were used for the internal control. Real-time PCR amplifications were performed in three independent replicates on an ABI7500 (Life Technologies), and the data were evaluated by the Delta Delta Ct method.[2] The SETBP1 expression ratio (patient versus normal control) was calculated in each of the three examinations.

Concentrations of SETBP1 in the cell lysates of skin fibroblasts from patient 1 and the control were also analysed by western blotting using the SETBP1 MaxPab mouse polyclonal antibody (B01), catalogue number H00026040-B01 (Abnova, Taipei City, Taiwan) as described previously.[3]

Cohort study of *SETBP1*

A total of 142 Japanese patients with developmental delay, without genomic copy number aberrations as determined by aCGH, participated in the cohort study.[4] *SETBP1* sequences were analysed by the standard PCR-direct sequencing method. The primers used for PCR and the big-dye sequencing reaction (Life Technologies) were designed using Primer3 (http://primer3.sourceforge.net/) (supplemental online table 1). When we identified nucleotide changes in samples for which parental samples were available, trio analyses were performed to check whether the changes were de novo or familial. The nucleotide sequences of *SETBP1*, in which nucleotide alterations were found in the cohort study, were compared with homologues in species including *Callithrix jacchus*, *Gorilla gorilla*, *Macaca mulatta*, *Pan troglodytes*, *Pongo pygmaeus*, *Tarsius syrichta* and *Tupaia belangeri*, which were identified using Gene Tree (http://www.ensembl.org). DNA samples from 70 Japanese volunteers were used for the control cohort of normal Japanese.

RESULTS

Cytogenetic analyses

In patient 1, aCGH analysis revealed an aberration in the contiguous 11 probes at 18q12.3 with the mean $\log_2$ ratio of −1.02306 (figure 2A). This indicated a 986 kb loss of genomic copy number at 18q12.3; molecular karyotyping was determined as arr chr18q12.3q12.3 (40 282 934–41 269 199)x1. The deletion exclusively contained *SETBP1* and was confirmed by FISH analysis showing only one signal from the targeted probes (supplemental online figure S1). FISH analysis using the same probes showed no abnormality in either parent, indicating a de novo deletion (data not shown).

In patient 2, aCGH showed an 850 kb deletion within the chromosomal region 18q12.3 (chr18:40 233 803–41 088 224) (figure 2B). The deletion was confirmed by FISH, and both parents were found to be normal by conventional chromosome analysis and FISH analysis with the same locus-specific probe, indicating a de novo occurrence (data not shown). The only referenced gene within the deleted region was *SETBP1*. The two neighbouring genes, *BC051727* and *AK123972*, were non-coding. *TSLC14A2* (NM_007163) encodes a renal tubular urea transporter of the solute carrier family 14, not related to the phenotype of the patient.

Expression of SETBP1

In comparison with the normal control, SETBP1 RNA expression in the skin fibroblasts derived from patient 1 was reduced to 0.53, 0.60 and 0.41 (mean 0.51), and the lower SETBP1

