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Research Letter

No Detectable Genomic Aberrations by BAC Array CGH in Kabuki Make-Up Syndrome Patients

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To the Editor:

Kabuki make-up syndrome (KMS, OMIM 147920), independently established by Niikawa et al. [1981] and Kuroki et al. [1981], is characterized by characteristic facial features resembling the Kabuki actor's make-up, mild to moderate mental retardation, postnatal growth retardation, skeletal abnormalities, and unusual dermatoglyphic patterns [Matsumoto and Niikawa, 2003]. The multisystem involvement of the KMS phenotype suggests that KMS is caused by a microdeletion or microduplication involving several genes. Milunsky and Huang [2003] reported that all of the six KMS patients they examined had approximate 3.5-Mb duplication at 8p22–p23.1 revealed by comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). They also suggested that a paracentric inversion in mothers, detected by RP11-122N11, might contribute to the occurrence of the syndrome. At least three groups, including us, failed to replicate their results by FISH and/or array CGH analysis [Miyake et al., 2004; Engelen et al., 2005; Hoffman et al., 2005]. Schoumans et al. [2005] reported that they observed no chromosomal abnormalities in 10 affected Caucasian individuals with typical KMS using the 1.2-Mb-resolution whole genome BAC array.

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Using a newly developed BAC array covering the whole genome with a 1.5-Mb resolution, in this study, array CGH (aCGH) was performed in 38 patients with typical KMS to find genomic imbalances in relation to KMS. Detailed experimental protocols are described elsewhere [Miyake et al., 2005]. Peripheral blood leukocytes or lymphoblastoid cell lines from the 38 patients (20 males and 18 females) and their parents when available were sent to us after informed consent. All KMS cases were sporadic and had a normal karyotype according to G-banded chromosomal analysis at the 400-band level. Their metaphase chromosomes for FISH and DNA for aCGH were prepared either from immortalized lymphoblastoid cell lines or peripheral blood leukocytes according to standard protocols.

We detected a total of 115 clones implying occult genomic imbalances, which were all subsequently confirmed by FISH (14 clones as deletions and 101 clones as duplications) (Fig. 1). Heterozygous deletion/duplication might be associated with the pathogenesis of KMS, because an autosomal (or pseudoautosomal) dominant inheritance is suspected in KMS [Matsumoto and Niikawa, 2003]. All copy number changed loci (clones) observed in this series of KMS patients, except four loci, were unlikely to be associated with KMS, because the same changes were also observed in either of their healthy parents, and/or non-KMS individuals with non-syndromic mental retardation [Miyake et al., 2005], and/or were registered in the Database of Genomic Variants (http://projects.tcag.ca/variation/). One heterozygous deletion at RP11-97F19 (2p11.2) in two patients (KMS5 and KMS17), and two heterozygous duplications at RP11-418N20 (Xp22.33) and RP4-617A9 (Xp22.3) in a patient (KMS14) remain inconclusive, because parental samples were unavailable. These changes in the three patients are described in detail below. Among 37 loci showing copy number changes in KMS patients, a total of 11 loci were already registered as copy number polymorphisms (CPNs) in the Database of Genomic Variants (checked on July 19, 2005) (Fig. 1), confirming that these changes are unlikely pathogenic.

RP11-97F19 Deletion at 2p11.2 in KMS5 and KMS17

We delimited the deletion to an approximate 1-Mb region from RP11-15J7 to RP11-136K15 by detailed

