Short Report
Unmasking Kabuki syndrome: chromosome 8p22-8p23.1 duplication revealed by comparative genomic hybridization and BAC-FISH

by Dr. Jeff Milunsky and Huang, XL

Clinical Genetics Volume 64 Issue 6 Page 509 - December 2003

Kabuki syndrome (KS) is a multiple congenital anomalies/mental retardation syndrome that heretofore has had an unknown etiology. Although several cases with KS features have been reported with different chromosome anomalies, none have had an autosomal cytogenetic aberration in common. We found an 8p22-8p23.1 duplication, using comparative genomic hybridization (CGH) in six unrelated patients diagnosed with KS. This observation was confirmed using BAC-FISH in all cases that delimited the duplicated region to approximately 3.5 Mb. No duplication of this region was found in two parents or 20 controls by either CGH or BAC-FISH. Two out of two mothers of KS patients and one out of 20 controls were found to have a heterozygous submicroscopic inversion at 8p23.1. As the six patients with KS represent different races, this duplication may represent a common etiologic basis for this disorder.

Kabuki syndrome (KS [MIM147920]) is a multiple congenital anomalies/mental retardation syndrome that is now well recognized worldwide with more than 350 cases reported in the literature (1, 2). Niikawa et al. (3) and Kuroki et al. (4) both initially described KS in 1981. Heretofore, the etiology of KS was undetermined. Hence, clinicians had to rely upon cardinal clinical manifestations of this syndrome to make a diagnosis. Niikawa et al.(5) delineated 5 cardinal manifestations of KS 1) craniofacial anomalies characterized by long palpebral fissures with lateral eversion of the lower eyelids, arched eyebrows, depressed nasal tip, and prominent ears; 2) dermatoglyphic abnormalities including persistence of fetal fingertip pads and increased digital ulnar loops; 3) skeletal anomalies including brachydactyly and scoliosis; 4) postnatal growth deficiency; and 5) mild to moderate mental retardation.

Most cases of KS are sporadic with a nearly equal male-to-female ratio, but several familial cases have been reported with likely dominant transmission (6, 7). Most patients with KS have normal karyotypes, although several different chromosome anomalies in individuals with features of KS have been reported (1, 2), including some with ring X. The cardinal signs in those cases were not fulfilled, were Kabuki-like, and may represent a different clinical entity. There have been, thus far, no autosomal cytogenetic aberrations in common.

The range of multiple anomalies and mental retardation in KS raise the possibility of a contiguous gene syndrome. Given the incidence of heart defects, cleft palate, and the occurrence of lower lip pits in KS, microdeletions involving chromosome 22q11 and chromosome 1q32-q41 have been investigated. Microdeletions involving these two regions have been excluded (8-11).

Comparative genomic hybridization (CGH) has proven useful for demonstrating chromosome deletions and duplications (12-15). In the past, the sensitivity of CGH was considered to be relatively low; however, more recently, high-resolution CGH has increased the sensitivity and specificity of the technique and has enabled detection of imbalances as small as 3 Mb (12). Finding a microdeletion or microduplication would be consistent with the mostly sporadic occurrence as well as the few reported cases of dominant inheritance of KS.

Materials and Methods

Subjects

We performed CGH analysis on six unrelated patients who met the cardinal clinical diagnostic criteria for KS and in whom normal high- resolution chromosome analysis was documented. Table 1 summarizes the major clinical findings in the six KS patients. The characteristic face of two of our KS patients is pictured in Fig. 1. Four of the patients are Caucasian, one is African American, and one is Haitian (16). All individuals involved in this study were diagnosed with KS by a clinical geneticist. Clinical information was furnished by each family who filled out the Kabuki syndrome clinical database survey (17) (http://www.kabukisyndrome.com/KSN.html) and who provided several pictures of the affected child. Blood was collected after Institutional Review Board approval for high-resolution cytogenetic analysis, CGH, and BAC-FISH analysis and submitted to the Center for Human Genetics at Boston University School of Medicine. Twenty anonymized controls for BAC-FISH testing were selected. Ten samples (five males and five females) were selected from those submitted to our laboratory for subtelomeric FISH analysis due to unexplained mental retardation. These individuals with mental retardation did not meet the cardinal diagnostic criteria for KS. Another 10 samples (five males and five females) were selected from those submitted to our laboratory for routine chromosome analysis due to either multiple miscarriages or infertility.

DNA isolation

Genomic DNA was isolated from peripheral blood using the Pure Gene kit (Gentra Systems, Minneapolis, MN).

Cytogenetic analysis

Metaphases from patient, two sets of parents, and control peripheral blood lymphocytes were prepared according to standard procedures using methotrexate for synchronization

of the cell cycle. Good quality metaphases of chromosomes were karyotyped by conventional G-banding at approximately 500-550 bands level.

