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ORIGINAL ARTICLE

Age-dependent change in behavioral feature in Rubinstein-Taybi syndrome

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ABSTRACT **Rubinstein-Taybi syndrome (RTS) is characterized by developmental delay, postnatal growth retardation, typical facial appearance, and broad thumbs and big toes. The behavioral phenotype of children with RTS has been described as friendly and having good social contacts; however, a short attention span and hyperactivity are sometimes present. Little attention has been paid to the behavioral aspects of adults with RTS. We conducted an observational study focusing on behavioral problems in adolescents and adults with RTS compared with children with RTS. A total of 63 patients with RTS and their caretakers answered self-administered questionnaires regarding behavioral features including the Child Behavior Checklist (CBCL). High total CBCL scores were observed, and the mean score was beyond the clinical cut-off point. After stratification into two groups according to age, the older group (≥14 years) displayed statistically significant higher scores for *Anxious/Depression* ($P = 0.002$) and *Aggressive Behavior* ($P = 0.036$) than the younger group (≤13 years). In analyses of single items, statistically significant differences between the younger group and the older group were found for 'Nervous, high-strung, or tense' (31.3% vs 67.7%, $P = 0.004$) and 'Too fearful or anxious' (37.5% vs 64.5%, $P = 0.032$). Here, we showed that the specific behavioral phenotypes of RTS change during adolescence, with anxiety, mood instability, and aggressive behavior emerging as patients age. A clear need exists to follow-up patients with RTS to catch the eventual emergence of psychiatric problems with age. If necessary, pharmacological treatment should be considered.**

Key Words: age-dependent change, behavioral problem, Child Behavior Checklist, depression, Rubinstein-Taybi syndrome

INTRODUCTION

Rubinstein-Taybi syndrome (RTS; OMIM 180849) is characterized by developmental delay, postnatal growth retardation, microcephaly, typical facial appearance, and broad thumbs and big toes (Rubinstein and Taybi 1963). Chromosomal or molecular abnormalities are found in about 55% of cases (Hennekam 2006; Stef et al. 2007). Its occurrence is generally sporadic, and the condition can be caused by a micro-deletion of chromosome 16p13.3 or by a mutation in either the gene encoding the transcriptional coactivator CREB-binding protein (CREBBP) on chromosome 16p13 or the EP300 gene on chromosome 22q13 (Bartsch et al. 2010). The diagnosis of RTS, however, remains primarily clinical.

In the original report by Rubinstein and Taybi (1963), the behavioral phenotype of RTS was described as hyperactivity and emotional lability. Later studies demonstrated that children with RTS were generally friendly and more readily accepted social contacts; however, RTS children sometimes have irritability and impulsivity (Gotts and Liemohn 1977; Stevens et al. 1990). The first epidemiological study to use a standardized psychometric tool, the Child Behavior Checklist (CBCL), focused on the psychological aspects of RTS. It showed that the most frequently reported behavioral problems were 'acts too young for age', 'can't concentrate', 'poorly coordinated, clumsy', and 'likes to be alone' (Hennekam et al. 1992). A recent comparative study between children with RTS and a control group concluded that a short attention span, stereotypical behavior, and poor coordination were common behavioral features in RTS (Galera et al. 2009).

There is emerging evidence that RTS may be associated with specific behavioral problems; however, little attention has been paid to the behavioral aspects of adults with RTS. Levitas and Reid (1998) performed psychiatric evaluations of adults with RTS who had been referred for behavioral problems. The resulting diagnoses were clustered into mood disorders and obsessive-compulsive disorder spectrum. Similarly, a case report described a 39-year-old woman who presented with symptoms of severe hyperactivity, short attention span, mood instability, and aggressive outbursts in a cyclical pattern (Hellings et al. 2002). Verhoeven et al. (2010) also reported another adult patient with RTS who was admitted because of depressive mood, impulsivity, and temper outbursts. Hennekam (2006) reported that short attention span, stubbornness, lack of persistence, and emotional lability became increasingly apparent during early adulthood, leading to uncertain behavior and occasional aggressiveness. These reports in adult patients support a potential link between RTS and mood instability/aggressive behavior. Whether a specific age-dependent behavioral pattern of RTS exists remains to be determined using a standardized psychometric tool. Here, we report an observational study focusing on behavioral problems in adolescents and adults with RTS, compared with children with RTS.

MATERIALS AND METHODS

Patients

Patients with RTS were recruited from the Rubinstein-Taybi Syndrome Family Support Group, Japan (Cosmos). This nationwide

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organization was formed in 1994 by and for families of individuals with RTS and presently includes about 100 family members. Inclusion in the present study required a confirmed diagnosis of RTS made by clinical geneticists based on the presence of diagnosis criteria, including abnormalities of the face, broad and angulated thumbs and big toes, growth retardation, and mental retardation. Molecular tests for the diagnosis of RTS were not required.

Study design

A postal questionnaire was sent in December 2010 to all individuals on the mailing list of the Rubinstein-Taybi Syndrome Family Support Group, Japan. The consent form and questionnaires were sent with a return envelope to each participant. They were invited to complete the self-administered questionnaires regarding the socio-behavioral features of their children, including the Child Behavior Checklist, and to return the forms to our hospital. The Ethics Committee at Keio University approved the study protocol.

Child Behavior Checklist (CBCL)

The CBCL is composed of questions related to a total of 113 items categorized into the following eight subscale items: (I) *Withdrawn*; (II) *Somatic Complaints*; (III) *Anxious/Depressed*; (IV) *Social Problems*; (V) *Thought Problems*; (VI) *Attention Problems*; (VII) *Delinquent Behavior*; and (VIII) *Aggressive Behavior*. *Internalizing* (I + II + III), *Externalizing* (VII + VIII), and *Total Problems* were also derived from these subscales. Caretakers answered each question by selecting one of three answer choices. The raw scores for 11 scales were calculated from the scores for the answers. Then, the raw scores were converted into T-scores according to the profile sheet based on a normal Japanese population (Achenbach 1991; Itani et al. 2001). In this study, the T-scores of patients aged under 4 years and over 15 years were estimated according to the standard profile of 4–11 years and 12–15 years, respectively. Previously, a higher score was found to be associated with a greater likelihood of problematic behavior for that scale (Achenbach 1991). A T-score > 63 in *Total Problems* and T-score > 67 in each subscale suggested that the patient should have some clinical problems.

Data analyses

The distributions of sex, age, presence or absence of genetic abnormalities, and CBCL scores were examined for all the subjects. The patients were then stratified according to age into two groups. Patients younger than the 50%tile (≤13 years) were classified as the younger group, and those who were older (≥14 years) were classified as the older group. We compared the T-scores for the 11 scales that were examined between the two groups (Mann-Whitney *U*-test). We also compared the two groups for the single items. For these single items and to assess clinically relevant problems, we constructed dichotomous dependent variables by considering a rating of 0 as 'No' versus a rating of 1 or 2 as 'Yes'. To examine a possible gene-behavior link, we finally compared the T-scores between the two age groups only in subjects with genetic abnormalities associated with RTS. An alpha level of 0.05 (two-tailed) was adopted as the criterion for statistical significance. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Background of the subjects

A total of 63 patients (37 men and 26 women), aged 1–38 years (median: 13 years) were studied. Molecular tests had been performed in 20 of 63 patients (32%). Molecular abnormalities (i.e. intragenic mutations or deletions in CREBBP or EP300 gene), had been identified in nine of 20 patients (45%). The mean T-scores were 63.5 for *Total Problems*, 57.2 for *Internalizing*, 56.1 for *Externalizing*, 58.1 for *Withdrawn*, 57.9 for *Somatic Complaints*, 56.6 for *Anxious/Depression*, 67.3 for *Social Problems*, 62.1 for *Thought Problems*, 67.1 for *Attention Problems*, 57.8 for *Delinquent Behavior*, and 56.1 for *Aggressive Behavior*. The T-score for *Total Problems* was beyond the clinical cut-off point of 63. Questions that were answered 'Yes' by more than 70% of patients included 'Acts too young for his/her age' (97%), 'Can't concentrate, can't pay attention for long' (92%), 'Poorly coordinated or clumsy' (87%), and 'Clings to adults or too dependent' (71%).

CBCL scores after stratification according to age

Figure 1 shows mean CBCL scale scores among subjects with RTS after stratification according to interquartile age (≤7, 8–13, 14–22, and ≥23 years). The mean *Total Problem* score in each group was 62, 63, 65, and 64, respectively. The scores for the three groups aged 8 years or older exceeded the cut-off value of 63 points. Two CBCL scales, *Social Problems*, and *Attention Problems*, were beyond the cut-off point of 67 in all the age groups. The scores for *Anxious/Depression* and *Aggressive Behavior* tended to increase with age (Fig. 1). After stratification into two groups according to age, the older group (≥14 years) displayed statistically significant higher scores of *Anxious/Depression* ($P = 0.002$) and *Aggressive Behavior* ($P = 0.036$) than the younger group (≤13 years) (Table 1a). Among nine subjects with identified genetic abnormalities, the older group tended to display higher scores for *Anxious/Depression* ($P = 0.037$) and *Aggressive Behavior* ($P = 0.157$) than the younger group (Table 1b). In analyses of single items, statistically significant differences between the younger group and the older group were found for 'Nervous, high-strung, or tense' (31.3% vs 67.7%, $P = 0.004$), 'Can't concentrate, can't pay attention for long' (84.4% vs 100.0%, $P = 0.022$), and 'Too fearful or anxious' (37.5% vs 64.5%, $P = 0.032$) (Table 2).

