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SHORT COMMUNICATION

Concomitant microduplications of *MECP2* and *ATRX* in male patients with severe mental retardation

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Investigations of chromosomal rearrangements in patients with mental retardation (MR) are particularly informative in the search for genes involved in MR. Here we report a family with concomitant duplications of methyl CpG binding protein 2 (*MECP2*) at Xq28 and *ATRX* (the causative gene for X-linked alpha thalassemia/mental retardation) at Xq21.1 detected by array-comparative genomic hybridization. The alterations were observed in a 25-year-old man who inherited them from his mother, who showed a normal phenotype and completely skewed X-chromosome inactivation, and also in his cousin, a 32-year-old man. The proband and his cousin showed severe MR, muscular hypotonia, recurrent respiratory infections and various other features characteristic of *MECP2* duplication syndrome. However, the proband also had cerebellar atrophy never reported before in *MECP2* duplication syndrome, suggesting that his phenotypes were modified through the *ATRX* duplication in an additive or epistatic manner.
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Duplication at Xq28 involving methyl CpG binding protein 2 (*MECP2*) has been detected at high frequency (1–2%) in males with unexplained X-linked mental retardation (XLMR).[1,2] *MECP2* duplication syndrome is now recognized as a clinical entity showing severe MR, muscular hypotonia, absence of speech, a history of recurrent infection and mild dysmorphic features.[3] In the course of a program to screen possible patients with XLMR for copy-number aberrations by array-comparative genomic hybridization (aCGH) using a bacterial artificial chromosome (BAC)-based X-tiling array (MCG X-tiling array),[2,4] we detected an ~0.4-Mb duplication at Xq28 involving *MECP2* together with an ~0.3-Mb duplication at Xq21.1 that included *ATRX*, the causative gene for ATR-X (X-linked alpha thalassemia/mental retardation) syndrome, in a 25-year-old man and his cousin, a 32-year-old man (Figure 1a).

The proband (III-1, Figure 1b) was born at 41 weeks after an uneventful pregnancy as the first child of non-consanguineous healthy parents. At birth, his weight and occipital–frontal circumference (OFC) were 3280 g (±0 s.d.) and 33.5 cm (0.3 s.d.), respectively. He was developmentally retarded: first smiling at 3 months, holding up his head at 5 months, rolling over at 7 months, sitting by himself at 12 months and crawling at 13 months. At 25 years, his height, weight and OFC were 160.8 cm (−1.7 s.d.), 50 kg (−1.2 s.d.) and 56.3 cm (−0.9 s.d.), respectively. The proband exhibited hypertelorism, microcephaly and synophrys (Figure 1c). At 28 years, magnetic resonance imaging (MRI) showed cerebral atrophy, cerebellar atrophy and a thin corpus callosum (Figure 1d). He could walk and communicate until he was 14 years old, but became unable to do either of this after developing epilepsy. At the age of 4 years and 10 months, his total Developmental Quotient was 22, calculated by using the Kyoto Scale of Psychological Development. A blood investigation showed that his IgA level was low. The HbH inclusion body that is detected frequently in patients with *ATRX* mutation was not found by brilliant cresyl staining. His younger brother (III-2) had intrapartum asphyxia and two maternal uncles (II-3, II-4) died immediately after birth.

The cousin of the proband (III-3) was born in 41 weeks after an uneventful pregnancy to non-consanguineous healthy parents by normal delivery. At birth, his weight and OFC were 2850 g (−1.2 s.d.) and 37 cm (+2.4 s.d.), respectively. He was characterized by macrocephaly. He had started smiling at 2–3 months, holding up his head at 4 months, sitting by himself at 12 months and walking at 40 months. At 32 years of age, his height, weight and OFC were in the normal range (164.5 cm, −1.1 s.d.; 57 kg, −0.5 s.d.; 59.4 cm, +1.8 s.d.). Information on his Developmental Quotient was unavailable. A blood investigation showed that his IgA level was low. He had been affected

