A case of epilepsy with myoclonic atonic seizures caused by *SLC6A1* gene mutation due to balanced chromosomal translocation

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Abstract

Introduction

Epilepsy with myoclonic atonic seizures (EMAtS) was previously thought to occur in normally developing children. We report a female case of EMAtS and mild developmental delay before onset. Importantly, a de novo balanced chromosomal translocation was recognized in the patient.

Case presentation

The patient was a 4-year-old girl. Mild developmental delay was observed during infancy. At the age of one and a half years, she developed atonic seizures once a month. At 4 years of age, her seizures increased to more than 10 times per hour. An ictal electroencephalogram (EEG) showed a 3–4-Hz spike-and-wave complex, which was consistent with atonic and myoclonic seizures of the trunk, eyelids, and lips. Therefore, EMAtS was diagnosed based on the symptoms and EEG findings. After administration of valproic acid (VPA), the epileptic seizures disappeared immediately. At the age of 5 years and 2 months, the seizures recurred but disappeared again when the dose of VPA was increased. Subsequently, no recurrence was observed until 6 years and 3 months of age on VPA and lamotrigine. Chromosome analysis of the patient disclosed 46,XX,t(3;11)(p25;q13.1)dn. Long-read sequencing of the the patient's genomic DNA revealed that the 3p25.3 translocation breakpoint disrupted the intron 7 of the *SLC6A1* gene.

Conclusion

The *SLC6A1* disruption by chromosome translocation well explains the clinical features of this patient. Long-read sequencing is a powerful technique to determine genomic abnormality at the nucleotide level for disease-associated chromosomal abnormality.

Keywords:

epilepsy with myoclonic atonic seizures (EMAtS), *SLC6A1,* balanced chromosomal translocation, long-read sequencing

Introduction

Epilepsy with myoclonic atonic seizures (EMAtS), also known as myoclonic-astatic epilepsy (MAE) or Doose syndrome, was first reported as "centrencephalic myoclonic-astatic petit mal" by Doose et al. in 1970 [1]. EMAtS accounts for 1%–2.2% of childhood-onset epilepsy cases and is characterized by normal development before seizure onset, which generally occurs between 7 months and 6 years of age [2]. In recent years, with the development of genetic testing for epilepsy, cases of EMAtS with pathogenic variants in the solute carrier family 6 member 1 gene (*SLC6A1*) have been reported [3].

Herein, we report a case of a female with EMAtS with mild developmental delay before epilepsy onset. We also detected a de novo balanced chromosomal translocation, 46,XX,t(3;11)(p25;q13.1)dn. We characterized the patient's chromosome by using long-read whole genome sequencing.

Materials/Subjects

The patient, an outpatient at Tokushima University Hospital, participated in the Initiative on Rare and Undiagnosed Diseases (IRUD, https://plaza.umin.ac.jp/irud/ [in Japanese]) for the purpose of examining the cause of her epilepsy. Genetic analyses of the patient and her parents were performed at the Department of Genetics, Yokohama City University. This study was approved by the Institutional Review Boards of Yokohama City University Faculty of Medicine and Tokushima University. Written informed consent for this study and publication of the results was obtained from the patient's legal guardians (her parents).

Methods

Exome sequencing (ES)

Proband-based ES was performed to confirm the presence of the pathogenic gene responsible for epilepsy and data were analyzed as previously described [4].

Copy number variation analysis

Copy number variation (CNV) analysis was performed using an exome

hidden Markov model (XHMM) and the jNord method [5, 6] as previously described [7].

Long-read whole genome sequencing

Nanopore sequencing using PromethION was performed as previously described [8]. In brief, output reads data after base-calling were aligned to the human reference genome (GRCh38) by using LAST and dnarrange [9]. The patient's structural variants (SVs) were called and subtracted with data from eight control samples. For consensus sequence construction, we used lamassemble (https://gitlab.com/mcfrith/lamassemble).

Confirmation of breakpoints by Sanger sequencing

Translocation breakpoints were amplified by PCR using genomic DNA from the patient and her parents. Sanger sequencing of a PCR product was performed using the Applied Biosystems 3500xL Genetic Analyzer 24-Capillary (Thermo Fisher Scientific, Tokyo, Japan).

Results

Clinical feature

The patient was born at term as a second child to healthy, non-consanguineous Japanese parents. According to her family history, her maternal aunt developed epilepsy in adulthood. Prior to the onset of epilepsy, she had shown psychomotor developmental delay (developmental quotient = 65, Kyoto Scale of Psychological Development 2001). She was stable in a sitting position at 9 months of age and was able to walk at 1 year and 4 months. She did not speak any words until 2 years of age. She developed epilepsy at 1 year and 6 months of age, with sudden atonic and myoclonic seizures of the trunk that lasted for 1–2 s. Subsequently, the same symptoms were observed once every few months. At the age of 3 years and 11 months, after the patient contracted the flu, her seizure frequency increased to more than 10 times per hour. Therefore, she was admitted to our hospital for further examination and treatment.

