Table 1 A total of 40 cases with CNV at subtelomeric region(s) among 54 positive cases in the first screening

	Position where CNV detected				
Gender	*Loss*	*Gain*	*Corresponding disorder*[a]	*OMIM or citation*	*Parental analysis*[b]
M	1p36.33		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
M	3q29		Chromosome 3q29 deletion syndrome	#609425	
F	5p15.33p15.32		Cri-du-chat syndrome	#123450	
M	5q35.2q35.3		Chromosome 5q subtelomeric deletion syndrome	Rauch *et al.*[25]	
F	6p25.3		Chromosome 6pter-p24 deletion syndrome	#612582	
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
F	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
F	9q34.3		Kleefstra syndrome	#610253	
F	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
F	16p13.3		Chromosome 16p13.3 deletion syndrome	#610543	
F	22q13.31		Chromosome 22q13 deletion syndrome	#606232	
M	22q13.31q13.33		Chromosome 22q13 deletion syndrome	#606232	
M		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
F		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
M		21q22.13q22.3	Down's syndrome (partial trisomy 21)	#190685	
M		Xp22.33	A few cases have been reported; e.g. V5-130 in Lu *et al.*[28]		
M		Xq28	Chromosome Xq28 duplication syndrome	#300815	
F	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
		8p23.2p23.3			
M	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		12p13.33p11.22			
F	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		16p13.3	Chromosome 16p13.3 duplication syndrome	#613458	
F	4q35.2		4q− syndrome[d]	Jones *et al.*[30]	
		7q36.3			
M	5p15.33		Cri-du-chat syndrome	#123450	
		20p13			
M	5p15.33p15.32		Cri-du-chat syndrome	#123450	
		2p25.3			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		11q25			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		8q24.3			
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	*dn*
		1q44			
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
		7q36.3			
F	10p15.3p15.2		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	*pat*
		7p22.3p22.2			
M	10p15.3		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	
		2p25.3			
M	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
		2q37.3	Distal trisomy 2q[d]	Elbracht *et al.*[33]	
M	18q23		Chromosome 18q deletion syndrome	#601808	
		7q36.3			
F	22q13.31q13.33		Chromosome 22q13.3 deletion syndrome	#606232	*pat*
		17q25.3	One case was reported	Lukusa *et al.*[34]	
M	Xp22.33/Yp11.32		Contiguous gene–deletion syndrome on Xp22.3[d]	Fukami *et al.*[35]	
		Xq27.3q28	Chromosome Xq28 duplication syndrome	#300815	

Abbreviations: F, female; CNV, copy-number variant; M, male; OMIM, Online Mendelian Inheritance in Man; *dn, de novo* CNV observed in neither of the parents.
[a] The name of disorder is based on entry names of OMIM, expect for entry names in DECIPHER and description in each cited article.
[b] *pat*, father had a balanced translocation involved in corresponding subtelomeric regions.
[c] Entry names in DECIPHER.
[d] Description in each cited article.

All the CNVs detected in the first screening were confirmed by FISH. Among the positive cases, in 24 cases one CNV was detected. All the CNVs corresponded to well-established syndromes or already described disorders (Table 1). In 16 cases two CNVs, one deletion and one duplication, were detected at two subtelomeric regions, indicating that one of parents might be a carrier with reciprocal translocation involved in corresponding subtelomeric regions, and at least either of the two CNVs corresponded to the disorders. We also performed parental analysis by FISH for three cases whose parental samples were available, and confirmed that in two cases the subtelomeric aberrations were inherited from paternal balanced translocation and in one case the subtelomeric aberrations were *de novo* (Table 1). In the other 14 cases, CNVs (25.9%) were detected in regions corresponding to known disorders (Table 2).

CNVs detected in the second screening and assessment of the CNVs
Cases were subject to the second screening in the order of subjects detected no CNV in the first screening, and until now we have analyzed 349 of 482 negative cases in the first screening. In advance, we excluded highly frequent CNVs observed in healthy individuals and/or in multiple patients showing disparate phenotypes from the present results based on an internal database, which contained all results of aCGH analysis we have performed using WGA-4500, or other available online databases; for example, Database of Genomic Variant (http://projects.tcag.ca/variation/). As a result, we detected 66 CNVs in 63 cases (Figure 1; Table 3). Among them, three patients (cases 36, 42 and 44) showed two CNVs. All the CNVs detected in the second screening were confirmed by other cytogenetic methods including FISH and/or X-array. For 60 cases, we performed FISH for confirmation and to determine the size of each CNV. For five cases, cases 13, 36, 48, 57 and 63, with CNVs on the X chromosome, we used the X-array instead of FISH. For cases 4, 6, 16–19 and 34, we also used Agilent Human Genome CGH Microarray 244K to determine the refined sizes of CNVs. The maximum and minimum sizes of each CNV determined by these analyses are described in Table 3.

Well-documented pCNVs emerged in the second screening
CNVs identified for recently established syndromes. We assessed the pathogenicity of the detected CNVs in several aspects (Figure 2).[21,37,38] First, in nine cases, we identified well-documented pCNVs, which are responsible for syndromes recently established. A heterozygous deletion at 1q41–q42.11 in case 2 was identical to patients in the first report of 1q41q42 microdeletion syndrome.[39] Likewise a CNV in case 3 was identical to chromosome 1q43–q44 deletion syndrome (OMIM: #612337),[40] a CNV in case 4 was identical to 2q23.1 microdeletion syndrome,[41] a CNV in case 5 was identical to 14q12 microdeletion syndrome[42] and a CNV in case 6 was identical to chromosome 15q26-qter deletion syndrome (Drayer's syndrome) (OMIM: #612626).[43] Cases 7, 8 and 9 involved CNVs of different sizes at 16p12.1-p11.2, the region responsible for 16p11.2-p12.2 microdeletion syndrome.[44,45] Although an interstitial deletion at 1p36.23-p36.22 observed in case 1 partially overlapped with a causative region of chromosome 1p36 deletion syndrome (OMIM: #607872), the region deleted was identical to a proximal interstitial 1p36 deletion that was recently reported.[46] Because patients with the proximal 1p36 deletion including case 1 demonstrated different clinical characteristics from cases of typical chromosome 1p36 deletion syndrome, in the near term their clinical features should be redefined as an independent syndrome.[46]

CNVs containing pathogenic gene(s). In four cases we identified pCNVs that contained a gene(s) probably responsible for phenotypes. In case 10, the CNV had a deletion harboring *GLI3* (OMIM: *165240) accounting for Greig cephalopolysyndactyly syndrome (GCS; OMIM: 175700).[47] Although phenotypes of the patient, for example, pre-axial polydactyly of the hands and feet, were consistent with GCS, his severe and atypical features of GCS, for example, MR or microcephaly, might be affected by other contiguous genes contained in the deletion.[48] Heterozygous deletions of *BMP4* (OMIM: *112262) in case 11 and *CASK* (OMIM: *300172) in case 13 have been reported previously.[49,50] In case 12, the CNV contained *YWHAE* (OMIM: *605066) whose haploinsufficiency would be involved in MR and mild CNS dysmorphology of the patient because a previous report demonstrated that haploinsufficiency of *ywhae* caused a defect of neuronal migration in mice[51] and a recent report also described a microdeletion of *YWHAE* in a patient with brain malformation.[52]

Table 2 Other cases among 54 positive cases in the first screening

	Position where CNV detected			
Gender	*Gain*	*Loss*	*Corresponding disorder*	*OMIM*
F		4p16.3 4q35.2	Ring chromosome	
M		3q22.323	BPES	#110100
M		2q22.3	ZFHX1B region	*605802
M		4q22.1	Synuclein (SNCA) region	*163890
F		7p21.1	Craniosynostosis, type 1	#123100
F		7q11.23	Williams syndrome	#194050
F		8q23.3q24.11	Langer–Giedion syndrome	#150230
M	15q11.2q13.1		Prader–Willi/Angelman	#176270/ #105830
F		17p11.2	Smith–Magenis syndrome	#182290
M		17q11.2	Neurofibromatosis, type I	+162200
M	22q11.21		DiGeorge syndrome	#188400
F		22q11.21	DiGeorge syndrome	#188400
F	Xp22.31		Kallmann syndrome 1	+308700
F	Whole X		Mosaicism	

Abbreviations: CNV, copy-number variant; F, female; M, male; OMIM, Online Mendelian Inheritance in Man.

Recurrent CNVs in the same regions. We also considered recurrent CNVs in the same region as pathogenic; three pairs of patients had overlapping CNVs, which have never been reported previously. Case 16 had a 3.3-Mb heterozygous deletion at 10q24.31–q25.1 and case 17 had a 2.0-Mb deletion at 10q24.32–q25.1. The clinical and genetic information will be reported elsewhere. Likewise, cases 14 and 15 also had an overlapping CNV at 6q12–q14.1 and 6q14.1, and cases 18 and 19 had an overlapping CNV at 10p12.1–p11.23. Hereafter, more additional cases with the recurrent CNV would assist in defining new syndromes.

