



Analysis of IKBKG/NEMO gene in five Japanese cases of incontinentia pigmenti with retinopathy: fine genomic assay of a rare male case with mosaicism

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論文題目

Analysis of *IKBKG/NEMO* gene in five Japanese cases of incontinentia pigmenti with retinopathy: fine genomic assay of a rare male case with mosaicism

(網膜症を伴う色素失調症の日本人5症例における *IKBKG/NEMO* 遺伝子の解析: モザイクを示す稀な男性症例の精密ゲノム分析)

論文の内容の要旨

[Introduction]

Incontinentia pigmenti (IP) is an X-linked dominant genodermatosis involving many systemic abnormalities and caused by the mutation in *IKBKG/NEMO* gene; that is usually lethal *in utero* in males, though exceptionally they survive very rarely either with Klinefelter syndrome or a somatic mosaicism consisting of normal cells and mutant cells. An intrachromosomal rearrangement that deletes *NEMO* exon 4–10 accounts for 90% of the IP mutations. In this study, we did genomic analysis of five Japanese IP patients together with three patients' parents (both fathers and mothers). Among those five cases, one was a very rare boy case, and therefore it was subjected to further detailed genomic analysis for the mosaicism ratio and the structure of mutant allele in a sequence level.

[Materials and Methods]

This study was approved by the Institutional Review Board of the National Center for Child Health and Development (518 and 1676) and the Ethics Committee of Hamamatsu University School of Medicine (14-040). Informed consent of the patients, their parents and the healthy control were taken.

The elaborate ocular clinical manifestations and treatment outcomes of P262, P280, and P295 were described in the paper by Nakao S *et al.* in the Japanese Journal of Ophthalmology (2020). Severe posterior retinopathy occurred bilaterally in two patients (P280 and P295) and unilaterally in one patient (P301), whereas unilateral mild peripheral retinopathy developed in one patient (P356). In the boy case of P262, severe posterior retinopathy appeared in the left eye but minimal avascular retina was detected in the right eye. Marked left-right difference was seen in the boy case and one of the four girl cases (P301).

Analysis of five patients and their parents were confirmed by various methods specific PCR techniques, which includes multiplex PCR to detect the exon 4–10 deletion in *NEMO* and $\Delta NEMO$ (a pseudogene of *NEMO* with highly homologous genomic structure), specific detection of the exon 4–10 deletion in *NEMO*, specific detection of the exon 4–10 deletion in $\Delta NEMO$, DNA sequencing for the deletion breakpoints, and real-time PCR for determination of the gene copy number with $\Delta\Delta CT$ method.

[Results]

Four patients including the boy revealed the recurrent exon 4–10 deletion in the sole known causative gene *IKBKG/NEMO*. The girl P295 is a *de novo* and the girl P356 is an inherited case. For the girl P280, an extensive sequencing analysis was done for all exons, exon-surrounding intronic regions, and promoter region of *NEMO*, but no pathogenic mutation was found.

The boy's saliva DNA showed a mosaicism consisting of the deletion and intact alleles,

but his blood DNA did not. Relative quantification analysis of the real-time PCR data by $\Delta\Delta\text{CT}$ method estimated the mosaicism ratio of the boy's saliva as 45:55 (deletion:intact). A genomic analysis for the recurrent deletion at the nucleotide sequence level has been performed directly using patient's DNA and it has been clarified that the breakpoints are within two MER67B repeats in the intron 3 and downstream of exon 10.

[Discussion]

In this study, 80% (= 4/5) IP patients showed the typical exon 4–10 deletion, though the number of patients is very small to discuss. Of these five cases, one was a rare boy case (P262), for which extensive molecular genetical analyses was performed. His saliva DNA showed the recurrent exon 4–10 deletion of *NEMO*, but blood DNA did not. Since the *NEMO* allele with the deletion and its intact allele were together detected in his saliva, it was indicated that a mosaicism exists in his saliva tissue. It is considered that, he survives because significant *NEMO* deletion is not in the essential tissues to maintain the life such as blood, and the intact *NEMO* partially exists in other tissues such as saliva.

Next, the mosaicism ratio of the boy's saliva obtained at 4 months age was elucidated 45:55 (deletion:intact) by usual real-time PCR method. The assay method of the mosaicism ratio using real-time PCR reported in this study is less cost- and labor-consuming, and is considered to be suitable for regular monitoring of patients with recurrent exon 4–10 deletion.

Since most of the ocular tissue is of ectodermal origin, which is similar to saliva, it is considered that the boy's eyes would have the mosaicism, probably causing his retinopathy. Their marked left–right difference in the severity of his retinopathy might be related to the possible different mosaicism ratio in both eyes. For the boy, the sample was collected only once at 4 months age. Therefore, this mosaicism ratio 45% in his saliva might be changed with age. It is also possible that other tissues might have the difference in the mosaicism ratio as well as in its changing speed if samples could be collected.

[Conclusion]

To our best knowledge, this is the first report of the assay for the mosaicism ratio of a male IP case with a recurrent exon 4–10 deletion and the direct sequencing analysis of the breakpoints of the deletion.