Common origin of the Val30Met mutation responsible for the amyloidogenic transthyretin type of familial amyloidotic polyneuropathy

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ONLINE MUTATION REPORT

The amyloidogenic transthyretin (TTR) type of familial amyloidotic polyneuropathy (FAP [MIM 176300], http://www.ncbi.nlm.nih.gov/OMIM/) is the most common form of hereditary systemic amyloidosis. It is well known that amyloidogenic TTR resulting from gene mutations is a major constituent of amyloid deposits in tissues of patients with FAP. To date, more than 80 mutations of the TTR gene have been associated with human amyloidosis. Of those mutations, a mutation changing valine at amino acid 30 to methionine (Val30Met) is the most common, and only patients with this mutation are found in large foci throughout the world.

The clinical manifestations of the TTR Val30Met type of FAP in Japan and Portugal are as follows: (1) the disease is inherited as an autosomal dominant trait with equal sex distribution and high penetrance; (2) the age at onset is late twenties to early forties; (3) polyneuropathy with sensory distribution and high penetrance; (4) manifestations of various autonomic dysfunctions such as severe orthostatic hypotension, disturbed bowel movement with constipation and diarrhoea, impotence, and urinary incontinence invariably appear during the course of the disease; and (5) amyloid deposition in loco causes dysfunction of various organs. In contrast, the clinical profile of Swedish patients is different: the average age at onset is the middle fifties, and penetrance of the disease is very low. Also, the disease progresses more slowly in Swedish patients than in Japanese and Portuguese patients.

The disorder was first described in Portugal in 1952. In the 1960s, large foci of patients were found in Japan and Sweden. In Japan, two large foci of TTR Val30Met type FAP exist: one in Kumamoto prefecture of Kyushu, and the other in Nagano prefecture on the Mainland of Japan. However, these two foci are geographically distant, and a consanguineous relationship between the foci has not been identified. The issue of whether there is a common origin for a mutant allele in the two foci has not been resolved. Furthermore, Continho11 hypothesised that a mutant allele in the Portuguese kindred could be the origin of the mutation for FAP foci throughout the world, including Japan, Europe, North and South America, and Africa. This hypothesis was based only on well-known historical bonds, and thus far it has not been scientifically tested.

Recently, estimation of haplotype in the absence of DNA of related individuals became possible by means of the maximum-likelihood method algorithm.12 In addition, the human genome project has continuously provided human genome resources. Almost 200,000 reliable single-nucleotide polymorphisms (SNPs) for the entire genome of the Japanese population are now available (IMS JSNP database, http://snp.im.s.u-tokyo.ac.jp/index.html). Microsatellite information is now also available (GenBank, http://www.ncbi.nlm.nih.gov/Entrez/); assay of this information provides a powerful method for determining the origin of the gene because heterozygosity is usually much higher than that for SNPs. Statistical software can be used to construct a haplotype using SNPs and microsatellite information together (Arlequin, http://lbh.unige.ch/arlequin/). Thus, we decided to reanalyse the origin of Val30Met mutation with the newly developed methods and resources.

We first collected DNA samples from five foci—two foci in Japan and one each in Portugal, Majorca Island (Spain), and Sweden—and then analysed haplotype structures with four SNPs and five microsatellite markers covering a 215 kb TTR gene region. We observed major haplotypes close to 50% in frequency in each focus of the world, which indicated that each focus originated from one founder because the disease is inherited in an autosomal dominant fashion. Furthermore, we obtained support for the hypothesis that the origin of the mutation is common in the Spanish, Portuguese, and Japanese foci but not in the Swedish foci.

Key points

- Our work aimed to identify the origin of Val30Met mutation, responsible for the amyloidogenic transthyretin (TTR) type of familial amyloidotic polyneuropathy (FAP) found in Japan and Europe as foci.
- We analysed the TTR gene haplotype of FAP patients from Japan, Portugal, Spain, and Sweden, and of Japanese and Caucasian control subjects with four single nucleotide polymorphisms (SNPs) and five microsatellite markers in the neighbouring gene region, spanning 215 kb (loci 1–9).
- We observed that the disease-causing haplotype is the same in two major foci in Japan, indicating the existence of a common founder.
- By comparing haplotype among foci, we obtained results indicating that there could be a common founder for Japanese and Portuguese patients, and for Portuguese and Spanish patients, but not for Swedish or other patients.
- These data, plus the history of 16th century trade, lead to the plausible hypothesis of a mutant allele of a Portuguese kindred being the origin of the mutation in most Japanese FAP patients.