CNVs reported as pathogenic in previous studies. Five cases were applicable to these criteria. A deletion at 3p21.2 in case 20 overlapped with that in one case recently reported.[53] The following four cases had CNVs reported as pathogenic in recent studies: a CNV at 7p22.1 in case 21 overlapped with that of patient 6545 in a study by Friedman *et al.*,[14] a CNV at 14q11.2 in case 22 overlapped with those of patients 8326 and 5566 in Friedman *et al.*,[14] a CNV at 17q24.1–q24.2 in case 23 overlapped with that in patient 99 in Buysse *et al.*[54] and a CNV at 19p13.2 in case 24 overlapped with case P11 in Fan *et al.*[55]

Large or gene-rich CNVs, or CNVs containing morbid OMIM genes. In cases inapplicable to the above criteria, we assessed CNVs

Table 3 Sixty-three cases with CNV in the 2nd screening

								Base position and size of the identified CNV[a]									
Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)	Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
1	M	MCA/MR		del	1p36.23p36.22	arr cgh 1p36.23p36.22 (RP11-81J7→ RP11-19901)x1	ish del(1)(p36.23p36.22) (RP11-462M3+, RP11-106A3-, RP11-28P4+)dn	8 585 127	8 890 860	10 561 097	11 143 717	1 670 237	2 558 590	*dn*	32	P	
2	M	MCA/MR		del	1q41q42.11	arr cgh 1q41 (RP11-135J2→ RP11-239E10)x1	ish del(1)(q41q42.11) (RP11-706L9+, RP11-224019-, RP11-36704-)dn	215 986 492	216 532 600	221 534 398	222 467 931	5 001 798	6 481 439	*dn*	35	P	
3	F	MCA/MR	Epilepsy	del	1q44	arr cgh 1q44 (RP11-156E8)x1	ish del(1)(q44) (RP11-56019+, RP11-156E8-)	241 996 973	243 177 632	243 251 660	244 141 010	74 028	2 144 037		11	P	
4	F	MCA/MR		del	2q22	arr cgh 2q23.1 (RP11-72H23)x1	ish del(2)(q23.1) (RP11-375H16-)	147 651 472	147 688 255	149 855 826	149 879 891	2 167 571	2 228 419		7	P	
5	F	MCA/MR		del	14q12q13.2	arr cgh 14q12q13.2 (RP11-36909→ RP11-26M6)x1	ish del(14)(q13.2) (RP11-831F6-)	28 768 137	29 297 829	34 689 412	35 489 337	5 391 583	6 721 200		25	P	
6	M	MCA/MR	CHD	del	15q26.2	arr cgh 15q26.2q26.3 (RP11-79C10→ RP11-80F4)x1	ish del(15)(q26.2) (RP11-308P12-)	93 199 415	93 214 053	96 928 421	96 942 334	3 714 368	3 742 919		6	P	
7	M	MCA/MR	CHD	del	16p12.1p11.2	arr cgh 16p12.1p11.2 (RP11-309I14→ RP11-150K5)x1	ish del(16)(p11.2) (RP11-75J11-)dn	25 795 340	27 008 538	29 825 404	31 443 492	2 816 866	5 648 152	*dn*	138	P	
8	M	MCA/MR	CHD	del	16p11.2	arr cgh 16p12.1p11.2 (RP11-360L15→ RP11-150K5)x1	ish del(16)(p11.2) (RP11-360L15-, RP11-388M20+, RP11-75J11+)dn	27 184 508	28 873 631	29 825 404	31 443 492	951 773	4 258 984	*dn*	134	P	
9	F	MCA/MR		del	16p11.2	arr cgh 16p11.2 (RP11-368N21→ RP11-499D5)x1	ish del(16)(p11.2) (RP11-388M20-, RP11-75J11-)	28 873 841	29 408 698	32 773 200	34 476 095	3 364 502	5 602 254		125	P	
10	M	MCA/MR		del	7p14.2p13	arr cgh 7p14.2p13 (RP11-138E20→ RP11-52M17)x1	ish del(7)(p14.1p13) (RP11-258I11+, RP11-2J17-, RP11-346F12-)dn	35 621 006	36 470 190	44 657 334	45 508 196	8 187 144	9 887 190	*dn*	70	P	*GLI3*
11	F	MCA/MR	Corneal opacity	del	14q22.1q22.3	arr cgh 14q22.1q22.3 (RP11-122A4→ RP11-172G1)x1	ish del(14)(q22.1) (RP11-122A4-, RP11-316L15+)dn	51 964 774	51 983 834	54 730 496	55 054 754	2 746 662	3 089 980	*dn*	18	P	*BMP4*
12	M	MCA/MR	Idiopathic leukodystrophy	del	17q13.3	arr cgh 17p13.3 (RP11-294J5→ RP11-35707)x1	ish del(17)(p13.3) (RP11-4F24-, RP11-26N6+)dn	1 008 128	1 146 211	2 077 151	2 026 967	930 940	1 018 839	*dn*	22	P	*YWHAE*
13	M	MCA/MR		del	Xp11.4p11.3	arr cgh Xp11.3p11.4 (RP11-1069J5→ RP11-245M24)x1	ish del(X)(p11.4p11.3) (RP11-95C16-, RP11-829C10-)dn	41 392 291	41 385 453	45 419 624	45 495 709	4 034 171	4 103 418	*dn*	9	P	*CASK*

Table 3 Continued

								Base position and size of the identified CNV^{a}									
Case	*Gender*	*Clinical diagnosis*	*Remarkable clinical features*	*CNV*	*Position*	*WGA-4500*^{b}	*FISH*^{b}	*Start (max)*	*Start (min)*	*End (min)*	*End (max)*	*Size (min)*	*Size (max)*	*Parental analysis*	*Protein-coding genes*^{c}	*CNV assessment*^{d}	*Corresponding or candidate gene(s)*
14	M	MCA/MR		del	6q12q14.1	arr cgh 6q12q14.2(RP11-502L6→ RP11-232L4)x1	ish del(6)(q13) (RP11-28P18-)dn	69 029 871	69 731 888	83 926 178	85 101 718	14 194 290	16 071 847	*dn*	56	P	
15	M	ZLS		del	6q14.1	arr cgh 6q14.1 (RP11-343P23→ RP11-217L13)x1	ish del(6)(q14.1) (RP11-5N7-,RP11-990K4-,RP11-1I6+)	75 484 004	76 145 436	79 474 428	79 851 528	3 328 992	4 367 524		10	P	
16	F	MCA/MR	CHD	del	10p12.1p11.23	arr cgh 10p12.1p11.23 (RP11-89D1→ 91A23)x1	ish del(10) (p12.1p11.23) (RP11-164A7-, RP11-110B21-)	27 045 285	27 054 002	29 057 401	29 088 950	2 003 399	2 043 665		18	P	
17	M	MCA/MR		del	10p12.1p11.23	arr cgh 10p12.1p11.23 (RP11-218D6→ RP11-RP11-181I11)x1	ish del(10)(p11.23) (RP11-15H10-)	28 121 596	28 131 608	30 559 024	30 577 807	2 427 416	2 456 211		12	P	
18	M	MCA/MR	CHD	del	10q24.31q25.1	arr cgh 10q24.31q25.1 (RP11-108L7→ RP11-108L7)x1	ish del(10)(q24.33) (RP11-416N2-)dn	102 560 783	102 568 462	105 914 057	105 929 608	3 345 595	3 368 825	*dn*	66	P	
19	M	MCA/MR		del	10q24.32q25.1	arr cgh 10q24.32q25.1 (RP11-21N23→ RP11-99N20)x1	ish del(10)(q24.33) (RP11-416N2-)dn	103 917 900	103 928 189	106 005 827	106 011 522	2 077 638	2 093 622	*dn*	41	P	
20	F	MCA/MR		del	3p21.31p21.2	arr cgh 3p21.31p21.2 (RP11-24F11→ RP11-89F17)x1	ish del(3)(p21.31) (RP11-3B7-)	46 150 261	46 359 965	51 390 597	52 571 544	5 030 632	6 421 283		175	P	
21	M	MCA/MR		del	7p22.1	arr cgh 7p22.1 (RP11-90J23→ RP11-2K20)x1	ish del(7)(p22.1) (RP11-2K20-)dn	3 185 609	5 892 225	6 233 987	6 409 277	341 762	3 223 668	*dn*	28	P	
22	F	MCA/MR	Corneal opacity, CHD	dup	14q11.2	arr cgh 14q11.2 (RP11-152G22→ RP11-84D12)x3	ish dup(14)(q11.2) (RP11-152G22++)	20 070 731	20 306 624	20 534 929	21 264 945	228 305	1 194 214		>30	P	
23	M	MCA/MR		del	17q24.1q24.2	arr cgh 17q24.1q24.2 (RP11-89L7→ RP11-79K13)x1	ish del(17) (q24.1q24.2) (RP11-93E5-, RP11-89L7-, RP11-79K13-)	60 576 365	60 936 391	64 592 701	64 587 782	3 656 310	4 011 417		29	P	
24	M	SMS susp.		del	19p13.2	arr cgh 19p13.2 (RP11-19704→ RP11-164D24)x1	ish del(19)(p13.2) (91021-)	9 248 377	10 248 853	11 968 772	12 553 279	1 719 919	3 304 902	*dn*		P	
25	M	MCA/MR	Epilepsy	dup	2q11.2q13	arr cgh 2q11.2q13(RP11-90G13→ RP11-79K7)x3	ish dup(2)(q11.2) (RP11-542D13++)	88 273 220	91 696 986	109 869 691	112 714 666	18 172 705	24 441 446		>30	P	
26	M	MCA/MR	CHD	dup	4p16.1	arr cgh 4p16.1 (RP11-1719)x3	ish dup(4)(p16.1) (RP11-301J10++)	8 202 790	8 520 479	9 793 705	10 638 054	1 273 226	2 435 264		17	P	