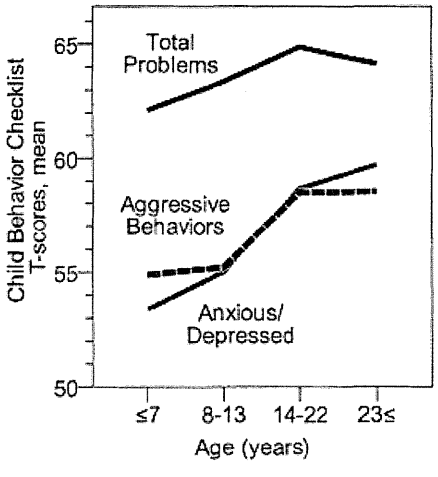

Fig. 1 Temporal scores for the Child Behavior Checklist scales in subjects with Rubinstein-Taybi syndrome. Line charts represent mean scores for CBCL scales in each age group. T-score, standardized score calculated from the raw score for each subject based on a normal population. Black solid line, *Total Problems*; grey solid line, *Anxious/Depressed*; dotted line: *Aggressive Behaviors*.

Table 1 T-scores for Child Behavior Checklist (CBCL) compared between younger and older groups with Rubinstein-Taybi syndrome

CBCL T-scores, median (interquartile range)	Younger group N = 32	Older group N = 31	*P*-value
All subjects (63 subjects)			
Total problems	63.0 (57.0–68.0)	64.0 (61.0–68.0)	0.268
Internalizing	57.0 (50.5–61.0)	61.0 (54.0–64.0)	0.072
Externalizing	54.0 (47.0–60.0)	57.0 (54.0–61.0)	0.069
Withdrawn	56.0 (53.0–63.0)	59.0 (50.0–63.0)	0.878
Somatic complaints	55.0 (50.0–67.0)	54.0 (54.0–64.0)	0.972
Anxious/depressed	52.0 (50.0–56.5)	60.0 (52.0–63.0)	0.002*
Social problems	65.0 (63.0–69.0)	68.0 (65.0–70.0)	0.150
Thought problems	62.5 (50.0–73.0)	56.0 (50.0–73.0)	0.829
Attention problems	69.0 (62.0–72.0)	67.0 (63.0–70.0)	0.756
Delinquent behavior	54.5 (52.0–65.0)	55.0 (50.0–63.0)	0.878
Aggressive behavior	52.5 (50.0–58.5)	57.0 (51.0–61.0)	0.036*
CBCL T-scores, median (interquartile range)	Younger group N = 5	Older group N = 4	*P*-value
Subjects with genetic abnormalities (9 subjects)			
Total problems	63.0 (61.0–66.0)	66.0 (63.0–69.5)	0.389
Internalizing	61.0 (56.0–61.0)	59.5 (56.5–63.0)	0.701
Externalizing	51.0 (48.0–57.0)	59.5 (54.5–62.0)	0.176
Withdrawn	53.0 (53.0–59.0)	62.0 (56.0–64.0)	0.385
Somatic complaints	67.0 (55.0–67.0)	57.0 (50.0–65.0)	0.211
Anxious/depressed	52.0 (52.0–58.0)	59.0 (58.0–61.5)	0.037*
Social problems	63.0 (63.0–70.0)	70.0 (68.0–71.5)	0.118
Thought problems	69.0 (56.0–70.0)	72.5 (60.0–76.5)	0.385
Attention problems	69.0 (67.0–69.0)	67.0 (65.0–74.0)	0.802
Delinquent behavior	65.0 (54.0–65.0)	55.0 (55.0–60.5)	0.800
Aggressive behavior	50.0 (50.0–54.0)	59.0 (53.5–61.0)	0.157

*$P < 0.05$ (Mann–Whitney *U*-test).
T-score, standardized score calculated from raw score of each subject based on a normal population.
Younger group, subjects aged <13 years; Older group, subjects aged >14 years.

DISCUSSION

Here, we showed that the specific behavioral phenotype of RTS changes during adolescence, with anxiety, depression, and aggressive behaviors emerging at that time. The present study represents the first comprehensive assessment of age-dependent changes in behavior using a standardized psychometric tool, the CBCL, and reinforces the observations of previous case reports or case series describing the emergence of nervousness, anxiety, stubbornness, sullenness, and irritability during adolescence or adulthood (Levitas and Reid 1998; Hellings et al. 2002; Verhoeven et al. 2010).

As demonstrated in studies of other congenital malformation syndromes, including Costello syndrome and 22q11.2 deletion syndrome (Galera et al. 2006; Jansen et al. 2007), the CBCL has been instrumental in delineating disease-specific behavioral patterns. However, a limitation of this study is the use of the CBCL in adults, because the T-score of the CBCL Japanese version was standardized only between 4- and 15-year-old children but not at older ages (Itani et al. 2001). More refined evaluations (e.g. standardized diagnostic interviews) are needed to confirm the diagnostic criteria of psychiatric diseases in the adolescent and adult patients. Furthermore, a close longitudinal follow-up of patients with RTS would clarify whether behavioral problems, including mood instability or temper tantrums, need subsequent psychiatric management (e.g. drug treatment with mood stabilizers).

The reason why these psychiatric traits arise during adolescence is unknown at present, although postnatal dysfunction of the CREBBP/EP300 genes in patients with RTS has been postulated to lead to neural alterations during adolescence (Alarcon et al. 2004; Hennekam 2006). Cyclic adenosine monophosphate response element binding protein (CREB) and its cofactor, CREBBP, regulates the expression of many genes involved in the development of the nervous system, learning, memory and cell survival (Viola et al. 2000; Hardingham et al. 2001; Lonze and Ginty 2002). The observations that the CREB protein expressions were significantly decreased in patients with depression speak towards CREB and CREBBP's potential involvement in the pathophysiology of psychiatric diseases (Yuan et al. 2010; Ren et al. 2011). These observations postulate that postnatal dysfunction of the CREBBP/EP300 genes in patients with RTS may play an important role in the

Table 2 Common behaviors compared between younger and older groups with Rubinstein-Taybi syndrome (N = 63)

CBCL single items, number (%)	Younger group N = 32		Older group N = 31		*P*-value
Nervous, high-strung, or tense	10	31.3%	21	67.7%	0.004**
Wets the bed	21	65.6%	9	29.0%	0.004**
Can't concentrate, can't pay attention for long	27	84.4%	31	100.0%	0.022*
Bowel movements outside toilet	18	56.3%	9	29.0%	0.029*
Doesn't seem to feel guilty after misbehaving	18	56.3%	9	29.0%	0.029*
Too fearful or anxious	12	37.5%	20	64.5%	0.032*
Can't sit still, restless, or hyperactive	20	62.5%	12	38.7%	0.059
Can't get his/her mind off certain thoughts; obsessions	5	15.6%	11	35.5%	0.070
Stubborn, sullen, or irritable	11	34.4%	17	54.8%	0.102
Poorly coordinated or clumsy	26	81.3%	29	93.5%	0.143
Acts too young for his/her age	32	100.0%	29	93.5%	0.144

*$P < 0.05$ (Mann–Whitney *U*-test); **$P < 0.01$ (Mann–Whitney *U*-test).
Younger group, subjects aged <13 years; Older group, subjects aged >14 years.

pathophysiology of subsequent behavioral features in RTS and that biological pathway mediated by CREB may well be an important target when pharmacological intervention is to be explored. Several different mouse models of RTS have been created in which CBP or p300 function is genetically altered, and these mutant mice exhibit deficits in synaptic plasticity and memory (Alarcon et al. 2004; Korzus et al. 2004). In addition, these studies have suggested that some of the cognitive deficits observed in individuals with RTS may not simply be due to the reduction of CBP during development but might also result from the continued requirement of specific enzymatic activities throughout life, as some deficits observed in CBP-mutant mice can be ameliorated using inhibitors of enzymes that compensate for a reduction in the functioning of CBP as a CREB co-activator, such as histone deacetylase inhibitors (HDACI) (Alarcon et al. 2004; Korzus et al. 2004).

These animal findings may open the possibility of pharmacological treatment using HDACI for the neurological deficits observed in RTS patients, enabling normal CBP function to be reestablished and alleviating some of their symptoms. In support of this hypothesis, a previous report has described an adult patient with RTS who was successfully treated with valproic acid: valproic acid is an antiepileptic drug that acts as a clinically available HDACI and is already established as a mood stabilizers to alleviate certain psychiatric symptoms such as mood instability, irritability, and aggressiveness even in the absence of seizures (Hellings et al. 2002; Abel and Zukin 2008). If further case reports are accumulated in the future, it may be worthwhile to test the effectiveness of HDACIs as disease-modifying drugs, which are used to modify the natural course of the disease, rather than curing the disease itself. These drugs might not be a cure for RTS, but they can reduce the deteriorations in cognitive deficits and subsequent psychiatric problems.

In summary, we found that the specific behavioral phenotype of RTS changes during adolescence in an age-dependent manner. Anxiety, mood instability, and aggressive behavior tended to emerge as individuals with RTS aged. There is a clear need to follow up patients with RTS to catch the eventual emergence of psychiatric problems with age. If necessary, patients with RTS should be referred to a psychiatrist and pharmacological treatment should be considered.

