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Letter to the editor

Title: Pseudo-homozygous mutation due to a primer site polymorphism in hereditary ATTR amyloidosis -A pitfall of PCR-based genetic testing-

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Hereditary ATTR amyloidosis is an autosomal dominant disease associated with mutations of the transthyretin (*TTR)* gene, which exists on the long arm of chromosome 18. TTR protein tetramer instability leads to systemic amyloid deposition, and causes multi-organ dysfunction, involving the peripheral nerves, autonomic nervous system, cardiac muscles, and gastrointestinal tract [1]. To date, more than 140 types of mutations in the *TTR* gene have been reported. In the endemic foci of Japan, the Val30Met (p.Val50Met) mutation is the most common. Although most cases of hereditary ATTR are attributable to heterozygous mutations of the *TTR* gene, a few cases with homozygous mutations have been reported. Homozygous ATTR may occasionally develop earlier in some cases [2]. Here, we report a pseudo-homozygous hereditary ATTR amyloidosis patient with a *TTR* Ala120Ser (p.Ala140Ser) mutation due to a primer site polymorphism.

A 65-year-old man visited our hospital due to progressive weakness and numbness of the extremities. The age of onset was 54. The patient is the third of three siblings, and he has two daughters. There has been no consanguineous marriage or family history of neurological diseases in his pedigree.

On visiting the Neurology department of our hospital in his 65th year, a

neurological examination revealed polyneuropathy without autonomic dysfunction. Mild liver dysfunction was identified. An echocardiogram showed left ventricular and leaflet thickening, left atrial dilation and a pseudonormalized pattern of transmitral flow velocity. Late gadolinium enhancement of cardiac MRI was identified in the ventricular septum. Amyloid deposition was detected by a myocardial biopsy. Subsequent *TTR* genetic testing was accordingly conducted.

The result of the *TTR* gene test revealed a homozygous Ala120Ser/c.418G>T missense mutation (Figure 1A). This type of mutation has previously been reported in only two cases, with no previous reports in Japan. Because of the homozygous nature of the mutation, it was considered almost certain that the patient's two daughters would have inherited this mutation. After the attending physician had carefully performed informed consent for the daughters about the aforementioned possibility, they duly requested genetic testing. In both case, however, the results of the test proved negative. The attending doctor subsequently consulted the Division of Clinical Genetics at Hokkaido University Hospital. Although there was the possibility of non-paternity, their similar facial appearance suggested that this was not the case. The patient was accordingly informed regarding the abnormal result and the need for re-examination. After redesigning the primers for PCR, re-sequencing of the PCR product and mass

spectroscopic analysis of the TTR protein were carried out.

In the initial analysis, the primers were designed to sandwich exon 4, including the Ala120Ser mutation, in the *TTR* gene. However, toward the 5' end of the primer (ATGGATCTGTCTGTCTTCTCT) was found to contain a polymorphism (rs36204272 G>C) that is reported in the dbSNP of the National Center for Biotechnology Information [3]. The allele frequency of this polymorphism in East Asians is relatively high (6.75%). In the "INTEGRATIVE Japanese Genome Variation Database" constructed by Tohoku University Tohoku Medical Megabank Organization (ToMMo) [4], which summarizes Japanese polymorphisms with allelic frequencies greater than 5%, this polymorphism is also described. It seemed this polymorphism has previously given rise to discrepancies in the results of genetic testing. New primers were accordingly designed from the site that contains the initial primers and exon 4, but does not include any polymorphisms with an allelic frequency greater than 1%. When genetic testing using the redesigned primers was performed on the proband and his daughters, the expected polymorphism was detected in the initial primer site. The results of the test confirmed that the proband has a heterozygous Ala120Ser mutation (Figure 1B), but that neither daughter has the mutation. Mass spectroscopy of the TTR protein also revealed the wild type peaks and the presence of the heterozygous Ala120Ser mutation

(Figure 1C, D). These results showed that the normal allele containing the polymorphism was not amplified in the initial analysis, and therefore the results for proband indicated a homozygous mutation. As a result, this series of investigations led to solutions to problems related to this genetic test. We followed up with the proband and his family with the correct interpretation.

In cases where children are normal homozygous individuals, even though one parent has a homozygous mutation, there may be several explanations: (i) no biological parent–child relationship, (ii) uniparental disomy, or (iii) hemizygous individuals who inherited the deletion-containing allele.

In the results of a survey in which whole genomes of 1,070 healthy Japanese people were analyzed, single-nucleotide variants (SNVs) with allele frequencies greater than 1% were found in 21.2 million locations, and SNVs with allele frequencies greater than 5% were found in 4.3 million locations [5] [6]. As a future countermeasure to avoiding incorrect genetic test results, use of databases such as the “INTEGRATIVE Japanese Genome Variation Database” [4] in designing primers is considered desirable. Additionally, it is better to not use the primers in regions containing highly frequent SNPs.

Compared with two previous cases in which patients harbored Ala120Ser

mutation, the patient in the present case had a younger age of onset [7] [8]. In cases where mutations are frequently reported, detailed reviews can be performed using large amounts of clinical information. However, when mutations are infrequently reported, it is difficult to make an adequate assessment due to a deficiency of clinical information. Therefore, in the present case, careful interpretation of the results of genetic testing was necessary.

