

Gene dosage change of *TPTE* and *BAGE2* and breakpoint analysis in Robertsonian Down syndrome

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Abstract Only 4% of Down syndrome (DS) cases have a Robertsonian translocation (ROB). The aim of this study was to define the possible breakage area in 21p where ROB occurs. We prospectively and consecutively collected ten cases ROB DS from three medical centers. Of the ten DS children, six were de novo (60%), and four were due to paternal or maternal inheritance (40%). They consisted of four der(21q;21q), four der(14q;21q), one der(13q;21q),

and one der(21q;22q). The origin of the extra chromosome 21q was maternal in five of six de novo ROB and paternal in one case. All four der(21;21) ROB DS were an isochromosome. The result of gene dosage change by real-time quantitative polymerase chain reaction (PCR) was compatible with array-comparative genomic hybridization in one case. We further used real-time PCR to detect the copy number of *TPTE* and *BAGE2* located on 21p11 and *SAMSN1* on 21q11. The ratio of copy number in 21p:21q was 1:3 in der(21q;21q) but 2:3 in der(13q;21q), der(14q;21q), and der(21q;22q). Our preliminary results demonstrated the critical breakpoint of chromosome 21 involving ROB might lie between *BAGE2* and the centromere, located from 10.1 to 12.3 Mb.

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Introduction

Down syndrome (DS) is the most common human aneuploidy at birth and is the most common genetic cause of mental retardation. DS occurs in approximately 1/700 live births (Korenberg et al. 1994). It was first identified as trisomy of chromosome 21 in 1959 (Lejeune et al. 1959). Nearly 95% of DS cases is due to a complete extra chromosome 21. About 90% of them arises from a maternal meiotic error (Yoon et al. 1996; Antonarakis et al. 2004). The remaining 4% results from a Robertsonian (ROB) translocation. Of those, 25% of ROB DS is familial whereas the remainder is de novo. Mosaic DS comprises 1–2% of cases (Shaffer et al. 1993; Wolff and Schwartz 1993; Mutton et al. 1996; Antonarakis 1998; Roizen and Patterson 2003).

ROB are whole-arm exchanges between the short arms of acrocentric chromosomes 13, 14, 15, 21, and 22 (Bandyopadhyay et al. 2002; Berend et al. 2003). In these particular short arms, satellite DNA is present in the p11 region, nucleolar organizer region (NOR) in p12, and telomeric sequence in p13 (Page et al. 1996; Bandyopadhyay et al. 2001). We are aware of three possible mechanisms of formation of ROB: (1) centric fusion at the centromere, (2) fusion following breakage in one short arm and one long arm, and (3) fusion following breakages in two short arms (Guichaoua et al. 1986). The first two mechanisms that form a translocation chromosome with one centromere, known as a monocentric chromosome, occur very rarely. The third results in a chromosome with two centromeres, or a dicentric chromosome.

The breakpoint of ROB, as with other chromosomal rearrangements, predominately occurs in the pericentromeric and subtelomeric regions (Roth and Wilson 1986; Shaw and Lupski 2004). The proximal domain of the pericentromeric region of chromosome 21 is a patchwork of chromosome duplication containing two genes (*TPTE* and *BAGE2*) in 21p (Brun et al. 2003). These two genes belong to gene families, and they control the function of testis and cancer expression (Brun et al. 2003; Tapparel et al. 2003). No other genes are present in 21p; therefore, *TPTE* and *BAGE2* are excellent candidates for breakpoint analysis of ROB.

Array-based comparative genomic hybridization (CGH) allows identification of chromosome regions of gains and losses in cancers and genetics diseases (Pinkel et al. 1998; Pollack et al. 1999; Hodgson et al. 2001; Snijders et al. 2001; Greshock et al. 2004). To detect single-copy number changes or an unbalanced translocation, oligonucleotide-based high resolution microarray CGH contains the complete genome representation and most detailed probes (Solinas-Toldo et al. 1997; Lucito et al. 2003; Bignell et al. 2004). Array CGH is also a powerful tool for chromosome breakpoint analysis (Chao et al. 2006; Peng et al. 2006). Then, the results of array CGH can be verified by quantitative polymerase chain reaction (PCR) or fluorescence in situ hybridization (FISH).

In this novel study, we used high-resolution genomic-wide array CGH to analyze the gene dosage change of chromosome 21 of ROB DS. Then, we arranged real-time quantitative PCR for confirmation. *TPTE* and *BAGE2* located in 21p were designated as the genes to analyze for the breakage points of ROB.