Abbreviations: FAP, familial amyloidotic polyneuropathy; SNPs, single nucleotide polymorphisms; TTR, transthyretin
METHODS

Study subjects

A total of 100 DNA samples were collected from FAP patients with TTR Val30Met in four countries. In Japan, in addition to patients in the two major foci (16 patients from Kumamoto and two from Nagano), 20 patients from other locations throughout the country agreed to participate in this study. In Europe, 21 samples from Majorca Island (Spain), 18 samples from Portugal, and 23 samples from Sweden were collected. A total of 54 healthy volunteers in Asahikawa Medical College were recruited to provide DNA samples for haplotype analysis of the Japanese population, and 96 Caucasian DNA samples were purchased from the Coriell Cell Repository (Coriell Institute, Camden, NJ, USA). The Ethical Committee of Kumamoto University School of Medicine approved this study, and all patients and Japanese volunteers gave written informed consent.

Microsatellite genotyping

We selected five informative microsatellite sequences (L1, L2, L4, L8, and L9) near the TTR gene region from the National Center for Biotechnology Information (NCBI) database GenBank (fig 1). PCR primers were designed to flank the repeat sequences for the analysis. The PCR primers were as follows: L1 forward: 5'-TCAAGAGGCTCTAAGAGA-3'; L1 reverse: 5'-AAGAATTGAAGTGGGAA-3'; L2 forward: 5'-AGGCAAGCTGATGTAGCTTG-3'; L2 reverse: 5'-GAGTCCCCTGGCTCAATAT-3'; L4 forward: 5'-TTCTCCTCGTGCGGAC TTATT-3'; L4 reverse: 5'-TAGTGTCCCAAACGGAGCT-3'; L8 forward: 5'-AACCTGAGATAGAGCTTC-3'; L8 reverse: 5'-TCTCCCTAATCTAAGAAAGCCACAT-3'; L9 forward: 5'-GGCCGTCACAGGTCTAGAA-3'; L9 reverse: 5'-CCACCGAG GAAAACCAACCT-3'. Fluorescence-labeled primers were used. PCR was performed in a volume of 7.5 μl containing 20 ng of genomic DNA, 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 μM dNTPs, each primer at 5 pmol, and 0.25 U AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). PCR amplification was performed for 40 cycles at 95°C for 30 s, at 53–60°C for 30 s, and at 72°C for 30 s, depending on the region analysed, with a final extension step of 5 min at 72°C in a Gene Amp PCR9700 System (Applied Biosystems, Foster City, CA). Electrophoresis was performed with an ABI 310 sequencing reaction and electrophoresis were performed with the BigDye Terminator kit (Applied Biosystems, Foster City, CA), according to the manufacturer’s protocol.

SNP genotyping

Four SNPs—L3, L5, L6, and L7—were identified from the JMS-JST JSNP database (fig 1). L1 was located about 90 kb upstream of L2, and L8 was about 103 kb downstream of L7. Thus, the gene region analysed covered 215 kb (fig 1). The heterozygosity of microsatellite markers of Japanese and Caucasian controls is shown in table 1. All but L2, whose name is D18S36, are anonymous in the GenBank database. All markers except L1 showed similar heterozygosities in Japanese and Caucasians subjects. Four SNPs used in this study (L3, L5, L6, and L7) were obtained from the Japanese SNP database (IMS-JST JSNP). Two of those (loci 3 and 5) were located in the 5' flanking region, and the remaining two (loci 6 and 7) were located in intron 3, as shown in fig 1 and table 2. Almost no ethnic difference in allele frequency between Japanese and Caucasian control subjects was found. The Val30Met mutation occurs in exon 2 (fig 1).