We present a case of a hereditary ATTR amyloidosis patient who had a primer site polymorphism that made it difficult to interpret the results of genetic testing. Consequently, when conducting genetic testing, the possibility that a primer site is polymorphic should be taken into consideration. When rarely reported mutations are detected, it is desirable to obtain evidence from multiple sources, such as that from DNA, RNA, and mass spectroscopic analyses, and, moreover, the results of genetic testing need to be carefully interpreted.

Conflict of Interest: The authors declare no conflict of interest.

References

1. Koike H, Sobue G. Diagnosis of familial amyloid polyneuropathy: wide-ranged clinicopathological features. Expert Opin Med Diagn 2010; 4: 323–331.

2. Tojo K, Sekijima Y, Machida K, Tsuchiya A, Yazaki M, Ikeda S. Amyloidogenic transthyretin Val30Met homozygote showing unusually early-onset familial amyloid polyneuropathy. Muscle Nerve 2008; 37: 796–803.

3. National Center for Biotechnology Information. dbSNP Short Genetic Variations. https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=36204272 Accessed 29 September 2016.

4. Tohoku University Tohoku Medical Megabank Organization (ToMMo). INTEGRATIVE Japanese Genome Variation Database (2015). https://ijgvd.megabank.tohoku.ac.jp/ Accessed 29 September 2016.

5. Kawai Y, Mimori T, Kojima K, Nariai N, Danjoh I, Saito R, Yasuda J, et al. Japonica array: improved genotype imputation by designing a population-specific SNP array with 1070 Japanese individuals. J Hum Genet 2015; 60: 581–587.

6. Yamaguchi-Kabata Y, Nariai N, Kawai Y, Sato Y, Kojima K, Tateno M, Katsuoka F, et al. iJGVD: an integrative Japanese genome variation database based on whole-genome sequencing. Hum Genome Var 2015; 2:15050.

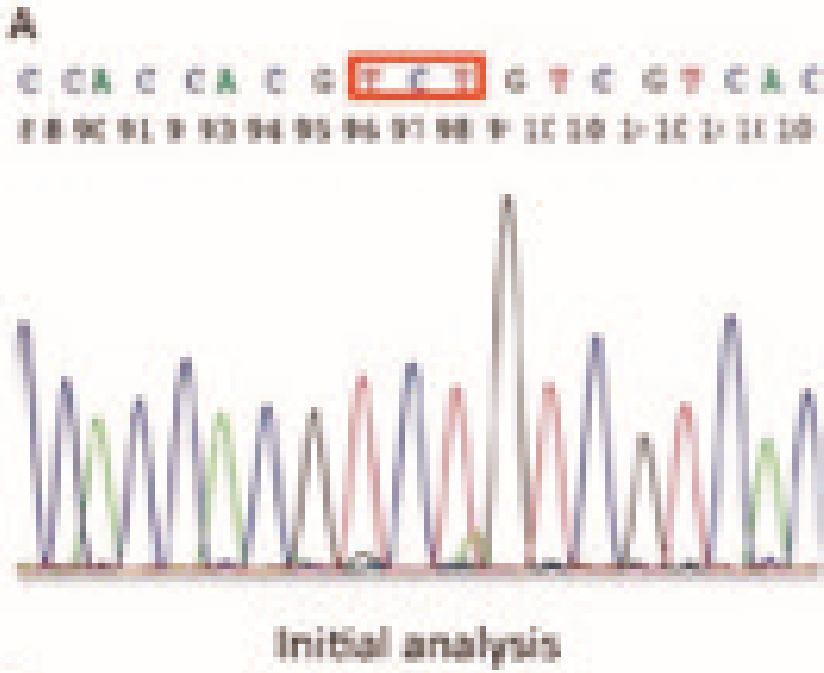
7. Luigetti M, Conte A, Del Grande A, Bisogni G, Madia F, Lo Monaco M, Laurenti L, et al. TTR-related amyloid neuropathy: clinical, electrophysiological and pathological findings in 15 unrelated patients. Neurol Sci 2013; 34: 1057–1063.

8. Lachmann HJ, Booth DR, Booth SE, Bybee A, Gilbertson JA, Gillmore JD, Pepys MB, et al. Misdiagnosis of hereditary amyloidosis as AL (primary) amyloidosis. N Engl J Med 2002; 346: 1786–1791.

Titles and legends to figure

Figure 1. The Sanger sequence of the initial analysis and redesigned analyses of exon 4 of the *TTR* gene, and the mass spectra.

The result of the *TTR* gene test revealed a homozygous Ala120Ser (p.Ala140Ser)/c.418G>T missense mutation (red square) (A). The Sanger sequence of the redesigned analysis (B), together with the mass spectra showing the pattern for a normal subject (C) and that of the patient (D).


A
Initial analysis
B
G/T
Redesigned analysis
C
WT
WT+Cys
Normal pattern
D
WT
WT+Cys
p.A120S
Patient