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SHORT REPORT

Genome-wide screening using array-CGH does not reveal microdeletions/microduplications in children with Kabuki syndrome

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Kabuki syndrome (KS) is a rare multiple congenital anomaly/mental retardation syndrome. It is characterized by a distinct facial appearance, mental retardation, postnatal growth retardation, skeletal anomalies, unusual dermatoglyphics and fetal fingertip pads. It has previously been speculated that KS is caused by a microdeletion or duplication. In a recent report, an interstitial microduplication of 8p22–23.1 was presented in several cases with this disorder. We investigated 10 Caucasian patients diagnosed with KS by fluorescence *in situ* hybridization and microsatellite markers located on 8p22–23.1. Using the same clones that were previously reported to be duplicated on chromosome 8p, we could exclude the duplication in all our patients. In addition, we performed a genome-wide screening on this group of patients using array-based comparative genomic hybridization containing BAC clones spaced at approximately 1 Mb intervals across the genome and could not find any evidence for gene dose alterations. The characteristics of KS are variable, a fact that complicates the diagnosis of this disorder. It is possible that we will find genetic heterogeneity among Kabuki patients, and therefore it is unlikely that all patients have an interstitial 8p duplication. We conclude that the etiology of KS remains to be solved and further genetic studies are necessary to delineate its genetic cause.
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Introduction

Kabuki syndrome (KS) was first independently described by Kuroki *et al*[1] and Niikawa *et al*.[2] It was initially believed to be fairly uncommon outside of Japan, but is now well recognized, and more than 350 cases have been reported worldwide.[3,4] Although clinical manifestations of KS are well established, it is probably greatly underdiagnosed and the number of medical professionals who are familiar with the syndrome is still growing. Diagnosis is further

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complicated by the fact that the spectrum of symptoms is very diverse. Several chromosome abnormalities have been reported to be associated with KS. Matsumoto and Niikawa[3] speculated on a possible microdeletion or microduplication as the cause of the disorder.

For successful detection of submicroscopic chromosome aberrations in patients with idiopathic mental retardation,[5-10] different genome-wide high–resolution screening technologies have been applied during the past few years. Recently, Milunsky and Huang[11] presented a 8p22–23.1 duplication as a common etiologic basis for KS as a result of a screening of six patients using HR comparative genomic hybridization (HR-CGH). This finding prompted us to investigate 10 KS patients for 8p22–23.1 duplications using fluorescence *in situ* hybridization (FISH) and microsatellite markers. In addition, we performed a whole genome screen on the same cases using array-based comparative genomic hybridization (array-CGH) in order to detect microdeletions or microduplications.

Materials and methods

Patient selection

Blood samples were collected from 10 patients who met cardinal clinical diagnostic criteria for KS. All patients were Caucasian of Swedish or Danish origin and all of them were evaluated by a clinical geneticist. Table 1 summarizes the major clinical findings.

Cytogenetic analysis

Metaphases from patient peripheral blood lymphocytes were prepared according to standard procedures. Routine chromosome analysis (450–500 bands) was performed in all cases without revealing any abnormalities.

Array-CGH

Genomic DNA was extracted from peripheral venous blood samples or cultured fibroblasts using Puregene blood kit (Gentra Systems Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. Genomic DNA from the patients was digested into fragments of 100–2000 bp by overnight incubation with *Dpn*II (New England Biolabs Inc., Beverly, MA, USA) at 37°C and checked on a 2% agarose gel. The DNA fragments were purified using Qiaquick PCR purification kit (Qiagen GmbH, Hilden, Germany).