Blood tests and general biochemical tests performed on admission showed no abnormalities. Karyotype analysis revealed a de novo balanced chromosomal translocation, 46,XX,t(3;11)(p25;q13.1)dn as parental

karyotypes were normal (Figure 1). Interictal electroencephalogram (EEG) during wakefulness showed 4–5-Hz occipital- and parietal-dominant slow waves that were not suppressed by eye opening (Figure 2A). Light stimulation did not induce changes in the EEG or seizures. Immediately after falling asleep, her interictal EEG showed continuous high-voltage slow waves without humps or sleep spindles (Figure 2B). Ictal EEG revealed a diffuse 3–4-Hz spike-and-wave complex, consistent with the atonic seizure (Figure 2C). Furthermore, atypical absence seizures with loss of consciousness consistent with irregular 3–4-Hz spike-and-wave complexes were also identified in the ictal EEG. Based on the abovementioned clinical course and EEG findings, a clinical diagnosis of EMAtS was established and valproic acid (VPA) was administered. When the VPA dose was increased to 15 mg/kg/day, the epileptic seizures disappeared with improvement in EEG findings. At the age of 5 years and 2 months, the epilepsy recurred with atonic seizures (only once) and myoclonic seizures of the upper extremities and around the lip with exacerbation of EEG abnormalities. After the VPA dose was increased to 20 mg/kg/day, the seizures disappeared. Lamotrigine was added to treat the residual EEG abnormalities. The patient is now 6 years and 3 months old and has been seizure-free for the past 1 year. An overview of the clinical course of this case is shown in Supplementary Figure 1.

Genetic analysis

Proband-based ES did not identify any pathogenic variants or copy number variations. Long-read whole genome sequencing was performed on the patient's genomic DNA, using the PromethION. We obtained 27.0 Gb of long-read data with an averaged 12.1-Kb read length (~9× read coverage), and identified the patient's unique SVs using dnarrange. All candidate SVs were manually inspected. We found that *SLC6A1* was disrupted by the 3p25.3 translocation breakpoint (Figure 3). Sanger sequencing of the breakpoint-specific PCR confirmed the intron 7 disruption (Figure 3 and Supplementary Figures S2 and S3). The other breakpoint was identified in the *TBC1D10C* gene at 11q13.2 (Supplementary Figure S2). We found a mammalian-wide interspersed repeat (MIR) 90 bp away from the 3p25.3 breakpoint and an MIR3 375 bp away from the 11q13.2 breakpoint; the latter, however, may not have directly contributed to the formation of the

balanced translocation.

Discussion

Voltage-dependent gamma-aminobutyric acid (GABA) transporter 1 (GAT-1), encoded by *SLC6A1*, is a major GABA transporter in the human central nervous system [10]. GABA acts to suppress excessive neural activity in the central nervous system. GAT-1 is expressed mainly on the nerve terminals of GABA-mediated neurons but is also found in astrocytes and is essential for GABA reuptake and removal from the extracellular domain [11]. Recently, the concept of *SLC6A1*-related neurodevelopmental disorders was proposed. The expected molecular mechanism by which variants lead to SLC6A1-related disorders is loss of function or haploinsufficiency. This disease-model is supported by *in vivo* and *in vitro* experiments in both wild-type and GAT-$1^{-/-}$ mice as well as by studies on recombinant GAT-1 proteins from individuals with *SLC6A1* variants [3]. In the case presented here, a chromosomal translocation with a breakpoint in the *SLC6A1* gene could also have caused haploinsufficiency of GAT-1.

According to a report of 116 cases by Goodspeed et al., the main symptoms of *SLC6A1*-related neurodevelopmental disorders are epilepsy (92/101, 91.1%), developmental delay and cognitive impairment (46/56, 82.1%), and autistic traits (20/92, 22.8%) [3]. The authors observed developmental delay in 60% of patients before seizure onset, mostly in the mild to moderate range (35/55, 63.6%), and the most common epileptic syndrome was EMAtS (20/82, 24.3%). Our patient also showed developmental delay before the onset of EMAtS, which is consistent with the features of *SLC6A1*-related neurodevelopmental disorders. Katrine et al. also reported cases of pathogenic *SLC6A1* variants, where 31/34 patients (91%) had epilepsy, with an average onset age of 3.7 years; 16 patients met the EMAtS criteria and seizures were resolved in 20/31 (65%) patients, with VPA being the most effective agent. Furthermore, EEG revealed an irregular 2.5–3.5-Hz spike-and-wave complex in 25/31 (81%) patients [12]. These characteristics of a high seizure-free rate, good responsiveness to VPA, and EEG findings, are consistent with those of our case and differ from the conventional diagnosis criteria of MAE or Doose syndrome. Patients with MAE or Doose syndrome do generally not have presymptomatic intellectual disabilities, and these conditions are resistant to treatment. However, comparison of the

