# Molecular diagnosis of transthyretin Met$^{30}$ mutation in an Italian family with familial amyloidotic polyneuropathy

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**Abstract** **We report the molecular analysis of the transthyretin gene in a large Italian pedigree with familial amyloidotic polyneuropathy and demonstrate the presence of a Met$^{30}$ mutation. The usefulness of the genetic analysis in the identification of presymptomatic persons and the diagnosis of individuals with partial symptoms is discussed.**

*Key words:* Transthyretin; Familial amyloidotic polyneuropathy; Polymerase chain reaction; DNA sequencing

## 1. Introduction

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disorder characterized by extracellular deposition of mutated protein fibrils, such as transthyretin (TTR), apolipoprotein A I and gelsolin [1,2]. FAP associated with mutations in the plasma TTR gene is the most frequent form [3]. Defined mutations in individual exons have been correlated with clinical presentations in several ethnic groups, including Italian families [4–8]. A new large Italian pedigree in the province of Molise was characterized clinically and pathologically [9]. Nineteen individuals were affected in six generations and presented with peripheral sensory neuropathy involving the lower extremities, dysautonomia, cardiopathy and variable presence of ocular involvement and nephropathy. Furthermore two individuals presented with only dysautonomia and one with scalloped pupils.

We report the molecular analysis of the transthyretin gene in this Italian pedigree with familial amyloidotic polyneuropathy and demonstrate the presence of a Met$^{30}$ mutation. The usefulness of the genetic analysis in the identification of presymptomatic persons and the diagnosis of individuals with partial symptoms is discussed.

## 2. Materials and methods

### 2.1. Patients

The FAP family studied is a large kindred with nineteen affected individuals in six generations (Fig. 1). The clinical features and pathological characterization have been reported [9]. Two affected persons IV-25 (propositus) and IV-15 have biopsy proven endoneurial deposition of amyloid. Blood samples were obtained from IV-25 and IV-15, two individuals with only dysautonomia (IV-24 and IV-19), one with scalloped pupils (V-23) and three clinically asymptomatic individuals (V-30, IV-31 and V-31).

### 2.2. DNA isolation and amplification

Genomic DNA was extracted from peripheral blood white cells of the individuals described in the previous section (IV-15, IV-25, IV-24, IV-19 and V-23, V-30, IV-31 and V-31) and was amplified by polymerase chain reaction (PCR) using two 22 bases long oligonucleotide primers complementary to intronic sequences flanking TTR exon 2, 5′-CGCTCCAGATTTCTAATACCAC-3′ and 5′-AGTGAGGGGC-AAACGGGAAGAT-3′ [10]. Reaction mixtures containing 25 ng of template and 5 pmol of each primer were amplified using Gene Amp PCR core kit (Perkin-Elmer Cetus). The first cycle was 95°C for 3 min, 56°C for 30 s, 72°C for 1 min and subsequent cycles were at 95°C for 1 min, 56°C for 30 s, 72°C for 1 min with a final extension step at 72°C for 5 min. After thirty cycles of amplification the yield of PCR product containing exon 2 was approximately 20 μg/ml of DNA. Approximately 300 ng of PCR product DNA was digested overnight with ten units of *Nsi*I enzyme at 37°C, fractionated by electrophoresis through a 1.7% agarose gel and bands were visualized by ethidium bromide staining.

### 2.3. DNA sequencing

Sequence analysis was done using the AmpliTaq cycle sequencing kit (Perkin-Elmer Cetus). Prior to sequencing the PCR product DNA was purified through Qiaquick spin PCR purification kit (Qiagen) to remove nucleotides and primers. Hundred fmol of template were annealed with 1.6 pmol of one of the primers used for amplification that was end labeled with 50 μCi of [γ-$^{33}$P]ATP (3,000 Ci/mmol, Dupont/NEN). Sequencing products were separated through a 6% acrylamide, 8 M urea gel and bands were visualized by autoradiography.

## 3. Results and discussion

This pedigree shows an autosomal dominant pattern of inheritance (Fig. 1) and clinical features similar to those described for FAP associated with mutations in TTR exon 2 [1,11]. The hallmark of the disease in this family is the presence of a peripheral neuropathy starting in the lower extremities with sensory and autonomic involvement and severe cardiac dysfunction. Variability in clinical expressivity in the affected individuals was noted as well as the presence of partial symptomatology in three individuals who are siblings of affected members [9].