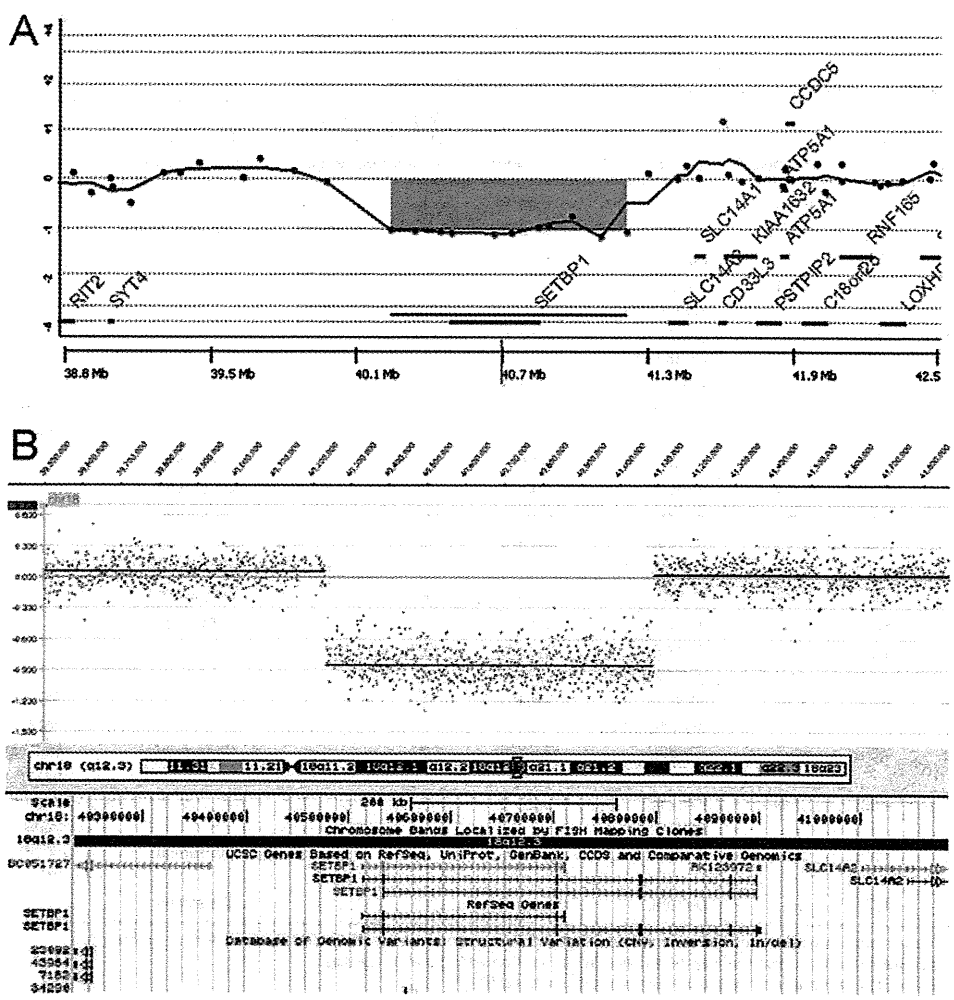

Figure 2 Microarray-based comparative genomic hybridisation identifies small deletions including *SETBP1* in patient 1 (A) and patient 2 (B). DNA copy number changes are represented by the negative $\log_2$ ratio below the baseline showing the deletions. (B) The square in the chromosome ideogram indicates the chromosomal position of the deletion; genes contained within the deletion are depicted below (http://genome.ucsc.edu).

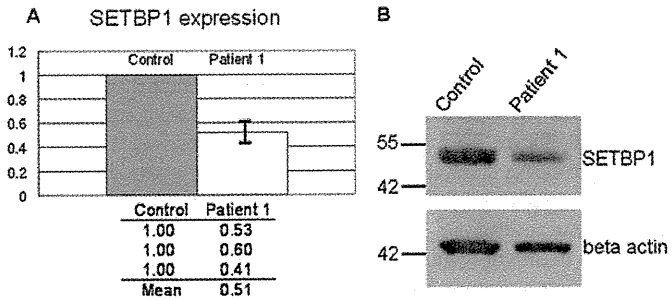

Figure 3 Expression studies. (A) SETBP1 RNA expression ratio analysed by real-time PCR. Raw data are given beneath the histogram. SETBP1 expression in the patient was about half that found in the control. (B) Western blotting of SETBP1. A total of 10 μg protein was separated in the gels. SETBP1 protein can be seen to be decreased in the patient. Beta actin was used as the internal control. Molecular mass (kDa) is indicated on the left of the gel.

protein concentration was also confirmed by western blotting (figure 3A,B).

Cohort study for *SETBP1* mutations

We identified 18 nucleotide changes including 11 non-synonymous and seven synonymous mutations, but no nonsense and no truncation mutations (table 1). The seven synonymous and four non-synonymous mutations, V231L, A390V, V1101I and P1130T, which were already listed in the single-nucleotide polymorphism database, were benign single-nucleotide polymorphisms. Four missense mutations (R627C, E958G, G1067S and W1242C; data not shown) located on the conserved sequence regions compared with the homologous genes from other species were not identified in normal control samples. However, W1242C was found in a healthy parent. Q1558L was also inherited from a healthy parent. The codon positions of E1466D and P1526Q were conserved among species and included in the important regions, SET-binging region and PPLPPPPP repeat, respectively. However, the patients' phenotypes were not similar to the presenting patient or SGS. Thus there was no definite pathogenic mutation. The sequence of the remaining *SETBP1* allele in patient 1 contained no nucleotide alterations.