Array-CGH was performed using arrays containing 2600 BAC clones (Spectral Genomics Inc., Houston, TX, USA) according to the manufacturer's protocol with some modifications, previously described by Schoumans *et al*.[9]

Microsatellite analysis

Three microsatellite markers D8S550, D8S552 and D8S520 located on 8p22–23.1 were selected from Ensembl Genome Browser (http://ensembl.org, Build 34).[12] Oligonucleotide primers labeled with NED (fluorescent dye) were obtained from Applied Biosystems. Polymerase chain reaction (PCR) amplification of genomic DNA from patients (50 ng) was performed according to the manufacturer's protocol and analyzed using an automated sequencer (ABI PRISM® 3100 Avant Genetic analyzer, Applied Biosystems, Foster City, CA, USA). Resulting genotype data were analyzed using Genemapper® 3.5 software from ABI (Applied Biosystems). Alleles from patients and their

Table 1 Clinical findings

Case	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Age (years)	4	15	6	14	7	2	5	8	9	7
Gender	F	M	F	M	F	F	F	F	F	F
Prenatal growth retard.	+	−	−	+	−	−	−	+	+	NA
Postnatal growth retard.	+	+	+	+	−	−	−	+	(+)	(+)
Mental retardation	MMR	ModMR	MMR/N	ModMR	SMR	SMR	SMR	MMR	MMR	ModMR
Seizures	+*	+	−	+	+	+	+	−	+	−
Hypotonia	+	+	+	−	+	+	+	(+)	+	+
Long palpebral fissures	+	+	+	+	+	+	+	+	+	+
Eversion of lower eyelid	+	+	+	−	+	+	+	+	+	+
Arched eyebrows	+	+	+	+	+	+	−	+	+	+
Large ears	+	+	+	+	+	+	+	+	+	+
Depressed nasal tip	+	+	+	+	+	+	+	(+)	+	−
Abnormal dentition	−	+	+	+	+	NA	−	+	NA	+
Skeletal abnormalities	+	−	+	+	−	−	−	+	NA	NA
Brachydactyly V	+	+	+	+	+	NA	NA	−	−	NA
Fetal finger pads	+	+	+	+	+	+	+	NA	+	+
Congenital heart defect	+	−	−	−	−	−	−	−	+	−
Lip pits	−	−	−	−	+	−	−	−	−	−
Renal abnormality	−	−	+	+	−	NI	NI	−	−	−

ModMR = moderate MR; MMR = mild MR; SMR = severe MR; N = normal; F = female; M = male; NI = not investigated; (+) = slightly; *febrile convulsion; NA = data not available.

parents were scored, and the microsatellite allele signal intensities were in addition compared to normal DNA controls.

FISH analysis

The following clones located on 8p22–23.1 were used for FISH analysis: RP11-92C1, RP11-31B7 and RP11-112G9. Clone RP11-23H1 was used as control. For investigation of polymorphic clones, RP11-259N12, RP11-316O14, RP11-100C24 and RP11-125A5 were used. All clones were obtained from The Welcome Trust Sanger Institute (Cambridge, UK). Bacterial cultures and DNA isolation were performed according to the BAC-PAC miniprep protocol (http://www.biologia.uniba.it/rmc). Probes were labeled with FITC–dUTP (NEN Life Science Products, Boston, MA, USA) or SpectrumOrange–dUTP (Vysis Inc, Downers Grove, IL, USA) by nick translation, and FISH analyses were performed according to a standard protocol on metaphase slides that were prepared from lymphocyte cultures of peripheral blood. The slides were analyzed on a Zeiss Axioplan 2 (Carl Zeiss, Göttingen, Germany) epifluorescence microscope and images were captured using a cooled CCD camera (Sensys Photometrics, München, Germany) and SmartCapture 2 software (DigitalScientific Ltd, Cambridge, UK). FISH signals were examined on both metaphase chromosomes and interphase nuclei. Inverted DAPI staining was used for chromosome identification during FISH analysis.

Results

The results of all experiments are summarized in Table 2.

