an apparently balanced (2;8)(q35;q21.2) translocation. We have used a battery of molecular cytogenetic and molecular techniques to detect and characterise a cryptic deletion at the translocation breakpoint and to ensure that no other chromosomal abnormalities could be detected.

PATIENT, MATERIALS, AND METHODS

The proband was ascertained as part of a study of patients with developmental delay and apparently balanced translocations within the Mendelian Cytogenetics Network.[12] He is the third child of healthy, non-consanguineous, white parents. He is the second twin, born at 32 weeks by caesarean section for fetal distress and intrauterine growth retardation. He was very small for dates, weighing 1130 g, but had few problems in the neonatal period and needed ventilation for under a day, for transient tachypnoea. There was no intracranial haemorrhage. He was a very quiet baby and showed poor socialisation and eye contact from an early age. His parents noted that he was slower than his twin sister from the age of 3 months. At the corrected age of 19 months, he had only just started walking, speech was non-existent, and he was delayed in personal social skills and was difficult to feed. The latter necessitated prolonged hospital admissions, as at times he would completely refuse to drink or eat in spite of there being no physical or mechanical abnormalities. An MRI scan of the brain showed moderately enlarged lateral and third ventricles, but there was no increased intracranial pressure. A diagnosis of arrested hydrocephalus was made and the head circumference continued to grow along the 50th centile. Fundi were reported as normal after examination under anaesthesia. Routine hearing tests at 2 years and 6 years were difficult to carry out owing to the child's lack of cooperation, but were reported as being apparently normal. No auditory evoked potential measurements have been carried out. The proband was reported as being sensitive to high pitched sounds and was more receptive when he was given clear signing as well as verbal explanations and instructions. An assessment by a paediatric neurosurgeon at 4 years ruled out the child's learning difficulties as being the result of the hydrocephalus. A

metabolic screen, endocrine function, urine amino acids, and routine blood investigations were normal. Chromosome analysis showed a balanced rearrangement between chromosomes 2 and 8, namely, 46,XY,t(2;8)(q36;q21.3). His mother's karyotype was normal, whereas his father was found to have an unrelated balanced rearrangement 46,XY,t(5;20)(p10;p10). His twin sister, who carries the paternal translocation, was normal for dates at birth (1900 g) and had made entirely normal progress; his older sister was also developing normally. There was no family history of developmental delay or mental retardation. Based on the persistent abnormal social, communicative, and behavioural functioning, a diagnosis of autism in addition to his learning difficulties was made at the age of 6 years.

When we saw the proband at the age of 11 years, his parents reported significant learning difficulties with behavioural problems still centred on feeding difficulties. He was eating a very small variety of foods and would use his sense of smell in determining preference. He was very particular about routines and showed obsessional behaviour. He was still not toilet trained. He was attending a school for special needs and was using sign language (Makaton) to communicate; he had only started saying some words since the age of 9. He was unable to maintain eye contact and had a poor concentration span. He did not utter any words. He played repeatedly with his parents' credit cards, flicking them between his hands and occasionally smelling them. He was growing along the 3rd centile for weight, the 10th for height, and along the 50th centile for head circumference. Minor facial dysmorphic features included upward slanting palpebral fissures, mild dystopia canthorum, and prominent antihelices, but he was not dysmorphic overall. The rest of the physical examination was normal. The clinical and developmental history and current function met the DSM-IV diagnostic criteria for autism. An autism diagnostic interview (ADI) was not carried out as the parents declined this. However, it was not felt that the ADI was crucial in confirming the diagnosis, since throughout his development four experienced paediatricians had seen him and independently reached the same diagnosis.

Fluorescence in situ hybridisation

Standard protocols were used in the preparation of metaphase chromosomes and for fluorescence in situ hybridisation (FISH) studies. Cell line culture preparations from the patient were used to sort the normal and aberrant chromosomes 2 and 8 as well as the X chromosome, using bivariate flow karyotype analysis. Subtelomeric probes were from the Cytocell Chromoprobe-T kit. Whole chromosome paints were prepared from flow sorted chromosomes using degenerate oligonucleotide primed PCR (DOP-PCR) as described by Telenius et al[13] and Carter et al.[14] A 24 colour paint probe was prepared in house from five chromosome pools and the appropriate dUTPs and fluorochromes were again incorporated by DOP-PCR. Cross species colour (RxFISH) paint was also prepared in house.[15]

Breakpoint mapping used CEPH YAC probes selected from the Whitehead Institute contigs, and BACs and PACs from UCSC "Golden Path" (tables 1-3). These were prepared by standard techniques, labelled with appropriately coupled dUTPs by nick translation or directly labelled by DOP-PCR, and used as probes in FISH as before.[16]

Locus specific PCR

Finer mapping of chromosomal breakpoints and investigation of parental origins of the derivative chromosomes used PCR on sorted chromosome DNA with primers specific for STS markers close to the breakpoints identified by FISH. Normal and derivative chromosomes from the proband were isolated by flow sorting and DNA amplified by DOP-PCR in 100 µl reactions as before[17] and 0.2 µl DOP-PCR products were used

Table 1 FISH results using CEPH YAC clones on 2q and 8q. Clones bordering or deleted at breakpoint are in bold type.