DISCUSSION

In this study, we identified two patients with de novo chromosomal microdeletions in 18q12.3 that included *SETBP1* exclusively. *SETBP1* haploinsufficiency was suggested to be pathogenic. The patients exhibit moderate developmental delay and distinctive facial features, including prominent forehead, sparse eyebrows, mild ptosis with periorbital fullness. Patient 2 in particular showed a striking discrepancy between expressive speech impairment and conserved receptive speech, which has also been previously observed in patients with larger deletions in del(18)(q12.3q12.3). The complete and exclusive loss of one copy of *SETBP1* in our patient in correlation thus suggests an essential role for *SETBP1* in expressive speech development.

Schinzel *et al* reported on three patients with del(18)(q12.2q21.1) showing muscular hypotonia, seizures, behavioural disorders, and a pattern of minor dysmorphic features including prominent forehead, ptosis of the upper eyelids, full periorbital tissue, epicanthic folds and strabismus.[5] These phenotypic characteristics are similar to those in the cases presented here. Tinkle *et al* reported on a patient with del(18)(q12.2q21.1) with

Table 1 Identified nucleotide alteration in the cohort study

Nucleotide position*	Change	Amino acid change*	Location	Number of alleles that showed nucleotide changes	Conserved/ not conserved†	Function‡	Trio analyses	Results of population study	In silico database
c. 691	G>C	V231L	Exon 4	4	Not conserved				rs11082414
c. 1169	C>T	A390V	Exon 4	1	Not conserved				rs8091231
c. 1879	C>T	R627C	Exon 4	3	Conserved			None	None
c. 1911	G>A	P637P (synonymous)	Exon 4	1	-				None
c. 1932	C>T	S644S (synonymous)	Exon 4	2	-				rs3744824
c. 2607	C>T	S869S (synonymous)	Exon 4	12	-	The Ski homology region			None
c. 2873	A>G	E958G	Exon 4	1	Conserved			None	None
c. 3199	G>A	G1067S	Exon 4	1	Conserved			None	None
c. 3301	G>A	V1101I	Exon 4	90	Conserved				rs3744825
c. 3372	C>T	G1124G (synonymous)	Exon 4	1	-				None
c. 3388	C>A	P1130T	Exon 4	66	Conserved				rs1064204
c. 3726	G>C	W1242C	Exon 4	1	Conserved		Familial	None	None
c. 3825	A>G	S1275S (synonymous)	Exon 4	2	-				rs8096662
c. 4010	G>C	S1337S (synonymous)	Exon 5	1	-	SET-binding region			None
c. 4398	G>T	E1466D	Exon 6	3	Conserved	SET-binding region			None
c. 4563	C>G	P1521P (synonymous)	Exon 6	1	-	PPLPPPPP repeat			None
c. 4577	C>A	P1526Q	Exon 6	1	Conserved	PPLPPPPP repeat			None
c. 4673	A>T	Q1558L	Exon 6	1	Conserved		Familial		None

*Nucleotide and amino acid positions indicate NM_015559 sequence with the first initiation codon ATG at position 1.
†Conserved or not conserved was determined by comparison with the other species.
‡Functional domains were obtained from Minakuchi *et al* (2001).[12]

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long-term survival, and concluded that life expectancy is minimally reduced.[6]

Although the previously reported chromosomal anomalies were identified at chromosomal G-banded levels, in more recent reports deletions in 18q12.2q21.1 were characterised by molecular techniques, and common features in the patients' phenotypes were reported.[7 8] The critical region for the phenotype of patients was narrowed to the 18q12.3-q21.1 region by Cody *et al*[9] and Buysse *et al*[10] (figure 4), who proposed a new syndrome involving expressive speech delay. They hypothesised that genes within the region may be specific to the neural and motor planning domains necessary for speech. However, deletions described so far contain numerous genes, including *SETBP1*, not allowing a phenotype–genotype correlation for haploinsufficiency of *SETBP1* exclusively.