We investigated in detail chromosome region 8p22–23.1 in all 10 patients diagnosed with KS using the same clones reported by Milunsky and Huang.[11] We performed metaphase and interphase FISH analysis on five patients (case 1, 2 and 4–6). In the other five cases (case 3 and 7–10), cells were not available for FISH analysis and we therefore examined these patients using three microsatellite markers located in the same region. None of the clones or microsatellite markers revealed a duplication in our KS patients.

In addition, we used a whole genome array containing 2600 clones with an average resolution of 1 Mb to screen the patients for microdeletions or microduplications. Among these, three clones were covering >3 Mb fragment at 8p22–8p23.1 (RP11-252K12, RP11-80B8 and RP11-90O17). None of these clones revealed a duplication in any of the patients investigated, nor did we observe any other chromosomal imbalances.

However, in some patients we observed gains or losses on four BAC clones (listed in Table 3).

Discussion

We have studied 10 cases with KS for gene dose alterations. We were unable to repeat the observation of chromosome band 8p22–23.1 duplications with FISH or microsatellite analysis in our patients. Furthermore, array-CGH analysis with 2600 clones evenly distributed over the human genome with 1 Mb intervals did not reveal any alterations, except for four polymorphic clones. Variations in the genetic material among phenotypically normal individuals have been known to occur in the form of single base pair changes. However, very recently Iafrate *et al*[13] reported large-scale copy-number variations involving gains or losses of several kilobases to hundreds of kilobases of genomic DNA. One of the polymorphic clones observed in our study (RP11-259N12) was also reported by Iafrate *et al*

Table 3 Polymorphic clones

Clone ID	*Cytogenetic location*	*Gain*	*Loss*	*Case*
RP11-259N12	1p21.1	1	2	1, 3, 8
RP11-316O14	2q35	4	0	3, 4, 6, 7
RP11-100C24	13q21.1	2	0	3, 8
RP11-125A5	14q12	0	3	1, 4, 6

Table 2 Summary of gene dose analysis

	Microsattelite			*FISH*			
Case	*D8S550*	*D8S552*	*D8S520*	*RP11-92C1*	*RP11-31B7*	*RP11-112G9*	*Array-CGH 2600 clones*
1				ND	ND	ND	NA
2				ND	ND	ND	NA
3	NI	ND	ND				NA
4				ND	ND	ND	NA
5				ND	ND	ND	NA
6				ND	ND	ND	NA
7	ND	ND	ND				NA
8	ND	ND	ND				NA
9	ND	ND	ND				NA
10	NI	ND	NI				NA

NA = no abnormality; ND = no duplication; NI = not informative.

and was only confirmable by FIBER–FISH analysis. We experienced the same difficulties as Iafrate *et al* to confirm gains or losses in our patients by conventional metaphase- and interphase-FISH analysis using the four clones listed in Table 3. However, the same clones are listed in the public accessible Genome Variation Database at http://projects.tcag.ca/variation, since they are frequently polymorphic in healthy individuals and they are therefore not considered to contribute to the cause of KS.

Taken together, our results suggest that gene dose alterations in 8p are unlikely to be the cause in our series of KS cases. Miyake *et al*,[14] who performed a thorough investigation of region 8p22–23.1 in 26 KS patients, came to the same conclusion.

The previously reported 8p duplication in six KS patients was relatively large in size (3.5 Mb), which should be easily detected. Our inability to detect the duplication might have different explanations. Firstly, the 8p region is prone to genetic rearrangements due to large olfactory receptor (OR)-gene clusters causing unequal crossovers between two OR clusters in this region.[15] Imbalances of the 8p region have been frequently reported with a wide range of different phenotypes, and also in individuals without clinical manifestations.[16,17] The duplication reported by Milunsky and Huang could thus be a normal variant present in their patient population.

Secondly, the wide spectrum of characteristics of KS complicates the diagnosis and it is therefore likely that KS is clinically and genetically heterogeneous.

In conclusion, our data demonstrate that the etiology of KS remains unknown. It may be worthwhile to further investigate this group of patients using a microarray covering the whole genome at a much higher resolution.[18]

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