Genetic distance	STS marker	YAC clone	Size (kb)	FISH result
2q				
223	D2S295	817-B-12	950	Proximal
226	D2S163	708-C-9	1600	Proximal
229	D2S339	906-C-3	910	Proximal
229	**D2S339**	**879-H-9**	**1110**	**Proximal**
230	**D2S2323**	**743-C-9**	**1500**	**Deleted**
232	**D2S133**	**802-A-9**	**1140**	**Deleted**
233	**D2S353**	**703-E-9**	**1160/1610**	**Deleted**
233	**D2S351**	**886-H-7**	**1700**	**Deleted**
234	**D2S2204**	**873-B-12**	**510**	**Distal**
236	D2S2158	933-C-7	1690	Distal
236	D2S159	929-G-1	1220	Distal
8q				
95	D8S1764	790-A-1	1720	Proximal
	D8S1760			
97	D8S275	762-B-3	1480	Proximal
101	D8S271	718-D-11	350,540	Proximal
102	D8S1811	820-C-9	1680	Proximal
102	**D8S1724**	**759-G-8**	**1730**	**Proximal**
102	**D8S270**	**725-A-6**	**1620**	**Distal**
105	D8S1794	751-D-3	1620	Distal
109	D8S506	913-G-5	NA	Distal
	D8S1778			
112	D8S1808	811-A-6	1350	Distal
115	D8S521	662-E-12	NA	Distal

NA: not available.

as substrates with STS specific primers (as identified in the Uni-STS database) in 20 µl reactions. The presence of a particular STS marker in a chromosome was assessed by the ability to amplify it from the DOP-PCR products of the proband's flow sorted normal chromosome and of one or other of the derivative chromosomes. STS primer pairs that failed to amplify the normal chromosome after DOP-PCR were discarded from the analysis. Markers used for Xp were DXYS14, DXYS20, DXYS28, DXYS60, DXYS129, DXYS130, DXYS131, DXYS132, and DXYS153; markers used for the der(2) and der(8) chromosomes are tabulated (table 4).

Contigs

We have used the UCSC Golden Path contigs (Aug 06, 2001 freeze), in compiling figures and tables for this paper, but in STS mapping studies we have also tested STS derived from the NCBI (build 26) and EnsEMBL (Aug 2001 freeze) datasets, where these are different (table 4).

RESULTS

Karyotype defined by G banding, chromosome painting, and subtelomeric probes

Re-evaluation of the patient's G banded karyotype at the 550 band level redefined the breakpoint on chromosome 2 at q35 and that on chromosome 8 at q21.2 (fig 1A). RxFISH and spectral karyotyping (SKY FISH) showed this translocation clearly, but failed to show any further rearrangements (fig 1B, C). The derivative chromosomes were isolated by flow cytometry (fig 1D shows the flow karyotype). Conclusions from G banding were confirmed by both forward and reverse chromosome painting (not shown). The latter failed to show any deletions, suggesting a balanced reciprocal translocation. Subtelomeric fluorescent probes were used to screen telomeric regions of all chromosomes. This screen confirmed the presence of the t(2;8) translocation and also the presence of both p and q telomeres of the translocated chromosomes (fig 1E-G). An apparent terminal deletion (absence of hybridisation signal) on Xp was observed. FISH with cosmid 34F5 (containing *SHOX* exons I-IV and extending distally) showed

Table 2 Summary of BAC FISH results using clone order from August 2001 freeze of the Human Genome Browser. Clones bordering breakpoints or deleted in rearrangement are in bold type. Position on chromosome 2q: 232 635 159 bp-238 902 300 bp