CGH

Slides with normal lymphocyte metaphase chromosomes for CGH analysis were stored at 20 °C before hybridization. CGH was performed as described by Kirchhoff et al. (13). The CGH hybridization slides were analyzed using the CytoVision System Version 2.72 High Resolution CGH analysis software (Applied Imaging, Santa Clara, CA). Ten to fifteen metaphases were captured using a Zeiss fluorescent microscope with an integrating CCD camera (Photometrics, Tucson, AZ). The green (patient DNA) to red (reference DNA) fluorescence ratio along the length of the chromosomes was calculated. The CGH profiles were compared to a dynamic standard reference interval based on an average of normal cases, as described by Kirchhoff et al. (12, 13). The intervals were scaled automatically to fit the test case. The mean ratio profile of each case with 99.5% confidence intervals was compared to the average ratio profile of the normal cases with similar confidence intervals. The aberrations found were those where the confidence intervals of the patient profile and normal averaged profile did not overlap.

FISH

Blood was set up for FISH analysis by standard protocols. Ten BAC clones (RP11-11P7, RP11-140K14, RP11-122N11, RP11-235F10, RP11-112G9, RP11-252K12, RP11-31B7, RP11-92C1, RP11-23H1, and RP11-141K9) were selected from a human genomic library at the Children's Hospital Oakland Research Institute (Oakland, CA). BAC probes were isolated by standard procedure and labeled with FITC-12-dUTP or Texas Red-5-dUTP by standard nick translation. The probe (60 ng) was precipated with Human Cot-1 DNA (1 μg) and resuspended in above hybridization buffer, then, denatured and hybridized with prepared subject's metaphase slide overnight at 37 °C. After postwash, the chromosomes were counterstained with DAPI II.

Results

CGH revealed a chromosome 8p22-8p23.1 duplication in all of the patients studied (Fig. 2). We employed BAC-FISH to confirm and further define the duplicated region. We have, thus far, delimited the duplicated region between BAC probes RP11-112G9 and RP11-92C1 (approximately 3.5 Mb; Table 2). CGH and high-resolution chromosome analysis from two sets of available parents, (paternity demonstrated by Identifiler [Applied Biosystems, Foster City, CA] according to manufacturer's instructions) all yielded normal results. However, the two mothers studied by BAC-FISH revealed an inversion of BAC probe RP11-122N11 at 8p23.1 (Fig. 3; Table 2). The remainder of the BAC-FISH studies from these parents was normal. No duplications were detected. Further BAC-FISH studies using different fluorochromes (RP11-235F10, RP11-122 N11, and RP11-252K12) revealed that the two KS children inherited their mother's submicroscopic inversion at 8p23.1 and have a duplication involving the 8p22-8p23.1 region (Fig. 3). BAC-FISH studies of the remaining four KS patients also revealed an

identical submicroscopic inversion at 8p23.1 (Table 2). We demonstrated that the inversion and duplication of the 8p22-8p23.1 region is on the same chromosome (Fig. 4). One out of 20 controls studied by BAC-FISH was found to have a heterozygous submicroscopic inversion at 8p22-8p23.1 (Fig. 5; Table 2). This inversion is larger than that found in the KS patients and the two mothers studied but involves the common BAC-FISH probe RP11-122N11. None of the controls showed duplication of the 8p22-8p23.1 region.

Discussion

We have demonstrated an 8p22-8p23.1 duplication using CGH in six unrelated KS patients. This observation was confirmed using BAC-FISH in all cases that delimited the duplicated region to approximately 3.5 Mb. No duplication of this region was found in the two parents or 20 controls by either CGH or BAC-FISH. Two out of two mothers of KS patients and one out of 20 controls were found to have a heterozygous submicroscopic inversion at 8p23.1. An inversion polymorphism in this region between two 8p olfactory receptor gene clusters has been found in 26% of a population of European descent (18) and 27% of a population of Japanese descent (19). The precise mechanism by which the described duplication occurs remains unproven. Repeat gene clusters have been identified, flanking multiple microdeletion and several microduplication syndromes. Unequal crossing over between these flanking repeats has been implicated as the mechanism underlying these genomic disorders. The most well studied prototypic submicroscopic genomic disorder is the reciprocal Charcot-Marie-Tooth 1 A (CMT1A) duplication/hereditary neuropathy with liability to pressure palsy deletion. Complex low-copy repeats have now been identified at each of the inversion breakpoints at chromosome 8p23 (19). Each of the two low-copy repeats contains several olfactory receptor genes and has been implicated as a potential cause of an 8p23 interstitial duplication (18). Our candidate-duplicated region is between the two recently identified complex low-copy repeats [REPD and REPP (19)]. Finding a paracentric inversion without duplication of this region in the mothers of our tested KS patients, as well as the inversion and duplication on the same chromosome in all the tested KS patients implicates its involvement in the underlying etiology of the duplication. Alternatively, the polymorphic inversion may not be related to the observed duplication. Further studies will be necessary to determine whether a causal relationship exists. Several genes are identified in the candidate region and include B lymphoid tyrosine kinase (BLK), GATA-binding protein 4 (GATA4), farnesyl-diphosphate farnesyl transferase (FDFT1), and cathepsin B (CTSB).