traditional definition of MAE with the International League Against Epilepsy (ILAE) 2022 definition of EMAtS reveals that the prognostic description of epileptic seizures has changed. In the 2022 definition of EMAtS, despite initially experiencing drug-resistant seizures, two-thirds of children achieve remission, usually within 3 years of onset, and can be weaned off antiseizure therapies [13]. Classification of syndromes is important, however, it is equally important to accumulate cases for each causative gene in determining treatment strategies.

VPA promotes the increase of gamma-aminobutyric acid levels, which contributes to the activation of an intracerebral suppression network. This activation mechanism probably contributes to seizure suppression in patients with *SLC6A1*-related neurodevelopmental disorders, because *SLC6A1* encodes GAT-1. Therefore, if EMAtS is clinically suspected, genetic testing may aid in determining optimal therapeutic strategies and predicting the prognosis more accurately. To date, only one other case of a balanced chromosomal translocation, 46,XX,t(3;4)(p25.3;q31.1) has been reported to disrupt the intron 1 of *SLC6A1* [14].

In the case presented here, the other breakpoint was detected in the *TBC1D10C* gene at 11q13.2, which has an N-terminal Rab-GTPase domain and a C-terminal binding site for calcineurin and inhibits both the Ras signaling pathway and calcineurin, a phosphatase regulated by calcium and calmodulin [15]. According to the Human Gene Mutation Database, only one variant in *TBC1D10C*, c.659G>A p.R220Q, has been associated with autism spectrum disorder.

Although short- or long-read WGS sequencing would be a straightforward approach to identify breakpoint-associated genes, here we first applied exome sequencing to rule out pathogenic variants unrelated to chromosomal abnormalities.

Conclusion

We encountered a case of a girl with EMAtS in which a de novo balanced translocation disrupted the *SLC6A1* gene. Long-read sequencing is a powerful approach to determine the translocation breakpoints in which a culprit gene is indeed disrupted.

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Author Contributions

TMo and MS are co-first authors. TMo, TT, AG, YT, and MU were involved in the patient treatment and contributed to the preparation of the original draft describing the clinical course. MS, AF, TMi, and NM were responsible for the genomic analysis. All authors contributed to the writing of the final manuscript.

Conflict of interest Disclosures

The authors declare no potential conflicts of interest regarding the research, authorship, or publication of this article.

References

[1] Doose H, Gerken H, Leonhardt R, Völzke E, Völz C. Centrencephalic myoclonic-astatic petit mal. Clinical and genetic investigation. Neuropädiatrie 1970;2:59–78. https://doi.org/10.1055/s-0028-1091841.

[2] Hinokuma N, Nakashima M, Asai H, Nakamura K, Akaboshi S, Fukuoka M, et al. Clinical and genetic characteristics of patients with Doose syndrome. Epilepsia Open 2020;5:442–50. https://doi.org/10.1002/epi4.12417.

[3] Goodspeed K, Pérez-Palma E, Iqbal S, Cooper D, Scimemi A, Johannesen KM, et al. Current knowledge of *SLC6A1*-related neurodevelopmental disorders. Brain Commun 2020;2:fcaa170. https://doi.org/10.1093/braincomms/fcaa170.

[4] Sakamoto M, Sasaki K, Sugie A, Nitta Y, Kimura T, Gursoy S, et al. De novo *ARF3* variants cause neurodevelopmental disorder with brain abnormality. Hum Mol Genet 2021;31(1):69-81. https://doi.org/10.1093/hmg/ddab224

[5] Fromer M, Moran JL, Chambert K, Banks E, Bergen SE, Ruderfer DM, et al. Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. Am J Hum Genet 2012;91:597-607. https://doi.org/10.1016/j.ajhg.2012.08.005.

[6] Nord AS, Lee M, King MC, Walsh T. Accurate and exact CNV identification from targeted high-throughput sequence data. BMC Genomics 2011;12:184. https://doi.org/10.1186/1471-2164-12-184.

[7] Uchiyama Y, Yamaguchi D, Iwama K, Miyatake S, Hamanaka K, Tsuchida N, et al. Efficient detection of copy-number variations using exome data: Batch- and sex-based analyses. Hum Mutat 2021;42:50-65. https://doi.org/10.1002/humu.24129.