All nine polymorphisms were employed to construct haplotypes for Japanese patients and controls. To clarify the origin of the mutant allele, Japanese patients were classified into three groups: 16 patients from Kumamoto, two from Nagano, and 20 from the rest of Japan. As shown in table 3, patients from Kumamoto showed a major haplotype frequency of 40% (which is close to 50%), but the same haplotype in the controls was very rare, in fact, it was very rare.

RESULTS AND DISCUSSION

Nine polymorphisms were employed in the present study. Of those, five (L1, L2, L4, L8, and L9) are microsatellite markers (fig 1). L2 and L4 were in the 5' flanking region of the gene. L1 was located about 90 kb upstream of L2, and L8 was about 103 kb downstream of L7. Thus, the gene region analysed covered 215 kb (fig 1). The heterozygosity of microsatellite markers of Japanese and Caucasian controls is shown in table 1. All but L2, whose name is D18S36, are anonymous in the GenBank database. All markers except L1 showed similar heterozygosities in Japanese and Caucasians subjects. Four SNPs used in this study (L3, L5, L6, and L7) were obtained from the Japanese SNP database (IMS-JST JSNP). Two of those (loci 3 and 5) were located in the 5' flanking region, and the remaining two (loci 6 and 7) were located in intron 3, as shown in fig 1 and table 2. Almost no ethnic difference in allele frequency between Japanese and Caucasian control subjects was found. The Val30Met mutation occurs in exon 2 (fig 1).

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that in other countries such as Japan and Portugal.371 3

onset of the disease in patients in Sweden is much later than
FAP patients with other mutations? For example, the age at
causes the significant differences in clinical symptoms among
the large foci of patients throughout the world? Third, what
Caucasian controls, with other foci of patients.

13 kb region (loci 2–7), which is also a major haplotype in the
was common. However, Swedish patients shared only the
103 kb region (loci 1–7) was common between the two

was compared with that of Japanese patients, all loci except

(equation 4). When the major haplotype of Portuguese patients
represent a disease-causing allele, as for the Japanese focus

of these cases were thought to result from an independent
mutational event; 20 however, an unexpectedly high fre-
of these cases were thought to result from an independent

In diseases inherited in an autosomal dominant fashion, an
allele responsible for the disease in the patient population
would theoretically have a frequency of 50%. The fact that the
frequency of the major haplotype observed in the Kumamoto
focus (237–307-T-271-C-G-A-299–311) was 40%, which is
considered to be close to 50%, whereas that of Japanese
focus (237–307-T-271-C-G-A-299–311) was 40%, which is
predicted to be about 10^{-13} per locus per generation,18 which is negligibly low.
If the duration of one generation is assumed to be 30 years, a
mutation at a certain microsatellite locus would occur at a
rate of only one in 30,000 years. In view of the high
dered in one 215 kb region, which occurred over a
substantial time period, as is usual (table 4).

In the present study, we used, in addition to four SNPs (two
the same as those analysed before by researchers), four
highly polymorphic microsatellite markers covering 215 kb of
the TTR gene region. A microsatellite repeat is generally
considered to have a relatively high mutation rate when
compared with SNPs, but its mutation rate is estimated to be
about 10^{-13} per locus per generation,18 which is negligibly low.
If the duration of one generation is assumed to be 30 years, a
mutation at a certain microsatellite locus would occur at a
rate of only one in 30,000 years. In view of the high
heterozygosity of microsatellite markers, such a microsatellite
would be a powerful marker for our study.

To determine whether all major FAP foci throughout the
world could be derived from a Portuguese mutant allele,11 we
analysed the haplotype pattern of patients from Portugal,
Spain, and Sweden. We found that all European foci have
their own major haplotypes, whose frequencies were close to
50% (40.9–47.6%), which indicated that these haplotypes
represent a disease-causing allele, as for the Japanese focus
(table 4). When the major haplotype of Portuguese patients
was compared with that of Japanese patients, all loci except
L8 were exactly the same, which indicated that at least the
103 kb region (loci 1–7) was common between the two
populations. Also, comparison of Portuguese and Spanish
patients revealed that at least the 116 kb region (loci 2–8)
was common. However, Swedish patients shared only the
13 kb region (loci 2–7), which is also a major haplotype in the
Caucasian controls, with other foci of patients.