Materials and methods

Patients with ROB translocations of DS including der(14q;21q), der(21q;21q), der(13q;21q), der(15q;21q),

and der(21q;22q) were enrolled in our study. The first two are the most common form of ROB found in DS. We prospectively collected ten cases of ROB DS, either familiar type or de novo. Due to the rarity of ROB DS, we reviewed the databases of the cytogenetics laboratory from Chang Gung Memorial Hospital (CGMH), National Taiwan University Hospital (NTUH), and Mackay Memorial Hospital (MMH) from 1996 to 2005. These children were all followed in the pediatric department of those medical centers. We selected consecutive cases without any selection bias. The study closed when the tenth case was enrolled. Genetic counselors and a research nurse recorded the phenotype of each case. Medical history was obtained from chart review or direct history. Informed consent was given to by parents, and the research and ethics committees approved the study design.

Genomic DNA isolation

Genomic DNA was extracted with the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA), DNA quality was confirmed by gel electrophoresis, and the amount of DNA was quantified with a spectrophotometer (GeneQuant; Amersham Biosciences, Piscataway, NJ, USA).

Molecular genetic analysis

DNA was isolated from peripheral blood. Quantitative fluorescent polymerase chain reaction (QF-PCR) using polymorphic small tandem repeat (STR) markers was carried out to verify the parental origin of all DSs with Robertsonian translocation. Polymorphic STR markers specific to chromosomes 21 were used to determine the parental origin of the extra chromosome 21 (Table 1). Further STR markers specific to chromosome 14 (four cases) and chromosome 13 (one case) were applied to detect uniparental disomy (UPD).

Array experiment

Genomic DNA was fragmented by AluI and RsaI restriction digest for a minimum of 2 h at 37°C. After purification with the QIAquick PCR purification kit (Qiagen, USA), digested DNA was visualized using the 2100 BioAnalyzer (Agilent Technologies, USA) with DNA 500 chip. Labeling reactions were performed with 10 µg of purified DNA and a Bioprime labeling kit (Invitrogen), according to the manufacturer's instructions, in a volume of 50 µl with a modified deoxyribonucleotide triphosphate (dNTP) pool

Table 1 General characteristics of ten cases of Robertsonian Down syndrome

Case	Karyotype	Age (years)	Maternal age (years)	Inheritance	STR marker	Position	Father	Mother	Child	Origin	UPD study	Meiosis error
1	46,XX,der(21;21),+21	6.4	22	De novo	D21S1443	21q21.2–21.3	238/ 258	238/266	258/266/266	M	i(21q)	II
2	46,XX,der(14;21),+21	1.4	29	De novo	D21S1411	21q22.3	277/ 293	285/293	277/285/285	M	N	II
3	46,XX,der(13;21),+21	9.9	26	Inheritance	D21S11	21q21.1	28/31.2	29/30	29/30/31.2	M	N	–
4	46,XY,der(21;21),+21	4.0	24	De novo	D21S11	21q21.1	30/30	31.2/ 32.2	30/31.2/31.2	M	i(21q)	II
5	46,XX,der(14;21),+21	6.0	22	De novo	D21S1437	21q21.1	135/ 135	119/127	119/127/135	M	N	I
6	46,XY,der(14;21),+21	2.7	21	Inheritance	D21S1409	21q21.2	180/ 180	196/196	180/196/196	M	N	–
7	46,XX,der(21;21),+21	2.3	31	De novo	D21S1437	21q21.1	113/ 121	121/129	113/129/129	M	i(21q)	II
8	46,XY,der(21;22),+21	5.6	24	Inheritance	D21S11	21q21.1	30/30	29/32.2	29/30/32.2	M	–	–
9	46,XY,der(21;21),+21	4.7	29	De novo	D21S11	21q21.1	28/30	28/32.2	30/30/32.2	P	i(21q)	II
10	46,XY,der(14;21),+21	1.7	23	Inheritance	D21S1411	21q22.3	285/ 298	289/293	285/285 / 289	P	N	–

STR short tandem repeat, *i* isochromosome, *M* maternal origin, *P* paternal origin, *N* negative, *UPD* uniparental disomy

containing 120 µl each of dATP, deoxyadenosine triphosphate (dGTP), and deoxycytidine triphosphate (dCTP), 60 µl deoxythymidine triphosphate (dTTP), and 60 µl Cy5-labeled deoxyuridine triphosphate (Cy5-dUTP) (for the experimental sample), or Cy3-dUTP (for the reference) (PerkinElmer). Labeled targets were subsequently purified by using a Centricon YM-30 column (Millipore). Each sample for hybridization was compared against normal pooled human female as a reference (Promega). Experimental and reference targets for each hybridization were pooled and mixed in a 500 µl hybridization mixtures of 50 µg of human Cot-1 DNA (Invitrogen) in 1× hybridization buffer (Agilent Technologies). Before hybridization to the array, hybridization mixtures were denatured at 95°C for 3 min and incubated at 37°C for 30 min. To remove any precipitate, the mixture was centrifuged at ≥14,000 g for 5 min and the supernatant was transferred to a new tube. The labeled and denatured DNA target was then hybridized to human genome CGH 244A microarray (G4411B, Agilent Technologies, USA) at 65°C for 40 h. The arrays were then washed in 0.5× SSC/0.005% Triton X-102 (wash 1) at room temperature for 5 min, followed by 5 min at 37°C in 0.1× sodium chloride, sodium citrate (SSC)/0.005% Triton X-102 (wash 2).

