***RBPJ* is disrupted in a case of proximal 4p deletion syndrome with epilepsy**

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Abstract

Proximal 4p deletion syndrome is characterized clinically by mental retardation, minor dysmorphic facial features, and is occasionally complicated with epilepsy. More than 20 cases of proximal 4p deletion syndrome have been reported, but the causative gene(s) remain elusive. We describe here a 2-year-old female patient with a common manifestation of proximal 4p deletion syndrome and infantile epileptic encephalopathy possessing a *de novo* balanced translocation t(4;13)(p15.2;q12.13). The patient was diagnosed as infantile spasms at 9 months of age. She presented with dysmorphic facial features and global developmental delay, compatible with proximal 4p deletion syndrome. Using fluorescence *in situ* hybridization, we determined the translocation breakpoint at 4p15.2 to be within *RBPJ*. *RBPJ* is a transcription factor in the Notch/*RBPJ* signaling pathway, playing a crucial role in the developing human brain, and particularly telencephalon development. Our findings, combined with those of previous studies, strongly suggest that *RBPJ* is causative for proximal 4p deletion syndrome and epilepsy in this case.

Key Words: balanced translocation; Infantile spasms; proximal 4p deletion syndrome; *RBPJ*

1 Introduction

2 Deletions involving the proximal portion of human chromosome 4p15 result in
3 a clinically distinct syndrome characterized by variable degrees of intellectual
4 disability, unusual faces, and minor dysmorphic features [1-3]. More than 20 cases of
5 proximal 4p deletion have been reported. The critical region for proximal 4p deletion
6 syndrome has been localized to 4p15.2–15.33, but the causative gene remains elusive.

7 Infantile spasms (IS), also known as West syndrome, is the most frequent type
8 of epileptogenic encephalopathy. Various types of epileptic spasms, hypsarrhythmia
9 on electroencephalography (EEG), and psychomotor deterioration constitute the basis
10 for a diagnosis of IS. IS is not a nosological entity but rather constitutes a
11 heterogeneous group of conditions that share this clinical triad. Several genetic
12 abnormalities have been identified among the diverse possible causes of IS. Although
13 the number of mutations identified is small, it gives rise to the intriguing possibility
14 that genetic abnormalities underlie a proportion of IS etiology.

15 Here, we present a patient with IS and profound psychomotor delay with a *de*
16 *novo* reciprocal translocation t(4;13)(p15.2;q12.13) disrupting the gene that encodes
17 recombination signal binding protein for immunoglobulin kappa J (RBPJ).

19 Case Report

20 The patient, a 2-year-old girl, was born after an uneventful pregnancy to
21 unrelated healthy parents at term without asphyxia. Neither parent had intellectual
22 impairment or epilepsy. She experienced clonic convulsions of the extremities at day
23 20 after birth. An initial EEG performed at 1 month was normal. Her seizures were
24 not well controlled by a combination of phenobarbital and carbamazepine, and
25 gradually evolved into recurrent brief tonic spasms. IS was diagnosed at 9 months of

age by intellectual disability, a series of tonic spasms, and modified hypsarrhythmia described as periodic independent polyspikes-wave bursts on EEG.

On examination at 2 years, her weight was 10.7 kg (-0.8 SD) and her height was 86 cm ($+0.6$ SD). She presented with dysmorphic facial features; upslanted palpebral fissures, epicanthal folds, a large beaked nose, thick lower lips, and micrognathia (Figure 1a; Table 1). Other minor anomalies included bilateral fifth finger mild brachydactyly (Figure 1b). A physical examination revealed mild axial hypotonia. She showed global developmental delays with motor skills equivalent to those normally observed at 5 months of age. She was unable to speak any recognizable words. Routine laboratory and neurometabolic investigations, computed tomography scans, and magnetic resonance imaging findings were normal. No abnormalities were seen on an echocardiogram and electrocardiogram. EEG showed diffuse high voltage slow waves with independent spike waves over the bilateral frontal and parieto-occipital area (Figure 1c, d).