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BRIEF REPORT

Human Mutation

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KDM6A Point Mutations Cause Kabuki Syndrome

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ABSTRACT: Kabuki syndrome (KS) is a rare congenital anomaly syndrome characterized by a unique facial appearance, growth retardation, skeletal abnormalities, and intellectual disability. In 2010, *MLL2* was identified as a causative gene. On the basis of published reports, 55–80% of KS cases can be explained by *MLL2* abnormalities. Recently, de novo deletion of *KDM6A* has been reported in three KS patients, but point mutations of *KDM6A* have never been found. In this study, we investigated KDM6A in 32 KS patients without an *MLL2* mutation. We identified two nonsense mutations and one 3-bp deletion of *KDM6A* in three KS cases. This is the first report of *KDM6A* point mutations associated with KS.

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KEY WORDS: Kabuki syndrome; *KDM6A*; point mutations; chromosome X

Kabuki syndrome (KS; MIM# 147920), first described by Niikawa and Kuroki in 1981, is a rare congenital anomaly syndrome with the characteristic facial features of a long palpebral fissure and eversion of lateral third of the inferior eyelids [Kuroki et al., 1981; Niikawa et al., 1981]. Individuals with KS also show mild to severe intellectual disability, growth retardation, skeletal abnormalities, and a variety of visceral malformations. Although KS is thought to inherit in autosomal dominant fashion, other inheritance patterns have also been considered [Matsumoto and Niikawa, 2003]. In 2010, whole exome sequencing successfully identified loss-of-function mutations in *MLL2* in KS. *MLL2* maps to 12q13.12 and consists of at least 54 coding exons. *MLL2* encodes a histone H3 lysine 4 (H3K4)-specific methyl transferase and plays important roles in the epigenetic control of active chromatin states. On the basis of recent reports of *MLL2* mutations in KS, the mutation detection rate of *MLL2* in KS is 55–80% [Banka et al., 2012]. Among the published mutations, 73.2% (170/232) were truncation type, and pathogenic missense mutations were mainly localized in exon 48 [Banka et al., 2012].

X-linked inheritance has also been implicated in KS. Sex chromosome abnormalities in KS have been reported many times and some of the clinical manifestations are shared with Turner syndrome; patients showing overlapping features, called "Turner–Kabuki" syndrome, have been reported [Bianca et al., 2009; Dennis et al., 1993; Niikawa et al., 1988; Rodriguez et al., 2008; Stankiewicz et al., 2001; Wellesley and Slaney, 1994]. Common structural abnormalities (inversion, translocation, and ring chromosome) involving Xp11 and Yp11 in the pseudoautosomal region were observed in KS, implying the potential involvement of the regions for pathogenesis in KS [Matsumoto and Niikawa, 2003]. In addition, two unrelated KS patients with ring X (p11.2q13) have been reported [McGinniss et al., 1997; Niikawa et al., 1988]. However, an X-linked gene for KS has not been identified until recently. In 2012, complete or partial de novo deletions of *KDM6A* (MIM# 300228) were identified in three patients with KS [Lederer et al., 2012]. *KDM6A* resides at Xp11.3 and encodes the lysine demethylase 6A (KDM6A) demethylating di- and trimethyl-lysine 27 on histone H3 (H3K27) [Lee et al., 2007]. H3K4 methylation by MLL2/3 is linked to the demethylation of H3K27 by KDM6A [Lee et al., 2007]. These authors sequenced *KDM6A* in their series of 22 patients, but found no point mutations [Lederer et al., 2012]. In this study, we investigated *KDM6A* with regard to point mutations in KS after obtaining written informed consents from families of patients. The institutional review board of Yokohama City University School of Medicine approved this study.

To identify *KDM6A* mutations in KS, we examined this gene's 29 coding exons along with its exon–intron boundaries (NM_021140.2) in 32 KS individuals with no *MLL2* mutation, using high-resolution melting analysis combined with direct sequencing. We identified three mutations: c.3717G>A (p.Trp1239*) in patient 1 (male, hemizygous), c.1555C>T (p.Arg519*) in patient 2 (male, hemizygous), and c.3354_3356delTCT (p.Leu1119del) in patient 3 (female, heterozygous) (Fig. 1). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_021140.2), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. One mutation (c.3354_3356delTCT) occurred de novo; parental samples were unavailable for the other two. Because the two nonsense mutations were outside of the last

Additional Supporting Information may be found in the online version of this article.

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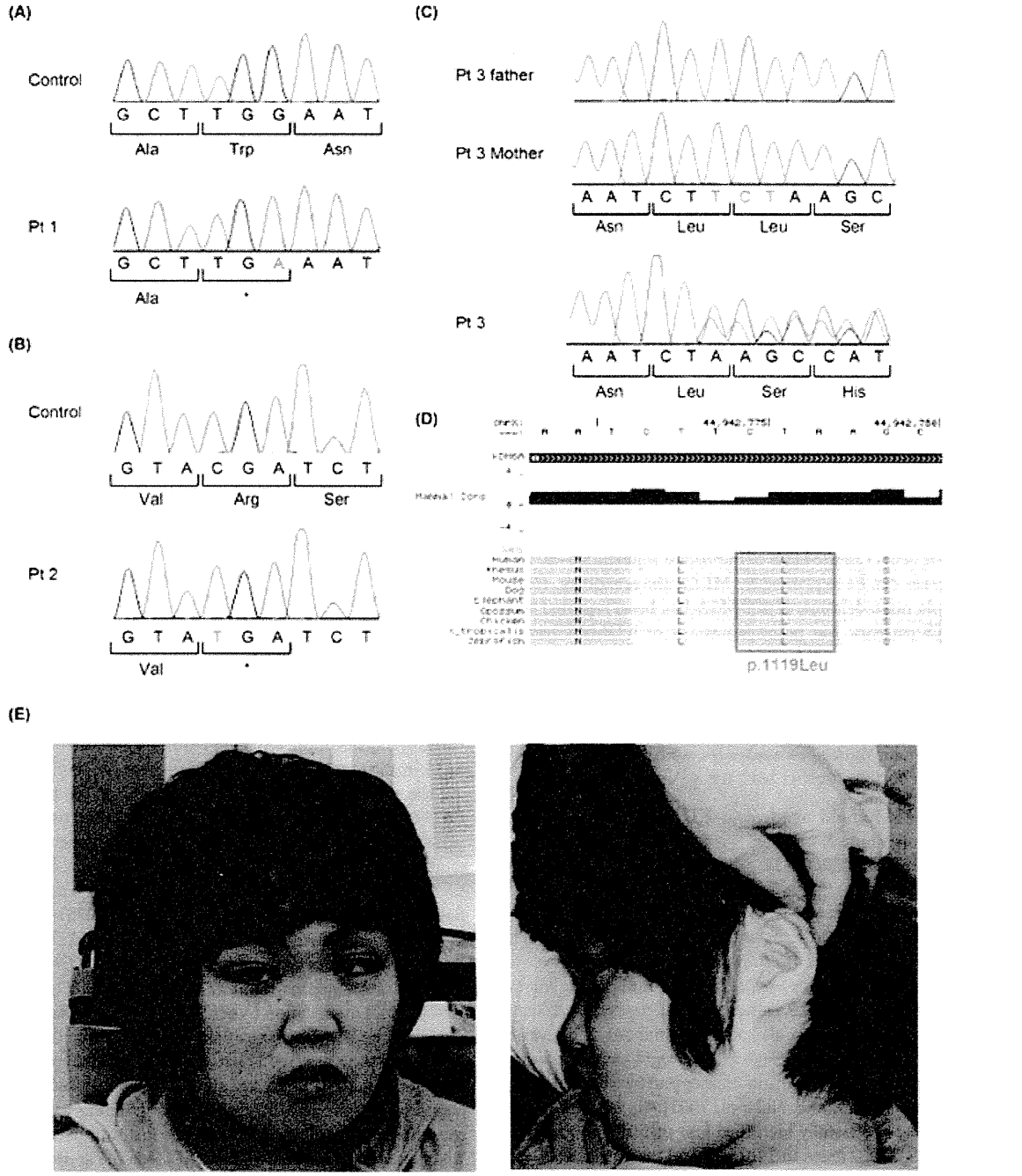

Figure 1. *KDM6A* mutations in three Kabuki syndrome patients. **A–C:** Electropherogram of patient 1: c.3717G>A (p.Trp1239*) (**A**), patient 2: c.1555C>T (p.Arg519*) (**B**), and patient 3: c.3354_3356delTCT (p.Leu1119del) (**C**). Hemizygous changes (**A** and **B**) and a heterozygous change (**C**) can be seen. The altered or deleted nucleotides are written in red. **D:** p. Leu1119 is evolutionarily conserved from zebrafish to human. The position of p.Leu1119 is boxed in red. **E:** Facial photographs of patient 3.

coding exon, and in an exon 55 bp from the 3′ most exon–exon junction, the mutant alleles could be subjected to nonsense-mediated mRNA decay (unfortunately living cells from the patients were unavailable, so we could not test this hypothesis). c.3354_3356delTCT in patient 3 would lead to deletion of one amino acid within the functionally important catalytic Jumonji C (JmjC) domain [Lee et al., 2007]. The amino residue p.Leu1119 is evolutionarily conserved from zebrafish to human (Fig. 1D) and plays an important role in hydrophobic core formation with p.Ile1126 and p.Met1129 to stabilize the JmjC domain [Sengoku and Yokoyama, 2011]. This amino acid deletion may impair helix formation around the mutated residue, resulting in domain destabilization.