Image acquisition and raw data processing

After drying, hybridized arrays were scanned on an Agilent DNA microarray scanner at 535 nm for Cy3 and 625 nm for Cy5 at a resolution of 5 µm. Scanned images were analyzed by Feature Extraction 8.1 software (Agilent Technologies, USA), and image analysis and normalization software used to quantify signal and background intensity for each feature substantially normalized the data by linear normalization method. Data analysis was performed on the CGH Analytics 3.3 (Agilent Technologies, USA).

PCR primers

Specific oligonucleotide primer pairs for *TPTE* (21p11, locus 9.93–10.01 Mb on UCSC hg 17 map, 5′ → 3′ sense primer: GGTTCATGCTACTAGGAAAGTCTCA, 5′ → 3′ antisense primer: TGTTTGTCACATTACCTTTCTGTG), *BAGE2* (21p11, locus 10.08–10.12 Mb on UCSC hg 17 map, 5′ → 3′ sense primer: TGATCTCTTTTGGCTCA CCGTA, 5′ → 3′ antisense primer: TTGAGTATTCCCCC AATTTTT) and *SAMSN1* (21q11, locus 14.78–14.84 Mb on UCSC hg 17 map, 5′ → 3′ sense primer: TGAAAA TCTGTCTGACATGGTACA, 5′ → 3′ antisense primer: TAGTTGGGAATGCGTGTTC) were selected from the Beacon designer for real-time PCR assays. The specificity

of each primer pair was validated by performing a PCR reaction using normal female DNA (cat.# G1521, Promega, USA) as DNA template, and the size of the PCR product was validated by a DNA 1,000-chip (Agilent Technologies, USA) run on Bioanalyzer 2100 (Agilent Technologies, USA). Primer pairs of generating predicted product size and no other side products were chosen for the following real-time PCR reaction.

Real-time quantitative PCR and calculation of gene dosage

Real-time PCR reactions were performed on the Roche LightCycler Instrument 1.5 using LightCycler[®] FastStart DNA Master^{PLUS} SYBR Green I kit (Roche Cat. 03 515 885 001, Castle Hill, Australia). Briefly, 10- μ l reactions contained 2 μ l of Master Mix, 2 μ l of 0.75 μ M forward primer, 6 μ l of 0.75 μ M of reversed primer and 10 ng of template DNA. Each sample was run in triplicate. The PCR program was 95°C for 10 min, 50 cycles of 95°C for 10 s, 60°C for 15 s, and 72°C for 10 s. At the end of the program, a melt curve analysis was done. At the end of each PCR run, data were automatically analyzed, and an amplification plot was generated for each cDNA sample. From each of these plots, the LightCycler3 data analysis software automatically calculated the CP value (crossing point; the turning point that corresponds to the first maximum of the second derivative curve), which implied the beginning of exponential amplification. The fold change of the target gene relative to internal control gene *ATP2B4* (1q32, locus 201.86–201.98 Mb on UCSC hg 17 map) in each sample was then calculated by the following formula:

$$2^{-\Delta\Delta CP} \text{ where } \Delta CP = CP_{\text{target gene}} - CP_{\text{internal control}} \text{ and,} \\ \Delta\Delta CP = \Delta CP_{\text{test sample}} - \Delta CP_{\text{control sample}}$$

Results

Ten cases of ROB DS were collected. Seven cases were from CGMH, two from NTUH, and one from MMH. Patients' mean age was 53 months. Average maternal age at delivery was 25.1 years. Of the ten children, six had a de novo ROB translocation (60%), and four were inherited (40%). Karyotypes included four der(21q;21q), four der(14q;21q), one der(13q;21q), and one der(21q;22q). All cytogenetic results were confirmed by review or reculturing. Molecular cytogenetic analysis demonstrated that the origin of the extra chromosome 21q was maternal in 5/6 de novo ROB and paternal in one. Most (5/6, 83.3%) de novo ROB DS revealed homozygosity through STR marker analysis and that the error occurred in meiosis II. Case 5

showed three different peaks in STR result, thereby indicating that formation of the translocation occurred in meiosis I with crossover. For the four cases of parental inheritance, three ROB were maternally inherited, and one was paternally inherited (Table 1). Therefore, eight (80%) cases were maternally inherited compared with paternal inheritance (2/10, 20%). No cases of UPD for chromosomes 13 and 14 were detected. All four der(21;21) ROB translocations were an isochromosome, i(21q), instead of a true translocation, t(21q;21q). One of them was paternal in origin and the other three were maternal.