G-banded chromosomal analysis revealed a balanced translocation $t(4;13)(p15.2;q12.13)$ in the patient. Her parents showed a normal karyotype (data not shown), indicating that the translocation occurred *de novo*. Array CGH, according to a method described elsewhere [4] (Supplementary information), did not show any significant copy number variations (data not shown), suggesting that the balanced translocation did not involve occult changes. FISH, performed as previously described [5] (Supplementary information), demonstrated that the putative breakpoint on chromosome 4 was located within the *RBPJ* gene (Figure 2a, b; Supplementary Figure 1). The breakpoint on chromosome 13 resided in the intergenic region between *GPR12* and *USP12* (Figure 2c, d). Thus, the balanced translocation in this patient disrupted only one gene, *RBPJ*; this is assumed to lead to hemizygous *RBPJ* loss.

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2 Discussion

3 Proximal 4p deletion syndrome is characterized by a long face, upslanted
4 palpebral fissures, epicanthal folds, a thick lower lip, micrognathia, a large beaked
5 nose, a tall and thin body habitus, broad hands and feet, and varying degrees of mental
6 retardation. There are so far 15 reports describing a total of 24 individuals with
7 deletions involving the proximal portion of human chromosome 4p15 (see Table 1 for
8 details) [1-3, 6-17]. Our patient shares a number of cardinal manifestations of
9 proximal 4p deletion syndrome. The critical region for all of those features has been
10 suggested to be 4p15.2–p15.33 [3]. The disrupted *RBPJ* gene at 4p15.2 in this patient
11 clearly implicates *RBPJ* as a causative gene for proximal 4p deletion syndrome.

12 Our patient also demonstrated IS followed by intractable childhood epilepsy,
13 with no obvious brain MRI abnormality. The prevalence of epilepsy in cases with
14 proximal 4p deletion is approximately 20%, which is higher than the prevalence rate
15 of epilepsy in general population. The clinical manifestations of previous reported
16 cases are heterogeneous; the seizure types associated with these deletions have been
17 described for five cases, including focal, generalized tonic clonic seizures, and
18 complex partial seizures [2, 13-15]. Cerebral abnormalities, such as periventricular
19 heterotopias, polymicrogiria, and arachnoid cyst have been reported in some of these
20 patients with epilepsy [14, 15]. While these cerebral abnormalities may account for
21 the presence of epilepsy, the current patient exhibiting epilepsy show no distinctive
22 abnormality in neuro images, which is common for proximal deletion of 4p. Our
23 present case supports the notion that proximal deletion of 4p could lead to variable
24 epileptic phenotypes.

25 *RBPJ* is a transcription factor in the Notch/*RBPJ* signaling pathway. The role

of Notch signaling in neuronal development has been intensively analyzed by conditional inactivation of *Rbpj* in mice; this has revealed that Notch signaling is required for the maintenance of neuronal stem cells [18]. Inactivation of *Rbpj* induces complete loss of neural stem cells in the developing and adult telencephalon, revealing that *RBPJ* is likely to play a crucial role in the developing human brain, and particularly telencephalon development [19]. Although we could not confirm the exact pathological role of *RBPJ* abnormalities in epileptogenicity, *RBPJ* haploinsufficiency might increase susceptibility to particular types of epilepsy including IS. Further studies of similar cytogenetically proven cases are needed for a better understanding of the pathogenesis of proximal 4p deletion syndrome with epilepsy.

A recent study identified *RBPJ* missense mutations in two families affected by Adams-Oliver syndrome (AOS) [20]; a rare multiple-malformation disorder consisting primarily of congenital cutis aplasia of the scalp vertex, transverse terminal limb defects, and congenital heart defects.. Although our current case with hemizygous *RBPJ* disruption presented with bilateral fifth finger brachydactyly, which might be a milder form of terminal limb malformation, no other clinical symptoms were suggestive of AOS. The absence of typical AOS manifestations in our current patient is consistent with previous reports that the phenotypes of patients with proximal 4p deletion syndrome are clinically distinctive from those of AOS. Importantly, proximal 4p deletion syndromes involve at least hemizygous deletion of *RBPJ*, supporting the notion that *RBPJ* haploinsufficiency is associated with proximal 4p deletion syndrome. Accordingly, our current observations shed light on an aspect of *RBPJ* function in the central nervous system. Further study of *RBPJ* aberrations will be required to establish it in a causative role in proximal 4p deletion syndrome as

well as epileptic syndromes.