[8] Ohori S, Tsuburaya R, Kinoshita M, Miyagi E, Mizuguchi T, Mitsuhashi S, et al. Long-read whole-genome sequencing identified a partial MBD5 deletion in an exome-negative patient with neurodevelopmental disorder. J Hum Genet 2021;66(7):697-705. https://doi.org/10.1038/s10038-020-00893-8

[9] Mitsuhashi S, Ohori S, Katoh K, Frith M, Matsumoto N.A pipeline for complete characterization of complex germline rearrangements from long DNA reads. Genome Med 2020;12(1):67.

https://doi.org/ 10.1186/s13073-020-00762-1.
[10] Madsen KK, Hansen GH, Danielsen EM, Schousboe A. The subcellular localization of GABA transporters and its implication for seizure management. Neurochem Res 2015;40:410–9. https://doi.org/10.1007/s11064-014-1494-9.
[11] Zhou Y, Danbolt NC. GABA and glutamate transporters in brain. Front Endocrinol (Lausanne) 2013;4:165. https://doi.org/10.3389/fendo.2013.00165.
[12] Johannesen KM, Gardella E, Linnankivi T, Courage C, de Saint Martin A, Lehesjoki AE et al. Defining the phenotypic spectrum of SLC6A1 mutations. Epilepsia 2018;59:389–402. https://doi.org/10.1111/epi.13986.
[13] Nicola S, Elaine CW, Ingrid ES, Rima N, Kate R, Pauline S, et al. International League Against Epilepsy classification and definition of epilepsy syndromes with onset in childhood: Position paper by the ILAE Task Force on Nosology and Definitions. Epilepsia 2022;63:1398-442.
[14] Pesz K, Pienkowski VM, Pollak A, Gasperowicz P, Sykulski M, Kosińska J, et al. Phenotypic consequences of gene disruption by a balanced de novo translocation involving SLC6A1 and NAA15. Eur J Med Genet 2018;61:596–601. https://doi.org/10.1016/j.ejmg.2018.03.013.
[15] National center for biotechnology information [Internet]. TBC1D10C TBC1 domain family member 10C [Homo sapiens (human)] [updated 2022 Dec 4]. Available from://www.ncbi.nlm.nih.gov/gene/374403.

Figure legends

Figure 1. Partial karyotype of the patient

Karyotype analysis demonstrating a de novo balanced chromosomal translocation, 46,XX,t(3;11)(p25;q13.1)dn.

Figure 2. Electroencepahlogram (EEG) of the patient at 4 years of age

A: EEG during wakefulness. Occipital- and parietal-dominant slow waves of 4–5 Hz were observed, which were not suppressed by eye opening.
B: Interictal EEG during sleep. Continuous high-voltage slow waves without humps or sleep spindles were observed.
C: Ictal EEG revealing a diffuse 3–4-Hz spike-and-wave complex consistent with the occurrence of an atonic seizure.

Figure 3. Balanced chromosomal translocation in the patient

A: Top: scheme of a patient's balanced translocation. Middle: consensus sequence of reads obtained from the PromethION data, indicating translocation breakpoints. Bottom: Sanger sequencing electropherograms of the breakpoints.
B: Entire *SLC6A1* gene and location of the breakpoint (red line).

Supplementary Figure S1. An overview of the clinical course of the patient

Frequency of epileptic seizures and course of treatment. VPA: valproic acid, LTG: lamotrigine.