Table 3 Continued

							Base position and size of the identified CNV[a]							Protein-	CNV	Corresponding
Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV Position	WGA-4500[b]	FISH[b]	Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)	Parental analysis	coding genes[c]	assessment[d]	or candidate gene(s)
27	F	MCA/MR		del 7q22.1q22.2	arr cgh 7q22.1q22.2 (RP11-10D8→ RP11-72J24)x1	ish del(7)(q22.1q22.2) (RP11-124G15+,RP11-188E1-,RP11-95P19-)	97 314 215	98 261 079	105 604 920	106 451 506	7 343 841	9 137 291		135	P	
28	F	MCA/MR	Epilepsy	del 12q13.13	arr cgh 12q13.13 (RP11-74I8→ RP11-624J6)x1	ish del(12)(q13.13) (RP11-624J6-)	50 987 232	51 016 427	51 956 291	52 180 088	939 864	1 192 856		44	P	
29	M	MCA/MR		dup 16q22.3	arr cgh 16q22.3 (RP11-90L19→ RP11-89K4)x3	ish dup(16)(q22.3) (RP11-115E3++, RP11-90L19++)	70 355 260	70 848 592	72 328 913	73 785 124	1 480 321	3 429 864		25	P	
30	M	RTS susp.		dup 16q24.1	arr cgh 16q24.1 (RP11-140K16→ RP11-442O1)x3	ish dup(16)(q24.1) (RP11-770B4++, RP11-140K16++)	82 699 729	82 797 548	83 749 375	84 123 857	951 827	1 424 128		16	P	
31	M	MCA/MR	Epilepsy	del 2q24.2q24.3	arr cgh 2q24.2 (RP11-89L13→ RP11-79L13)x1	ish del(2)(q24.2) (RP11-638N12-)	160 407 234	161 072 815	162 883 584	166 923 475	1 810 769	6 516 241		28	P	*TBR1*
32	M	MCA/MR		del 3p26.2	arr cgh 3p26.2 (RP11-32F23)x1	ish del(3)(p26.2) (RP11-32F23-)	3 943 353	4 016 797	4 198 468	4 329 970	181 671	386 617		2	P	*SUMF1*
33	M	MCA/MR	IgA deficiency	del 7q21.11	arr cgh 7q21.11 (RP11-22M18)x1	ish del(7)(q21.11) (RP11-115M2+, RP11-353O4-, RP11-22M18-)	83 597 839	83 601 541	84 549 609	84 788 160	948 068	1 190 321		3	P	*SEMA3A*
34	M	MCA/MR		dup 14q32.2	arr cgh 14q32.2 (RP11-128L1)x3	ish dup(14)(q32.2) (RP11-177F8++)	99 330 486	99 337 358	99 841 558	99 845 472	504 200	514 986		7	P	*EML1, YY1*
35	M	MCA/MR	Epilepsy	dup 16p13.3	arr cgh 16p13.3 (RP11-349I11)x3	ish dup(16)(p13.3) (RP11-349I11++)	4 851 459	5 678 447	5 906 909	6 165 923	228 462	1 314 464		9	P	*A2BP1*
36	M	MCA/MR		dup Xp22.2p22.13	arr cgh Xp22.2p22.13 (RP11-2K15→ RP11-115I10)x3	not performed (X-tiling array)	16 874 735	16 952 121	17 596 600	17 638 351	644 479	763 616		2	P	
				dup Xp21.3	arr cgh Xp21.3 (RP11-438J7)x3	not performed (X-tiling array)	28 704 076	28 704 076	28 868 075	28 868 075	163 999	163 999		1	P	*IL1RAPL1*
37	F	MCA/MR		del 1p34.3	arr cgh 1p34.3 (RP11-89N10→ RP11-416A14)x1	ish del(1)(p34.2) (RP11-195A8+, RP11-166F21-)dn	37 830 131	38 338 265	39 466 349	39 583 645	1 128 084	1 753 514	*dn*	7	P	
38	M	MCA/MR	Hyper IgE	dup 1q25.2	arr cgh 1q25.2 (RP11-177A2→ RP11-152A16)x3	ish dup(1)(1q25.2) (RP11-177A2++, RP11-152A16++)	177 088 480	177 196 858	177 535 659	177 859 828	338 801	771 348	*dn*	9	P	
39	M	MCA/MR		del 2p24.1p23.3	arr cgh 2p24.1p23.3 (RP11-80H16→ RP11-88F6)x1	ish del(2)(p23.3) (RP11-88F6-, RP11-373D23+)dn	20 037 821	23 094 244	26 815 794	28 414 457	3 721 550	8 376 636	*dn*	86	P	
40	F	MCA/MR	CHD	del 3p26.1p25.3	arr cgh 3p26.1p25.3 (RP11-128A5→ RP11-402P11)x1	ish del(3)(p26.1p25.3) (RP11-936E1-, RP11-402P11-, RP11-1079H21+) dn	8 190 557	8 497 949	9 930 973	10 026 217	1 433 024	1 835 660	*dn*	18	P	

Table 3 Continued

							Base position and size of the identified CNV[a]									
Case	*Gender*	*Clinical diagnosis*	*Remarkable clinical features*	*CNV Position*	*WGA-4500*[b]	*FISH*[b]	*Start (max)*	*Start (min)*	*End (min)*	*End (max)*	*Size (min)*	*Size (max)*	*Parental analysis*	*Protein-coding genes*[c]	*CNV assess-ment*[d]	*Corresponding or candidate gene(s)*
41	M	MCA/MR		del 3p22.1p21.31	arr cgh 3p22.1p21.31 (RP11-241P3→ RP11-88B8)x1	ish del(3)(p22.1) (RP11-61H16+, RP11-241P3-, RP11-78O10+)dn	41 365 663	42 284 365	48 177 538	49 198 542	5 893 173	7 832 879	*dn*	123	P	
42	M	MCA/MR	Corneal opacity	del 3p14.3p14.2	arr cgh 3p14.3p14.2 (RP11-80H18→ RP11-79J9)x1	ish del(3)(p14.2) (RP11-79J19-, RP11-230A22+)mat	57 370 434	58 149 199	58 742 633	58 887 574	593 434	1 517 140	*mat*	11	B	
				del 8q21.11q21.13	arr cgh 8q21.11q21.13 (RP11-225J6→ RP11-214E11)x1	ish del(8) (q21.11q21.13) (RP11-225J6-, RP11-48B3+)dn	75 722 961	75 821 163	81 110 557	81 493 446	5 289 394	5 770 485	*dn*	12	P	
43	M	MCA/MR		del 3q26.31q26.33	arr cgh 3q26.31-q26.33 (RP11-292L5→ RP11-355N16)x1	ish del(3)(q26.32) (RP11-300L9+, RP11-105L6-)dn	175 650 310	176 531 688	180 613 203	181 653 281	4 081 515	6 002 971	*dn*	12	P	
44	M	MCA/MR	CHD	del 13q13.2q13.3	arr cgh 13q13.2 (RP11-269G10→ 90F5)x1	ish del(13)(q13.2) (RP11-142E9+, RP11-381E21-, RP11-98D3+)dn	33 451 136	33 895 560	34 813 379	34 909 905	917 819	1 458 769	*dn*	1	P	
				del 22q11.21	arr cgh 22q11.21 (RP11-155F20→ 54C2)x1	ish del(22)(q11.21) (RP11-155F20-, RP11-590C5-, RP11-54C2-)pat	19 310 307	19 310 307	19 590 642	19 590 642	280 335	280 335	*pat*	15	B	
45	F	aRS		del 18q21.2	arr cgh 18q21.2 (RP11-89B14)x1	ish del(18)(q21.2) (RP11-159D14+, RP11-186B13-, RP11-111C17-)dn	48 218 621	49 166 752	51 288 665	51 861 143	2 121 913	3 642 522	*dn*	9	P	
46	M	MCA/MR		dup 19p13.3	arr cgh 19p13.3 (RP11-49M3→ RP11-268O21)x3		1 095 485	2 418 857	3 499 581	4 460 252	1 080 724	3 364 767	*dn*	113	P	
47	F	MCA/MR	Autism	del 19p13.3	arr cgh 19p13.3 (RP11-30F17→ RP11-330I7)x1	ish del(19)(p13.3) (RP11-330I7-)dn	4 844 383	6 043 505	6 859 584	6 881 792	816 079	2 037 409	*dn*	23	P	
48	M	MCA/MR		del Xp11.3	arr cgh Xp11.3 (RP11-151G3→ RP11-48J14)x0	ish del(X)(p11.3) (RP11-203D16-)mat	44 403 077	44 433 162	46 795 584	46 795 588	2 362 422	2 392 511	*mat*	18	P	
49	M	MCA/MR		dup 3p26.3	arr cgh 3p26.3 (RP11-63O1)x3	ish dup(3)(p26.3) (RP11-63O1++)pat	2 377 366	2 443 357	2 619 407	2 628 216	176 050	250 850	*pat*	1	B	
50	M	MCA/MR		dup 5p14.3	arr cgh 5p14.3 (RP11-91A5)x3	ish dup(5)(p14.3) (RP11-91A5++)pat	19 046 234	19 485 530	19 656 108	20 798 445	170 578	1 752 211	*pat*	1	B	
51	M	MCA/MR		dup 5q13.3	arr cgh 5q13.1 (RP11-40N8→ RP11-91C10)x3	ish dup(5)(q13.1) (RP11-105A11++)mat	66 417 271	66 481 371	67 501 700	67 838 977	1 020 329	1 421 706	*mat*	3	B	

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV Position	WGA-4500^b	FISH^b	Base position and size of the identified CNV^a						Parental analysis	Protein-coding genes^c	CNV assess-ment^d	Corresponding or candidate gene(s)
							Start (max)	*Start (min)*	*End (min)*	*End (max)*	*Size (min)*	*Size (max)*				
52	M	MCA/MR		dup 7p22.3	arr cgh 7p22.3 (RP11-23D23)x3	ish dup(7)(p22.3) (RP11-23D23++, RP11-1133D5+)mat	1	954 016	954 584	1 101 944	568	1 101 943	*mat*	12	B	
53	F	MCA/MR		dup 8p23.2	arr cgh 8p23.2 (RP11-79I19→ RP11-89I12)x3	ish dup(8)(p23.2) (RP11-89I19++, RP11-89I12++)pat	3 324 954	3 726 061	4 564 671	5 973 493	838 610	2 648 539	*pat*	1	B	
54	M	MCA/MR		dup 9q33.1	arr cgh 9q33.1 (RP11-150L1)x3	ish dup(9)(q33.1) (RP11-150L1++)pat	118 980 752	119 452 372	119 614 984	120 011 559	162 612	1 030 807	*pat*	2	B	
55	F	MCA/MR		dup 10q22.3	arr cgh 10q22.3 (RP11-79M9)x3	ish dup(10)(q22.3) (RP11-79M9++)mat	77 356 915	77 718 484	77 873 148	78 230 039	154 664	873 124	*mat*	1	B	
56	M	MCA/MR	ELBW, hepato-blastoma	dup 12q21.31	arr cgh 12q21.31 (RP11-91C4)x3	ish dup(12)(q21.31) (RP11-91C4++, RP11-142L2+)pat	80 924 954	82 678 148	82 830 190	85 768 388	152 042	4 843 434	*pat*	3	B	
57	M	GS		del Xp11.23	arr cgh Xp11.23 (RP11-876B24) x0 mat	not performed (X-tiling array)	47 752 808	47 747 918	47 852 109	47 868 412	104 191	115 604	*mat*	3	B	
58	M	MCA/MR		dup 8q11.23	arr cgh 8q11.23 (RP11-221P7)x3	ish dup(8)(q11.23) (RP11-221P7++, RP11-26P22++)	53 665 974	53 717 675	54 235 229	54 576 654	517 554	910 680		3	VOUS	
59	F	MCA/MR	Micro-cephaly	dup 10q11.21	arr cgh 10q11.21 (RP11-178A10)x3	ish dup(10)(q11.21) (RP11-178A10++)	41 986 946	42 197 693	42 320 775	43 603 027	123 082	1 616 081		15	VOUS	
60	M	MCA/MR		dup 11p14.2p14.1	arr cgh 11p14.2p14.1 (RP11-1L12)x3	ish dup(11) (p14.2p14.1) (RP11-1L12++)	26 723 462	27 033 270	27 213 374	27 445 504	180 104	722 042		4	VOUS	
61	F	MCA/MR		dup 12p11.1	arr cgh 12p11.1 (RP11-88P4)x3	ish dup(12)(p11.1) (RP11-472A10++)	33 333 493	33 359 944	33 572 956	33 572 956	213 012	239 463		2	VOUS	
62	F	aRS		dup 12q21.31	arr cgh 12q21.31 (RP11-91I24→ RP11-91C4)x3	ish dup(12)(q21.31) (RP11-91C4++, RP11-142L2++)	79 949 648	82 172 368	83 968 319	85 768 388	1 795 951	5 818 740		12	VOUS	
63	F	MR	Congenital myopathy	dup Xq12	arr cgh Xq12 (RP11-90P17→ RP11-383C12)x3	Not performed (X-tiling array)	66 212 661	66 216 353	66 921 699	66 948 538	705 346	735 877		1	VOUS	