Accession No	BAC clone	Sequence Status	Size (bp)	Known genes or transcripts	FISH result
AC009302	RP11-71J24	Finished	180970		Not hybridised
AC012591	**RP11-296A19**	**Finished**	**141950**		**Proximal**
AC010980	**RP11-384O8**	**Finished**	**171374**	*PAX3*	**Proximal**
AC012251	RP11-16P6	18 fragments	169980	*FARSLB1**	Chromosome 15
AC016843	RP11-3122	7 fragments	169272	*FARSLB1*, AF085977*	**Deleted**
AC016712	RP11-266F17	Finished	159748		**Deleted**
AC013476	RP11-525G12	3 fragments	194222	*FACL3*	**Deleted**
AC017014	RP11-142D10	Finished	182366		**Deleted**
AC068035	RP11-799L6	2 fragments	177008		Not hybridised
AC079120	RP11-345M24	1 unordered	181388		Not hybridised
AC013448	RP11-551D18	Finished	129553		**Deleted**
AC012512	RP11-561O7	Finished	179460	*SCG2*	**Deleted**
AC016701	RP11-183N7	15 fragments	160196	*FLJ21685 fis*	**Deleted**
AC068343	RP11-660K21	19 fragments	190220	*FLJ21685 fis*	**Deleted**
AC073641	RP11-368E14	3 fragments	199083	*FLJ12701 (MRLP44), FLJ12826, M11783*	Not hybridised
AC012460	RP11-536G14	9 fragments	200885	*FLJ12701 (MRLP44) FLJ12826, M11783*	**Deleted**
AC012664	RP11-3C18	9 fragments	163421	*FLJ12701 (MRLP44) FLJ12826, M11783*	Not hybridised
AC019109	RP11-488C22	3 fragments	184298		**Deleted**
AC023458	RP11-456I21	24 fragments	199134		Not hybridised
AC008072	RP11-408N22	Finished	206177	*FLJ22746*	Not hybridised
AC079836	RP11-99K18	25 fragments	153903	*FLJ22746/CUL3*	Not hybridised
AC073052	RP11-92F20	1 unordered	152982	*CUL3*	Not hybridised
AC092679	RP11-676D22	2 fragments	182396	*CUL3*	Not hybridised
AC025339	RP11-626D22	51 fragments	185492	*CUL3*	**Deleted**
AC023985	RP11-811O14	29 fragments	168684	*FLJ20220*	Not hybridised
AC011739	RP11-68H19	Finished	171811	*FLJ20220, KIAA0694*	Not hybridised
AC027805	RP11-327P24	25 fragments	145642	*KIAA0694*	Not hybridised
AC018382	RP11-5I19	41 fragments	196556	*KIAA0694*	Not hybridised
AC017095	RP11-516C4	4 fragments	185804	*KIAA0694*	Not hybridised
AC015974	RP11-467A18	Finished	196544		Not hybridised
AC067961	RP11-59F3	1 unordered	159890	*KIAA1486*	**Deleted**
AC011035	RP11-7D20	31 fragments	165199	*KIAA1486*	**Deleted**
AC019231	RP11-573O16	Finished	187268	*KIAA1486*	**Deleted**
AC016717	RP11-314B1	Finished	210558	*KIAA1486*	**Deleted**
AC068138	RP11-813K12	7 fragments	218165		**Deleted**
AC069383	RP11-86O17	20 fragments	200323		**Deleted**
AF107045	N.A.	Finished	45043	*RRM1*	Not hybridised
AC062015	CTD-2031E17	Finished	144223		Not hybridised
AC08002	RP11-231F9	26 fragments	141182		Not hybridised
AC026588	**RP11-211K17**	**19 fragments**	**160341**		**Distal**
AC079402	**RP11-597E15**	**10 fragments**	**159078**		**Distal**
AC079769	RP11-235I3	31 fragments	147289		Not hybridised
AC010735	**RP11-395N3**	**Finished**	**211980**	*IRS1*	**Distal**
AC073149	RP11-803F15	44 fragments	175504		Not hybridised
AC079235	RP11-495O23	51 fragments	200847	*COL4A4*	Not hybridised

Not hybridised: FISH not carried out with these clones.
NA: not available.
*Phenylalanyl-tRNA synthetase beta subunit: provisional nomenclature, HUGO, Dec 2001. Locus link designation PheHB.

that this sequence was not deleted. PCR experiments using primer pairs located between 0 and 79cR$_{10k}$ on the Stanford G3 radiation hybrid map within the pseudo-autosomal region of Xp, to amplify the patient's isolated X chromosomal DNA, gave no evidence of deletion. The gastrin releasing peptide receptor gene (*GRPR*) was also studied by PCR and was not found to be deleted. Subsequent chromosome analysis of the phenotypically normal mother showed the same cytogenetic and molecular findings.

Breakpoint mapping
Breakpoint mapping using FISH with cytogenetically and genetically anchored CEPH YAC probes was carried out (table 1, fig 2A, B). These showed a cryptic deletion within band 2q35 at the breakpoint of the translocation chromosomes. YAC clones from proximal to the deletion hybridised to normal chromosome 2 and to der(2) while those distal to the deletion hybridised to normal chromosome 2 and to der(8). By locating markers known to be within the boundary YACs, we were able to refine measurement of this deletion using FISH with appropriate BACs (table 2, fig 2C-E). The BAC clone immediately proximal to the breakpoint was AC010980, which includes the promoter and most of the coding sequence of the paired box 3 (*PAX3*) gene. AC026588 was the most proximal clone derived from chromosome 2 which hybridised on der(8), while no hybridisation to either derivative chromosome was seen from clones between these two. These results define a deletion of 4-4.8 Mb on 2q35.

The breakpoint on chromosome 8 was characterised similarly by FISH with appropriate YACs (table 1) and BACs (table 3). In this case, no evidence of a deletion was found by FISH (fig 3), with clones placed proximal and distal to the

Table 3 Summary of 8q BAC FISH results using clone order derived from the August 2001 freeze of the Human Genome Browser. Clones bordering breakpoint are in bold type. Position on chromosome 8q: 103 750 000 bp-105 050 000 bp

Accession No	BAC clone	Sequence status	Size (bp)	Known genes	FISH result
AC068510	RP11-662E23	7 fragments	192155		Proximal
AC027258	RP11-72H9	23 fragments	159806		Proximal
AC023841	RP11-565L3	12 fragments	161297		Proximal
AC067967	**RP11-363H6**	**Finished**	**41972**	*SEPP1*	**Proximal**
AC017061	**RP11-353D5**	**8 fragments**	**172403**	*SLC26A7*	**Distal**
AC027374	RP11-656D12	4 fragments	202856	*SLC26A7*	Distal
AC020783	RP11-16M2	3 fragments	156973		Chromosome 8p22
AF268619	RP11-122C21	3 fragments	152233		Distal
AC015952	RP11-1B10	7 fragments	166991		Chromosome 5q14

Table 4 Presence of STS on chromosomes of the proband as determined by PCR of isolated chromosomes. Markers bordering breakpoints or deleted in rearrangement are in bold type