Supplementary Figure S2. Location of translocation breakpoints

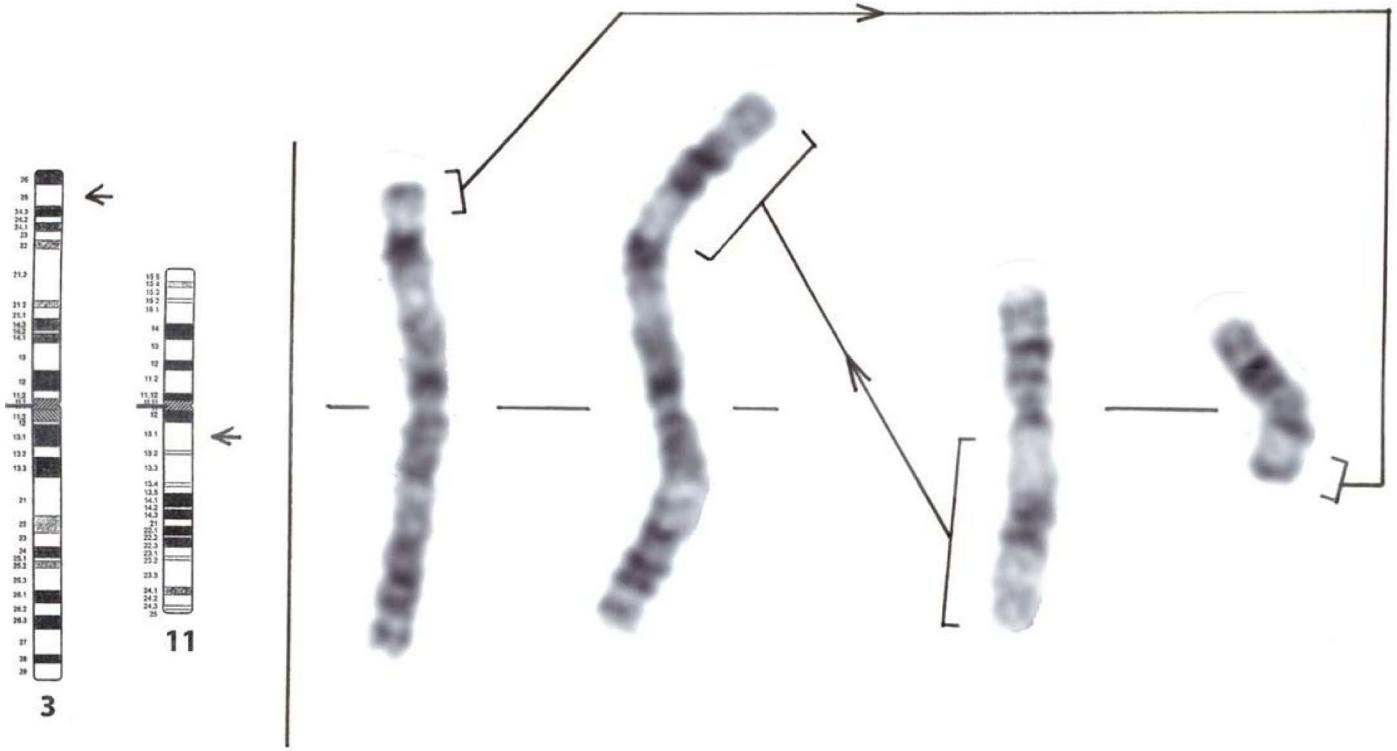
Based on the UCSC genome browser, translocation breakpoints are found in the intron 7 of *SLC6A1* at 3p25.3 and the intron 8 of *TBC1D10C*, Mammalian-wide interspersed repeat (MIR) 90 bp away from the 3p25.3 breakpoint