Abbreviations: aRS, atyplical Rett syndrome; B, benign; CNV, copy-number variant; *dn*: *de novo* CNV observed in neither of the parents; ELBW, extremely low birth weight; FISH, fluorescence *in situ* hybridization; GS, Gillespie syndrome; *mat*: CNV identified also in mother; P, pathogenic; *pat*: CNV identified also in father; RTS, Rubinstein–Taybi syndrome; SMS, Smith–Magenis syndrome; VOUS, variant of uncertain clinical significance; ZLS, Zimmermann–Laband syndrome.

[a] The sizes were estimated by WGA-4500, X-array, FISH or Agilent Human Genome CGH microarray 244K.

[b] The notation systems is based on ISCN2005.[36]

[c] The number of protein-coding genes contained in the respective CNVs.

[d] The result of CNV assessment.

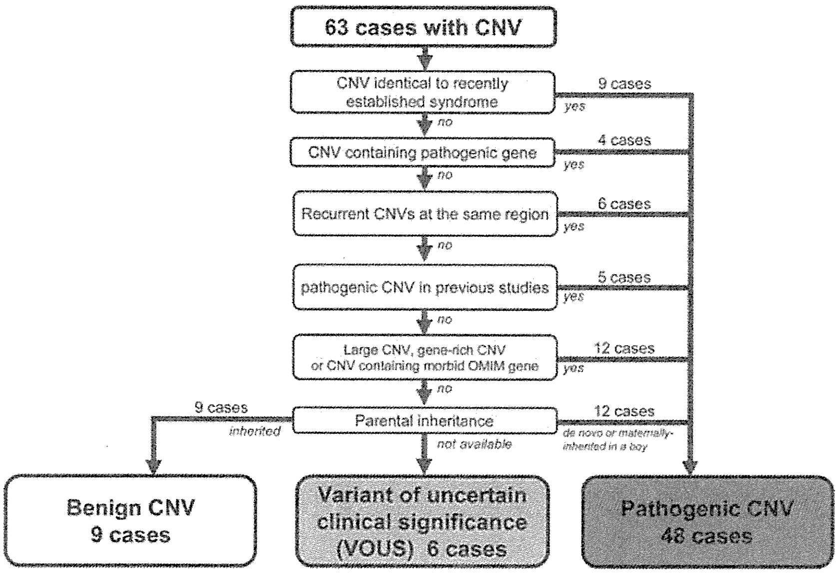

Figure 2 A flowchart of the assessment of CNVs detected in the second screening.

from several aspects. A CNV that contains abundant genes or is large (>3 Mb) has a high possibility to be pathogenic.[21] The CNVs in cases 25–30 probably correspond to such CNVs. Also, we judged a CNV containing a morbid OMIM gene as pathogenic:[21] *TBR1* (OMIM: *604616) in case 31,[56] *SUMF1* (OMIM: *607939) in case 32,[57,58] *SEMA3A* (OMIM: *603961) in case 33,[59] *EML1* (OMIM: *602033) and/or *YY1* (OMIM: *600013) in case 34,[60,61] *A2BP1* (OMIM: *605104) in case 35[62] and *IL1RAPL1* (OMIM: *300206) in case 36.[63] Several previous reports suggest that these genes are likely to be pathogenic, although at present no evidence of a direct association between these genes and phenotypes exists.

CNVs de novo or X maternally inherited. Among the remaining 27 cases, 12 cases had CNVs considered pathogenic as their CNVs were *de novo* (cases 37–47) or inherited del(X)(p11.3) from the mother (case 48). In the second screening we performed FISH for 36 CNVs of the 34 cases whose parental samples were available to confirm that 24 cases had *de novo* CNVs, which were probably pathogenic. A CNV in case 48, a boy with a nullizygous deletion at Xp11.3 inherited from his mother, was also probably relevant to his phenotype (Tables 3 and 4). Meanwhile, although case 57 was a boy with a deletion at Xp11.23 inherited from his mother, he was clinically diagnosed with Gillespie syndrome (OMIM: #206700) that was reported to show an autosomal dominant or recessive pattern,[64] thus we judged that the deletion was not relevant to his phenotype. As a result, cases 49–57 had only CNVs inherited from one of their parents which are likely to be unrelated to the phenotypes; that is, bCNV (Table 4).

As a result, we estimated that 48 cases among 349 analyzed (13.8%) had pCNV(s) in the second screening (Table 3; Figure 2). The CNVs of the remaining six cases, cases 58–63, were not associated with previously reported pathogenicity and their inheritance could not be evaluated, thus we estimated they were variants of uncertain clinical significance (VOUS).[38]

DISCUSSION

Because aCGH is a high-throughput technique to detect CNVs rapidly and comprehensively, this technique has been commonly used for analyses of patients with MCA and/or MR.[38,65–68] However, recent studies of human genomic variation have uncovered surprising properties of CNV, which covers 3.5–12% of the human genome even in healthy populations.[18–20,69] Thus analyses of patients with uncertain clinical phenotypes need to assess whether the CNV is pathogenic or unrelated to phenotypes.[21] However, such an assessment may diminish the rapidness or convenience of aCGH.

In this study, we evaluated whether our in-house GDA can work well as a diagnostic tool to detect CNVs responsible for well-established syndromes or those involved in subtelomeric aberrations in a clinical setting, and then explored candidate pCNVs in cases without any CNV in the first GDA screening. We recruited 536 cases that had been undiagnosed clinically and studied them in a two-stage screening using aCGH. In the first screening we detected CNVs in 54 cases (10.1%). Among them, 40 cases had CNV(s) at subtelomeric region(s) corresponding to the well-established syndromes or the already described disorders and the other 14 cases had CNVs in the regions corresponding to known disorders. Thus about three quarters of cases had genomic aberrations involved in subtelomeric regions. All the subtelomeric deletions and a part of the subtelomeric duplications corresponded to the disorders, indicating that especially subtelomeric deletions had more clinical significance compared to subtelomeric duplications, although the duplication might result in milder phenotypes and/or function as a modifier of phenotypes.[70] Moreover, parental analysis in three cases with two subtelomeric aberrations revealed that two of them were derived from the parental balanced translocations, indicating that such subtelomeric aberrations were potentially recurrent and parental analyses were worth performing. Recently several similar studies analyzed patients with MCA/MR or developmental delay using a targeted array for subtelomeric regions and/or known genomic disorders and detected clinically relevant CNVs in 4.4–17.1% of the patients.[28,65,70,71] Our detection rate in the first screening was equivalent to these reports. Although such detection rates depend on the type of microarray, patient selection criteria and/or number of subjects, these results suggest that at least 10% of cases with undiagnosed MCA/MR and a normal karyotype would be detectable by targeted array.