Sequence analysis of exon 2 PCR amplified DNA showed both G and A at nucleotide position 79 in an affected (IV-15) and a presymptomatic (V-30) individual (Fig. 2). The transition of G to A causes a Val to Met mutation at position 30 and both individuals are heterozygous for Val$^{30}$ and Met$^{30}$. Since the G to A transition creates a new *Nsi*I restriction site, the mutated allele can be identified by digestion of exon 2 DNA with *Nsi*I. The mutation in two affected and a presymptomatic individual was confirmed with this analysis (Fig. 3). After digestion of exon 2 PCR amplified DNA with *Nsi*I two bands of 165 and 112 bp respectively were generated in addition to the normal uncut allele of 277 bp. PCR products from DNA of two individuals with only dysautonomia and one with scalloped pupils (IV-19, IV-24 and V-23) did not show the mutation after digestion with *Nsi*I (Fig. 4).

Thus the Val to Met$^{30}$ mutation is associated with the disease

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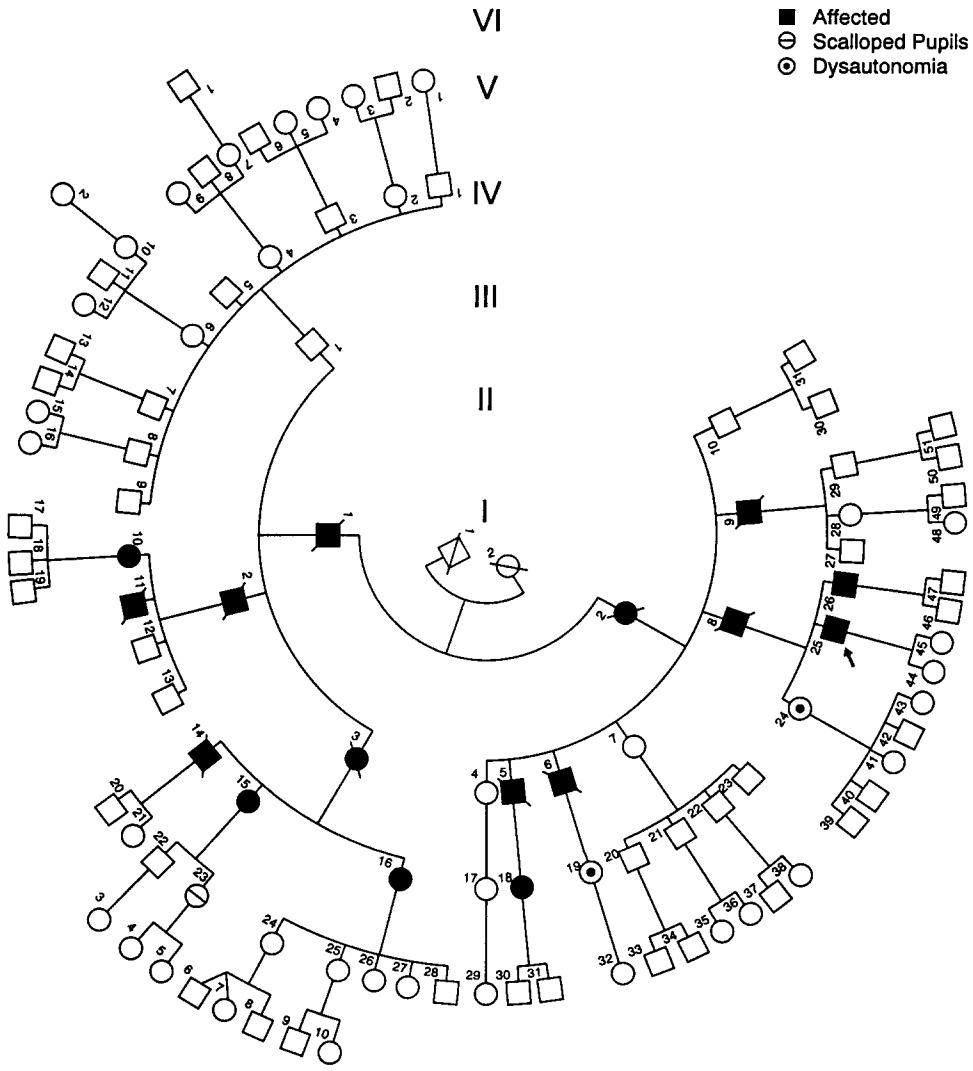


Fig. 1. Pedigree of an Italian kindred with autosomal dominant familial amyloidotic polyneuropathy. Affected individuals are designated by black symbols. All the individuals in generation IV had full neurological and ophthalmological examinations as well as electrophysiological studies; IV-25 and IV-15 have biopsy proven endoneurial deposition of amyloid. No data are available for generation I.

in the affected members of this family and in a presymptomatic individual who has inherited the mutation from the mother (Fig. 1). He is only 32 years old and has not yet developed clinical signs of the disease that begins in the early forty's in this branch of the family [9]. By contrast, two individuals who presented with only dysautonomia and one with scalloped pupils did not carry the mutation. This shows the importance of the molecular analysis in families with FAP. The two individuals with only dysautonomia have affected siblings and could have been carriers of the mutation but not have developed the full blown clinical syndrome. Given the great clinical heterogeneity in organs involvement, age of onset and duration of FAP [12], the diagnosis of this disease based exclusively on clinical grounds is difficult and requires identification of the mutation at the DNA level.