Basically, *KDM6A*/*Kdm6a* escapes X-inactivation in humans and mice [Greenfield et al., 1998; Xu et al., 2008]. However, its expression from the inactive X chromosome is lower (15–35%) than that from the active X chromosome in female mice; thus, *Kdm6a* expression

Table 1. Clinical Features of Patients with a *KDM6A* Mutation

	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Female
Mutation	c.3717G>A	c.1555C>T	c.3354_3356delTCT
Protein change	p.Trp1239*	p.Arg519*	p.Leu1119del
De novo status	NA	NA	De novo
Paternal age at birth	34	42	27
Maternal age at birth	33	40	26
Characteristic face	+	+	+
Microcephaly	+	+	–
Long palpebral fissures	+	+	+
Epicanthus	+	–	–
Lower palpebral eversion	+	+	+
Prominent ear	+	+	–
Auricular deformity	+	+	
Depressed nasal tip	+	+	NA
Short nasal septum	+	+	NA
Abnormal dentition	+	+	–
Hypodontia	+	+	–
High-arched palate	+	+	–
Micrognathia	+	–	–
Short fifth finger	+	–	+
Developmental delay	+ (Severe)	+ (Severe)	+ (Severe)
Intellectual disability	+ (Severe)	+ (Severe)	+ (Severe)
Short stature	+	+	+
Prenatal growth retardation	+ (−1.96 SD)	+	–
Postnatal growth retardation	+	+	+
Cardiovascular abnormality	+	–	–
Joint laxity	+	+	–
Recurrent otitis media	+	–	–
Deafness	+[a]	–	NA
Karyotype	46,XY	46,XY	46,XX

[a]The deafness in patient 1 is conductive because of recurrent otitis media.
KDM6A gene variants were deposited in a gene-specific database (http://www.lovd.nl/KDM6A).
NA, not analyzed.

in female mice was not twice that in male mice [Xu et al., 2008]. In addition, *UTY* (Yq11.221), a paralog of *KDM6*, has been suspected to partially compensate in males while its function is not well known [Lederer et al., 2012; Xu et al., 2008]. Patient 3 in our study showed a random pattern of X-inactivation with the ratio 57:43 in genomic DNA of peripheral leukocytes. Interestingly, marked skewing of X-inactivation was observed in two female patients reported by Lederer et al. (2012). In their lymphoblast, *KDM6A* deletion was recognized at inactive X chromosome in all 70 mitoses. Here, we propose the threshold model for the pathogenicity of *KDM6A* abnormality (Supp. Fig. S1). The two female patients with a *KDM6A* deletion might not attain the appropriate level of *KDM6A* expression allowing normal development due to existence of specific cells with unfavorable inactivation, whereas male and pure Turner syndrome female with appropriate *KDM6A* expression do not show KS phenotype under assumption of unknown partial functional compensation of *KDM6A* by *UTY* in Y chromosome (only for male) (Supp. Fig. S1).

We reviewed the clinical details of the three patients (Table 1; Supp. Text). All patients were born to unrelated healthy parents. All the three showed severe developmental delay and intellectual disability. Interestingly, patient 3 (female) presented less dysmorphic features and the two male patients 1 and 2 showed a much more severe phenotype with multiple organ involvement (Table 1; Fig. 1E). Null expression of *KDM6A* in males and residual *KDM6A* expression from active X chromosome may explain sex-biased severity (Supp. Fig. S1). Alternatively, it could be explained by a lesser effect of the in-frame mutation in female patient. However, in a previous study, the severity of clinical symptoms varied also among two female patients and a male with a *KDM6A* deletion [Lederer et al., 2012]. More studies of KS patients with *KDM6A* abnormality are necessary. It is likely that the mutation type as well as the X-inactivation pattern in affected organs in females may determine the severity of KS.

In conclusion, we have described the first three point mutations of *KDM6A* in KS. Our three patients out of 32 *MLL2*-negative patients (mutation detection rate: 9.3%) are comparable to the three patients out of 22 *MLL2*-negative patients (13.6%) previously described [Lederer et al., 2012], regardless of the mutation type. The mutation detection rates for *MLL2* (55–80%) plus *KDM6A* (9–13%) in KS suggest that other gene(s) may be found. Because both MLL2 and KDM6A are histone modifiers, the other pathogenic genes might have related functions. Further research is needed to understand the pathomechanisms of KS as well as the role of histone modification in human disease.

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Fused teeth, macrodontia and increased caries are characteristic features of neurofibromatosis type 1 patients with *NF1* gene microdeletion

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Abstract. Neurofibromatosis type 1 (NF1) is the most common genetic condition caused by *NF1* gene alteration. A 1.5 Mb submicroscopic deletion encompassing the entire *NF1* gene, is known to be responsible for approximately 5% of NF1 cases. Patients with *NF1* deletion, compared to those with *NF1* mutation tend to exhibit more severe phenotypes. To know the possible differences in oral/dental features between *NF1* deletion and *NF1* mutation patients, we examined four patients with *NF1* deletion and three with *NF1* mutation to compare their oral manifestations. Fused teeth in the mandibular anterior region were found only in the patients with deletion (2/4). Macrodontia was noted in all four patients with an *NF1* deletion. Although macrodontia was also found in one patient with a mutation, it was relatively mild compared to the deletion patients. Dental caries were observed in both *NF1* deletion (4/4) and mutation (2/3) patients. However, patients with *NF1* deletions showed more apparently severe caries (average number of dental caries 12.8) than those with *NF1* mutation (average number 5.5). Other features also noted in patients with both deletions and mutations were high-arched palate, hypodontia and malocclusion. Our study might suggest that fused teeth, macrodontia and increased dental caries are distinctive manifestations of *NF1* deletion. Providing comprehensive dental care from early infancy would be very important to prevent dental caries especially in patients with *NF1* deletion.

Keywords: Neurofibromatosis type 1, *NF1* gene deletion, fused teeth, macrodontia, caries

1. Introduction

Neurofibromatosis type 1 (NF1) is the most common autosomal dominant genetic condition caused by *NF1* gene alteration. It affects 1 in 3,000 individuals and is characterized by multiple café au lait spots, neurofibromas, and axillary/inguinal freckling [1,2]. Other features associated with the disease include iris Lisch nodules, optic glioma, skeletal dysplasia, plexiform neurofibromas, and mental retardation/learning disability. Although tumors in the oral and facial areas have been frequently reported in association with NF1, the dental manifestations have not been fully characterized so far. To our knowledge, impacted teeth, displaced teeth, missing teeth, supernumerary teeth, increased dental caries, early primary tooth eruption, malocclusion, and periapical cemental dysplasia (in adult female patients) have been reported in patients with NF1 [3–7].

A submicroscopic deletion which is usually 1.5 Mb in size and involves the entire *NF1* gene and more than 20 genes adjacent to *NF1* gene is known to be responsible for approximately 5% of NF1 cases [8,9]. Patients

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with *NF1* deletions, compared to those with *NF1* mutations, tend to exhibit characteristic phenotypes such as facial dysmorphism, mental retardation and learning disability, plexiform neurofibromas, skeletal anomalies and cardiovascular defects, likely due to the involvement of contiguous genes around *NF1* [8,10–14]. However, the possible differences in oral/dental features between *NF1* deletion and *NF1* mutation patients have not been investigated in detail. We examined four patients with *NF1* deletion and three with *NF1* mutation to compare their oral manifestations.

2. Materials and methods

2.1. Patients

A total of seven patients were examined at Saitama Children's Medical Center; four (one male, three females; aged 5–12 yr) were identified as having a microdeletion including the *NF1* gene, and three (two males, one female; aged 5-12 yr) were identified as having a mutation of the *NF1* gene. Microdeletions were analyzed by fluorescence in situ hybridization analysis of metaphase chromosomes from peripheral blood, using a total of seven bacterial artificial chromosome clones comprising the bacterial artificial chromosome clone including *NF1* (RP11-876L22) and six neighboring clones (RP11-96L17, RP11-946G8, RP11-525H19, RP11-278E4, RP11-164M10, and RP11-55J8). The results showed that a deletion of approximately 1.5 Mb was detected in all four patients. Mutation analysis using genomic DNA extracted from peripheral blood was performed by means of polymerase chain reaction and direct sequencing of the coding regions for all exons. The results identified a splice mutation (c.1185+1G<A) in patient 5 and nonsense mutations (c.574C>T and c.3986C>G, respectively) in patients 6 and 7. Clinical manifestations are shown in Table 1. Neurofibromas of the oral and maxillofacial region were not present in any patient. This study protocol was approved by the Ethics Committee of Saitama Children's Medical Center and proper informed consents were obtained from the patients and their legal guardians of the patients.

2.2. Examination of craniofacial and oral condition by dental casts and radiographs

Palate morphology, occlusion, tooth size, and dental arch were evaluated by intraoral examination and dental cast studies. The relationship of the skeletal and dental structures and congenital hypodontia were evaluated on lateral cephalograms and orthopantomographs. The dimensions of the crown and dental arch were measured using a caliper with a resolution accuracy of 0.01 mm. Lateral cephalometric analysis was performed based on the method developed by Iizuka and Ishikawa [15] (Fig. 1). All data in this study (tooth size, dental arch form size, cephalometric findings) were compared with normal values in Japanese individuals.