Table 4 Parental analysis of 34 cases in the second screening

			CNV		*Size of CNV (bp)*				
Case	*Gender*	*Clinical diagnosis*	*del/dup*	*Position*	*Min.*	*Max.*	*Protein-coding genes*	*Parental analysis*	*Pathogenicity*
1	M	MCA/MR	del	1p36.23p36.22	1 670 237	2 558 590	32	*de novo*	P
2	M	MCA/MR	del	1q41q42.11	5 001 798	6 481 439	35	*de novo*	P
7	M	MCA/MR	del	16p12.1p11.2	2 816 866	5 648 152	138	*de novo*	P
8	M	MCA/MR with CHD	del	16p11.2	951 773	4 258 984	134	*de novo*	P
10	M	MCA/MR	del	7p14.2p13	8 516 513	9 421 233	70	*de novo*	P
11	F	MCA/MR	del	14q22.1q22.3	2 746 662	3 089 980	18	*de novo*	P
12	M	MCA/MR	del	17q13.3	930 940	1 018 839	22	*de novo*	P
13	M	MCA/MR	del	Xp11.4p11.3	4 034 171	4 103 418	9	*de novo*	P
14	M	MCA/MR	del	6q12q14.1	14 194 290	16 071 847	56	*de novo*	P
18	M	MCA/MR	del	10q24.31q25.1	3 345 595	3 368 825	66	*de novo*	P
19	M	MCA/MR	del	10q24.32q25.1	2 077 638	2 093 622	41	*de novo*	P
21	M	MCA/MR	del	7p22.1	341 762	3 223 668	28	*de novo*	P
24	M	SMS susp.	del	19p13.2	1 719 919	3 304 902	23	*de novo*	P
37	F	MCA/MR	del	1p34.3	1 128 084	1 753 514	7	*de novo*	P
38	M	MCA/MR	dup	1q25.2	338 801	771 348	9	*de novo*	P
39	M	MCA/MR	del	2p24.1p23.3	3 721 550	8 376 636	86	*de novo*	P
40	F	MCA/MR	del	3p26.1p25.3	1 433 024	1 835 660	18	*de novo*	P
41	M	MCA/MR	del	3p22.1p21.31	5 893 173	7 832 879	123	*de novo*	P
42[a]	M	MCA/MR	del	8q21.11q21.13	5 289 394	5 770 485	12	*de novo*	P
42[a]	M	MCA/MR	del	3p14.3p14.2	593 434	1 517 140	11	Maternal	B
43	M	MCA/MR	del	3q26.31q26.33	4 081 515	6 002 971	12	*de novo*	P
44[b]	M	MCA/MR	del	13q13.2q13.3	917 819	1 458 769	1	*de novo*	P
44[b]	M	MCA/MR	del	22q11.21	917 819	1 458 769	15	Paternal	B
45	F	Rett syndrome	del	18q21.2	2 121 913	3 642 522	9	*de novo*	P
46	M	MCA/MR	dup	19p13.3	2 041 395	2 404 096	113	*de novo*	P
47	F	MCA/MR	del	19p13.3	816 079	2 037 409	23	*de novo*	P
48[c]	M	MCA/MR	del	Xp11.3	2 362 422	2 392 511	18	Maternal	P
49	M	MCA/MR	dup	3p26.3	176 050	250 850	1	Paternal	B
50	M	MCA/MR	dup	5p14.3	170 578	1 752 211	1	Paternal	B
51	M	MCA/MR	dup	5q13.3	1 020 329	1 421 706	3	Maternal	B
52	M	MCA/MR	dup	7p22.3	568	1 101 943	12	Maternal	B
53	F	MCA/MR	dup	8p23.2	838 610	2 648 539	1	Paternal	B
54	M	MCA/MR	dup	9q33.1	162 612	1 030 807	2	Paternal	B
55	F	MCA/MR	dup	10q22.3	154 664	873 124	1	Maternal	B
56	M	MCA/MR	dup	12q21.31	152 042	4 843 434	3	Paternal	B
57	M	Gillespie syndrome	del	Xp11.23	104 191	115 604	3	Maternal	B

Abbreviations: B, benign; CNV, copy-number variant; F, female; M, male; MCA/MR, multiple congenital anomalies and mental retardation; P, pathogenic.
[a] Two CNVs were detected in case 42.
[b] Two CNVs were detected in case 44.
[c] Nullizygous deletion inherited from his mother probably affected the phenotype.

Another interesting observation in the first screening was that subtelomeric rearrangements frequently occurred even in patients with MCA/MR of uncertain whose karyotype had been diagnosed as normal. This result may be consistent with a property of subtelomeric regions whose rearrangements can be missed in conventional karyotyping,[72] and in fact other techniques involving subtelomeric FISH or MLPA also identified subtelomeric abnormalities in a number of patients with MCA and/or MR in previous reports.[70,73,74] Our result may support the availability of prompt screening of subtelomeric regions for cases with uncertain congenital disorders.

In the second screening we applied WGA-4500 to 349 cases to detect 66 candidate pCNVs in 63 cases (18.1%), and subsequently assessed the pathogenicity of these CNVs. The pCNVs included nine CNVs overlapping identical regions of recently recognized syndromes (cases 1–9; deletion at 1p36.23–p36.22, 1q41–q42.11, 1q43–q44, 2q23.1, 14q12, 15q26-qter and 16p11.2–p12.2, respectively), four CNVs containing disease-associated genes (cases 10–13; *GLI3*, *BMP4*, *YWHAE* and *CASK*, respectively), three pairs of CNVs of recurrent deletions (cases 14, 15: at 6q12–q14.1 and 6q14.1; case 16, 17: at 10p12.1–p11.23 and case 18, 19: at 10q24.31–q25.1 and 10q24.32–q25.1), five CNVs identical to pCNVs in previous studies (cases 20–24), six large and/or gene-rich CNVs (cases 25–30) and six CNVs containing a morbid OMIM gene (cases 31–36). For the remaining cases, we estimated the pathogenicity of the CNVs from a parental analysis (Table 4). We judged the 11 *de novo* CNVs (cases 37–47) and 1 CNV on chromosome Xp11.3 inherited from the mother (case 48) as probably pathogenic. And nine inherited CNVs (cases 49–57) were probably benign. The clinical significance of CNVs in the other six cases, cases 58–63, remains uncertain (VOUS). As a result we estimated CNVs as pathogenic in 48 cases among 349 cases (13.8%) analyzed in the second screening. None of the pCNVs corresponded to loci of well-established syndromes. This may suggest that our two-stage screening achieved a good balance between rapid screening of known syndromes and investigation of CNV of uncertain pathogenicity.

Among the cases with parental analyses, the 25 pCNVs had larger sizes and contained more protein-coding genes (average size, 3.1 Mb at minimum to 4.4 Mb at maximum; average number of genes, 44) as compared with the 11 inherited bCNVs that were probably unrelated to phenotypes (average size, 0.39 Mb at minimum to 1.5 Mb at maximum; average number of genes, 5) (Table 5). Although all of the 25 pCNVs except 2 were deletions, about three quarters (8 of 11 cases) of the inherited bCNVs were duplications (Table 5). These findings are consistent with previously reported features of pCNVs and bCNVs.[21,38]

We also compared our current study with recent aCGH studies meeting the following conditions: (1) a microarray targeted to whole genome was applied; (2) patients with MCA and/or MR of uncertain etiology, normal karyotype and the criteria for patients selection were clearly described; (3) pathogenicity of identified CNVs were assessed. On the basis of the above criteria, among studies reported in the past 5 years, we summarized 13 studies (Table 6).[10,14,15,17,54,55,75–81] Diagnostic yield of pCNVs in each study was 6.3–16.4%, and our current diagnostic yield of the second screening was 13.8%. Though cases with subtelomeric aberration detected in the first screening had been excluded, our diagnostic yield was comparable to those of the reported studies. It is not so important to make a simple comparison between diagnostic yields in different studies as they would depend on the conditions of each study, for example, sample size or array resolution,[38,82] however it seems interesting that the higher resolution of a microarray does not ensure an increase in the rate of detection of pCNVs. One recent study showed data that may explain the discrepancy between the resolution of microarray and diagnostic yield.[54,83] The authors analyzed 1001 patients with MCA and/or MR using one

Table 5 Summary of parental analyses

		Average size (bp)		The average number of protein-coding genes
		Min.	Max.	
Pathogenic CNVs[a]				
del	23	3 309 267	4 597 689	43
dup	2	1 190 098	1 587 722	61
Total	25	3 139 733	4 356 892	44
Benign CNVs[b]				
del	3	538 481	1 030 504	10
dup	8	334 432	1 740 327	3
Total	11	390 082	1 546 739	5

Abbreviation: CNV, copy-number variant.
[a]Twenty-four *de novo* CNVs and case 48.
[b]Eleven inherited CNVs other than case 48.

Table 6 Previous studies of analyzing patients with MCA and/or MR using aCGH targeted to whole genome

	Applied array			Patients		Pathogenic CNV	
Author (year)	Type	Number[a]	Distribution[b]	Number	Type of disorders	Number	%
Schoumans *et al.*[75]	BAC	2600	1.0 Mb*	41	MCA and MR	4	9.8
de Vries *et al.*[76]	BAC	32 477	Tiling	100	MCA and/or MR	10	10.0
Rosenberg *et al.*[77]	BAC	3500	1.0 Mb*	81	MCA and MR	13	16.0
Krepischi-Santos *et al.*[78]	BAC	3500	1.0 Mb*	95	MCA and/or MR	15	15.8
Friedman *et al.*[14]	SNP	Affymetrix 100K	23.6 kb**	100	MR	11	11.0
Thuresson *et al.*[79]	BAC		1.0 Mb*	48	MCA and MR	3	6.3
Wagenstaller *et al.*[80]	SNP	Affymetrix 100K	23.6 kb**	67	MR	11	16.4
Fan *et al.*[55]	Oligo	Agilent 44K	24 kb–43 kb**	100[c]	MCA and MR, Autism	15[d]	15.0
Xiang *et al.*[15]	Oligo	Agilent 44K	24 kb–43 kb**	40[e]	MR, DD and autism	3	7.5
Pickering *et al.*[10]	BAC	2600	1 Mb*	354[f]	MCA and/or MR	36[g]	10.2
McMullan *et al.*[17]	SNP	Affymetrix 500K	2.5 kb–5.8 kb**	120	MCA and/or MR	18	15.0
Bruno *et al.*[81]	SNP	Affymetrix 250K	2.5 kb–5.8 kb**	117	MCA and/or MR	18	15.4
Buysse *et al.*[54]	BAC	3431	1 Mb*	298	MCA and/or MR	26	8.7
	Oligo	Agilent 44K	24 kb–43 kb**	703	MCA and/or MR	74	10.5
Our current study	BAC	4523	0.7 Mb	349	MCA and MR	48	13.8
Total				2613		305	11.7

Abbreviations: BAC, bacterial artificial chromosome; CNV, copy-number variant; DD, developmental delay; MCA, multiple congenital anomalies; MR, mental retardation; SNP, single nucleotide polymorphism.
[a]The number of clones or name of array is described.
[b]Each distribution referred to each article (*) or manual of each manufacturer (**).
[c]All cases were analyzed by both a targeted array and a genome-wide array.
[d]In five cases, CNVs were also identified by a targeted array.
[e]Ten cases with an abnormal karyotype were excluded.
[f]Only cases studied with an array throughout the genome are described. Ninety-eight cases were also analyzed by a targeted array.
[g]Seventeen cases with an abnormal karyotype were excluded.

of two types of microarray, BAC array and oligonucleotide array. The BAC array was applied for 298 patients to detect 58 CNVs in 47 patients, and among them 26 CNVs (8.7%) were determined to be causal (pathogenic). Conversely, the oligonucleotide arrays were applied for 703 patients to detect 1538 CNVs in 603 patients, and among them 74 CNVs (10.5%) were determined to be pathogenic. These results may lead to the following idea: a lower-resolution microarray detects a limited number of CNVs likely to be pathogenic, because such CNVs tend to be large, and a higher-resolution microarray detects an increasing number of bCNVs or VOUS.[38] Indeed, in studies using a high-resolution microarray, most of the CNVs detected were smaller than 500 kb but almost all pCNVs were relatively large.[54,81,83] Most of the small CNVs were judged not to be pathogenic, and the percentage of pCNVs stabilized at around 10%. This percentage may suggest a frequency of patients with MCA/MR caused by CNV affecting one or more genes, other than known syndromes and subtelomeric aberrations. The other patients may be affected by another cause undetectable by genomic microarray; for example a point mutation or microdeletion/duplication of a single gene, aberration of microRNA, aberration of methylation states, epigenetic aberration or partial uniparental disomy.