Although not observed in our cases, inverted duplications of 8p are well described and are often accompanied by a telomeric deletion (8p23.1-8pter) (20, 21). Subtelomeric FISH, CGH, and BAC-FISH probes from 8p23.1 to 8pter did not demonstrate a deletion in any of our KS patients studied (Table 2). Duplication of 8p23.2 has been reported as a normal variant (22) and was not found in any of our KS patients. Controversy still exists as to the clinical significance of 8p23.1 duplications. These duplications have been documented in those with no or minimal pathology, as well as those with congenital heart defects, facial dysmorphism, and developmental delay (23-25). Duplications that include

8p22 have consistently resulted in phenotypic abnormalities that may vary with the extent of the duplication. These abnormalities have included mental retardation, facial dysmorphism, congenital heart defects, skeletal anomalies including scoliosis, and cleft lip and palate, all of which have been described in KS (1, 2, 26, 27).

Based on our findings, we propose that KS, with its varied clinical phenotype, may be a contiguous gene duplication syndrome of 8p22-8p23.1, whose molecular mechanism appears to be gene dosage, similar to CMT1A (28).

Further studies defining the molecular etiology of this duplication are in progress, aiming to identify the precise molecular defect, contiguous genes, and their expression patterns, and to determine genotype/phenotype correlations with potentially different-sized duplications. A larger cohort of KS patients is being studied to identify the proportion of cases with duplication of our candidate region. Point mutations, genetic heterogeneity, and KS-like phenotypes may explain those patients without a duplication in this region. The previously reported cases with other chromosome aberrations may represent coincidental findings, genetic heterogeneity, or different clinical entities with KS-like features.

We have developed a protocol using CGH and BAC-FISH that could be used in the diagnosis of KS, if these observations are confirmed. As KS is thought to be an underdiagnosed syndrome (29), testing will potentially increase the number of patients recognized with this disorder. Identification of the specific genes responsible for KS will increase our understanding of human growth and mental development.

In summary, we have demonstrated an approximately 3.5 Mb duplication of chromosome 8p22-8p23.1 in multiple cases of KS from different races, suggesting a possible common etiologic basis for this disorder.

Acknowledgements

We would like to thank all the participating families, the Kabuki Syndrome Network, and Applied Imaging for use of the High Resolution CGH software. We are very appreciative of the thoughtful comments and suggestions from Aubrey Milunsky, MD, DSc, Herman Wyandt, PhD, and Thomas A Maher, BS. We also thank Yanmei Huang, PhD for her help in preparing the figures.

References

1. Wessels MW, Brooks AS, Hoogeboom J, Niermeijer MF, Willems PJ. Kabuki syndrome: a review study of three hundred patients. Clin Dysmorphol 2002: 11: 95-102.
2. Matsumoto N, Niikawa N. Kabuki make-up syndrome. A Rev Am J Med Genet 2003: 117C: 57-65.