Table 1
Clinical manifestations of the seven patients with neurofibromatosis type 1

	Deletion				Mutation		
Patients	1	2	3	4	5	6	7
Gender	F	M	F	F	M	F	M
Age (Years)	12	5	5	6	12	5	6
Height (SD)	−0.68	1.02	−0.41	0.57	−1.02	−1.50	−0.08
Occipito-frontal circumference	−1.27	1.90	0.31	0.50	0.00	0.29	2.80
Mental retardation	−	+	+	−	−	−	−
Facial dysmorphism	+	+	+	+	−	+	−
Café au leit spots	+	+	+	+	+	+	+
Neurofibroma	+	+	−	−	−	+	−
Plexifrom neurofibroma	−	−	−	−	−	+	−
Optic glioma	−	−	−	−	+	−	−
Brain MRI	UBO	UBO	UBO	UBO	UBO astrocytoma	UBO	UBO
Others		Calcifying epithelioma	VUR, urachal cyst	Preauricular tag			Pes planovalgus

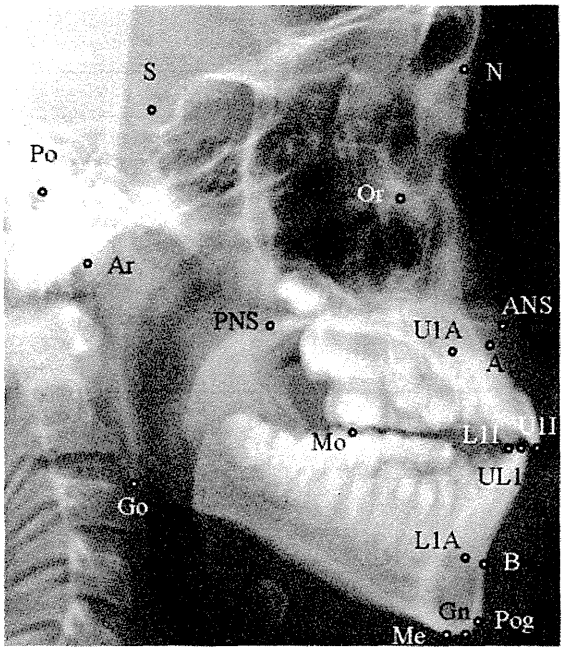

Fig. 1. Landmark points, angles and lines used in cephalometric analysis. Landmarks: N = Nasion; Or = Orbitale; S = Sella turcica; Po = Porion; Ar = Articulare; Go = Gonion; Me = Menton; Gn = Gnathion; Pog = Pogonion; B = B-point; A = A-point; ANS = Anterior nasal spine; Mo = Molar occlusion; U1A = Upper central incisor root apex; U1I = Upper central incisor edge; L1A = Lower central incisor root apex; L1I = Lower central incisor edge; UL1I = Middle of U1I and L1I. Angles: Convexity = N-A line to the A-Pog line; A-B plane = N-Pog line to the A-B line; SNA = S-N line to the N-A line; SNB = S-N line to the N-B line; Facial angle = Po-Or line to the N-Pog line; SNP = S-N line to the N-Pog line; Y axis = Po-Or line to the S-Gn line; SN-S.Gn = S-N line to the S-Gn line; Mandibular plane = Po-Or line to the Me-the lower border of the mandible line; Gonial angle = Ar-the posterior border of the ramus of the mandible line to the Me-the lower border of the mandible line; GZN = S-N line to the Ar-the posterior border of the ramus of the mandible line; FH to SN = Po-Or line to the S-N line; U-1 to FH plane = U1I-U1A line to the Po-Or line; U-1 to SN plane = U1I-U1A line to the S-N line; L-1 to mandibular = L1I-L1A line to the Me-the lower border of the mandible; Interincisal = U1A-U1I line to the L1A-L1I line; Occlusal plane = Po-Or line to the Mo-UL1I line.

3. Results

Oral manifestations noted in seven patients are summarized in Table 2. The prevalence of high-arched palate was high in both the *NF1* deletion and mutation patients (deletion 3/4, mutation 2/3). Dental caries occurred more frequently in all four patients with *NF1* deletion and two of the three patients with *NF1* mutation, but tooth decay was more severe in the patients with deletion compared with those with mutation, with an average of 12.8 affected teeth [10–16] in the former and 5.5 teeth (4 and 7) in the latter type. While mutation type patients showed caries only in the posterior teeth with less dental plaque, deletion type patients had caries both in the anterior and posterior teeth and also had more dental plaque as well (Fig. 2). Fused teeth were present in the mandibular anterior region in two out of four of the patients with *NF1* deletion (Patients 2 and 3). Panoramic radiographs showed congenital hypodontia of the permanent teeth in both these patients (Patient 2: bilateral second mandibular premolars and left mandibular first premolar; Patient 3: bilateral maxillary and mandibular second premolars and left mandibular incisor), with hypodontia of the succeeding permanent teeth for the fused teeth in patient 3. In the patients with *NF1* mutation, hypodontia (bilateral maxillary second premolars) was present in one out of three patients (Fig. 3). In terms of tooth size, macrodontia was present in all four *NF1* deletion patients and in one out of three mutation patients. The number of teeth greater than 2 SD larger than normal averaged 7.8 in patients with *NF1* deletion (4–13 teeth). Only three teeth exhibited macrodontia in the single *NF1* mutation patient (Table 3). Malocclusion was present in one out of four patients with *NF1* deletion (Patient 1: crowding) and one of the patients with *NF1* mutation (Patient 6: open bite). The dental crowding in patient 1 (deletion type) was associated with the patient's narrow dental arch and severe macrodontia (Table 4). In patient 6 (mutation type), it was judged to be caused by tongue thrusting. Lateral cephalometric analysis showed a tendency toward a dolichofacial pattern in the patients with *NF1* deletion with maxillary protrusion, and a tendency toward labioclination of the maxillary central incisors in those with *NF1* mutation (Table 5).

4. Discussion

The intraoral characteristics seen commonly in both deletion and mutation patients studied were high-arched palate, hypodontia, macrodontia, malocclusion and increased dental caries. Fused teeth were found only in the patients with deletion. Fusion of teeth is a relatively rare dental anomaly observed in the general population at a frequency of 4.10% [16]. Therefore, the fact that we observed fused teeth in two of four patients with *NF1* deletion suggests that it may be a characteristic feature of *NF1* deletion. It is noteworthy that both patients (Patients 2 and 3) who exhibited hypodontia had fused teeth. Macrodontia was noted in all four patients with *NF1* deletion. Macrodontia is a rare dental anomaly which may occur in isolation or

Table 2
Oral anomalies in seven patients

	Deletion				Mutation			Total	
Patient	1	2	3	4	5	6	7	Deletion	Mutation
High-arched palate	+	+	–	+	+	–	+	3/4	2/3
Fused teeth	–	+	+	–	–	–	–	2/4	0/3
Hypodontia	–	+	+	–	–	–	+	2/4	1/3
Macrodontia	+	+	+	+	–	+	–	4/4	1/3
Dental caries	+ (10/23)	+ (10/19)	+ (15/19)	+ (16/22)	+ (7/22)	+ (4/24)	– (0/22)	4/4	2/3
Malocclusion	+ Crowding, narrow dental arch	–	–	–	–	+ Open bite	–	1/4	1/4

Parenthesis represents the number of dental caries/the total of present teeth.

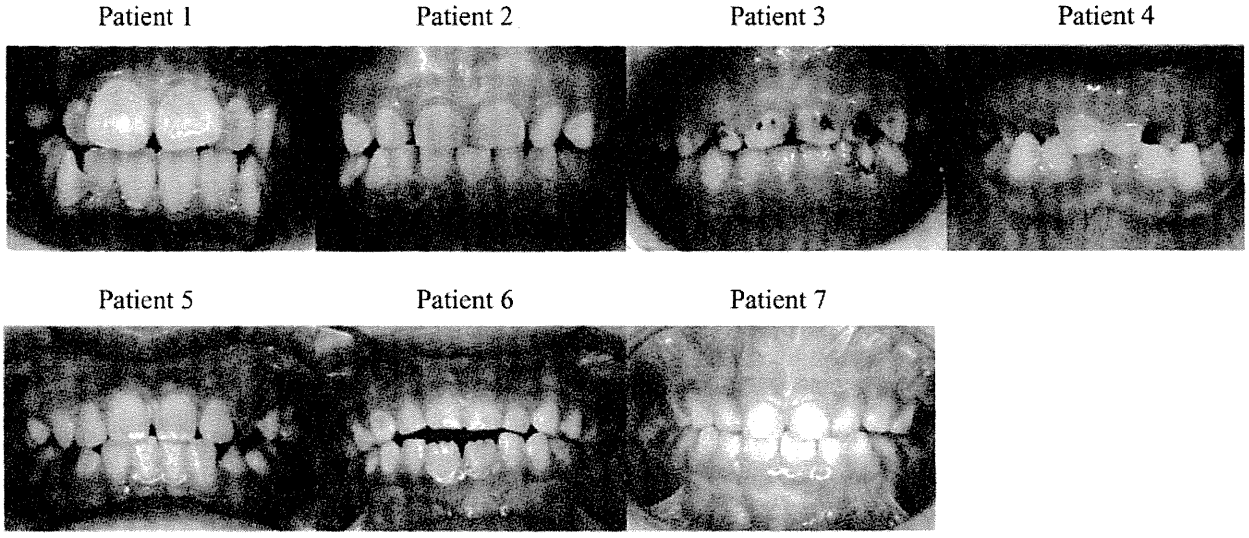

Fig. 2. Oral photographs of seven patients. Patients 1–4: deletion, patients 5–7: mutation.