The phenotype of these ten cases were recorded carefully. At least 90% of ROB DS had features of epicanthal folds, short stature, muscle hypotonia, and mental retardation. The incidence of congenital heart disease was 80%, which was higher than reported in the literature. One child had duodenal atresia. No child in our study had been diagnosed with an imperforate anus, Hirschsprung disease, or leukemia.

The 244-K-array CGH (Agilent Technologies) was applied to case 2 by simple random sampling, drawing of lots. Result revealed diploidy (gene dosage was nearly equal to 1.0) for every chromosome except chromosome 21. With whole q-arm amplification in chromosome 21, the average gene dosage was 1.5. However, the gene dosage in 21p was near 1 (Fig. 1a). *TPTE* and *BAGE2* are the two genes in 21p, with six probes for *TPTE* and seven probes for *BAGE2*. The physical map of both genes is located in 21p11. The average \log_2 ratio on *TPTE* was 0.2 and gene dosage was 1.1. The results were similar for *BAGE2*, $-0.1 \log_2$ ratio and 1.0 gene dosage (Table 2). Compared with *TPTE* and *BAGE2*, *SAMSNI* (eight probes) located in 21q11 showed 0.7 \log_2 ratio with 1.6 gene dosage. We performed real-time PCR, and the result was compatible with array CGH. Thus, the critical breakpoint might be located between the centromere and *BAGE2* (Fig. 1b).

Table 3 shows the results of real-time quantitative PCR for all ten cases, plus one positive control and one negative control. The gene dosage of *SAMSNI* was increased in all ROB DS and free trisomy 21. The gene dosage of *TPTE* and *BAGE2* was decreased in cases 1, 4, 7, and 9. These four cases involved isochromosome 21, and the ratio of DNA copy number 21p:21q was 1:3. The other six ROB DS revealed no gene dosage change in *TPTE* and *BAGE2* with the ratio of 2:3. The gene dosage on free trisomy 21 was near 1.5; therefore, the copy number of 21p:21q was 3:3. No gene dosage change with 2:2 DNA copy number ratio was observed in the negative control.

Discussion

De novo duplication of chromosome 21, also known as isochromosome 21 DS, is the second most common

Fig. 1 **a** Agilent 244-K-array comparative genomic hybridization (CGH) demonstrated the 21p11 area from 9.86 to 10.26 Mb. There was no gene dosage change in *TPTE* (red arrow) or *BAGE2* (green arrow). **b** Possible breakpoint of Robertsonian translocation in chromosome 21. The breakpoint might be between *BAGE2* and centromere

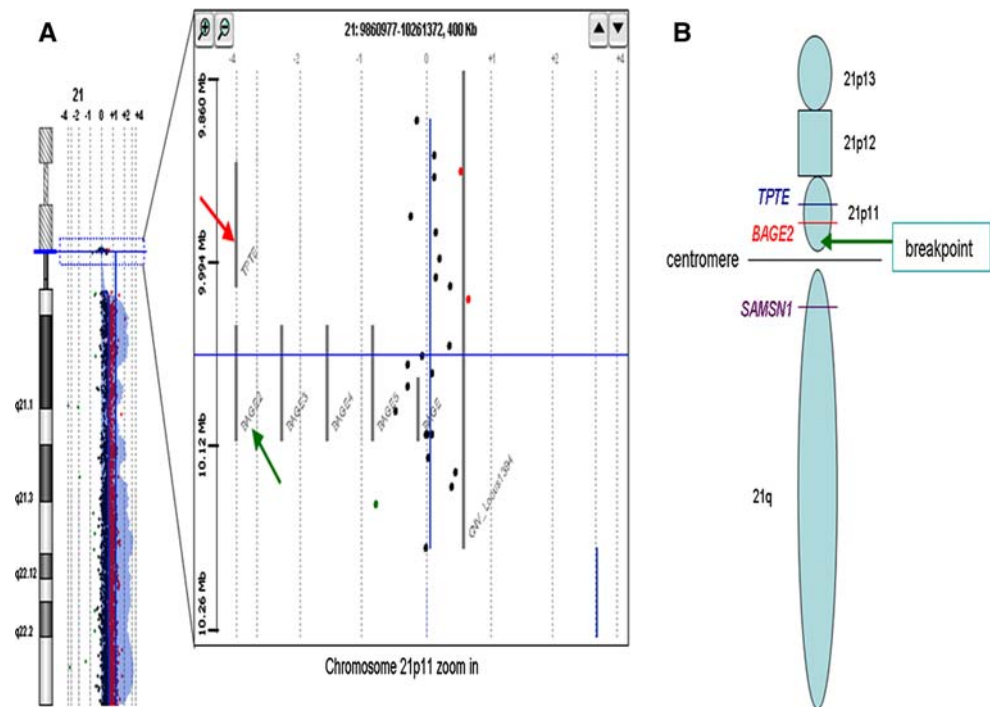


Table 2 Real-time PCR verification of gene dosage of *TPTE*, *BAGE2*, and *SAMSNI* by array CGH in case 2