Several major issues remain unsettled in the field of
research on FAP caused by the Val30Met mutation of the TTR
gene. First, although large foci of patients are located in
several restricted areas of the world, why is only the
Val30Met mutation found in those foci, even though more
than 80 different mutations of TTR gene have been
identified? Second, does any common founder exist among
the large foci of patients throughout the world? Third, what
causes the significant differences in clinical symptoms among
FAP patients with the Val30Met mutation compared with
FAP patients with other mutations? For example, the age at
onset of the disease in patients in Sweden is much later than
that in other countries such as Japan and Portugal.371 3

To answer the second question, haplotype analysis via gene
polymorphisms has been performed to clarify the founder
effect. Several researchers have discussed the origin of the
TTR Val30Met gene in FAP patients from Japan,16 North
America,13 and Europe.16 17 All these data were analysed by
using six or seven SNPs inside the gene, a region of about
7.0 kb, and the haplotype was determined by pedigree
analysis. Yoshioka et al16 analysed eight families, including
two from each major focus in Japan. They concluded that the
mutation had a multiple origin because two isolated families,
not from the two large foci, have different haplotypes,
although the remaining six have the same haplotype, which
was named “haplotype I”. Li and Sommer11 analysed six
unrelated FAP patients in North America and performed
haplotype analysis in the absence of DNA samples for
relatives. They found four different haplotypes and thus
agreed with a multiple origin hypothesis. For Europe,
however, Almeida et al16 and Reilly et al17 found only
haplotype I in patients from Portugal, Spain, and Sweden.
In the present study, we used, in addition to four SNPs (two
the same as those analysed before by researchers), four
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rate of only one in 30,000 years. In view of the high
heterozygosity of microsatellite markers, such a microsatellite
would be a powerful marker for our study.

To determine the haplotype profile in the general popula-
tion, 54 Japanese and 96 Caucasian control subjects under-
go genotyping, and haplotypes were constructed. As
expected, haplotypes constructed with all nine polymor-
phisms had a low frequency, the highest frequencies being 9.6%
in Japanese and 9.5% in Caucasians, which indicated
recombination in this 215 kb region, which occurred over a
substantial time period, as is usual (table 4).

In diseases inherited in an autosomal dominant fashion, an
allele responsible for the disease in the patient population
would theoretically have a frequency of 50%. The fact that the
frequency of the major haplotype observed in the Kumamoto
focus (237–307-T-271-C-G-A-299–311) was 40%, which is
considered to be close to 50%, whereas that of Japanese
controls was extremely low, indicates that the haplotype
represents the disease-causing allele. Although only two
patient samples were available from Nagano, the location of
another major focus of the disease in Japan, genotype data
revealed that they had exactly the same haplotype as that of
the Kumamoto patients (table 3). These data strongly
support the existence of a relatively recent common founder.
As for other FAP patients from the rest of Japan,11 a majority
of these cases were thought to result from an independent
mutational event;20 however, an unexpectedly high fre-
quency, 31.8%, of the same haplotype was found, which