Thirteen Italian families with a clinical presentation consistent with FAP have been reported. Seven families, including ours carry the $Met^{30}$ mutation whereas the remainder carry different mutations in exons 2 and 3 [4–7]. The prevalence of the $Met^{30}$ mutation in the Italian pedigrees is not surprising, since this is the most frequently found mutation in various ethnic backgrounds [13–14] and reflects the high mutation rate at CpG islands where the C is methylated and then converted

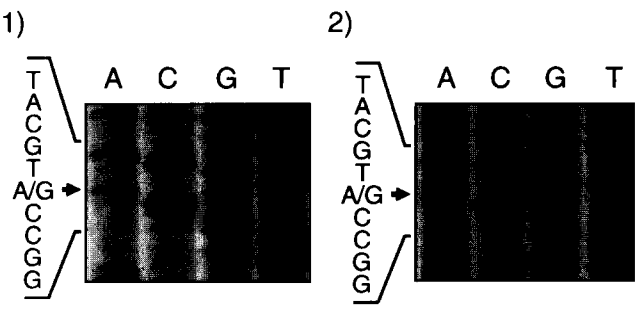


Fig. 2. Val to $Met^{30}$ mutation in exon 2: sequence analysis of PCR amplified exon 2 from DNA of individuals V-30 (1) and IV-15(2) showing the G to A transition at nucleotide 79.

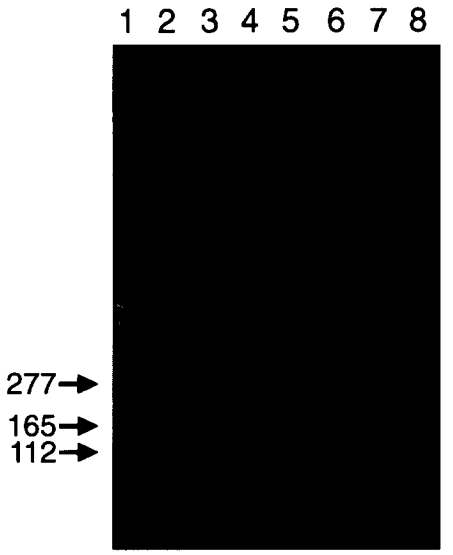


Fig. 3. Agarose gel electrophoresis of PCR amplified exon 2 from genomic DNA of the propositus IV-25 (lanes 2–3), an affected individual IV-15 (lanes 4–5) and a presymptomatic individual V-30 (lanes 6–7) before and after digestion with *Nsi*I respectively. Lanes 1 and 8 are *Msp*I cut pBR322 markers. Bands are visualized by ethidium bromide staining. After *Nsi*I digestion two bands of 165 and 112 nucleotides are generated from the mutated allele, while the uncut exon is 277 nucleotides in length.

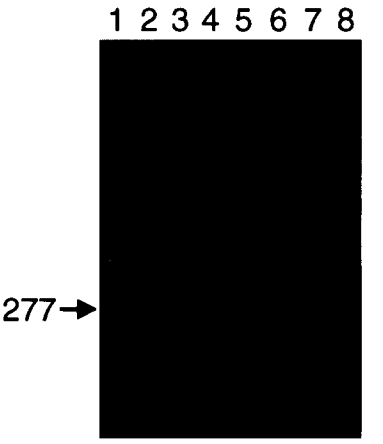


Fig. 4. Agarose gel electrophoresis of PCR amplified exon 2 from genomic DNA of two individuals with dysautonomia IV-19 and IV-24 (lanes 2–3 and 4–5) and one with scalloped pupils V-23 (lanes 6–7) before and after digestion with *Nsi*I respectively. Lanes 1 and 8 are *Msp*I cut pBR322 markers. Bands are visualized by ethidium bromide staining. After *Nsi*I digestion only the uncut exon of 277 nucleotides in length is visualized.

to T by deamination [15]. The G of the Val codon is part of such CpG island and thus is a mutation 'hot-spot'. In summary, we report the molecular analysis of the TTR gene in this new Italian family and demonstrate the presence of a $Met^{30}$ mutation. In addition this study emphasizes the usefulness of the genetic analysis in the identification of presymptomatic persons and characterization of individuals with partial clinical symptoms.

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