Gene	Physical map	Probe number	Locus (Mb)	Array CGH		Real-time PCR	
				Log ₂ ratio	Gene dosage	Log ₂ ratio	Gene dosage
Case 2							
<i>TPTE</i>	21p11	6	9.93–10.01	0.2	1.1	−0.24	1.18
<i>BAGE2</i>	21p11	7	10.08–10.12	−0.1	1.0	0.13	0.91
<i>SAMSNI</i>	21q11	8	14.78–14.84	0.7	1.6	−0.76	1.69

CGH comparative genomic hybridization, PCR polymerase chain reaction

chromosomal form of DS. Sometimes, it has been called a “21q21q” ROB. However, the two 21q components are usually identical (Antonarakis et al. 1990; Shaffer et al. 1993). In our study, four cases of der(21q;21q) involved in isochromosome and molecular study suggested that the isochromosome originate at early postzygotic mitosis. Seventy-five percent (3/4) of isochromosome 21 cases was of maternal and 25% of paternal origin. Two of four der(14q;21q) translocations were de novo, and the other two were of inherited. The extra chromosome 21 in these two cases of de novo der(14q;21q) translocation was maternally derived. From the literature, all cases of der(14q;21q) translocation were of maternal origin (Peteresen et al. 1991; Shaffer et al. 1992; Berend et al. 2003).

The UPD study results for chromosomes 13 and 14 were negative, meaning that the two alleles were of biparental origin. When a Robertsonian translocation occurs, trisomy or monosomy rescue could occur, resulting in UPD. If

there are regions of genome imprinting on a uniparental chromosome pair, phenotypic consequences can arise. Testing for UPD should be performed, especially in case of any parent who is a balanced carrier of ROB (Kim and Shaffer 2002).

All cases of ROB DS were phenotypically similar to those with free trisomy 21 (Antonarakis et al. 2004). The higher incidence of brachycephaly and epicanthal folds in our group might be due to Asian ancestry. Congenital heart diseases were also more commonly found in our cases (80% vs. 40% in the literature). Although translocation occurred in chromosome 21p, the severity of DS was almost similar. Most DS features appear when the DS critical region (DSCR) (located around the D21S55 marker and roughly comprising from distal 21q22.1 to proximal 21q22.3) is trisomic (Delabar et al. 1993; Korenberg et al. 1994; Valero et al. 1999; Bandyopadhyay et al. 2001). In our patients with ROB DS, we recognized every case with

Table 3 Result of gene dosage and DNA copy number ratio of 21p:21q in 12 cases (plus positive and negative control) by real-time polymerase chain reaction

Case	Karyotype	Gene dosage of <i>TPTE</i> (21p11)	Gene dosage of <i>BAGE2</i> (21p11)	Gene dosage of <i>SAMSNI</i> (21q11)	Ratio of DNA copy number (21p: 21q)
1	46,XX,der(21;21),+21	0.57	0.62	1.36	1:3
2	46,XX,der(14;21),+21	1.18	0.91	1.69	2:3
3	46,XX,der(13;21),+21	1.06	0.85	1.45	2:3
4	46,XY,der(21;21),+21	0.76	0.79	1.82	1:3
5	46,XX,der(14;21),+21	1.25	0.85	1.65	2:3
6	46,XY,der(14;21),+21	1.18	1.01	1.54	2:3
7	46,XX,der(21;21),+21	0.81	0.78	1.41	1:3
8	46,XY,der(21;22),+21	1.09	0.96	1.59	2:3
9	46,XY,der(21;21),+21	0.83	0.71	1.39	1:3
10	46,XY,der(14;21),+21	1.08	1.18	1.75	2:3
P	47,XY,+21	1.50	1.49	1.52	3:3
N	46,XX	1.05	0.93	1.03	2:2

P positive control, N negative control

21q involved in the trisomy, including DSCR. It is therefore understandable that these patients display most of the phenotypic features associated with free trisomy 21 DS.