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Heterozygosity of microsatellite markers above Japanese and Caucasian subjects</th>
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<tbody>
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<td>L1</td>
<td>0.497 0.704</td>
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<tr>
<td>L2</td>
<td>0.766 0.702</td>
</tr>
<tr>
<td>L4</td>
<td>0.349 0.448</td>
</tr>
<tr>
<td>L8</td>
<td>0.737 0.761</td>
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<tr>
<td>L9</td>
<td>0.871 0.856</td>
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<th>Table 2</th>
<th>Positions and allele frequencies of SNPs</th>
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<td>SNP</td>
<td>ID</td>
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<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>L3</td>
<td>IMS-JST 118368</td>
</tr>
<tr>
<td>L5</td>
<td>IMS-JST 118365</td>
</tr>
<tr>
<td>L6</td>
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</tr>
<tr>
<td>L7</td>
<td>IMS-JST 152576</td>
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suggests that most were also derived from the same founder of the foci, rather than from independent sporadic mutations. Each focus of European FAP patients has its own major haplotype, with a frequency close to 50% (40.9–47.6%) (table 4). The major haplotypes for Portuguese and Japanese patients are the same except for locus 8; in other words, at least the 103 kb region from locus 1 to locus 7 is common. One plausible explanation for this would be that the Portuguese Val30Met mutation was brought to Japan in recent times. Portuguese merchants arrived in Japan during the 16th century, and active trading was pursued in all areas in Kyushu (the island on which Kumamoto is located) until the beginning of the 17th century, when Japan became a closed country. The most likely explanation is thus as follows: in the early phase of transfer of the allele into the Japanese population, recombination with the Japanese allele (L8–L9; 299–311, which was found in Japanese controls) occurred once between L7 and L8, after which the allele with the Val30Met mutation spread to Nagano and other areas of Japan. Comparison of the major haplotypes for Portuguese and Spanish patients showed that a 116 kb region (loci 2–8) was common. This could also be explained by the existence of a common founder. Swedish patients, however, had only a 13 kb region (loci 3–7) in common with patients of other foci. Although the possibility of a common founder of Swedish and Portuguese cases cannot be ruled out, the fact that the haplotype of this 13 kb region is the same as the major haplotype found in Caucasian controls might indicate that the Val30Met mutation of the Swedish focus is due to an independent mutational event. However, further molecular genetic studies have to be performed focusing on the Swedish cases because of a trade history including the Swedish Viking invasion of Portugal in the 10th century, and because far milder symptoms occur in Swedish patients than in Japanese or Portuguese patients. The cause of the discrepant clinical phenotypes of Swedish patients and patients from other foci—the third question mentioned above—could be a difference in genetic background such as the existence of modifying gene(s) and/or other known or unknown mechanisms. The answer to this question would be quite valuable for development of therapeutic measures for FAP patients. Because the haplotypes of Spain and Portugal are somewhat diverse and that of Japan has a single disease-causing allele, the European haplotype could be older than the Japanese haplotype. This suggestion would support the hypothesis that the Portuguese mutant allele was brought to Japan, rather than transferred in an opposite direction, from Japan to Portugal. In summary, our data provide the molecular evidence that the Val30Met mutation that is widespread among patients in Portugal, Spain, and Japan, results from an ancient mutation of common origin rather than from multiple recurrent mutational events in a common haplotype. The data also

<table>
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<th>Table 3</th>
<th>Major haplotype frequencies for Japanese patients and controls</th>
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<tr>
<td>Population</td>
<td>L1</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
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<tr>
<td>Kumamoto patients</td>
<td>237</td>
</tr>
<tr>
<td>235</td>
<td>307</td>
</tr>
<tr>
<td>235</td>
<td>311</td>
</tr>
<tr>
<td>Nagano patients</td>
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<tr>
<td>237/237</td>
<td>305/307</td>
</tr>
<tr>
<td>Patients from other locations</td>
<td>237</td>
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<tr>
<td>237</td>
<td>307</td>
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<td>237</td>
<td>235</td>
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<td>237</td>
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<th>Table 4</th>
<th>Major haplotype frequencies for foci of FAP patients and for controls</th>
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<td>Population</td>
<td>L1</td>
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<tr>
<td>Kumamoto patients</td>
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<td>235</td>
<td>307</td>
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<tr>
<td>235</td>
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<tr>
<td>Japanese controls</td>
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<td>241</td>
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<td>313</td>
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<td>Portuguese patients</td>
<td>237</td>
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<td>235</td>
<td>307</td>
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<td>241</td>
<td>307</td>
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<td>Swedish patients</td>
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<td>241</td>
<td>307</td>
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<td>241</td>
<td>307</td>
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<tr>
<td>Caucasian controls</td>
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clearly demonstrate that microsatellite analysis must be applied in combination with SNPs when the origin and distribution of mutations are studied.

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