Marker name	Position (bp)*	NCBI	UCSC	Gene†	Product size	Amplified chromosom es§
2q35						
SHGC-104436	232636k			*PAX3* (intron)	298	N2, der(2)
WI-7962 (D2S2599)	**232668k**			*PAX3* (cds)	**330**	**N2, der(2)**
GDB: 603639	**232673k**			*PAX3* (5′UT)	**278**	**N2, del**
CP3PR	**232674k**			*PAX3* (promoter)	**148**	**N2, del**
SHGC-102627	**232694k**			-	**343**	**N2, del**
D2S313	**232784k**			-	**149**	**N2, del**
RH110169	**232873k ‡**			-	**303**	**N2, del**
stSG27208	233036k			*FARSLB1***	131	N2, del
SHGC-85602	**236902k**			-	**308**	**N2, del**
SHGC-148539	**237059k**			-	**332**	**N2, der(8)**
D2S2354	237237k			**-**	255-275	N2, der(8)
WI-9260 (D2S2647)	237454k			*IRS-1*	105-106	N2, der(8)
8q21.3						
stSG46229		89631k	104070k	**-**	141	N8, der(8)
WI-16423		89678k	104030k	**-**	129	N8, der(8)
SGC35860		89771k	Not in UCSC database‡	*LOC51633*‡	109	N8, der(8)
WI-4229		90674k	101612k‡	**-**	274	N8, der(8)
SHGC-10609		90018k	101402k‡	**-**	152	N8, der(8)
SHGC-155293		**90151k**	**101255k‡**	-	**283**	**N8, der(8)**
SHGC-101603		**90264k**	**101160k‡**	*MMP16*	**325**	**N8, del**
SHGC-85429		**90377k**	**100045k‡**	*MMP16*	**327**	**N8, del**
RH110076		**90808k**	**Not in UCSC database‡**	-	**331**	**N8, der(2)**
D8S1476		90924k	104316k/104628k¶	*SLC26A7*‡	125	N8, der(2)
SHGC-84012		91187k	104735k	**-**	272	N8, der(2)
D8S1618 (WI-3120)		91227k	104700k	**-**	249	N8, der(2)
SHGC-105203		91360k	104960k	**-**	320	N8, der(2)

*Positions on UCSC Genome Browser build 06.08.2001.
†Genes assignments are from UCSC Genome Browser build 06.08.2001 except where noted.
‡Markers ordered or genes assigned according to NCBI contigs.
§N2 is proband's normal 2; N8 is proband's normal 8; der(2) is proband's derivative (2); der(8) is proband's derivative (8).
¶Appears twice in UCSC database.
**Phenylalanyl-tRNA synthetase beta subunit: HUGO provisional designation, Dec 2001. Locus link designation PheHB.

8q21 breakpoint found adjacent on the UCSC contig (although not on that compiled by NCBI, which in the most recent build has an additional group of clones at this site).

To refine the breakpoint further, STS primer pairs were used in PCR, with the DOP-PCR amplification products of isolated (flow sorted) chromosomes of the proband as template. Results (table 4) showed complete correlation with the findings of the FISH work. The size of the deletion at the breakpoint at 2q35 is defined by this method as between 4.23 and 4.41 Mb. The deletion starts in AC010980 within the transcribed regions of the *PAX3* gene (fig 4). STS GDB: 603639, which occurs in the 5′ leader sequence of the *PAX3* gene, is deleted, but WI-7962, which is in exon 4 of the gene, about 5 kb further upstream, is present on der(2). AC010980 hybridises to der(2) through more than 50 kb of sequence which remains proximal to the breakpoint. At least 13 transcription units are completely deleted from the rear-

ranged chromosome (see Discussion), but the breakpoint at the distal end of the deletion does not lie within or very close to any known gene or transcription unit.