During the last two decades, technology has enabled a higher resolution analysis of the human genome. Diagnosis of genomic rearrangement has seen a shift from cytogenetic techniques, such as G-banding to FISH. Recently, array CGH has been successfully used to identify genomic deletions and duplications (Bruder et al. 2001; Yu et al. 2003; Shaw et al. 2004). Array CGH is higher throughput than FISH and may be especially useful in identifying new genomic disorders or in detecting submicroscopic rearrangements not visible by traditional chromosome analysis (Vissers et al. 2003).

The mechanisms of ROB formation are not yet clearly identified. However, the most of common ROB's have a consistent breakpoint location, whereas the rare translocations could have highly variable breakpoints locations (Page et al. 1996; Sullivan et al. 1996). Some studies further demonstrated microdeletions or loss of sequences occurs in ROB's (Earle et al. 1992; Bonthron et al. 1993; Rogatcheva et al. 2000). That is why we used high-resolution-array CGH with 244-K Agilent chip for evaluation of the whole genome and detection of any possible submicroscopic chromosomal deletion or duplication. In case 2, which was evaluated by array CGH, no other unbalanced chromosome rearrangement, except for duplication in 21p, was found.

Although the high resolution of array CGH is a powerful tool to detect aneuploidy, it cost is prohibitive, thus limiting us to analysis of a single case. We focused on the genes in 21p, including *TPTE* and *BAGE2*. Six probes for *TPTE* and seven probes for *BAGES2* were built into 244-K chips. We expected that DNA copy number change in these two genes could yield us valuable information. The results of array CGH showed the breakpoint of chromosome 21 might be

located proximal to *BAGE2* from the centromere. To confirm this result, we further arranged real-time PCR and designed three primers for *TPTE*, *BAGE2*, and *SAMSNI*. The results of real-time PCR were comparable to array CGH.

The 14q21q translocation in case 2 spurred us to seek solid evidence of breakpoints involving chromosome 21 in a ROB translocation. Page et al. used FISH probes pTRI-6 to locate the breakpoint of 14q21q translocation between p11 (DNA satellites) and p12 (NOR, ribosome RNA) (Page et al. 1996). Their group also defined the possible breakpoints of all types of ROB. Unlike other acrocentric chromosomes, chromosome 21 was the only one that p arm has some genes (International Human Genome Sequencing Consortium 2004). Our data of real-time PCR (Table 3) showed the DNA copy number of ROB DS was two in the p arm and three in the q arm. For isochromosome 21, the DNA copy number was one in 21p and three in 21q. The breakpoint of chromosome 21 involving an ROB translocation might lie between *BAGE2* and the centromere, a location from 10.1 to 12.3 Mb (Fig. 1b).

TPTE and *BAGE2* are located in the juxtacentromeric region of chromosome 21 and have a cancer and/or testis expression profile (Brun et al. 2003). Gene expression level analysis should be performed. Isochromosome 21 might have only one copy of *TPTE* and *BAGE2* but three copies of *SAMSNI*. We should pay attention to the effect of the gene deletion, especially in male cases. A critical breakpoint of translocation was estimated by only one case in our work. Therefore, additional analyses should be arranged. Higher resolution of array CGH or customized CGH, or molecular FISH probes specific to *TPTE* and *BAGE2*, could offer more information about 21p in the near future. Our preliminary work requires more support to confirm the results and complete the project.

In conclusion, this is the first study regarding gene dosage change and breakpoint analysis of ROB DS using

array CGH and real-time PCR. The breakpoint of chromosome 21 involving an ROB translocation might be located between *BAGE2* and centromere, located from 10.1 to 12.3 Mb. Further strategies to confirm the initial result should be planned.