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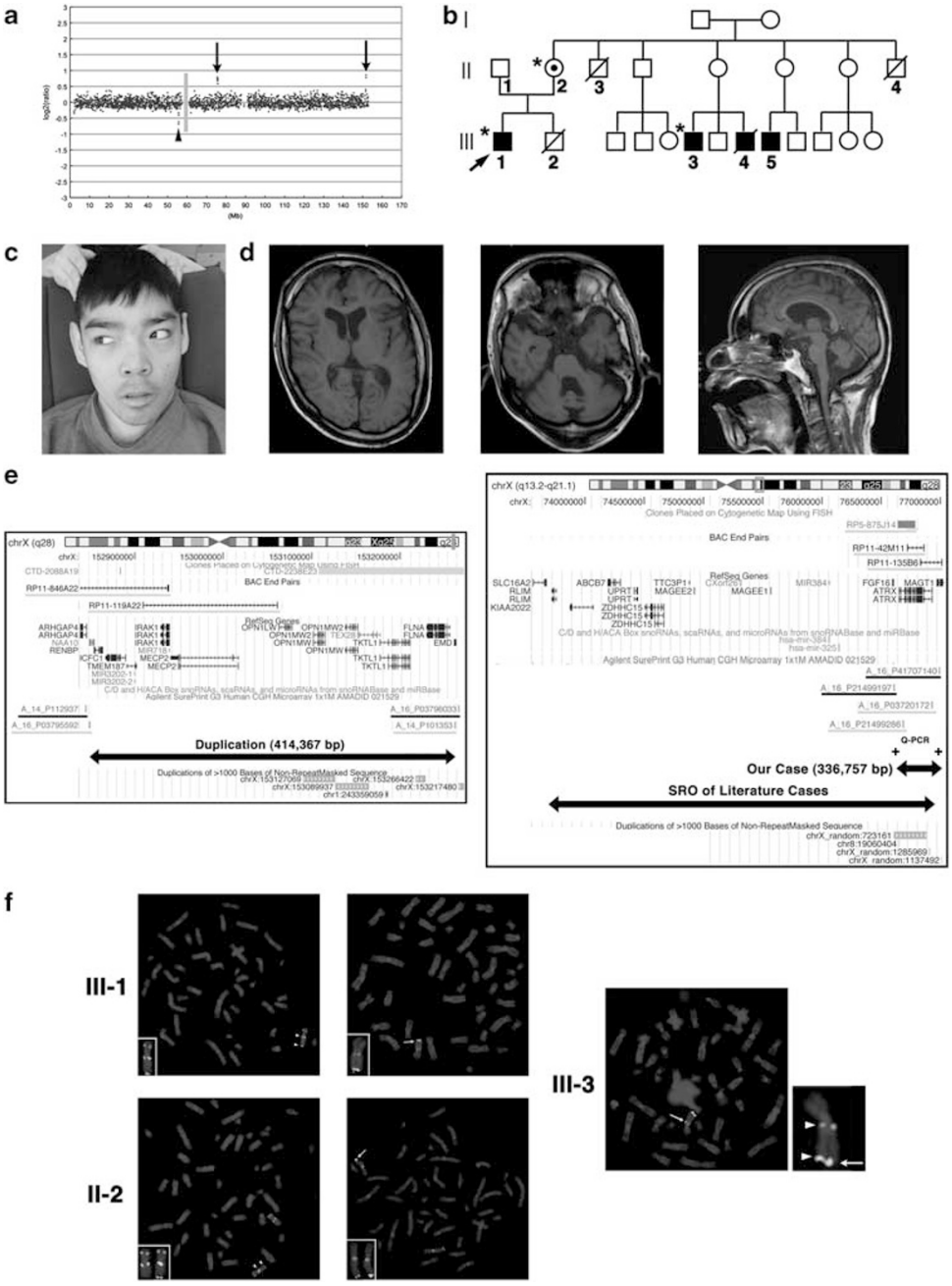