As recently hypothesized secondary insult, which is potentially another CNV, a mutation in a phenotypically related gene or an environmental event influencing the phenotype, may result in clinical manifestation.[84] Especially, in two-hit CNVs, two models have been hypothesized: (1) the additive model of two co-occurring CNVs affecting independent functional modules and (2) the epistatic model of two CNVs affecting the same functional module.[85] It also suggests difficulty in selecting an optimal platform in the clinical screening. Nevertheless, information on both pCNVs and bCNVs detected through studies using several types of microarrays is unambiguously significant because an accumulation of the CNVs will create a map of genotype–phenotype correlation that would determine the clinical significance of each CNV, illuminate gene function or establish a new syndrome.

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Clinical and Genomic Characterization of Siblings With a Distal Duplication of Chromosome 9q (9q34.1-qter)

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We report herein on two female siblings exhibiting mild intellectual disability, hypotonia in infancy, postnatal growth retardation, characteristic appearance of the face, fingers, and toes. Their healthy mother had a translocation between 9q34.1 and the 13pter. FISH and array CGH analysis demonstrated that the two children had an additional 8.5 Mb segment of the 9q34.1-qter at 13pter. The clinical features of the present cases were similar to those of previously reported 9q34 duplication cases; however, the present cases did not exhibit other abnormal behaviors, such as autistic features or attention deficit disorders, those are reportedly associated with 9q34 duplications. A 3.0 Mb region (9q34.1-q34.3) within 9q34 duplication in our patients are overlapped with duplication region of previously reported cases and is proposed to be critical for the presentation of several phenotypes associated with 9q34 duplications. © 2011 Wiley-Liss, Inc.

Key words: 9q34 duplication; intellectual disability; array CGH; dysmorphism

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INTRODUCTION

Duplications of a distal region of the long arm of chromosome 9 (9q34) are rare and few cases have been reported. The first association between 9q34 duplications and phenotypic abnormalities were demonstrated in seven cases in a large pedigree [Allderdice et al., 1983]. The patients had low birth weight, initial poor feeding and thriving, slight psychomotor retardation, characteristic appearance of the face, fingers, and toes. Hyperactive behavior, heart murmur, and ptosis and strabismus were also noted. In another case, a girl of 3 years and 2 months carried a 9q34 duplication and a deletion of 3p26-pter due to a balanced translocation in her mother [Hodou et al., 1987]. This patient presented with dolichocephaly, characteristic facial appearance, and long thin fingers and toes, all of which are phenotypes noted in previous cases of 9q34 duplication; she also exhibited features associated with 3p terminal monosomy. In addition, duplication of 9q34-qter and monosomy of a small region on 12p13.3 in a male infant was described by Spinner et al. [1993]. The same patient was followed up at 18 years of age, and the duplicated and deleted regions were determined in detail by array-based comparative genomic hybridization (array CGH) analysis [Youngs et al., 2010]. The patient exhibited autistic features, hyperactivity, and attention deficit disorder in addition to the features associated with 9q34 duplications reported previously. Gawlik-Kuklinska et al. [2007] reported the case of a 17-year-old girl with an interstitial 7.4 Mb duplication of 9q34.1-q34.3 determined by array CGH analysis and compared the clinical features of the patient with those of previous cases. This patient exhibited the features common to patients with 9q34 duplications and three additional phenotypes of food-seeking behavior, obesity, and secondary amenorrhea.

In this report, we present two female siblings with 9q34.1-qter duplications and compare the clinical features and 9q34 duplication region of these patients with those of two previously reported cases using array CGH analysis. We also discuss the loci potentially responsible for the several phenotypes associated with a specific segment of 9q34.

Additional supporting information may be found in the online version of this article.

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CLINICAL REPORTS

Patient 1. The patient was a 4-year-old girl and the first child of healthy, non-consanguineous Japanese parents. The family history was unremarkable. She was born at 40 weeks of gestation weighing 2,564 g and measuring 47.3 cm in length with an occipitofrontal circumference (OFC) of 33 cm, all within the standard range (10th–90th centile) for female Japanese neonates. The child was first evaluated at a cardiology clinic to investigate a heart murmur in the neonatal period. She was diagnosed with Ebstein anomaly, which was surgically repaired when she was 2-month old. At the age of 4 months, she was referred to our hospital due to generalized hypotonia and developmental delay. She rolled over at 12 months and sat up at 18 months. She stood with support at 24 months and started to walk unaided at 2.5 years. At 3 years of age, her height was 84 cm (−2.2 SD), body weight was 12.4 kg (−0.7 SD), and OFC was 49 cm (−0.2 SD). She could speak several meaningful words and understand simple sentences. Her developmental quotient (DQ) was 67, indicating mild intellectual disability. She was a sociable and friendly girl.

Clinical examination revealed that she had a characteristic facial appearance, including a round face, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1A). Her fingers were slender but not tapered (Fig. 1C). Neurological examination revealed that the cranial nerves were intact except for strabismus. Ocular fundi were normal. She walked slowly, but no ataxia was evident. Muscle tonus of the extremities was normal. Tendon reflexes of extremities were normal, and pathological reflex was absent. There was no evidence of epilepsy. Routine laboratory investigations were normal.

Patient 2. The patient was a 3-year-old girl and was the second child of the parents of Patient 1. She was born at 40 weeks of gestation weighing 2,874 g, measuring 49 cm in length with an OFC of 34.3 cm (all normal values for female Japanese neonates). She exhibited generalized hypotonia, but no feeding problems were observed during the neonatal period. She was referred to our hospital at the age of 19 months due to developmental delay. She exhibited head control at the age of 4 months. She rolled over at 9 months, sat at 10 months, and cruised between 11 and 12 months. She started to walk unaided at 18 months. Her height at 3 years was 88 cm (−2.4 SD), body weight was 10.1 kg (−2.7 SD), and OFC was 47 cm (−0.7 SD). DQ at the age of 3 was 72, indicating mild intellectual disability. She routinely exhibited affectionate and sociable behavior. She also had a round face with full cheeks, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1B). Ultrasonography of the abdomen showed no urogenital defects. No ophthalmic anomalies other than strabismus were found on routine evaluation. Neurological examination was not remarkable except strabismus. No epileptic seizures were observed. Routine laboratory investigations were normal. The clinical features of both patients and two previously reported cases of 9q34 duplication are summarized in Table I.

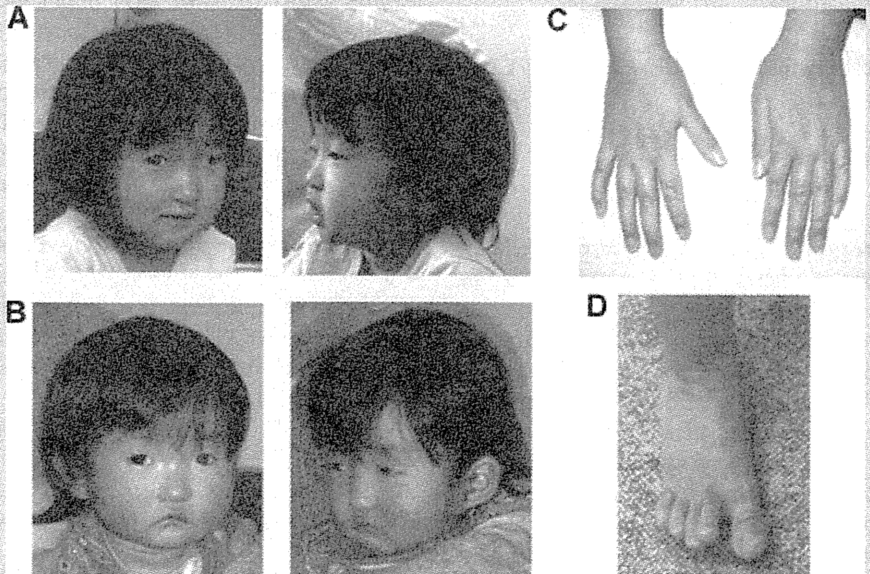

FIG. 1. A: Frontal and lateral views of Patient 1 at 3 years of age. Phenotypes include round face, hypertelorism, telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. B: Frontal and oblique view of Patient 2 at 2 years of age. Phenotypes include round face, hypertelorism, almond-shaped palpebral fissures with telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. C: Hands of Patient 1 with long and thin fingers. D: The right foot of Patient 1. She has long toes with increased space between the first and second toes.

TABLE I. Clinical Features of Patients With a 9q34.1-qter Duplication

Phenotypic features	Gawlik-Kuklinska et al. [2007]	Youngs et al. [2010]	Patient 1	Patient 2
General				
Hypotonia	+	+	+	+
Failure to thrive	+	−	−	−
Intellectual disability	Mild	Mild	Mild	Mild
Cardiac anomalies	−	+	+	−
Overweight/obesity	+	+	−	−
Scoliosis	+	−	−	−
Facial characteristics				
Dolichcephaly	+	+	−	−
Facial asymmetry	+	+	−	−
Narrow horizontal palpebral fissures	+	+	−	−
Deep-set eyes	+	+	−	−
Long nose	+	+	−	−
Prominent chin	+	+	−	−
Microstomia	+	+	+	+
Microretrognathia	+	+	+	+
Short philtrum	+	−	+	+
Round face	−	−	+	+
Hypertelorism	−	−	+	+
Depressed nasal bridge	−	−	+	+
Almond-shape palpebral fissures	−	−	+	+
Telecanthus	−	−	+	+
Short nose	−	−	+	+
Extremities				
Long and thin fingers	+	+	+	+
Increased space between first and second toes	+	+	+	+

+, present; −, absent.