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References

- International Human Genome Sequencing Consortium (2004) Finishing the euchromatic sequence of the human genome. *Nature* 431:931–945
- Antonarakis SE (1998) 10 years of genomics, chromosome 21, and Down syndrome. *Genomics* 51:1–16
- Antonarakis SE, Adelsberger PA, Petersen MB, Binkert F, Schinzel AA (1990) Analysis of DNA polymorphisms suggests that most de novo dup(21q) chromosomes in patients with Down syndrome are isochromosomes and not translocations. *Am J Hum Genet* 47:968–972
- Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S (2004) Chromosome 21 and down syndrome: from genomics to pathophysiology. *Nat Rev Genet* 5:725–738
- Bandyopadhyay R, Heller A, Knox-DuBois C, McCaskill C, Berend SA, Page SL, Shaffer LG (2002) Parental origin and timing of de novo Robertsonian translocation formation. *Am J Hum Genet* 71:1456–1462
- Bandyopadhyay R, McQuillan C, Page SL, Choo KH, Shaffer LG (2001) Identification and characterization of satellite III subfamilies to the acrocentric chromosomes. *Chromosome Res* 9:223–233
- Berend SA, Page SL, Atkinson W, McCaskill C, Lamb NE, Sherman SL, Shaffer LG (2003) Obligate short-arm exchange in de novo Robertsonian translocation formation influences placement of crossovers in chromosome 21 nondisjunction. *Am J Hum Genet* 72:488–495
- Bignell GR, Huang J, Greshock J, Watt S, Butler A, West S, Grigorova M, Jones KW, Wei W, Stratton MR, Futreal PA, Weber B, Shapero MH, Wooster R (2004) High-resolution analysis of DNA copy number using oligonucleotide microarrays. *Genome Res* 14:287–295
- Bonthron DT, Smith SJ, Fantes J, Gosden CM (1993) De novo microdeletion on an inherited Robertsonian translocation chromosome: a cause for dysmorphism in the apparently balanced translocation carrier. *Am J Hum Genet* 53:629–637
- Bruder CE, Hirvela C, Tapia-Paez I, Fransson I, Segreaves R, Hamilton G, Zhang XX, Evans DG, Wallace AJ, Baser ME, Zucman-Rossi J, Hergersberg M, Boltshauser E, Papi L, Rouleau GA, Poptodorov G, Jordanova A, Rask-Andersen H, Kluwe L, Mautner V, Sainio M, Hung G, Mathiesen T, Moller C, Pulst SM, Harder H, Heiberg A, Honda M, Niimura M, Sahlen S, Blennow E, Albertson DG, Pinkel D, Dumanski JP (2001) High resolution deletion analysis of constitutional DNA from neurofibromatosis type 2 (NF2) patients using microarray-CGH. *Hum Mol Genet* 10:271–282
- Brun ME, Ruault M, Ventura M, Roizes G, De Sario A (2003) Juxtacentromeric region of human chromosome 21: a boundary between centromeric heterochromatin and euchromatic chromosome arms. *Gene* 312:41–50
- Chao A, Lee YS, Chao AS, Wang TH, Chang SD (2006) Microarray-based comparative genomic hybridization analysis of Wolf-Hirschhorn syndrome in a fetus with deletion of 4p15.3 to 4pter. *Birth Defects Res A Clin Mol Teratol* 76:739–743
- Delabar JM, Theophile D, Rahmani Z, Chettouh Z, Blouin JL, Prieur M, Noel B, Sinet PM (1993) Molecular mapping of twenty-four features of Down syndrome on chromosome 21. *Eur J Hum Genet* 1:114–124
- Earle E, Shaffer LG, Kalitsis P, McQuillan C, Dale S, Choo KH (1992) Identification of DNA sequences flanking the breakpoint of human t(14q21q) Robertsonian translocations. *Am J Hum Genet* 50:717–724
- Greshock J, Naylor TL, Margolin A, Diskin S, Cleaver SH, Futreal PA, deJong PJ, Zhao S, Liebman M, Weber BL (2004) 1-Mb resolution array-based comparative genomic hybridization using a BAC clone set optimized for cancer gene analysis. *Genome Res* 14:179–187
- Guichaoua MR, Devictor M, Hartung M, Luciani JM, Stahl A (1986) Random acrocentric bivalent associations in human pachytene spermatocytes. Molecular implications in the occurrence of Robertsonian translocations. *Cytogenet Cell Genet* 42:191–197
- Hodgson G, Hager JH, Volik S, Hariono S, Wernick M, Moore D, Nowak N, Albertson DG, Pinkel D, Collins C, Hanahan D, Gray JW (2001) Genome scanning with array CGH delineates regional alterations in mouse islet carcinomas. *Nat Genet* 29:459–464
- Kim SR, Shaffer LG (2002) Robertsonian translocations: mechanisms of formation, aneuploidy, and uniparental disomy and diagnostic considerations. *Genet Test* 6:163–168
- Korenberg JR, Chen XN, Schipper R, Sun Z, Gonsky R, Gerwehr S, Carpenter N, Daumer C, Dignan P, Distech C et al. (1994) Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc Natl Acad Sci USA* 91:4997–5001
- Lejeune J, Gautier M, Turpin R (1959) [Study of somatic chromosomes from 9 mongoloid children.]. *C R Hebd Seances Acad Sci* 248:1721–1722
- Lucito R, Healy J, Alexander J, Reiner A, Esposito D, Chi M, Rodgers L, Brady A, Sebat J, Troge J, West JA, Rostan S, Nguyen KC, Powers S, Ye KQ, Olshen A, Venkatraman E, Norton L, Wigler M (2003) Representational oligonucleotide microarray analysis: a high-resolution method to detect genome copy number variation. *Genome Res* 13:2291–2305
- Mutton D, Alberman E, Hook EB (1996) Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. National Down Syndrome Cytogenetic Register and the Association of Clinical Cytogeneticists. *J Med Genet* 33:387–394
- Page SL, Shin JC, Han JY, Choo KH, Shaffer LG (1996) Breakpoint diversity illustrates distinct mechanisms for Robertsonian translocation formation. *Hum Mol Genet* 5:1279–1288
- Peng HH, Wang CJ, Wang TH, Chang SD (2006) Prenatal diagnosis of de novo interstitial 2q14.2–2q21.3 deletion assisted by array-based comparative genomic hybridization: a case report. *J Reprod Med* 51:438–442
- Petersen MB, Adelsberger PA, Schinzel AA, Binkert F, Hinkel GK, Antonarakis SE (1991) Down syndrome due to de novo Robertsonian translocation t(14q;21q): DNA polymorphism analysis suggests that the origin of the extra 21q is maternal. *Am J Hum Genet* 49:529–536
- Pinkel D, Segreaves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 20:207–211
- Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO (1999) Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat Genet* 23:41–46