On 8q, the breakpoint is in an area of provisional sequencing, and the contigs compiled by UCSC and NCBI are considerably different. The simplest interpretation of our results is that the marker order adopted in table 4 is correct. One marker, WI-4229, is displaced from its position in the NCBI contigs, but this displacement allows it to fit with the local order of assembly of the UCSC contigs. Otherwise, the order follows NCBI. Because of the uncertainty in contig assembly at this position, the STS amplification approach is less powerful, and the marker order may eventually prove to be different from that used in table 4. Our current evidence suggests that there is a small deletion at the 8q breakpoint (not detected by FISH) including part of the matrix metalloproteinase16
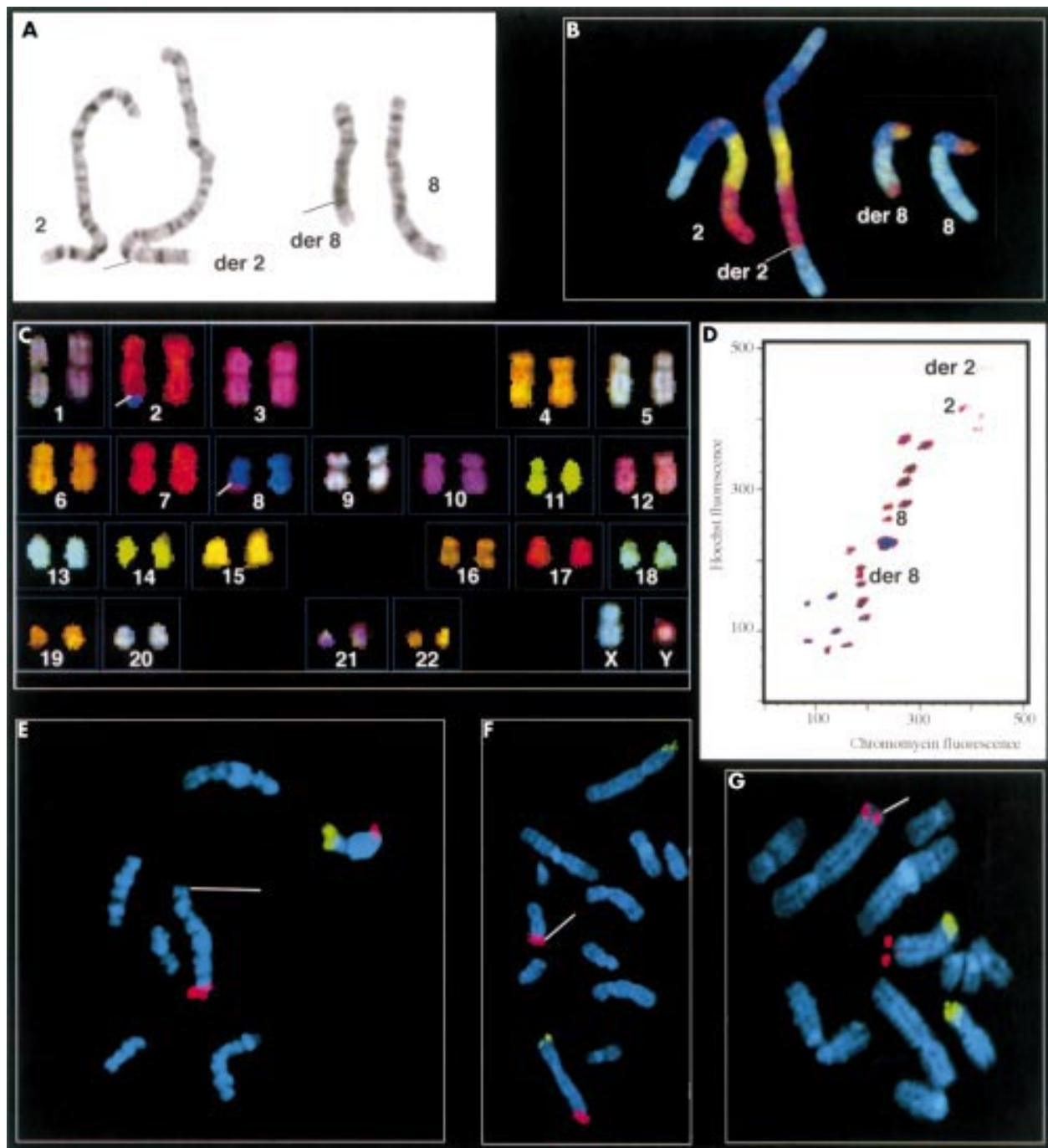
Figure 1 Initial characterisation of the proband's karyotype. (A) G banding of chromosomes 2 and 8 and derivative chromosome 2 and 8. (B) RxFISH of chromosomes 2, 8, and derivative chromosomes 2 and 8. (C) SKY FISH of the entire karyotype. Translocation breakpoints are indicated. (D) Flow karyotype. (E-G) Subtelomeric probes do not hybridise to Xp, but show the presence of both p and q telomeres of chromosomes 2 and 8. (E) Xp/Yp is green, Xq/Yq red. Note absent signal on Xp (arrow). (F) 2p is green, 2q red. Note the translocated 2q signal on derivative chromosome 8. (G) 8p is green, 8q red. Note the translocated 8q signal on derivative chromosome 2.

(*MMP16*) gene. The marker order used places this deletion at the junction with the 2q35 sequence.

DISCUSSION

The proband's twin sister has no developmental abnormalities, reducing the possibility that either prenatal or postnatal environmental factors are causative of autism or the other abnormalities seen in the proband. The twin sister also carries her father's balanced 5;20 translocation, verifying paternity and confirming the proband's 2;8 translocation as de novo. STS

analysis of paternal and maternal DNA showed that the derivative chromosomes are paternal in origin (data not shown). Moderate ventriculomegaly as well as intrauterine growth retardation could be the direct result of decreased placental blood flow to the proband as documented during pregnancy. The proband's other abnormalities are less likely to relate to this cause and we consider these to be of genetic origin.