as a component of syndromes such as KBG syndrome, "polydactyly, postaxial, with dental and vertebral anomalies", XXY and XYY male, and hemihyperplasia [17–21]. However, to our knowledge, macrodontia has not been described previously in NF1. Although macrodontia was also found in one patient with a mutation, it was relatively mild compared to the deletion patients, with only three large teeth having a width that exceeded 2 SD. Thus, macrodontia can be considered a distinctive feature of patients with *NF1* deletion. Dental caries was observed in both *NF1* deletion (4/4) and mutation (2/3) patients. However, patients with *NF1* deletion showed apparently severe caries (average number of dental caries 12.8) than those with *NF1* mutation (average number 5.5). Tucker et al. [5], on the basis of a questionnaire study of 37 families of children with NF1, reported that individuals with NF1 had a significantly higher average number of dental caries (8.1 ± 6.6) than their siblings without NF1 (5.5 ± 5.8). Unfortunately, no genetic investigation of the *NF1* gene was performed in their study. The authors mentioned some possibilities to account for increased dental caries in NF1 patients, including vitamin D deficiency, reduced bone mineralization (osteopenia or osteoporosis), and misregulation of various growth factor receptors. In addition, the author proposed that impaired mental capacity in NF1 patients might be a risk factor for excessive caries due to poor oral care. Our deletion patients tended to show poor oral hygiene indicated by the high plaque levels on oral examination. This could be explained by the reduced ability to perform dental care due to mental retardation. Nonetheless, the cause is still unknown and further studies are necessary. Malocclusion was not frequently seen in the patients examined. Patient 1 with

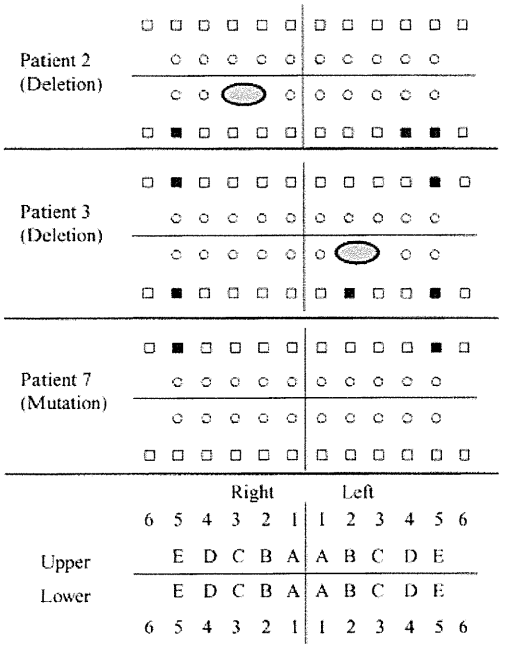

Fig. 3. Fused primary teeth and missing permanent teeth noted in patients 2, 3 with NF1 deletion, and missing permanent teeth observed in patient 7 with NF1 mutation. ⬭, Fused primary teeth. ■, Congenitally missing permanent tooth.

NF1 deletion had crowding, and patient 6 exhibited open bite. The former might be associated with macrodontia and a narrow dental arch, while the latter was likely due to tongue thrusting. Lateral cephalometric analysis showed a tendency toward a dolichofacial pattern in the patients with *NF1* deletion with maxillary protrusion, and a tendency toward labioclination of the maxillary central incisors in those with *NF1* mutation.

Grisart et al. [22] reported a family with microduplication of the identical *NF1* microdeletion region, in which patients showed moderate to borderline normal mental impairment, early onset of baldness and dental enamel hypoplasia. The author hypothesized that gene(s) responsible for dental enamel hypoplasia might reside in the deleted interval, although no candidate gene has been identified.

In conclusion, we evaluated seven NF1 patients, four with *NF1* deletion and three with *NF1* mutation, and found that fused teeth, macrodontia and excessive dental caries are distinctive manifestations of *NF1* deletion. Providing comprehensive dental care from early infancy would be very important to prevent dental caries especially in patients with *NF1* deletion.

Table 3
Size of the teeth in seven patients

Patient	Deletion								Mutation					
	1		2		3		4		5		6		7	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Primary teeth														
Maxillary														
Central incisor			2.05	2.00	2.97	1.43	−0.41	−0.57			0.30	−0.51	0.45	−0.08
Lateral incisor			1.37	1.40	1.22	1.08	0.46	−1.51			1.22	1.27	−0.37	−0.29
Cuspid			2.28	2.19	2.30	2.82	−0.21	−0.67			0.12	0.15	−0.93	−0.93
First molar			1.71	1.73	3.03	1.78	1.43	1.65			2.38	2.13	0.83	0.15
Second molar	2.16		1.29	1.12	2.30	2.78	0.48	−0.62	−0.78		1.24	1.00	−0.59	0.32
Mandibular														
Central incisor			0.67	0.93	2.14	2.14	0.55	−0.55						
Lateral incisor				1.26	1.76		2.00	2.31			0.21	0.86	−0.82	−0.97
Cuspid				2.44	0.71		−0.43	0.36			0.43	2.75	0.15	0.44
First molar			1.77	1.81	1.80	2.24	2.27	2.24		0.19	0.36	1.27	−1.56	−1.23
Second molar	1.33	1.13	0.71	0.71	1.24	1.78	−1.40	−1.67	−0.69	−0.61	−0.38	−0.55	0.94	0.78
Permanent teeth														
Maxillary														
Central incisor	4.78	4.39							−0.59	−0.74				
Lateral incisor	3.27	2.78							0.65	0.39				
Cuspid		3.77												
First premolar		3.69												
First molar	1.20	1.53							−2.59	−1.61	−0.57			
Mandibular														
Central incisor	4.03	4.19							−0.30	−0.40	1.14	1.33	−0.12	1.00
Lateral incisor	3.10	4.00							−0.33	0.09				
Cuspid	3.03	3.05												
First molar	1.82	1.52							−1.78	−0.98				

Tooth size represents the distance from the medial to distal. Unit, SD.

Table 4
Dental arch measurements in seven patients

	Deletion				Mutation		
Patient	1	2	3	4	5	6	7
Maxillary							
W_C		2.04	2.11	−1.09		0.15	−0.42
W_E		0.32	2.27	−2.10		−1.22	−1.73
L_{AE}		1.27	0.76	−0.37		0.77	0.25
W_3	No data				No data		
W_6	−2.19				1.77		
L_{16}	2.37				0.66		
Mandibular							
W_C		No data	No data	0.48		2.69	0.88
W_E		0.40	−0.12	0.34		0.53	−0.96
L_{AE}		−0.36	−0.68	−2.18		No data	No data
W_3	No data				No data		
W_6	−2.78				1.95		
L_{16}	2.07				1.51		

The W_C, W_3, W_E, W_6 represents the distance between the primary cuspids, the cuspids, the primary second molars, the first molars. The L_{AE} represents the length from the distal surface of the primary second molars to the primary incisors central point. The L_{16} represents the length from the mesial surface of the first molars to the incisors central point. Unit, SD.

Table 5
Lateral cephalometric analysis in seven patients

	Deletion				Mutation		
Patient	1	2	3	4	5	6	7
Skeletal							
Convexity	−0.83	2.35	5.63	3.14	−0.61	1.16	−1.49
A-B plane	1.53	−0.31	−0.52	−0.46	1.53	−1.98	1.68
SNA	−0.71	−0.32	2.74	1.49	1.24	−1.56	−0.49
SNB	−0.38	−1.02	1.29	1.02	1.74	−3.55	−0.18
Facial angle	0.14	−1.54	−1.08	−0.77	3.52	1.23	0.23
SNP	−0.74	−1.27	0.06	−0.16	1.41	−2.36	2.04
Y-axis	0.80	1.77	2.06	2.19	−2.38	−2.66	1.58
SN-S·Gn	1.89	1.82	1.09	1.40	−0.94	1.40	0.78
Mandibular plane	2.51	2.65	1.62	2.87	−2.91	−1.76	0.55
Gonial angle	0.39	0.96	−1.32	2.32	−1.12	−0.34	−0.11
GZN	1.85	0.61	1.51	0.39	0.63	1.44	0.13
FH to SN	0.96	−0.21	−1.56	−0.90	1.31	3.94	−1.22
Denture							
U-1 to FH plane	1.18	−0.31	0.07	−3.41	3.84	4.66	1.22
U-1 to SN plane	0.77	−0.34	0.66	1.30	3.14	2.10	1.87
L-1 to mandibular	−0.47	0.80	1.65	−1.85	−0.64	2.04	0.09
Interincisal	−1.36	−1.09	−1.41	−0.86	−1.21	−3.50	−1.33
Occlusal plane	−0.06	1.00	2.02	0.88	−2.64	−1.69	2.04

Unit, SD.