Our findings correlate the phenotypes of the two patients with the exclusive complete loss of one copy of *SETBP1*. There is significant phenotypic overlap with the previously reported del (18)(q12.2q21.1) syndrome, suggesting a major contribution of the deletion of *SETBP1* to these phenotypes, as it has been described in contiguous deletion syndromes. The discrepancy between expressive and receptive language abilities in our patients appears to be a unique characteristic in the *SETBP1* deletion phenotype. The complete and exclusive loss of one copy of *SETBP1* in our patients in correlation with their phenotypes suggests an essential role for *SETBP1* in expressive speech development, although the exact function of the gene remains unknown.

SETBP1 encodes SET binding protein 1 expressed in numerous tissues including fetal brain. Its fusion with nucleoporin 98 kDa (*NUP98*) by chromosomal translocation has been shown in acute T-cell lymphoblastic leukaemia,[11] and the SET binding protein has been proposed to play a key role in the mechanism of SET-related leukaemogenesis and tumorigenesis by regulatory function in the nucleus.[12] Hoischen *et al* recently identified mutations in *SETBP1* to be causative of SGS, which is

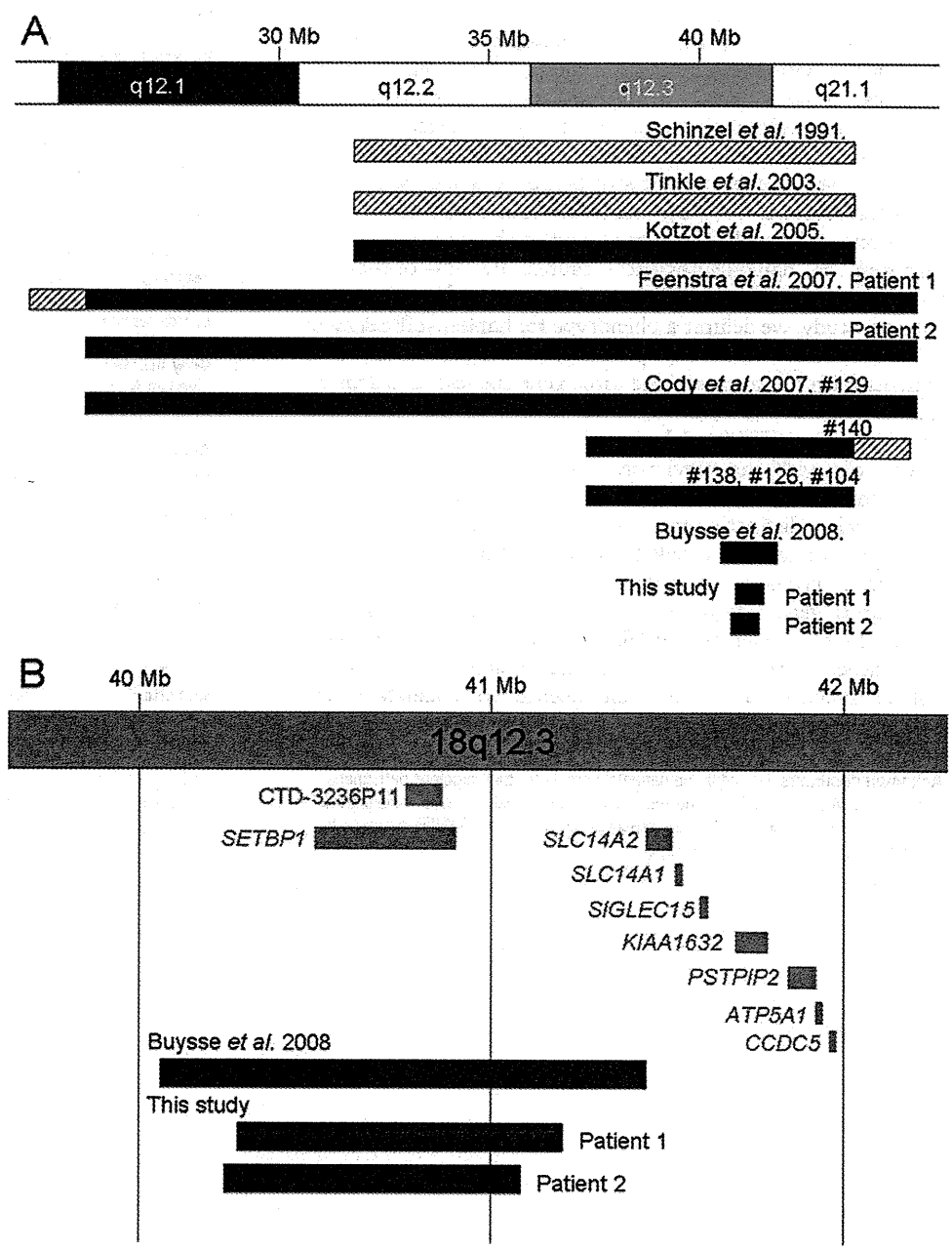

Figure 4 Comparison of the deletion regions. (A) Schematic representation of the previously reported deletions on a physical map of chromosome 18. (B) The deletion region of the patient is expanded. Bars filled with black and diagonal lines indicate definite and ambiguous deletion regions, respectively. Green and red bars indicate the position of the BAC clone used for fluorescence in situ hybridisation and the known genes, respectively.

characterised by severe mental retardation, distinctive facial features, and multiple congenital malformations.[1] Prognosis is poor, and most affected individuals die in the first decade of life. All reported mutations of *SETBP1* in patients with SGS were missense mutations in the important SET-binding region, and a gain-of-function or dominant-negative effect was suspected.[12]