MATERIALS AND METHODS

Cytogenetic Analysis

Cultured lymphoblastoid cells isolated from each patient were treated with colchicine (Sigma–Aldrich, St. Louis, MO) for 1 hr at a concentration of 20 ng/ml in culture medium, and then incubated in a hypotonic solution of 75 mM KCl at 37°C for 30 min. After incubation, cells were fixed with Carnoy's fixative (3:1 mixture of methanol and acetic acid), spread on glass slides in a humid atmosphere and air-dried. Chromosomal analysis was carried out on GTG banded chromosomes at a resolution of 400–550 bands. Fluorescence in situ hybridization (FISH) was performed on metaphase chromosome spreads from each patient. Commercial probes covering subtelomeric regions were used according to the manufacturer's protocols (ToTelVysion, Abbott Laboratories. Abbott Park, IL) [Flint et al., 1995]. In order to confirm the chromosomal rearrangement in detail, additional FISH analysis was carried out from the patients and their parents using a series of bacterial artificial chromosome (BAC) clones (Clontech Laboratories, Inc., Mountain View, CA) that map to chromosome regions 9q34 and 13q31.

Array CGH Analysis

Genomic DNA was isolated from peripheral blood lymphocytes of the two patients, their parents, and three normal controls by phenol/chloroform extraction. Array CGH analysis was performed using the Agilent Human Genome CGH 244K microarray platform (Agilent Technologies, Santa Clara, CA) according to standard protocols provided by the manufacturer. This array spans the entire human genome at a median resolution of approximately 8.9 kb. Genomic copy numbers were analyzed with Genomic Workbench (Standard Edition 5.0.14; Agilent Technologies).

Southern Blot Analysis

Genomic DNA samples (10 μg) from the patients, their parents, and the normal controls were digested with *Hin*dIII, separated on a 0.9% agarose gel, and transferred by the alkaline method to a nylon membrane (Hybond-N+; GE Healthcare, Tokyo, Japan). The membrane was sequentially hybridized with [α-^{32}P]dCTP-labeled *ABCA6* (exons 17–19) and *SP2* (exons 4–7) cDNA. A 301 bp *ABCA6* or a 798 bp *SP2* cDNA probe was prepared by amplifying the cDNA library of human lymphoblastoid cells with AmpliTaq-

Gold (Applied Biosystems, Foster City, CA) using specific primer pairs for *ABCA6* (sense: 5′-ATCTTTTCAGTGATCTGGATAAG-3′; antisense: 5′-AGGGTCAATAACACTTTAGTTT-3′), and for *SP2* (sense: 5′-GTCTACATCCGCACGCCTTC-3′; antisense: 5′-CCGCCGCAGTTGGCCTTA-3′), respectively. The PCR products were subcloned into pGEM-T easy vector (Promega, Madison, WI), and the nucleotide sequence of the probes was confirmed. Hybridization was performed in hybridization solution containing 5× standard saline citrate (SSC), 5× Denhardt's solution, and 0.5% SDS at 66°C overnight. The membrane was washed three times with 2× SSC containing 0.1% SDS at 37°C for 20 min and once with 0.1× SSC containing 0.1% SDS at 55°C for 10 min, and then radioactivity was quantified with a BAS 1800 image analyzer (FUJIFILM, Tokyo, Japan). The radioactivity of *ABCA6* versus *SP2* was determined for both patients and their parents (RP1, RP2, RF, RM) relative to the mean of the three normal controls (RC).

RESULTS

Additional 9q Subtelomeric Signal

The G-banding pattern of the both patients showed a 46,XX normal female karyotype. FISH with probes for subtelomeric regions revealed an additional 9q subtelomeric signal on the short arm of a D-group chromosome (chromosome 13, 14, or 15) in both patients (data not shown).

9q34 Duplication

To assess the chromosomal rearrangements in more detail, FISH analysis was performed in both patients and their parents with three BAC clones (RP11-40A7 and RP11-81N19) from chromosome 9q34 and RP11-524C15 from chromosome 13q31. The result indicated that the mother had a translocation; a 9q34.1-qter segment from one chromosome 9 was translocated to the terminus of chromosome 13p (Fig. 2, lower panel, indicated by a yellow arrow). Both patients had two normal chromosomes 9 and the derivative chromosome 13, which had an additional 9q34.1-qter segment at the p-terminal (Fig. 2, lower panels, indicated by yellow arrows). The father did not show any abnormalities (data not shown). These results indicate that the additional 9q34.1-qter segment at the p-terminal of chromosome 13 was of maternal origin (Fig. 2). The breakpoint of the translocation fell between two BAC clones at RP11-81N19 (129.2 Mb from the 9p terminus) and RP11-40A7 (133.4 Mb). Detailed mapping of the 13p breakpoint is not necessary because 13p does not code any genes. Thus, the duplicated segment was estimated to be 6.8–11.0 Mb derived from the 9q-terminus at position 140.2 Mb [46,XX.ish der(13)t(9;13)(q34.1;pter)mat] (Fig. 2).

8.5 Mb Duplication of 9q34.1-qter

We performed array CGH using genomic DNA from each patient to determine the precise size of the additional 9q34 segment and

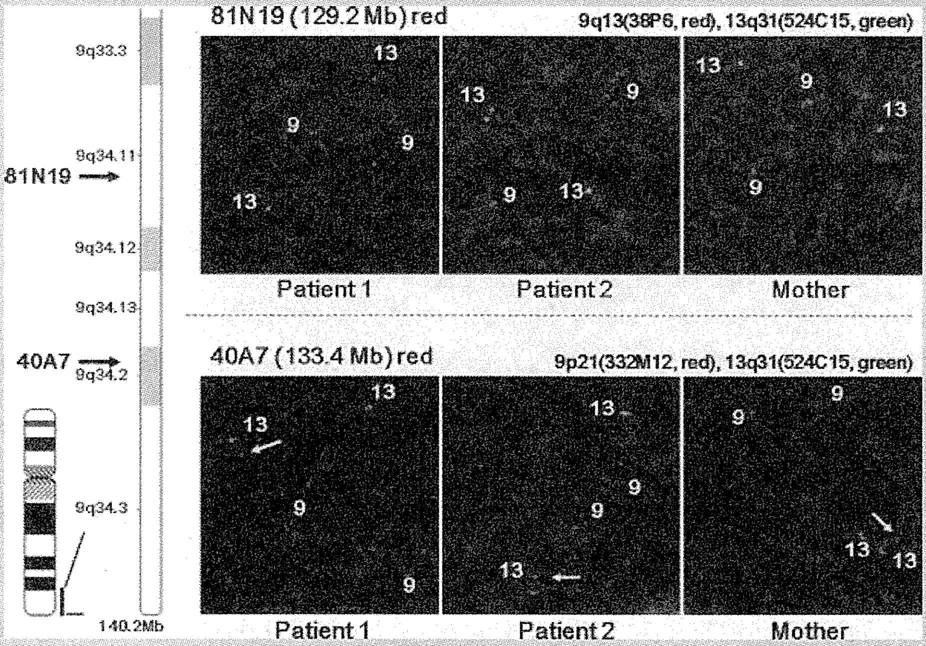

FIG. 2. Partial metaphases of FISH with BAC clone RP11-81N19 probe show two red signals on both 9q terminal regions of the mother and each patient (upper panel) and no signal on chromosome 13. Partial metaphases of FISH with BAC clone RP11-40A7 probe show a red signal on one 9q terminal region and the short arm of derivative chromosome 13 (yellow arrow) in the mother and three signals in both patients; two red signals on both 9q terminal regions and an additional signal on the short arm of derivative chromosome 13 (yellow arrow) (lower panel). RP11-38P6 (red), RP11-332M12 (red), and RP11-524C15 (green) are used as markers for 9q13, 9p21, and 13q31, respectively.

identify any other genomic abnormalities. Array CGH analysis of samples from Patients 1 and 2 demonstrated that the genomic copy number of 9q34.1-qter was 1.5-fold higher than the normal region (Fig. 3A,B). The size of the 9q34.1-qter duplication in both patients was approximately 8.5 Mb, from positions 131.7 to 140.2 Mb of chromosome 9 (Fig. 3). The breakpoint (position 131.7 Mb) of the 9q34 duplication in both patients was located in *FNBP1*, which encodes formin-binding protein 1. Analyses of Patients 1 and 2 revealed 12 and 15 copy number variations (CNVs), respectively (data not shown). CNVs are generally defined as the copy number differences of genomic DNA larger than 1 kb that vary in copy number between individuals. Patients 1 and 2 both had a 0.5-fold decrease in the genomic copy number of *ABCA6*, which encodes ATP-binding cassette, sub-family A, member 6; this is not recognized as a CNV (MIM 612504; Supplemental Fig. A and B).

ABCA6 Deletion in Both Patients and Their Mother

To confirm whether *ABCA6* was deleted in both patients and their parents, we performed Southern blot analysis using two cDNA probes against *ABCA6* (exons 17–19) and *SP2* (exons 4–7). *SP2* maps to 17q21, approximately 21 Mb proximal to *ABCA6*, and was not deleted in either patient based on the array CGH analysis. Southern blot analysis showed a decreased radioactive signal from *ABCA6* in family members (Supplemental Fig. C). When the mean ratio of *ABCA6* signal to *SP2* signal of the three normal controls was defined as 1.0, the ratio of *ABCA6* signal to *SP2* signal of the patients and their mother was approximately 0.5 and their father was 0.85 (Supplemental Fig. D). Thus, the both patients and their mother were heterozygous for an *ABCA6* deletion.

DISCUSSION

Duplications of 9q34 cause intellectual disability and multiple congenital anomalies. Reported cases presented with a variety of clinical features depending on the size of the duplication and the presence of other chromosomal abnormalities [Allderdice et al., 1983; Hodou et al., 1987; Spinner et al., 1993; Gawlik-Kuklinska et al., 2007; Youngs et al., 2010]. Our patients had a 9q34.1-qter duplication and partial 13p monosomy due to a translocation between 9q34.1 and 13pter in their healthy mother. Array CGH and Southern blot analyses confirmed that these patients had a 9q34.1-qter duplication and a heterozygous deletion of *ABCA6* (17q24). Because 13p does not code for any genes and the heterozygous deletion of *ABCA6* did not cause any phenotypic abnormalities in the mother, the present patients exhibited "pure" 9q34.1-qter duplications without any other chromosomal abnormalities involving coding genes.