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Focal Segmental Glomerulosclerosis in Patients With Complete Deletion of One *WT1* Allele

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Focal Segmental Glomerulosclerosis in Patients With Complete Deletion of One *WT1* Allele

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KEY WORDS
deletion, focal segmental glomerulosclerosis, WAGR syndrome, *WT1*

ABBREVIATIONS
ACEI—angiotensin-converting enzyme inhibitor
BUN—blood urea nitrogen
CrCl—creatinine clearance
DDS—Denys-Drash syndrome
DMS—diffuse mesangial sclerosis
FSGS—focal segmental glomerulosclerosis
WAGR—Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation

Each author contributed to the study as follows: Dr Iijima, patient management and manuscript writing; Dr Someya, patient management; Dr Ito, patient management; Dr Nozu, genetic analysis; Dr Nakanishi, genetic analysis; Dr Matsuoka, pathological analysis; Dr Ohashi, genetic analysis; Dr Nagata, pathological analysis; Dr Kamei, patient management; and Dr Sasaki, patient management.

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abstract

The renal prognosis of patients with Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation syndrome (WAGR) is poor. However, the renal histology and its mechanisms are not well understood. We performed renal biopsies in 3 patients with WAGR syndrome who had heavy proteinuria. The complete deletion of one *WT1* allele was detected in each patient by constitutional chromosomal deletion at 11p13 using G-banding, high-resolution G-banding, and fluorescence in situ hybridization. The patients exhibited proteinuria at the ages of 6, 10, and 6 years and were diagnosed as having focal segmental glomerulosclerosis (FSGS) at the ages of 7, 16 and 19 years, respectively. They exhibited normal or mildly declined renal function at the time of biopsy. Re-examination of a nephrectomized kidney from 1 patient revealed that some glomeruli showed segmental sclerosis, although he did not have proteinuria at the time of nephrectomy. The other 2 patients did not develop Wilms' tumor and thus did not undergo nephrectomy, chemotherapy, or radiotherapy, thereby eliminating any effect of these therapies on the renal histology. In conclusion, complete deletion of one *WT1* allele may induce the development of FSGS. Our findings suggest that haploinsufficiency of the *WT1* could be responsible for the development of FSGS. *Pediatrics* 2012;129:e1621–e1625

Miller et al[1] first described WAGR syndrome (Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation). Children with WAGR syndrome invariably have a constitutional chromosomal deletion at 11p13, the region where the *WT1* gene is located. Patients with Denys-Drash syndrome (DDS) usually have a germline missense mutation, which is predicted to result in an amino acid substitution in the eighth or ninth exon of *WT1*. Little et al[2] suggested that the severe nephropathy associated with DDS, which frequently leads to early renal failure, might result from the dominant-negative action of altered *WT1*. By contrast, because of the less severe genital anomalies and apparent lack of nephropathy associated with WAGR, a reduced *WT1* dosage during embryogenesis is thought to have a less pronounced effect on development, especially on renal system development.[3] Breslow et al[4] reviewed nearly 6000 patients enrolled in 4 clinical trials administered by the US National Wilms Tumor Study Group between 1969 and 1995. Of 22 patients with DDS, 13 (59%) developed renal failure; of 46 patients with WAGR, 10 (22%) developed renal failure. The cumulative risks of renal failure at 20 years were 62% and 38%, respectively. These findings suggest that nephropathy is not uniquely associated with missense mutations in *WT1* and that patients with the WAGR syndrome should be followed up closely throughout life for signs of nephropathy.

The renal prognosis of patients with WAGR is poor. However, the renal histology and its mechanisms are not well understood. We therefore performed renal biopsies to reveal the renal pathology in 3 patients with WAGR syndrome who had heavy proteinuria.

CASE REPORTS

Patient 1

Patient 1 was a male diagnosed with bilateral microphthalmos at 1 month of age. Wilms' tumor developed bilaterally at 3 years of age. He also had undescended testes and mental retardation. Previous analysis of G-banded metaphase chromosomes revealed a deletion of chromosome 11p13-15.1 in one allele[5]; the diagnosis of atypical WAGR syndrome was therefore made.[6] Because of a large tumor in the right kidney after the first chemotherapy treatment, the right kidney was nephrectomized. A diagnosis of nephroblastoma (nephroblastic type) was made. At the same time, the contralateral left kidney was biopsied, but no tumor was detected. The nephrectomized kidney revealed that there were no immature glomeruli, and a few glomeruli showed segmental sclerosis (Fig 1 A and B). The patient did not have proteinuria at the time of nephrectomy although microalbuminuria could have been detected.

The patient then underwent a second session of chemotherapy and radiotherapy treatment with left kidney protection. He developed heavy proteinuria at 6 years of age. The left kidney was biopsied (open biopsy) at age 7 years. Renal biopsy findings were consistent with focal segmental glomerulosclerosis (FSGS) (Fig 1 C and D). At the time of biopsy, the patient's height was 107.3 cm (–2.9 SD), weight was 21.7 kg (–0.7 SD), and blood pressure was 120/80 mm Hg. Biochemical data were as follows: total protein, 6.5 g/dL; albumin, 3.3 g/dL; blood urea nitrogen (BUN), 12.9 mg/dL; creatinine, 0.43 mg/dL; 24-hour creatinine clearance (CrCl), 72.2 mL/min/1.73 m^2; early morning urinary protein, 3+ (as measured by using a dipstick test); urinary protein to urinary creatinine ratio, 3.6 (milligram/milligram); and urinary β-2 microglobulin, 0.44 mg/dL (normal range: <0.23 mg/dL). His renal function gradually deteriorated despite angiotensin-converting enzyme inhibitor (ACEI) treatment. At 14 years of age, he underwent a preemptive living-related renal transplantation from his father.

Patient 2

Patient 2 was a male with aniridia, bilateral undescended testes, hypospadias, grade III to IV bilateral vesicoureteral reflux, and mental retardation. High-resolution G-banding revealed deletion of chromosome 11p13-p14.2 in one allele (Fig 2A), and fluorescence in situ hybridization showed heterozygous deletions of *PAX6, D11S2163, PER,* and *WT1* (Fig 2B), indicating WAGR syndrome. He had a single febrile urinary tract infection at 2 years of age and underwent an antireflux operation at 4 years of age, which resolved his vesicoureteral reflux. A dimercaptosuccinic acid radionuclide scan showed several defects in his right kidney. His proteinuria was detected at 10 years of age by the school urinary screening program. His proteinuria gradually increased, and he underwent renal biopsy (right kidney) at age 16 years. Renal biopsy findings were consistent with FSGS (Fig 1 E and F). At the time of biopsy, the patient's height was 169.2 cm, weight was 67.4 kg, and blood pressure was 128/78 mm Hg. Biochemical data were as follows: total protein, 6.8 g/dL; albumin, 4.3 g/dL; BUN, 25.0 mg/dL; creatinine, 1.20 mg/dL; 24-hour CrCl, 91.0 mL/min/1.73 m^2; early morning urinary protein, 3+ (as measured by using a dipstick test); urinary protein to urinary creatinine ratio, 2.7 (milligram/milligram); daily urinary protein, 3.1 g; and urinary β-2 microglobulin, 0.064 mg/dL. At the latest follow-up (24 years of age), his renal function was stable (BUN: 25.0 mg/dL; creatinine: 1.20 mg/dL) with ACEI treatment, and he had not developed Wilms' tumor.

Patient 3

Patient 3 was a female with aniridia and mental retardation. G-banding revealed deletion of chromosome 11p13-p14 in one allele (Fig 2C), and she was therefore diagnosed with WAGR syndrome. The patient developed proteinuria at