3. Niikawa N, Matsuura N, Fukushima Y, Ohsawa T, Kajii T. Kabuki make-up syndrome. a syndrome of mental retardation, unusual facies, large and protruding ears, and postnatal growth deficiency. J Pediatr 1981: 99: 565-569.
4. Kuroki Y, Suzuki Y, Chyo H, Hata A, Matsui I. A new malformation syndrome of long palpebral fissures, large ears, depressed nasal tip, and skeletal anomalies associated with postnatal dwarfism and mental retardation. J Pediatr 1981: 99: 570-573.
5. Niikawa N, Kuroki Y, Kajii T et al. Kabuki make-up (Niikawa-Kuroki) syndrome: a study of 62 patients. Am J Med Genet 1988: 31: 565-589.
6. Tsukahara M, Kuroki Y, Imaizumi K, Miyazawa Y, Matsuo K. Dominant inheritance of Kabuki make-up syndrome. Am J Med Genet 1997: 73: 19-23.
7. Courtens W, Rassart A, Stene JJ, Vamos E. Further evidence for autosomal dominant inheritance and ectodermal abnormalities in Kabuki syndrome. Am J Med Genet 2000: 93: 244-249.
8. Li M, Zackai EH, Niikawa N, Kaplan P, Driscoll DA. Kabuki syndrome is not caused by a microdeletion in the DiGeorge/velocardiofacial chromosomal region within 22q11. 2. Am J Med Genet 1996: 65: 101-103.
9. Lees M, Prescott NJ, Winter RM, Wilson LC. Kabuki syndrome with lip pits: a possible microdeletion syndrome involving the van der Woude locus. J Med Genet 1999: 36 (Suppl. 1): 5172.
10. Makita Y, Yamada K, Miyamoto A, Okuno A, Niikawa N. Kabuki make-up syndrome is not caused by a microdeletion close to the van der Woude syndrome critical region at 1q32-q41. Am J Med Genet 1999: 86: 285-288.
11. Digilio MC, Marino B, Toscano A, Giannotti A, Dallapiccola B. Congenital heart defects in Kabuki syndrome. Am J Med Genet 2001: 100: 269-274.
12. Kirchhoff M, Gerdes T, Maahr J et al. Detection of chromosomal gains and losses in comparative genomic hybridization analysis based on standard reference intervals. Cytometry 1998: 31: 163-173.
13. Kirchhoff M, Rose H, Maahr J et al. High resolution comparative genomic hybridisation analysis reveals imbalances in dyschromosomal patients with normal or apparently balanced conventional karyotypes. Eur J Hum Genet 2000: 8: 661-668.
14. Joly G, Lapierre J-M, Ozilou C et al. Comparative genomic hybridization in mentally retarded patients with dysmorphic features and a normal karyotype. Clin Genet 2001: 60: 212-219.
15. Ness GO, Lybaek H, Houge G. Usefulness of high- resolution comparative genomic hybridization (CGH) for detecting and characterizing constitutional chromosome abnormalities. Am J Med Genet 2002: 113: 125-136.
16. Milunsky JM, Cheney SM. Kabuki syndrome in a Haitian patient. Am J Med Genet 2001: 100: 172-174.
17. Kabuki Syndrome Network. Kabuki syndrome clinical database survey. 2001: http://www.kabukisyndrome.com/KSN.html .
18. Giglio S, Broman KW, Matsumoto N et al. Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. Am J Hum Genet 2001: 68: 874-883.

19. Sugawara H, Harada N, Ida T et al. Complex low-copy repeats associated with a common polymorphic inversion at human chromosome 8p23. Genomics 2003: 82: 238-244.
20. Engelen JJ, de Die-Smulders CE, Fryns JP et al. Partial trisomy and monosomy 8p due to inversion duplication. Clin Genet 1994: 45: 203-207.
21. Guo WJ, Callif-Daley F, Zapata MC, Miller ME. Clinical and cytogenetic findings in seven cases of inverted duplication of 8p with evidence of a telomeric deletion using fluorescence in situ hybridization. Am J Med Genet 1995: 58: 230-236.
22. Harada N, Takano J, Kondoh T et al. Duplication of 8p23.2: a benign cytogenetic variant? Am J Med Genet 2002: 111: 285-288.
23. Barber JC, Joyce CA, Collinson MN et al. Duplication of 8p23.1: a cytogenetic anomaly with no established clinical significance. J Med Genet 1998: 35: 491-496.
24. Kennedy SJ, Teebi AS, Adatia I, Teshima I. Inherited duplication, dup (8) (p23.1p23.1) pat, in a father and daughter with congenital heart defects. Am J Med Genet 2001: 104: 79-80.
25. Tsai CH, Graw SL, McGavran L. 8p23 duplication reconsidered: is it a true euchromatic variant with no clinical manifestation? J Med Genet 2002: 39: 769-774.
26. Feldman GL, Weiss L, Phelan MC, Schroer RJ, Van Dyke DL. Inverted duplication of 8p: ten new patients and review of the literature. Am J Med Genet 1993: 47: 482-486.
27. de Die-Smulders CE, Engelen JJ, Schrander-Stumpel CT et al. Inversion duplication of the short arm of chromosome 8: clinical data on seven patients and review of the literature. Am J Med Genet 1995: 59: 369-374.
28. Lupski JR, Montes de Oca-Luna R, Slaugenhaupt S et al. DNA duplication associated with Charcot-Marie-Tooth disease type 1a. Cell 1991: 66: 219-232.
29. Burke LW, Jones MC. Kabuki syndrome: underdiagnosed recognizable pattern in cleft palate patients. Cleft Palate Craniofac J 1995: 32: 77-84.

Clinical Genetics Volume 64 Issue 6 Page 509 - December 2003