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1 **Figure legends**

2 **Figure 1** Characteristics of the patient

3 (a) Facial appearance of the patient at 4 years. Upslanted palpebral fissures,
 4 epicanthal folds, a large beaked nose, thick lower lips, and micrognathia can be
 5 observed. Other minor anomalies include strabismus and widely spaced teeth. (b)
 6 Digits of the patient at 4 years, showing bilateral fifth finger mild brachydactyly. The
 7 parents of the patient gave their consent to publish these photographs in an academic
 8 journal. (c) Interictal EEG at 2 years. EEG during wakefulness shows a diffuse
 9 high-voltage slow rhythm superimposed with irregular independent spike waves over
 10 the bilateral frontal and parieto-occipital area. (d) Ictal EEG during tonic spasms. A
 11 diffuse irregular high-voltage slow rhythm is followed by diffuse voltage attenuation
 12 with rhythmic fast-wave bursts when tonic axial spasms with symmetric contraction
 13 of the deltoid muscles occur. Her daily tonic spasms and subsequent tonic seizures
 14 were refractory to pyridoxine, ACTH, valproic acid, clonazepam, lamotrigine,
 15 topiramate, and levetiracetam. After undergoing total corpus callosotomy at 2 years
 16 and 3 months of age, she showed fewer axial tonic seizures. EOG;
 17 electrooculography; ECG; electrocardiography; EMG1; electromyography of left
 18 deltoid muscle; EMG2; electromyography of right deltoid muscle.

20 **Figure 2** Characterization of breakpoints by FISH

21 (a) Schematic presentation of bacterial artificial chromosome probes at the 4p15.2
 22 breakpoint, based on the National Center for Biotechnology Information build 37.
 23 Both RP11-239C17 and RP11-450B14 contain *RBPJ* exons. The tan-shaded portion
 24 indicates the putative breakpoint region. Horizontal arrows indicate gene orientation.
 25 (b) FISH on the patient's chromosomes using RP11-239C17 (red) and RP11-450B14

(green) counterstained with 4',6-diamidino-2-phenylindole (DAPI) (light blue). Merged signals for RP11-239C17 and RP11-450B14 are seen on the normal chromosome 4 (arrowhead) and the der(4) (double arrowhead); an additional weak signal for RP11-450B14 is detected on the der(13) (arrow), indicating that the *RBPJ* coding region was disrupted by the breakpoint. An artifactual green signal is indicated by the dark gray arrow. **(c)** Schematic presentation of bacterial artificial chromosome probes at the 13q12.13 breakpoint. Note that RP11-78D7 does not contain any known genes. The tan-shaded box indicates the putative 13q breakpoint region. **(d)** FISH using RP11-88J11 (red; located at 27.0 Mb on chr. 13; not illustrated in Fig 2C) and RP11-78D7 (green), counterstained with DAPI (light blue). Merged signals for RP11-88J11 and RP11-78D7 are observed on the normal chromosome 13 (arrowhead) and the der(13) (double arrowhead); an additional weak signal for RP11-78D7 is detected on the der(4) (arrow), indicating that the putative breakpoint (tan-shaded box) is located between the telomeric ends of RP11-949A11 and RP11-78D7.

Table 1 Clinical features seen in patients with proximal 4p deletion syndrome

Patient findings	Proband	Francke	Nielsen	Ray	Romain	Fryns	Ishikawa
Publication		1977	1977	1984	1985	1989	1990
Breakpoint	p15.2	p11-p15.2	p12-p15	p12-p15	p15.2-p15.33	p14-p15.3	p15.2-p15.32
Sex	F	F	F	F	F	M	M
Age	2y	30y	12y	17y	32y	12mo	1mo
Long face	–	+	–	–	–	+	–
Upslanted palpebral	+	–	n/r	+	–	+	–
Epicanthal folds	+	–	+	+	–	+	–
Large beaked nose	+	+	+	+	–	–	–
High or cleft palate	–	+	–	–	–	–	+
Thick lower lip	+	–	n/r	+	–	+	+
Micrognathia	+	–	+	+	–	+	+
Broad short neck	+	+	–	–	–	–	n/r
Broad hands and feet	–	+	–	+	–	–	n/r
Tall, thin habitus	–	+	+	+	+	+	–
Epilepsy	+	–	–	–	–	–	–
Mental retardation	Sev.	Mod.	Sev.	Mod.	Mild	Mod.	Mild
Neuro image abnormality	–	n/r	n/r	n/r	n/r	n/r	n/r

Studies are listed by the name of the first author. M: male; F: female; n/r: n/r; *: mild brain atrophy, **: polymicrogyria of left temporal lobe, left temporal atrophy

Figure 1

Figure 2