Chromosome breakpoint mapping using cytogenetically anchored or physically mapped YAC and BAC clones has been instrumental in detecting cryptic interstitial deletions at apparently balanced breakpoints in patients with mental
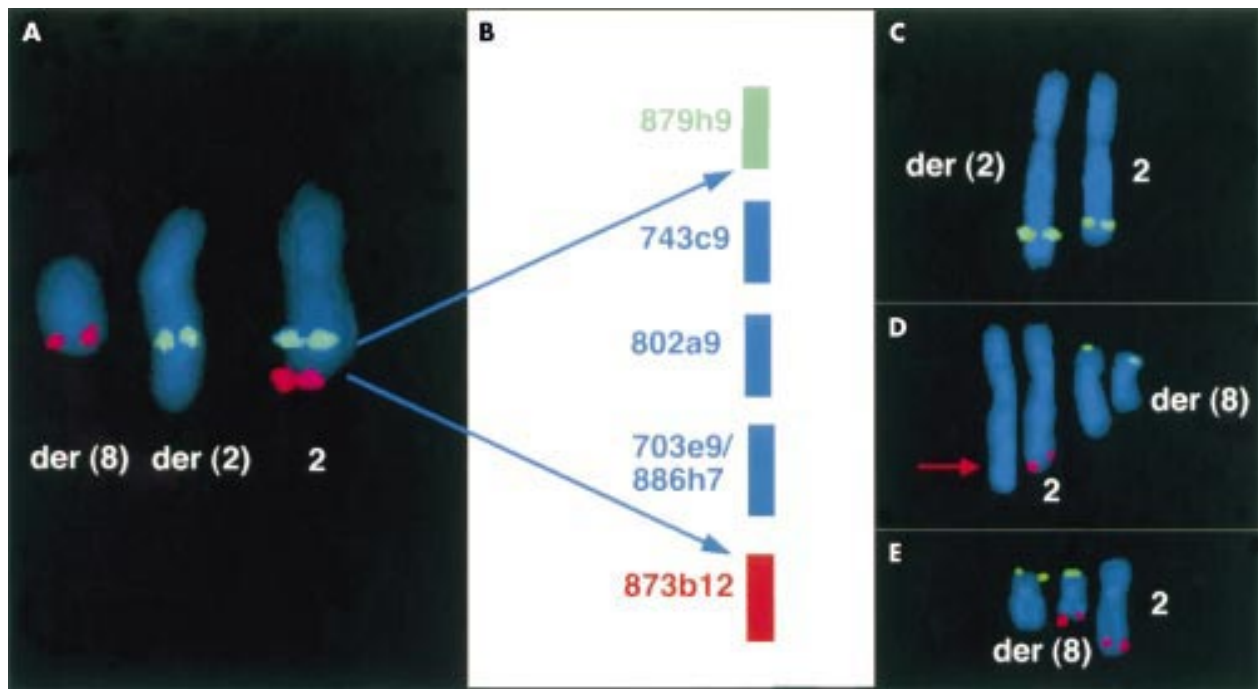
Figure 2 Characterisation of the t(2q;8q) breakpoint using chromosome 2 clones. (A) FISH using YAC clones. Clone 879h9 (green) hybridises to normal chromosome 2 and der(2) and is proximal to the breakpoint. Clone 873b12 (red) hybridises to chromosome 2 and der(8) and is therefore distal to the breakpoint. (B) Colour coded schematic representation of YAC clones; blue YACs were found to be deleted. (C-E) FISH using BAC clones. Marker 8p subtelomeric probes were cohybridised with the chromosome 2 BAC clones. (C) Clone RP11-384O8 hybridises proximal to the breakpoint; green signals are seen on der(2) and chromosome 2. (D) RP11-86O17 is a representative deleted BAC clone at the region of the chromosomal deletion on der(2) (red arrow). Normal hybridisation signals (red) are seen on chromosome 2. 8p probe is green. (E) Clone 211K17 is distal to the breakpoint with red hybridisation signals seen on chromosome 2 and der(8). 8p probe is green.
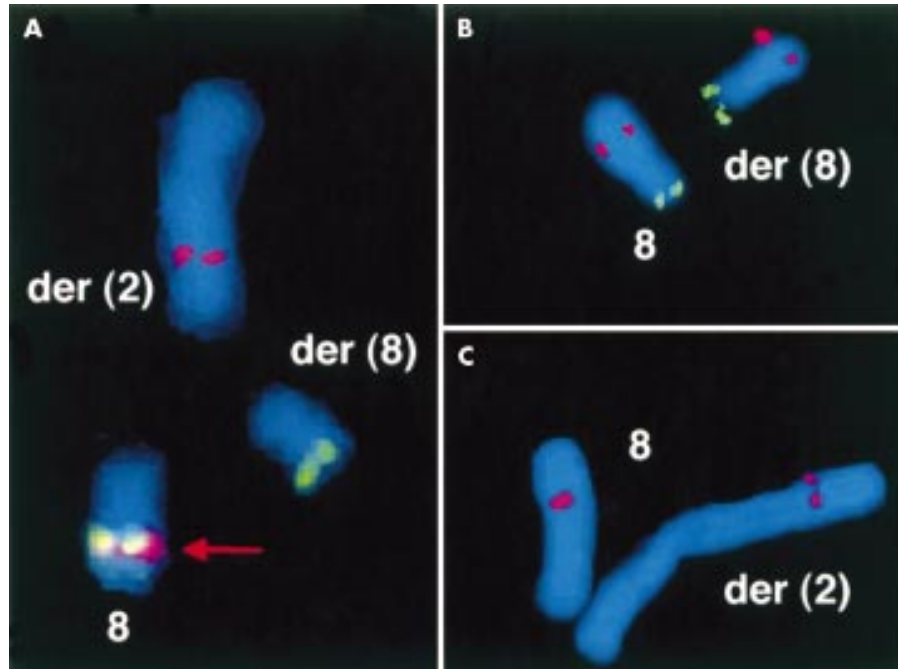
Figure 3 Characterisation of the t(2q;8q) breakpoint using chromosome 8 clones. (A) FISH using YAC clones. Clones 759g8 and 725a6 overlap (yellow signal/red arrow) at 102 cM from 8p terminus. (B, C) FISH using BAC clones. (B) Clone RP11-662E23 is proximal and (C) clone RP11-353D5 is distal to the breakpoint on 8q (both red signals). An 8p telomeric probe (green) was cohybridised with clone RP11-662E23.