Figure 1 (**a**) Profile of the copy-number ratio on chromosome X in the proband (III-1) detected with aCGH using an MCG X-tiling array. Each dot represents the test/reference value after normalization and $\log_2$ transformation in each BAC clone, and arrows indicate duplications (ratio > 0.4). The gray vertical lines represent the centromeric region for which no clones were available. Arrowheads indicate benign CNVs (Supplementary Table 1). (**b**) Three-generation genealogy of the studied family. Closed squares and circles and dotted circles indicate MR and carriers, respectively. The proband (III-1) indicated by an arrow was used for aCGH with the X-tiling array. Asterisks indicate persons having the duplications at Xq21.1 and Xq28. A slash indicates a death. (**c**) The proband (III-1) at 27 years showed hypertelorism, microcephaly and synophrys. (**d**) Brain MRI findings of the proband (III-1) at age 27. Coronal (left and middle) and (right) sagittal T1w sequences show cerebral atrophy, cerebellar atrophy and a thin corpus callosum. (**e**) Mapping of the duplications at Xq28 (left) and Xq21.1 (right) on the basis of the UCSC Genome Browser according to NCBI Build 36.1, March 2006, hg18 (http://www.genome.ucsc.edu/). A chromosome ideogram is presented. The track setting in the UCSC genome browser was set up to look for 'Base Positions', 'FISH Clones', 'BAC End Pairs', 'RefSeq Genes', 'sno/miRNA', 'Agilent Array' and 'Segmental Duplications'. Underlines below the BAC clone ID and oligonucleotide probe ID show a high (green bars) and normal (black bars) ratio detected with the MCG X-tiling array and Agilent array 244 K. Plus signs in the right panel indicate the duplication at Xq21.1 confirmed by quantitative genomic PCR. Duplicated regions in our case and the smallest region of overlap in reported cases[9] in the right panel are indicated with closed arrows. (**f**) In the proband (III-1) and the carrier mother (II-2), representative results of FISH using the clone RP11-42M11 at Xq21.1 (left) and the clone RP11-119A22 at Xq28 (right) showed separate green signals (arrowheads) and strong green signals (arrows), respectively. The red signals are of clone RP11-16H4 at Xp22.12 (left) RP11-13M9 at Xq13.2 (right) as a reference. Enlarged images of chromosome X are shown in the lower left insets in each panel. Similarly, in the affected proband's maternal cousin (III-3), representative results of FISH demonstrated separate red signals of the clone RP11-42M11 at Xq21.1 and strong green signals of the clone RP11-119A22 at Xq28. An enlarged image of chromosome X is shown in the lower right panel, indicating that the duplicated sequence at Xq21.1 inserted into the duplication at Xq28 together with the original Xq21.1 (arrowheads), whereas the duplicated sequence at Xq28 was inserted in close proximity (arrow). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

by pneumonia frequently since 3 months after birth. No MRI analysis had been performed. His younger brother (III-4) died because of disseminated intravascular coagulation at the age of 29, and his other cousin (III-5) shows a similar clinical manifestation to the proband.

On the basis of the results of precise mapping with an oligonucleotide array (Agilent array 244K, Palo Alto, CA, USA; data not shown), these aberrations are as follows: arr Xq21.1 (76646979–76983735)×2, arr Xq28 (152847991–153262357)×2 (Figure 1e). Although some copy-number variants (CNVs) were detected in other regions simultaneously, all of them have been registered in the Database of Genomic Variants (http://projects.tcag.ca/variation/ assembly, March 2006, Supplementary Table 1) and in part in our CNV database (MCG CGH database, http://www.cghtmd.jp/CNVdatabase). Subsequent real-time quantitative genomic PCR (qPCR) using primer sets recognizing around dup(X)(q21.1) (Supplementary Table 2) narrowed down dup(X)(q21.1) to between positions 76646868 and 76973049, including all of *ATRX* and part of *MAGT1* (Figure 1d). Fluorescence *in situ* hybridization (FISH) detected these duplications in the proband's unaffected mother (II-2) and his affected maternal male cousin (III-3) (Figures 1b and f), indicating maternally inherited duplications in these patients. In addition, the duplicated segment at Xq21.1 inserted into the duplicated region at Xq28, by contrast the segment at Xq28 was duplicated in tandem (Figure 1f). Our finding that the mother, a presumptive obligate carrier, had completely skewed X inactivation (dup(X):X=50:0) in a lymphoblastoid cell line , as shown by the androgen receptor X-inactivation assay described previously[5] and a late replication assay[6] with FISH (Supplementary Figure 1), supported our assumption that skewed X-chromosome inactivation appears to be characteristic of carriers of *MECP2* duplication such as other reported cases.[3]

The two affected men showed severe MR, muscular hypotonia, recurrent respiratory infections and various other features characteristic of *MECP2* duplication syndrome (Table 1). Moreover, they did not show short stature, hypoplastic genitalia and early life feeding issues, which were reported to be characteristic of MR in patients with duplications encompassing *ATRX* (Table 1).[9] The smallest region of overlap (SRO) of the reported *ATRX* duplication cases contains 11 genes, including *ATRX* and two miRNAs,[9] whereas the duplicated region of the present family includes only *ATRX* (Figure 1e), suggesting that genes other than *ATRX* within the SRO contribute to phenotypes observed in previously reported cases (Table 1).[9]

ATRX interacts with MECP2 *in vitro* and colocalizes at pericentromeric heterochromatin in mature neurons of the mouse brain.[10] Recently, it was reported that ATRX, MECP2 and cohesin cooperate to silence a subset of imprinted genes in the postnatal mouse brain.[11] Those experimental findings suggest that abnormally expressed ATRX with MECP2 through their simultaneous duplications may modify the phenotypes usually observed in *MECP2* duplication syndrome. Although our patients showed neither notably different nor more severe phenotypes compared with reported patients with *MECP2* duplication syndrome, the proband was found to have cerebellar atrophy by MRI (Figure 1e), which has never been reported before in *MECP2* duplication syndrome.[1,3] It is possible that these phenotypes in the proband were modified through ATRX duplication in an additive or epistatic manner.