and MIR3 375 bp away from the 11q13.2 breakpoint.

Supplementary Figure S3. Breakpoint PCRs

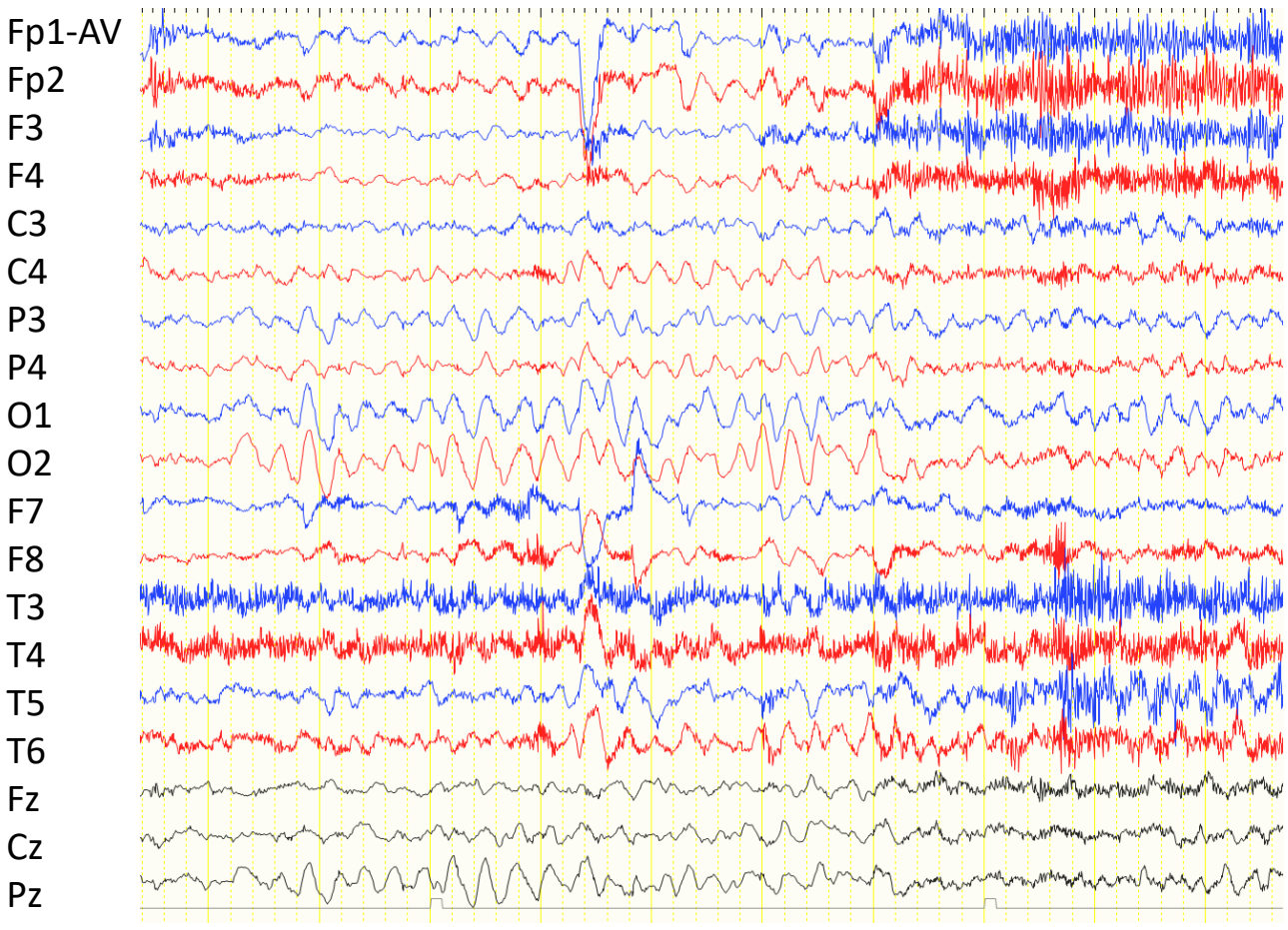
Top: Balanced translocation chromosomes, der(3) and der(11). Primer positions are indicated by blue arrows.
Middle: Breakpoint PCR successfully amplified the breakpoints only in the