As the phenotype of our two patients and the previously reported patients with del(18)(q12.2q21.1) including *SETBP1* does not resemble SGS and clinical features are generally milder, we conclude that haploinsufficiency of *SETBP1* does not cause SGS. We analysed the expression of SETBP1 by real-time PCR and western blotting, and found that SETBP1 was reduced in patient-derived skin fibroblasts, confirming that the effects of SETBP1 are allele dose-dependent. The deletion mainly affects speech, but the syndromic phenotype includes global development delay and recognisable facial dysmorphism underlying ubiquitous expression of SETBP1. As the phenotypic appearance of *SETBP1* haploinsufficiency is completely different from that of SGS, our findings support the proposed gain-of-function or dominant negative effect of the identified mutations in this gene.

There are various examples of phenotypic variability due to the different nature of mutations in the same gene. Mutations in fibroblast growth factor 3 (*FGFR3*) cause disproportionate growth in achondroplasia by gain-of-function, whereas terminal deletions of 4p including *FGFR3* cause Wolff–Hirschhorn syndrome, which does not show disproportionate growth at all, but small stature.[13 14] On the other hand, gain-of-function mutations of T-box 1 (*TBX1*) can result in the same phenotypic spectrum as haploinsufficiency caused by loss-of-function mutations or deletions in 22q11 including *TBX1*.[15]

In our study, we delimit a phenotype for haploinsufficiency of *SETBP1* distinct from the phenotype of SGS described in patients with mutations in the same gene suggesting a gain-of-function or a dominant negative effect of the mutations described. The *SETBP1* deletion phenotype seems to overlap extensively with the previously described del(18)(q12.2q21.1) syndrome, which has been characterised by moderate developmental delay, distinctive facial appearance, expressive language delay, and behavioural problems. Haploinsufficiency of *SETBP1* may thus primarily contribute to the phenotype of this contiguous gene syndrome. We did not identify pathogenic mutations on sequencing *SETBP1* in a cohort of 142 patients with developmental delay. Additional studies of the exact cellular function of *SETBP1* are needed to understand the pathogenic origin of the variable and distinct phenotypes.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Tokyo Women's Medical University and the University of Basel.

Provenance and peer review Not commissioned; externally peer reviewed.

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