- Rogatcheva MB, Ono T, Sonta S, Oda S, Borodin PM (2000) Robertsonian metacentrics of the house musk shrew (*Suncus murinus*, Insectivora, Soricidae) lose the telomeric sequences in the centromeric area. *Genes Genet Syst* 75:155–158
- Roizen NJ, Patterson D (2003) Down's syndrome. *Lancet* 361:1281–1289
- Roth DB, Wilson JH (1986) Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. *Mol Cell Biol* 6:4295–4304
- Shaffer LG, Jackson-Cook CK, Stasiowski BA, Spence JE, Brown JA (1992) Parental origin determination in thirty de novo Robertsonian translocations. *Am J Med Genet* 43:957–963
- Shaffer LG, McCaskill C, Haller V, Brown JA, Jackson-Cook CK (1993) Further characterization of 19 cases of re(21q21q) and delineation as isochromosomes or Robertsonian translocations in Down syndrome. *Am J Med Genet* 47:1218–1222
- Shaw CJ, Lupski JR (2004) Implications of human genome architecture for rearrangement-based disorders: the genomic basis of disease. *Hum Mol Genet* 13 Spec No 1:R57–R64
- Shaw CJ, Shaw CA, Yu W, Stankiewicz P, White LD, Beaudet AL, Lupski JR (2004) Comparative genomic hybridisation using a proximal 17p BAC/PAC array detects rearrangements responsible for four genomic disorders. *J Med Genet* 41:113–119
- Snijders AM, Nowak N, Segraves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hindle AK, Huey B, Kimura K, Law S, Myambo K, Palmer J, Ylstra B, Yue JP, Gray JW, Jain AN, Pinkel D, Albertson DG (2001) Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* 29:263–264
- Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Dohner H, Cremer T, Lichter P (1997) Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromosomes Cancer* 20:399–407
- Sullivan BA, Jenkins LS, Karson EM, Leana-Cox J, Schwartz S (1996) Evidence for structural heterogeneity from molecular cytogenetic analysis of dicentric Robertsonian translocations. *Am J Hum Genet* 59:167–175
- Tapparel C, Reymond A, Girardet C, Guillou L, Lyle R, Lamon C, Hutter P, Antonarakis SE (2003) The TPTE gene family: cellular expression, subcellular localization and alternative splicing. *Gene* 323:189–199
- Valero R, Marfany G, Gil-Benso R, Ibanez MA, Lopez-Pajares I, Prieto F, Rullan G, Sarret E, Gonzalez-Duarte R (1999) Molecular characterisation of partial chromosome 21 aneuploidies by fluorescent PCR. *J Med Genet* 36:694–699
- Visser LE, de Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, van der Vliet W, Huys EH, van Rijk A, Smeets D, van Ravenswaaij-Arts CM, Knoers NV, van der Burgt I, de Jong PJ, Brunner HG, van Kessel AG, Schoenmakers EF, Veltman JA (2003) Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 73:1261–1270
- Wolff DJ, Schwartz S (1993) The effect of Robertsonian translocation on recombination on chromosome 21. *Hum Mol Genet* 2:693–699
- Yoon PW, Freeman SB, Sherman SL, Taft LF, Gu Y, Pettay D, Flanders WD, Khoury MJ, Hassold TJ (1996) Advanced maternal age and the risk of Down syndrome characterized by the meiotic stage of chromosomal error: a population-based study. *Am J Hum Genet* 58:628–633
- Yu W, Ballif BC, Kashork CD, Heilstedt HA, Howard LA, Cai WW, White LD, Liu W, Beaudet AL, Bejjani BA, Shaw CA, Shaffer LG (2003) Development of a comparative genomic hybridization microarray and demonstration of its utility with 25 well-characterized 1p36 deletions. *Hum Mol Genet* 12:2145–2152