patient.
Bottom: Primer sequences of breakpoint PCR.

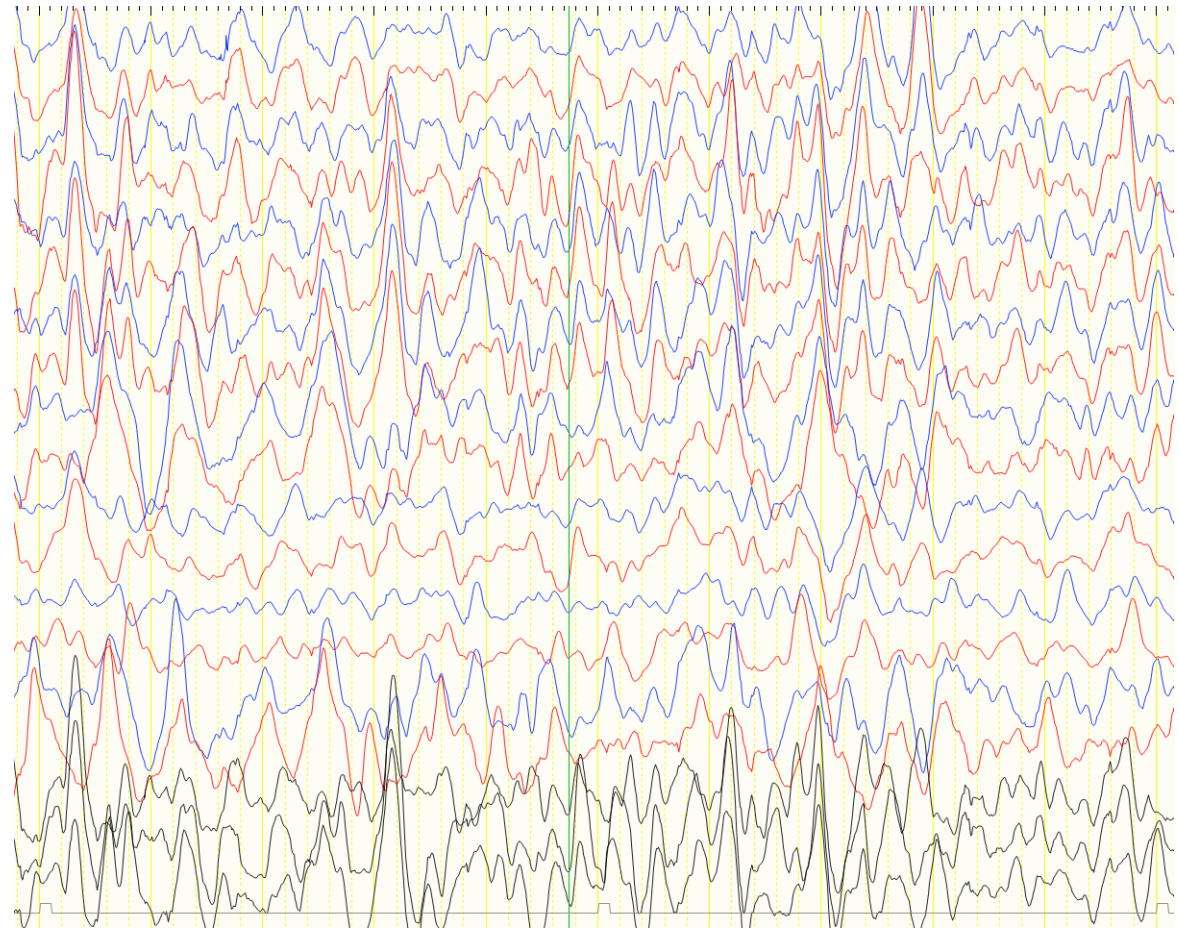
【Partial karyotyping】



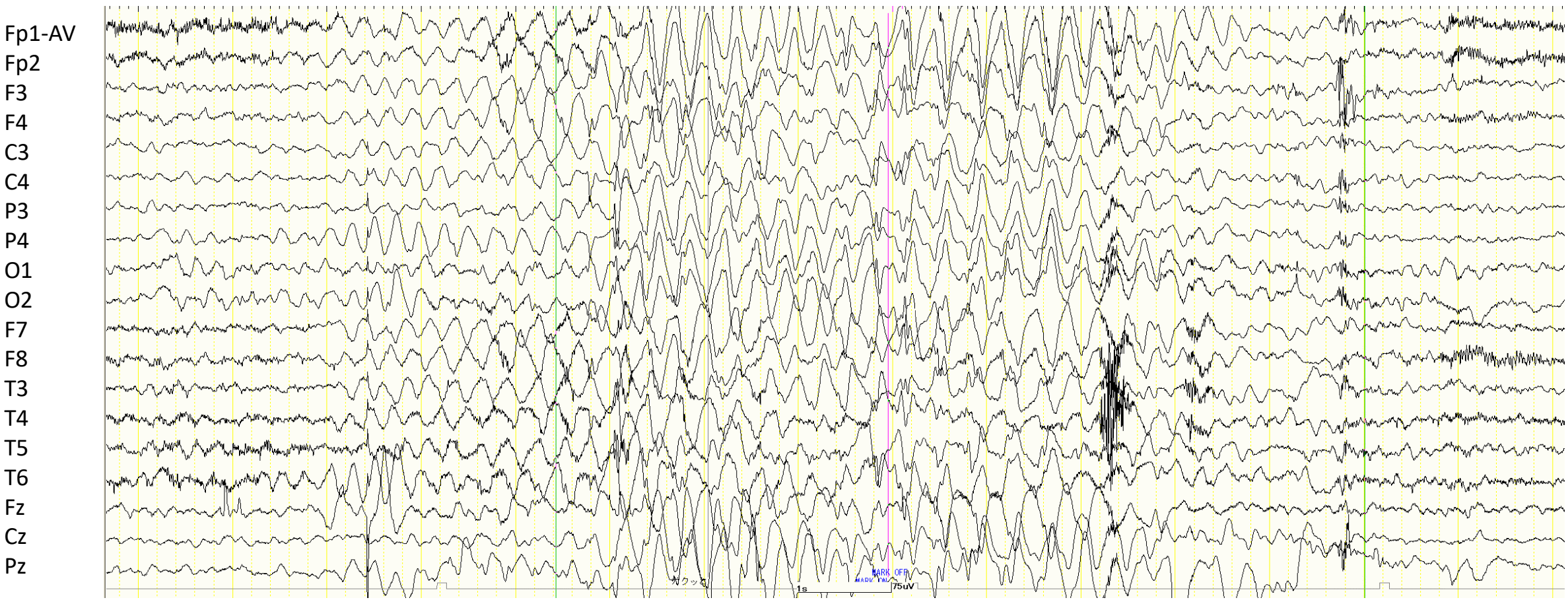
A: Awake