The mutations in *ATRX* give rise to changes in the pattern of methylation of several highly repeated sequences, including the ribosomal DNA (rDNA) arrays[12] and significantly altered mRNA expression in four ATRX targets (*NME4*, *SLC7A5*, *RASA3* and *GAS8*) relative to normal controls.[13] Although a Southern blot hybridization method reported previously[12] showed no change in the pattern of methylation at rDNA arrays compared with normal controls (Figure 2a), quantitative RT-PCR revealed that the expression of *ATRX* was upregulated in the present cases. Although *SLC7A5* expression showed no previous change compared with that in the healthy control (Figure 2b) and the expression of *GAS8* was too low for quantitative RT-PCR (data not shown), the expression of *NME4* and *RASA3* was similar to that in the patients with *ATRX* mutations. The alteration to the expression may be influenced by *MECP2* duplication or additive/epistatic effect between *ATRX* and *MECP2* duplication.

Table 1 Phenotype comparisons between our cases and MECP2 duplication syndrome patients or patients with *ATRX* duplication

Phenotype	*MECP2 duplication syndrome*[3,7,8]	*ATRX duplication cases*[9]	*Our Cases* III-1	*Our Cases* III-3
Mental retardation	118/119	11/11	+	+
Hypotonia	86/93	7/11	+	+
Absent speech	63/72	NA	+	+
Lack ambulation	20/71	NA	+	+
Recurrent infection	82/111	6/7	+	+
Breathing abnormalities	6/18	NA	–	+
Stereotyped hand movements	15/33	NA	+	+
Autistic features/autism	13/17	NA	+	+
Epilepsy	57/110	NA	+	+
GU abnormalities	29/67	7/10 (Hypoplastic genitalia)	–	+ (Bladder distention)
Death before 25 years	25/66	NA	–	–
Spasticity	42/71	NA	–	+
Ataxia	20/37	NA	+	+
GER	15/25	NA	–	+
Swallowing difficulty	23/45	NA	–	+
IPO or constipation	25/33	NA	–	–
IgA deficiency	4/10	NA	+	+
Short stature	NA	11/11	–	–
Early life feeding issues	NA	7/9	–	–
Failure to thrive	16/31	7/9	–	+
Broad thorax	NA	4/4	–	+
Pectus excavatum	NA	3/7	–	–
Short neck	NA	4/8	–	–
Simian crease	NA	4/5	–	–
Digital findings	22/52	6/7	–	–
Microcephaly	24/71	8/11	+	–
Hypertelorism	8/72	2/6	+	+
Epicanthal folds	4/72	6/8	–	–
Down-slanted palpebral fissures	NA	1/8	–	–
Ptosis	2/72	6/9	–	–
Flat nasal bridge	15/72	9/10	–	+
Down-turned corners of the mouth	NA	8/10	–	–
High-arched palate	3/72	4/4	–	–
Micro/retrognathia	NA	4/7	–	–
Low set ears	NA	4/10	–	–
Simple ears	NA	2/10	–	–
Cryptorchidism	2/4	9/10	+	–
Impaired social interaction	NA	5/6	+	+

Abbreviations: ATR-X, the causative gene for X-linked alpha thalassemia/mental retardation; GER, gastroesophageal reflux; GU, genito-urinary system; IPO, intestinal pseudo-obstruction; *MECP2,* methyl CpG binding protein 2; NA, not available.

