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Rapid Publication

On the Reported 8p22-p23.1 Duplication in Kabuki Make-Up Syndrome (KMS) and its Absence in Patients With Typical KMS

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To the Editor:

Kabuki make-up syndrome (KMS) was described originally and independently by Niikawa et al. [1981] and Kuroki et al. [1981]. KMS was characterized by a distinctive facial appearance resembling the Kabuki actor's make-up, mild to moderate mental retardation, skeletal abnormality, postnatal growth retardation, and dermatoglyphic abnormality. (See a review by Matsumoto and Niikawa [2003].) Multiple organ involvement implies that KMS is a contiguous syndrome, but its cause remains unknown. Milunsky and Huang [2003] recently reported that all of six KMS patients they examined had an approximate 3.5-Mb duplication at 8p22-p23.1 demonstrated by comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) using four BAC clones (RP11-112G9, RP11-252K12, RP11-31B7, and RP11-92C1) as probes. They also suggested that a paracentric inversion, detected by RP11-122N11, separated from the duplicated region may contribute to the occurrence of the condition.

We analyzed a total of 26 Japanese and 2 Thai patients with KMS and 52 phenotypically normal controls regarding such duplication and inversion by FISH using 15 BAC clones covering 8p22-8p23.1 after obtaining written informed consent and with the approval by IRB of Nagasaki University. All of these patients were referred to us after making a definitive diagnosis of KMS. Their metaphase chromosomes were prepared for FISH from immortalized lymphoblastoid cell lines or peripheral blood lymphocytes according to standard protocols. Eight BAC clones (GS-77L23, RP11-245H16, RP11-5E15, RP11-399J23, RP11-403C10, RP11-589N15, RP11-252C15, RP11-45O16) selected from the UCSC Genome Browser version July 2003 (http://genome.ucsc.edu/cgi-bin/hgGateway?org=human) in addition to 7 clones (RP11-122N11, RP11-235F10, RP11-112G9, RP11-252K12, RP11-31B7, RP11-92C1, RP11-23H1) used by Milunsky and Huang [2003] were labeled with SpectrumGreen™-11-dUTP or Spectrum Orange™-11-dUTP (Vysis, Downers Grove, IL) and used as probes for FISH. FISH signals of all clones were carefully examined on both of metaphase chromosomes and interphase nuclei. Duplication was determined if signal intensity of probe A was much stronger than that of probe B in metaphases and two dots were surely observed in interphases using two-color FISH. At least 10 metaphases and 10 interphases were scored in each experiment.

None of the four clones reported to be duplicated in patients by Milunsky and Huang [2003] revealed any duplication in our 28 KMS patients examined (Fig. 1). All other clones, but RP11-122N11, also showed a single-copy signal, not duplicated (Fig. 1). The RP11-122N11 locus was reported to be inverted in six KMS patients and two of their mothers [Milunsky and Huang, 2003]. However, using RP11–122N11 as a probe we observed "duplicated" rather than "inverted" signals (Fig. 1). As the signal of two green signals for this probe looked similar in size and intensity (Fig. 1a), duplication is more likely. Of 22 KMS patients analyzed with this probe, 15 had a homozygous duplication and 7 a heterozygous one, and thus the allele frequency for the duplication is 88.5%. Similarly, of 52 normal persons, 40 and 12 had a homozygous and a heterozygous duplication, respectively, the frequency for the duplication being 84.1%, which is not statistically different in the patients by χ^2 analysis ($P = 0.47$). Thus, findings of RP11-122N11 signal were likely to be a polymorphism (Fig. 2A–D).

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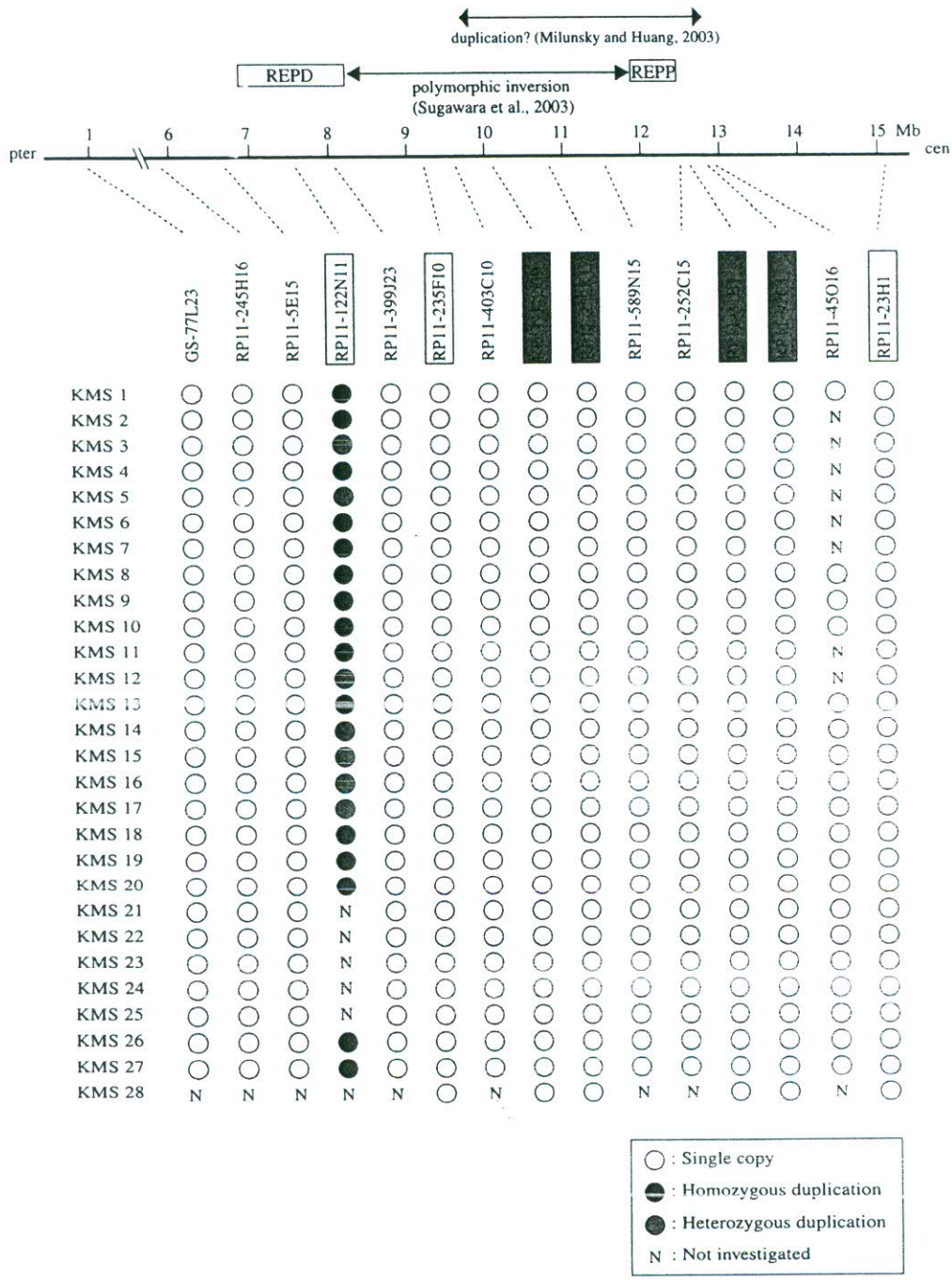