retardation,[16] with about 6.5-7.4% of patients with moderate or profound mental retardation showing such deletions in two recent FISH based surveys.[18] [19] Autistic children with 2q deletions ranging from 2q31 to 2qter have been reported, but where smaller deletions have been identified these have been of 2q37-2qter.[10] [11] [20] [21] The same region has been associated with mental retardation in patients with phenocopies of Albright's syndrome.[22]
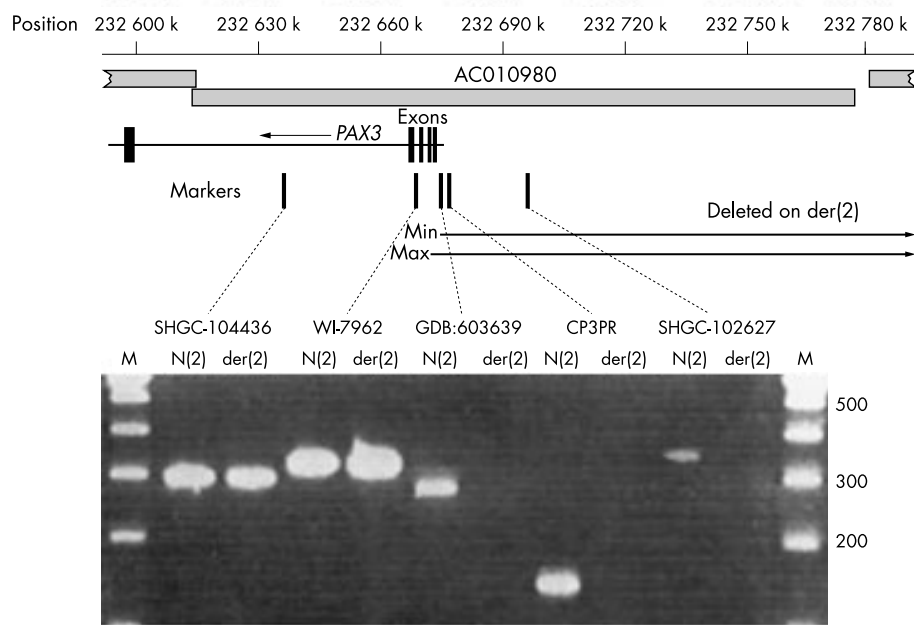
Figure 4 STS mapping of the chromosome 2 breakpoint. Amplification products of PCR using STS primers at different points along the BAC clone AC010980 are shown for normal and der(2) chromosomes. All products are of the expected sizes. There is no signal from der(2) amplifications using the last three primer pairs. (There was no signal with der(8) using any primer pair in this region, not shown.) The implied maximum and minimum end points for the deletion are shown, as well as the location of *PAX3*.

In our patient, both 2q telomeres were present, but FISH identified an apparent subtelomeric deletion of Xp. However, the use of subtelomeric probes is not by itself sufficient to identify telomeric deletions. Polymorphisms in subtelomeric probe hybridisation have recently been reported on Xp in phenotypically normal subjects, as have reductions in repeat length and signal strength on 2q.[23] The absence of signal from Xp subtelomeric probe on one maternal X chromosome as well as in the proband and the lack of a measurable associated deletion make it likely that the phenotype of the patient is not associated with Xp polymorphism. Instead, we hypothesise that the phenotype is the result of genetic damage associated with the 2;8 translocation. This includes deletion of genetic material at 2q35, potentially together with genetic damage on 8 and/or production of a fusion gene and position effects on gene expression arising from the translocation.

In the UCSC golden path, the 2q35 deletion completely removes five genes of known function. These are phenyl-alanine tRNA synthetase-like, beta subunit, (referred to as *PheHB* in genome databases and provisionally named *FARSLB1* by HUGO, Dec 2001), fatty acid coenzyme A ligase long chain 3 (*FACL3*), secretogranin II (*SCG2*), cullin 3 (*CUL3*), and ribonucleotide reductase M1 polypeptide (*RRM1*). In the equivalent ensEMBL and NCBI contigs, *RRM1* does not appear and it appears to be misplaced judging by somatic cell mapping studies and in situ hybridisation.[24] In addition, there are nine transcription units of unknown function in the deleted region (table 2). None of these 13 genes has been associated with any inherited disorder, but the first four are all expressed in brain, while secretogranin II is a constituent of secretory vesicles which has only been found in brain tissues.