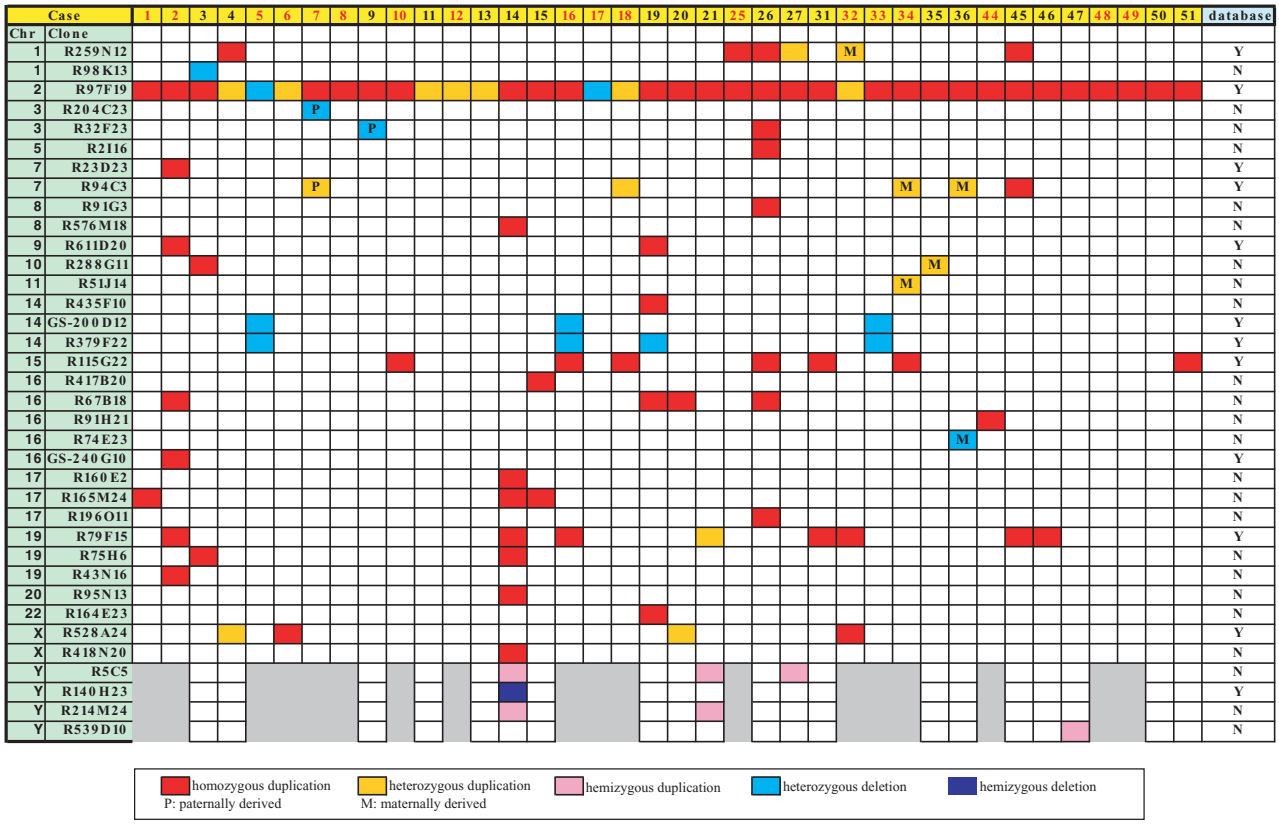

FIG. 1. Summary of BAC array CGH and FISH studies on KMS patients. Top yellow row indicates KMS patients (male in black, and female in red). Left green columns show information of clone IDs and their chromosomal locations. Duplications are shown as in red (homozygous) and orange (heterozygous). Deletions are in pale-blue (heterozygous) and dark-blue (hemizygous). Parental origin of changes is indicated as P (paternal) and M (maternal). No de novo changes were confirmed. Y in right column indicates clone/locus registered as copy number variation (CPN) locus in the Database of Genomic Variants (http://projects.tcag.ca/variation/). N clone/locus not registered in the database.

FISH analysis [UCSC Genome Browser NCBI build 35 (May 2004) coordinates, chromosome 2 nucleotide 88979594–89962288 bp] (data not shown). The gain of this region was reported previously [Sebat et al., 2004] and is described at the Database of Genomic Variants (http://projects.tcag.ca/variation), but the loss has never been reported. We did not find any cases with the same deletion in 200 chromosomes of normal Japanese controls. Regarding the duplication, homozygous and heterozygous duplication were found in 92 and 8 controls, respectively. The allele frequencies of the duplication in KMS and normal controls were 87.5% and 96%, respectively. No established genes exist within the deletion.

RP4-617A9 and RP11-418N20 Duplication at Xp22.3 in KMS14

RP4-617A9 and RP11-418N20 are closely located ~0.12 Mb apart. The heterozygous duplication of RP4-617A9 and RP11-418N20 in KMS14 was observed by aCGH. FISH analysis revealed that the duplication spans about 0.7 Mb from RP11-794A12 (distal) to RP11-418N20 (proximal) (UCSC coordinates, chromosome X nucleotide 2341315–3106243). None of 98 chromosomes in normal Japanese controls possessed the duplication. In addition, the gain of this region has not been reported yet at Database of Genome Variants. This region was overlapped with a part of the pseudoautosomal region 1 (PAR1). Among seven genes mapped to the duplication, *ZBED1*and *CD99* were in PAR1. Though they are attractive candidate genes according to a pseudoautosomal dominant inheritance hypothesis [Matsumoto and Niikawa, 2003], we could only find three SNPs in *CD99* [68A > G (D23G), 496A > G (M166V), 518A > T (N173I)], but no pathological nucleotide changes of the two genes in 37 other KMS patients (data not shown).

In conclusion, our study of 38 KMS patients did not show any pathological copy number changes, similar to the previous report [Schoumans et al., 2005]. Thus, it is less likely that microdeletions/duplications are frequent pathological changes in KMS. KMS may be caused by defects of a single gene that regulates various target genes/organs.

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