Fig. 1. Results of FISH analysis on 28 patients with Kabuki make-up syndrome (KMS). From top to bottom, a duplicated region reported by Milunsky and Huang [2003], polymorphic inversion reported by Sugawara et al. [2003], a scale from the 8p telomere to the centromere, BAC/PAC clones used for FISH study and their locations, and results of FISH studies in all patients. REPD, repeat distal; REPP, repeat proximal. BAC clones in square and grey square were those used for the study and reported to be duplicated, respectively, by Milunsky and Huang [2003]. Open, black and gray circles indicate a single-copy FISH signal, homozygous duplication, and heterozygous duplication, respectively. N, not investigated.

Unlike the data by Milunsky and Huang [2003], we were not able to detect any interstitial duplication at 8p22-8p23.1 in our series of 28 KMS patients. There must be some reasons for these discrepant results. The patient populations studied in two investigations may be different clinically. From our examination of the facial photographs of cases 1 and 2 in the report by Milunsky and Huang [2003], they may not have typical KMS and could be "8p23.1-p22 duplication syndrome." Alternatively, the discrepancy may be due to the complexity of the 8p23 region. We have constructed a comprehensive physical map covering low copy repeats (LCRs), and a common inverted region at 8p23. Although we did not incorporate the clone, RP11-122N11, to our previous map or evaluate it [Sugawara et al., 2003], we now assign it within one of LCRs

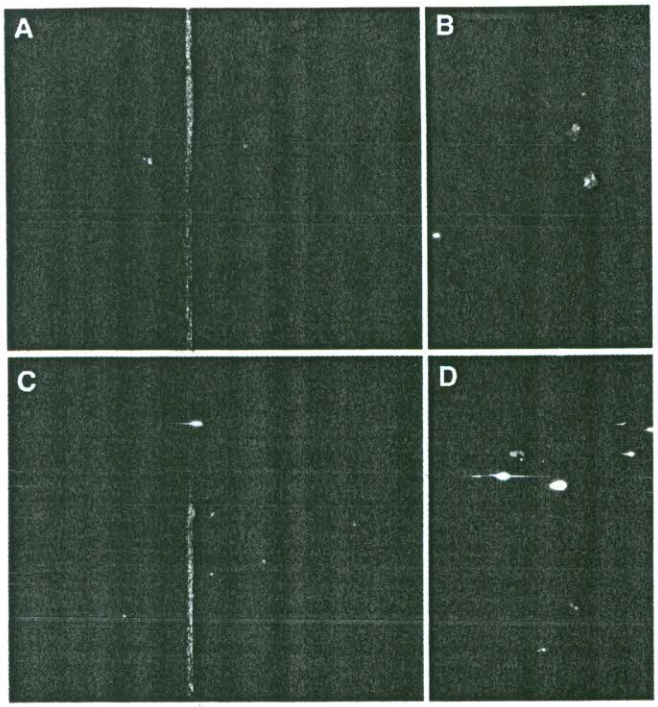

Fig. 2. FISH analysis using RP11–122N11 labeled with Spectrum-Green™ and RP11–235F10 labeled with Spectrum-Orange™ in normal controls, showing a homozygous duplication (**A**: An interphase nucleus and (**B**) metaphase chromosomes) and a heterozygous duplication (**C**: An interphase nucleus and (**D**) metaphase chromosomes).

according to both the UCSC database and our map. The clone would have shown seeming duplicated signals on both of homologous chromosomes, but the allele frequency of the RP11-122N11 duplication in the normal Japanese is 84.1%, not 100%. This implicates that this region has more complicated structure than expected, and should well be characterized.

In conclusion, our data suggest that the cause of KMS in most patients is still unknown, and further studies will be necessary absolutely.

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