Two known and two hypothetical genes have been mapped close to the 8q21.3 breakpoint. The known genes are selenoprotein P plasma 1 (*SEPP1*) and matrix metalloproteinase 16 (*MMP16*). *SEPP1* appears on the breakpoint proximal BAC on the UCSC 8q21.3 contig, but also on chromosome 5q31 in the same database (that is, it is assigned two positions), while the 5q31 position agrees both with the chromosome assignment on the NCBI databases and with the comparative mouse mapping data. (The gene is not assigned on ensEMBL.) The gene encod-

ing MMP16 protein, a membrane bound or secreted matrix metalloproteinase, is close to the breakpoint on the NCBI databases, but assigned a more proximal position on UCSC. Our STS data show disruption of *MMP16* on der(8). Deletion or damage to *MMP16* at the 8q breakpoint may be significant in the phenotype. *MMP16* mRNA is expressed at high levels in brain, as well as in other tissues, and is likely to function in processes such as development and tissue remodelling. Currently no diseases have been connected with mutation in this gene, and there are no reports of autism or mental retardation syndromes mapping in the immediate region of the 8q breakpoint. A well characterised (X;8)(p22.3;q22.1) translocation in a patient with autism and multiple exostoses has a breakpoint on chromosome 8, which is 30 kb distal to the syndecan 2 (*SDC2*) gene.[25] This is some 5 Mb distal to the breakpoint in the present patient. (The *GRPR* gene, which lies across the breakpoint on Xp in that patient, appears to be intact in our patient although we cannot rule out point mutations.) The present breakpoint also lies distal to the mapped Nijmegen breakage syndrome gene (*NBS1*) in the current genome map and is proximal to the Cohen syndrome defined region.[26]

Haploinsufficiency of *PAX3* has been shown to be causative of many cases of Waardenburg syndrome (WS).[27] WS is an autosomal dominant disorder that manifests as a complex of sensorineural deafness, pigmentation defects (characteristically a white forelock and heterochromia irides, but can include hypopigmented patches of skin and retinal fundus), and minor but characteristic facial abnormalities including dystopia canthorum. In a few WS patients, endolymphatic hydrops (raised pressure in endolymphatic spaces of cochlea and vestibular system caused by aplasia of the posterior semicircular canal) is associated with painful hyperacusis or tinnitus. Occasionally, Waardenburg syndrome has been observed combined with mental retardation. A case of mental retardation and WS with upper limb contractures (Klein-Waardenburg syndrome or WS type 3) was described in a patient with a deletion of 2q35-q36.[28] Most signs of WS may be more or less evident depending on the genetic background of the patient.[29] However, dystopia canthorum is characteristic of patients with WS1 and WS3, in all of whom *PAX3* mutation is considered to be responsible for the condition.

The 2q35 deletion mapped in this study spans the entire promoter and the leader sequence of *PAX3* so that it is very unlikely that functional *PAX3* mRNA will be produced from der(2).[30] The possibility that a fusion protein (MMP16-PAX3) could be produced at the breakpoint is under investigation. The proband shows mild dystopia canthorum, and his sensitivity to high pitched sounds may be a result of endolymphatic hydrops, but he has no pigmentary anomalies of the hair, iris, skin, or retinal fundus. His other abnormalities have not previously been associated with WS.

Four international or large linkage studies have associated autism with regions on chromosome 2q,[6–9] but these are slightly centromeric to our cryptic deletion, and probably too far away for the same genes to be involved as those deleted in our patient.[31] Two other studies have not reported linkage of autism to 2q.[32 33] The observed cryptic deletion may be responsible for the patient's phenotype, so that a gene (or genes) responsible for autism maps to a 4.3 Mb region of 2q35. In this case, it is likely that the phenotype is associated with haploinsufficiency, although we are examining genes on the patient's normal 2q35 for point mutation. *MMP16* on 8q21.2 and the region of 2q35 at, or immediately distal to, the *PAX3* gene should be considered when assessing the genetic background of autistic patients, especially those with developmental delay but no (or only subtle), dysmorphic features.

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Electronic database information. University of California Santa Cruz Human Genome Browser, August 06 2001 freeze (UCSC golden path): http://genome.cse.ucsc.edu/goldenPath/aug2001Tracks.html. NIH National Centre for Biotechnology Information, Entrez genome view, build 26, (NCBI contigs): http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search. European Molecular Biology Organisation Genome Server, August freeze (EnsEMBL): http://www.ensembl.org/

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ECHO ....

New mutations in hereditary pancreatitis

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Two new mutations in gene *PRSS*1, which causes hereditary pancreatitis, have been reported. This autosomal dominant condition, associated with recurring bouts of acute pancreatitis, developing chronic pancreatitis, and a high incidence of pancreatic cancer, is usually caused by mutations *R122H* and *N29I* in the cationic trypsinogen *PRSS1* gene.

By sequencing the gene in the index case in families without these mutations with sequential *Afl*III restriction endonuclease digestion (of exon 3, then exon 2, then exon 1, 3, 4, 5 if any of the preceding exons were negative) and new codon 29 RFLP analysis with *Bst*4CI, Pfützer *et al* discovered a mutation resulting in an amino acid substitution *R122C* in one family and an amino acid substitution *N29T* in another, unrelated, family. Each mutation showed autosomal dominant segregation within the family.

Neither new mutation was found in 58 patients with hereditary pancreatitis who did not have *R122H* or *N29I* mutation, 66 patients with familial or idiopathic pancreatitis, or 130 healthy controls.

*Afl*III digestion did not detect *R122C* mutation, *Bst*4CI digestion did detect *N29T* mutation, so non-standard techniques are needed to identify these new mutations.

▲ *Gut* 2002;**50**:271–273.