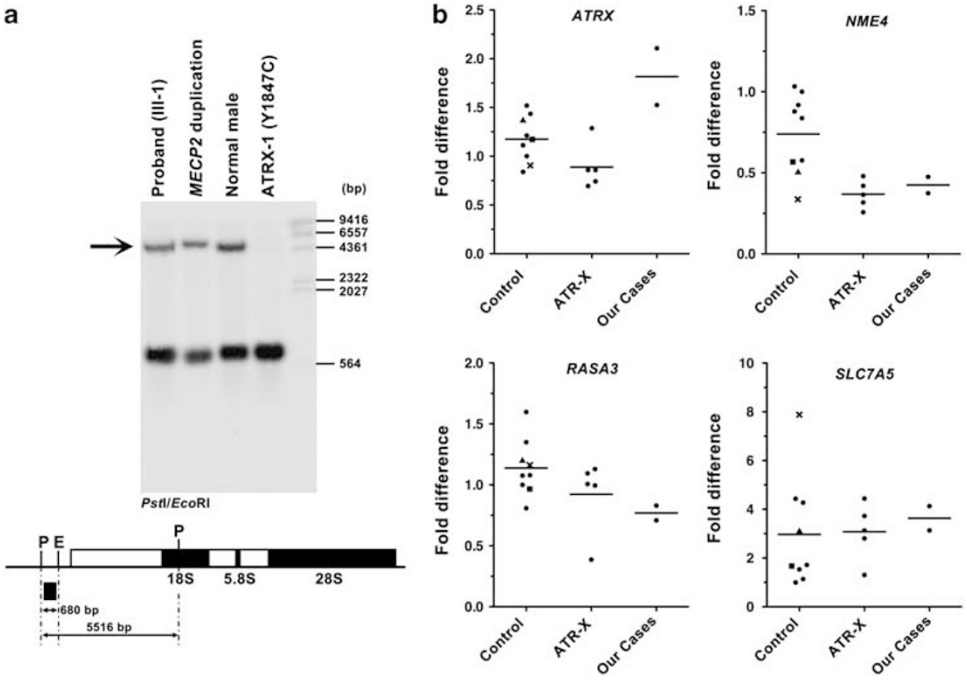

Figure 2 (**a**) Investigation of the methylation pattern of human rDNA repeats using Southern blotting. Genomic DNA from the lymphoblastoid cell line of our proband (III-1) with the *ATRX* duplication and the *MECP2* duplication, an MR male patient with *MECP2* duplication, an unrelated normal male, and an ATR-X patients with a missense mutation resulting in Y1847C in ATRX (Supplementary Table 3). DNA samples were digested with *Pst*I followed by the methylation-sensitive enzyme *Eco*RI. Hybridization is shown for probes corresponding to the region between restriction sites of the two enzymes in the 3′ end of the non-transcribed spacer. The methylated, uncut band is indicated (arrow). A restriction map of part of the rDNA repeat unit shown with the 18S, 5.8S and 28S genes in order and transcribed spacer as filled and open boxes, respectively, represents the sites for *Pst*I (P) and *Eco*RI (E). A black bar indicates the probe for the Southern hybridization. The size of the DNA segment resulting from the restriction enzymes is represented by closed arrows. (**b**) Real-time quantitative RT-PCR analysis of the mRNA expression of *ATRX* and three *ATRX* target genes (*NME4*, *RASA3* and *SLC7A5*) but not *GAS8*, the expression of which was too low to be estimated, in lymphoblastoid cells of our two patients, ATR-X patients whose *ATRX* mutations were identified through routine screening in a set of known XLMR genes by the Japanese Mental Retardation Consortium (unpublished data, n=5; Supplementary Table 3) and controls, including six healthy samples, the proband's parents, and a patient and a carrier with the *MECP2* duplication.[2] All the subjects provided written informed consent for the use of their phenotypic and genetic data. The proband's carrier mother, the patient and the carrier with the *MECP2* duplication are represented by a cross, triangle and square, respectively, in the control column. Data show the average values for fold differences relative to a normal male. Black bars represent mean values of each group.

The result of FISH suggests that *ATRX* duplication and *MECP2* duplication were occurred simultaneously resulting in complex genomic rearrangement. The proximal breakpoint of dup(X)(q21.1) and distal breakpoint of dup(X)(q28) were located on segmental duplications (Figure 1e) and the duplicated sequence at Xq21.1 existed near dup(X)(q28) (Figure 1f). Fork Stalling and Template Switching (FoSTeS) has been proposed as a replication-based mechanism that produces nonrecurrent rearrangements potentially facilitated by the presence of segmental duplications.[14] Previous reports suggested that complex genomic rearrangements at Xq28 such as an embedded triplicated segment and stretches of non-duplicated sequence within dup(X)(q28) were probably mediated by FoSTeS,[7,15] and a particular genomic architecture, especially low copy repeats at distal breakpoints of dup(X)(q28), may render the *MECP2* region unstable. Thus, the dup(X)(q28) and dup(X)(q21.1) detected in our patients might be generated simultaneously by FoSTeS or other mechanism in a segmental duplication-dependent manner, suggesting the structural analysis of the entire X chromosome in patients with dup(X)(q28) to be important for understanding their correct clinical condition and providing appropriate education.

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APPENDIX

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