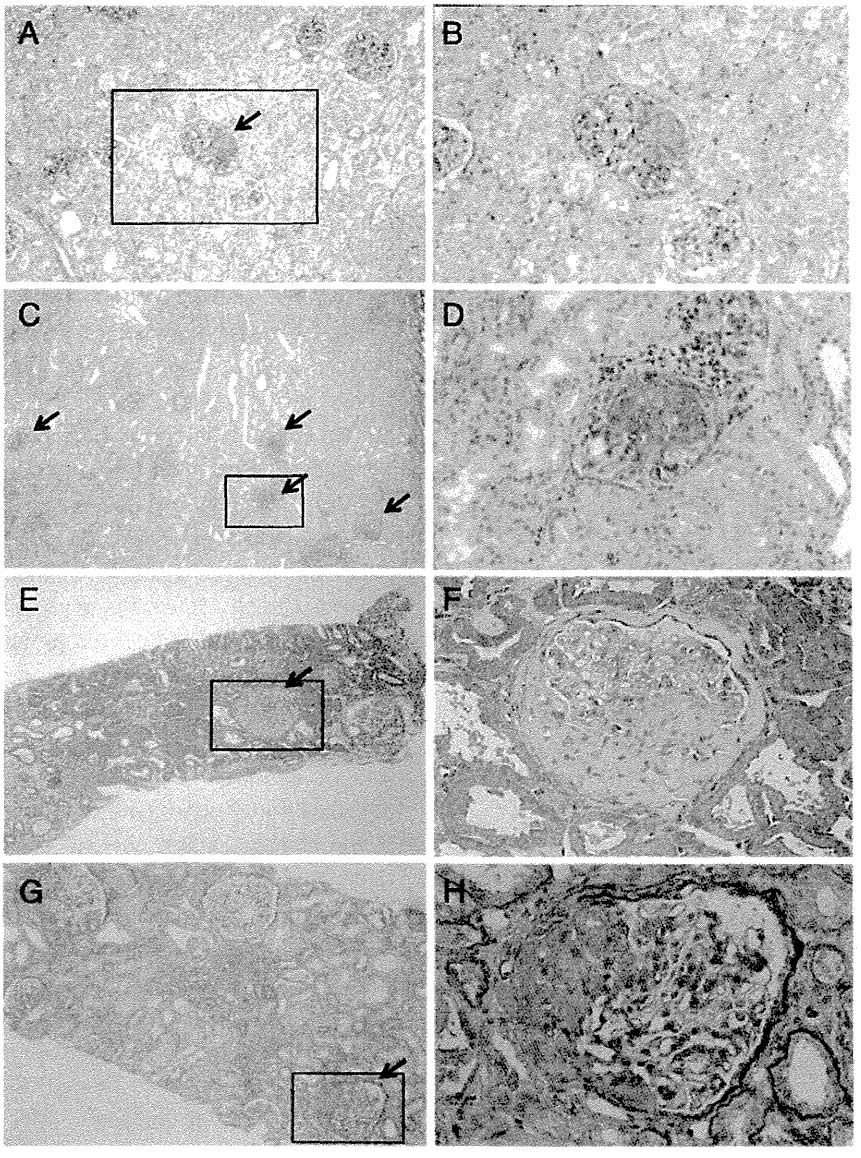

FIGURE 1

Renal histology. A, C, E, and G, Low magnification. B, D, F, and H, High magnification. Arrows show glomeruli with segmental glomerulosclerosis. A and B, Nephrectomized right kidney from patient 1. Patient 1 had no proteinuria at the time of nephrectomy. However, a few glomeruli exhibited segmental glomerulosclerosis although there were no immature glomeruli. C and D, Renal biopsy of left kidney from patient 1. Twenty-eight of 50 glomeruli showed segmental glomerulosclerosis. There were no tubulointerstitial lesions. E and F, Renal biopsy from patient 2. Two of eight glomeruli showed segmental glomerulosclerosis with interstitial fibrosis. G and H, Renal biopsy from patient 3. Ten of 30 glomeruli showed segmental glomerulosclerosis with interstitial fibrosis. All 3 patients exhibited FSGS (not otherwise specified).

the age of 6 years and nephrotic syndrome with normal renal function at age 15 years (urinary protein to urinary creatinine ratio, 10.6 [milligram/milligram]; total protein, 5.6 g/dL; albumin, 2.3 g/dL; BUN, 15.0 mg/dL; creatinine, 0.65 mg/dL; estimated glomerular filtration rate, 100.7 mL/min/1.73 m^2). We were unable to obtain her parents' consent for renal biopsy, and they chose to start drug treatment. However, treatment with prednisolone and ACEI was not effective, and her renal function gradually deteriorated. Therefore, she underwent renal biopsy at age 19 years. At the time of biopsy, her height was 144.5 cm, weight was 72.5 kg, and blood pressure was 130/83 mm Hg. Biochemical data were as follows: total protein, 5.5 g/dL; albumin, 2.5 g/dL; BUN, 30.0 mg/dL; creatinine, 1.40 mg/dL; 24-hour CrCl, 44.65 mL/min/1.73 m^2; early morning urinary protein, 3+ (as measured by using a dipstick test); daily urinary protein, 5.89 g; and urinary β-2 microglobulin, 0.495 mg/dL. Renal biopsy findings were consistent with FSGS (Fig 1 G and H). To date, she has not developed Wilms' tumor.

DISCUSSION

The current study demonstrated that 3 patients with atypical WAGR syndrome developed heavy proteinuria with FSGS, suggesting that the nephropathy seen in this syndrome is responsible for the FSGS lesion.

Patient 1 had possible bilateral Wilms' tumor and underwent unilateral nephrectomy, chemotherapy, and radiotherapy. Therefore, it is possible that the treatment of the remaining kidney for bilateral tumor or nephrogenic rest might account for the development of FSGS. However, the kidney nephrectomized after the first chemotherapy session but before radiotherapy treatment already showed segmental sclerosis in a few glomeruli, suggesting that radiotherapy was not the main cause of FSGS. Chemotherapeutic drugs such as adriamycin may induce FSGS as well as tubulointerstitial inflammation and fibrosis.[7] However, there were no tubulointerstitial lesions, suggesting that chemotherapy might not have been the main cause of FSGS. Nevertheless, it is possible that surgical renal ablation caused FSGS in patient 1.

Patients 2 and 3 did not develop Wilms' tumor during the course of clinical observation, and thus they did not undergo nephrectomy, chemotherapy, or radiotherapy, thereby eliminating any effect of these therapies on renal

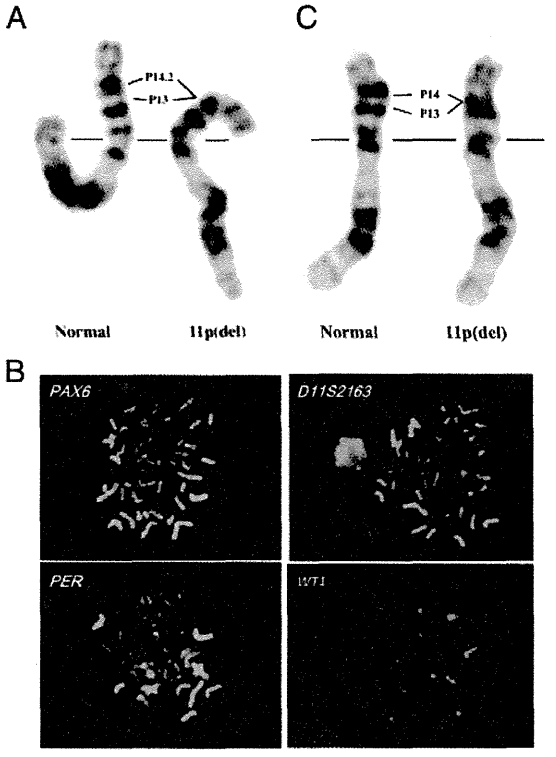

FIGURE 2

High-resolution G-banding of chromosome 11 and fluorescence in situ hybridization (FISH) in patient 2 and G-banding of chromosome 11 in patient 3. A, Patient 2 had deletion of chromosome 11p13-p14.2 in one allele. B, FISH using P1-derived artificial chromosome clones (1083G3 for *PAX6*; 65P5 for *D11S2163*; 685F3 for *PER*; and 104M13 for *WT1*) as probes was performed for patient 2, as previously reported.[6] Each FISH signal for *PAX6, D11S2163, PER, and WT1* was observed in only one chromosome 11 homolog, indicating heterozygous deletion of the WAGR region of 11p. C, Patient 3 had deletion of chromosome 11p13-p14 in one allele.

histology. The possibility of reflux nephropathy, however, could not be ruled out in patient 2. The perihilar variant with glomerular hypertrophy is particularly common in the secondary FSGS such as reduced renal mass–induced FSGS.[8] However, all 3 patients exhibited FSGS (not otherwise specified) without glomerular hypertrophy, suggesting that surgical renal ablation (patient 1) and reflux nephropathy (patient 2) may not have been the main cause of FSGS in these 2 patients. These findings suggest that the complete deletion of one *WT1* allele might have a pathogenetic role in the development of nephropathy.

The spectrum of glomerular diseases associated with *WT1* mutations has been reviewed.[9] *WT1* mutations can cause syndromic and nonsyndromic glomerular disease. The syndromic forms include DDS (early-onset nephrotic syndrome with diffuse mesangial sclerosis [DMS]); 46,XY disorders of sex development and Wilms' tumor; and Frasier syndrome (disorders of sex development, FSGS, and gonadoblastoma), which is caused by a mutation in the intron 9 splice site of *WT1* leading to the loss of the +KTS isoform of the protein. Mutations associated with both syndromic and nonsyndromic glomerular disease tend to cluster in exons 8 and 9 of *WT1*, which encode zinc fingers 2 and 3.[9,10] Orloff et al[11] reported that single-nucleotide polymorphisms in *WT1* may modulate the development of FSGS by altering *WT1* function. The current study suggests that complete deletion of one *WT1* allele may also induce the development of nephropathy.

Reduced expression levels of *Wt1*-induced glomerulopathies (crescentic glomerulonephritis or DMS) depending on gene dosage derived by combining *Wt1*-knockout mice and an inducible *Wt1* yeast artificial chromosome transgenic mouse model.[12] Eleven percent of mice heterozygous for the *Wt1* mutation showed severe proteinuria and DMS with tubular cysts, protein casts, and severe interstitial inflammation, although nephrogenesis was not delayed.[12] These findings indicate that the expression level of *WT1* plays an important role, not only during nephrogenesis but also in the homeostasis of normal kidney function. These findings also support our conclusion that complete deletion of one *WT1* allele in atypical WAGR syndrome could induce glomerulopathy without delayed nephrogenesis, although the reason for the discrepancy in histologic findings between man (FSGS) and mouse (DMS) is unclear.

CONCLUSIONS

Besides dominant-negative missense mutations in the eighth or ninth exon of *WT1* and mutations at the donor splice site of intron 9, complete deletion of one *WT1* allele may induce the development of FSGS. The findings in this study also suggest that haploinsufficiency of *WT1* could be responsible for the development of FSGS.

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