9q34 duplication has been analyzed in detail using array CGH in only two other patients. Gawlik-Kuklinska et al. [2007] reported the case of the female with a 7.4 Mb (RP11-269P11 to RP11-295G24; 127.3–134.7 Mb) duplication of 9q34.1-q34.3 (Fig. 4) and compared the patient's clinical features to those of previously reported 9q34 duplication cases [Spinner et al., 1993], including a male patient later shown to have a 13.8 Mb (126.4–140.2 Mb) duplication of 9q33.3-qter [Youngs et al., 2010] (Fig. 4). The following

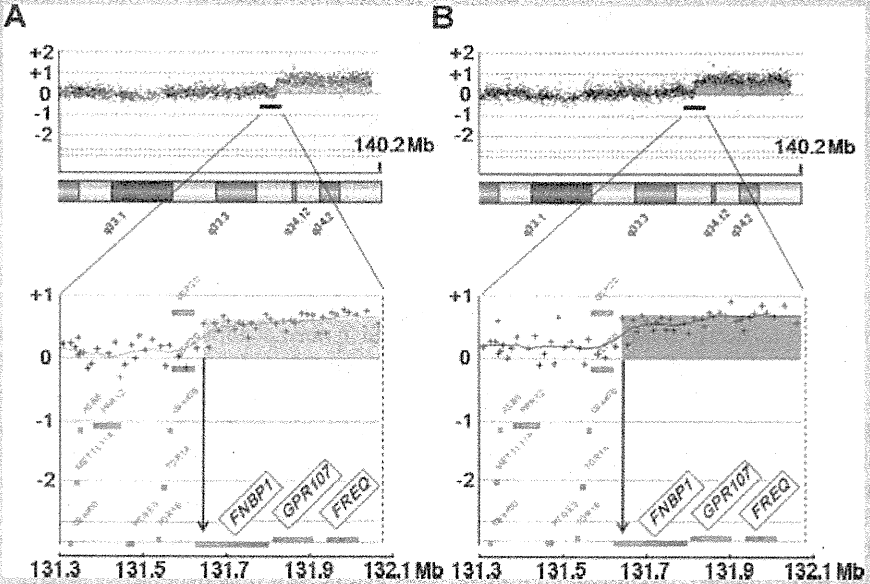

FIG. 3. A: Graphical representation of the results of the array CGH analysis (Agilent 244K oligonucleotide array) from Patient 1 shows the duplication of distal 9q34.1-qter (upper panel). The x- and y-axis denote genomic position and $\log_2$ ratio, respectively. B: Graphical representation of the results of the array CGH analysis from Patient 2 also shows the duplication of distal 9q34.1-qter (upper panel). The breakpoint in 9q34 was located in the *FNBP1* gene (131.7 Mb) in both patients (lower panels of A and B), which indicated that the size of the duplication was approximately 8.5 Mb (131.7–140.2 Mb) according to NCBI human genome build 36.3.

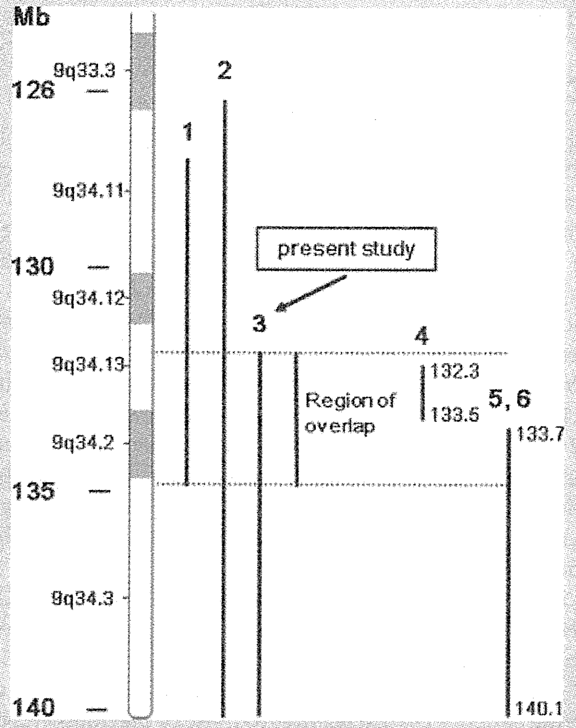

FIG. 4. A schematic illustration based on NCBI human genome build 36.3 of 9q34 duplications from two previously reported cases, the present patients, and three patients from DECIPHER. The duplications in the previously reported patients and our patients are denoted as 1 [127.3–134.7 Mb] [Gawlik-Kuklinska et al., 2007], 2 [126.4–140.2 Mb] [Youngs et al., 2010], and 3 [131.7–140.2 Mb] [present study]. The 3.0 Mb [131.7–134.7 Mb] overlapping region of all three 9q34 duplications is denoted as "Region of overlap." The duplications in the patients from DECIPHER are denoted as 4 [P253579; age 17, 46,XX] [132.3–133.5 Mb], 5 [P254131; age 2, 46,XX] [133.7–140.1 Mb], and 6 [P255167; age 2, 46,XY] [133.7–140.1 Mb].

features were common to both patients in these reports: hypotonia, intellectual disability, developmental delay, characteristic head and facial features associated with dolichocephaly, facial asymmetry, narrow palpebral fissures, deep-set eyes, long nose, prominent chin, microstomia, microretrognathia, and characteristic features of the extremities, including long thin fingers and toes and camptodactyly (Table I). Gawlik-Kuklinska et al. [2007] concluded a 7.4 Mb (127.3–134.7 Mb) duplicated region in their patient was critical for the phenotypes they observed (Fig. 4). Like these two previously reported cases, our patients also exhibited hypotonia, mild intellectual disability, developmental delay, microstomia, microretrognathia, and long thin fingers and toes. Thus, the 3.0 Mb region (131.7–134.7 Mb) of 9q34.13-q34.3 that overlapped in the cases reported by previous studies [Gawlik-Kuklinska et al., 2007; Youngs et al., 2010], and in our patients is most likely associated with the manifestation of the phenotypes observed in all four patients (Fig. 4, Table I). Unlike the other patients, our patients did not have dolichocephaly, facial asymmetry, narrow palpebral fissures, deep-set eyes, or long nose. The locus or loci associated with these phenotypes may be located in a region (127.3–131.7 Mb) that is proximal to the overlapping region (Fig. 4, Table I). Our patients exhibited other characteristic facial features, such as round faces, hypertelorism, almond-shaped palpebral fissures, telecanthus, and short nose; those were not observed in the previously reported cases (Table I). The distal-most segment of 9q34 (134.7–140.2 Mb) in our patients is the strongest candidate for the origin of these phenotypes (Fig. 4). However, these phenotypes were not observed in Patient 2 [Youngs et al., 2010], who had the same 9qter duplication. Therefore, the duplication of the proximal segment (127.3–131.7 Mb) of the overlapping region may have more impact on facial appearance than the duplication of the distal segment of the overlapping region. Clinical analyses of more patients with 9qter duplication (134.7–140.2 Mb) are necessary to determine the phenotypes caused by duplication of this region. It should be noted that DECIPHER (Database of Chromosomal Imbalance and Phenotype in Human using Ensembl Resources) includes two patients (P254131 and P255167) with the same 9q34.2-qter duplication (133.7–140.1 Mb) and heterozygous deletion of 17pter (0.01–0.41 Mb) (Fig. 4, numbers 5, 6). These patients exhibited hypotonia (non-myopathic), intellectual disability, developmental delay, patchy café au lait pigmentation spots on the skin, and speech delay. The heterozygous 17pter 0.4 Mb deletion has not been reported to cause any diseases, including intellectual disability. Another patient (P253579) presenting with facial abnormality, intellectual disability, and developmental delay had a 9q34.1-q34.2 duplication (132.3–133.5 Mb) in the 3.0 Mb overlapping region (Fig. 4, number 4). Notably, these two duplicated regions are included in the duplicated region in our patients, but they do not overlap with each other. These findings suggest the following correlations between duplicated chromosomal segments of 9q34 and phenotypes: (1) two duplicated segments (133.7–140.1 and 132.3–133.5 Mb) in 9q34 are associated with intellectual disability and developmental delay; and (2) the locus or loci associated with characteristic facial appearance may be within a duplicated region of 1.2 Mb (132.3–133.5 Mb), even though the detailed clinical features of P253579 are not available. Of the 18 genes that map to this 1.2 Mb region, individual duplications of 12 genes are reported in the Database of Genomic Variants (DGV; found in normal population). Thus, increased copy number of one or more of the other six genes (*FUBP3*, *EXOSC2*, *ABL1*, *NUP214*, *FAM78A*, and *PPAPDC3*) in this region could be the cause of the intellectual disability, developmental delay, and characteristic facial appearance observed in our patients and P253579.

Chromosomal rearrangements, arising from unequal recombination between repeated sequences, are found in a subset of patients with autism spectrum disorder [Marshall et al., 2008]. Abnormal behaviors, including hyperactive behavior [Allderdice et al., 1983], food-seeking behavior [Gawlik-Kuklinska et al., 2007], hyperactivity, attention deficit disorders, and atypical autism [Youngs et al., 2010], were also reported in some patients with 9q34 duplication. Unlike these patients, our patients exhibited friendly and affectionate social behaviors and did not exhibit autistic features or attention deficit disorder. It is important to repeatedly monitor the behaviors of our patients to determine whether the 9q34.1-qter duplication is associated with abnormal behaviors. In summary, our findings indicate that the duplication of 9q34 is a heterogeneous clinical condition and duplications of different segments of 9q34 are associated with a variety of symptoms. Genomic and clinical analyses of more patients carrying 9q34 duplications are necessary to better characterize the correlation between clinical phenotypes and specific 9q34 loci.

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