B: Sleep

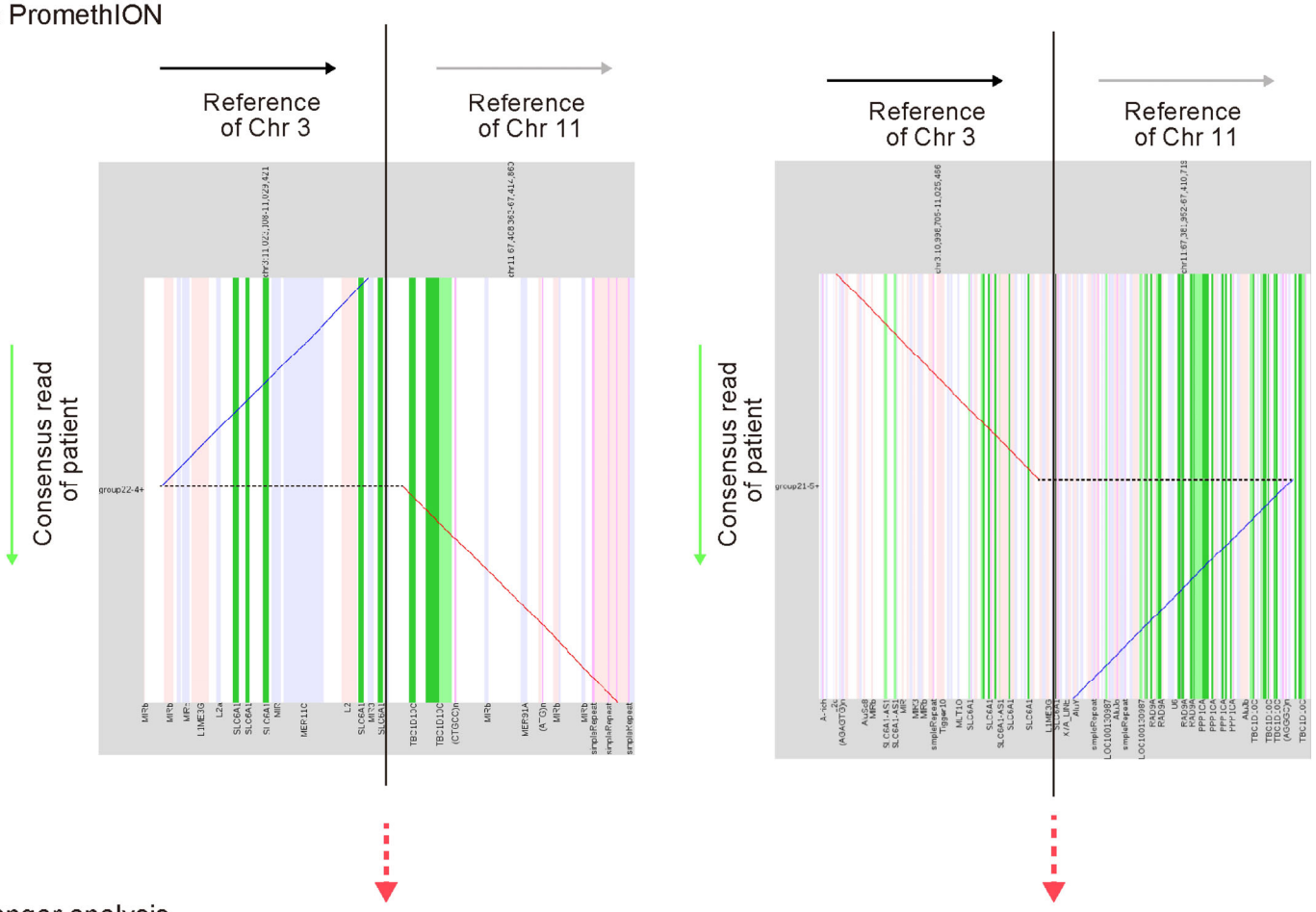


C: ictal

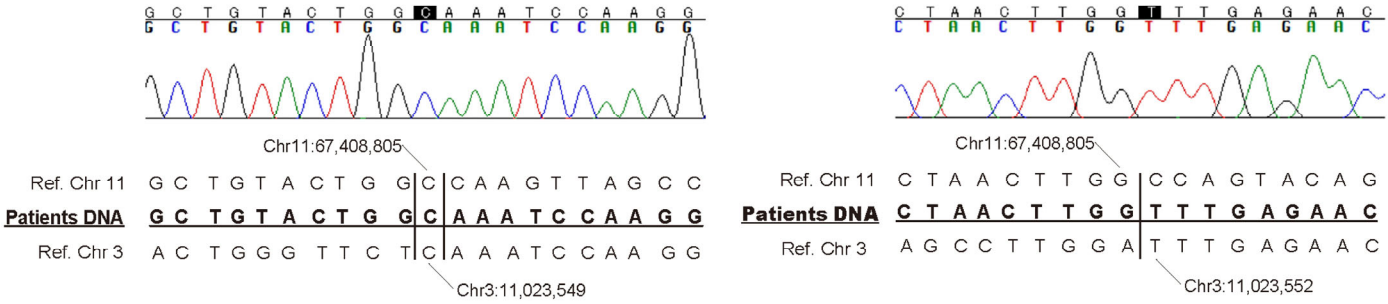


A

Consensus reads from PromethION



Sangar analysis



B