

Original articles

Geographical distribution of TTR met³⁰ carriers in northern Sweden: discrepancy between carrier frequency and prevalence rate

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Abstract

The first Swedish case of familial amyloidotic polyneuropathy (FAP) was published in 1965. The same transthyretin (TTR met³⁰) mutation as that seen in Japanese, Portuguese, and other populations was also found in Swedish FAP patients. More than 350 patients with clinical manifestations of FAP have been diagnosed in northern Sweden, most of them originating from the areas around Skellefteå and Piteå. The mean age of onset is 56 years, much later than in patients from Japan and Portugal. To estimate the frequency of the TTR met³⁰ mutation in the counties of Västerbotten and Norrbotten, sera from 1276 persons aged 24 to 65 years, randomly sampled from a health programme (MONICA), were screened with the monoclonal antibody FD6. In 19 persons, 13 females and six males, a positive reaction was seen in an Elisa test using this antibody. DNA analysis confirmed the TTR met³⁰ mutation and showed that 18 were heterozygous and one homozygous for this mutation. Other mutations were not looked for in this study. The mean TTR met³⁰ carrier frequency in the area was 1.5% ranging from 0.0 to 8.3% in 23 subpopulations. There was a notable discrepancy between the regional distribution of the TTR met³⁰ allele and the morbidity rate for FAP. The estimated number of TTR met³⁰ gene carriers in a total population of 500 000 in the area is approximately 7500. The penetrance of the TTR met³⁰ mutation shows considerable variation between families, and the overall diagnostic (predictive) value in this population is as low as around 2%.

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Amyloidosis comprises a group of diseases associated with the deposition of amyloid substance in various tissues of the body. All amyloid is built up by fine fibrils which are polymers of low molecular weight proteins in β

pleated sheet conformation.¹ The progressive build up of the amyloid leads to interference with normal function of the affected tissues. The protein differs between the various forms of amyloidosis. In several forms of familial amyloidotic polyneuropathy (FAP), mutated transthyretin (TTR) seems to be the main fibril component.²

Variant TTR with one amino acid substitution of methionine for valine at position 30 has been shown to be associated with type 1 FAP in patients of Japanese³ and Portuguese⁴ origin and North American patients of Swedish origin.⁵ The same transthyretin (met³⁰) mutation has also been found in Swedish FAP patients.^{6,7}

About 350 FAP patients have been diagnosed in northern Sweden and the gene frequency has been estimated to be around 2% in some areas.⁸

The first cases of homozygosity for the TTR met³⁰ gene were published in 1988 in a 56 year old man with polyneuropathy, gastrointestinal problems, and vitreous amyloid and in his 62 year old healthy sister.⁹ In 1990 homozygosity was found in two additional persons.¹⁰ By using the polymerase chain reaction (PCR) for the amplification of discrete regions of the TTR gene and the analysis of mutated regions by restriction enzyme digestion and agarose gel electrophoresis, three additional subjects homozygous for the TTR met³⁰ gene were found.¹¹

Recently homozygosity for the TTR met³⁰ gene has been detected in two Turkish brothers with FAP. The age of onset and the progression of symptoms were the same as in heterozygous patients.¹² The same clinical picture was seen in four Japanese patients homozygous for the met³⁰ gene.^{13,14} A homozygous patient has now also been identified in the Portuguese population (Costa, personal communication).

In the present investigation, the frequency of TTR met³⁰ carriers was studied in the two northernmost counties of Sweden by examining 1286 unselected healthy subjects with a monoclonal antibody and the Elisa technique.

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Table 1 Participation rates and comparison between participants and non-participants in the 1990 population survey of the northern Sweden MONICA study

	Men	Women
Proportion of participants (%)		
25-34 years	66.8	71.1
35-44 years	80.9	82.4
45-54 years	81.2	83.6
55-64 years	82.8	86.3
Married/cohabitants (%)		
Participants	81.7	80.9
Non-participants	56.7	68.1
Education above primary school (%)		
Participants	63.4	64.2
Non-participants	64.8	66.7

Area of investigation

The counties of Västerbotten and Norrbotten in northern Sweden have a population of about 500 000 with a higher population density in the coastal region compared with the mountainous inland region, which has a settlement of small and previously very isolated villages. The population of northern Sweden is a mixture of people of Finnish, Saamish, and central Swedish origin. The Finnish influence is strongest in the northern and north eastern parts of the area and the Saamish influence is strongest in the northern and western parts of the area.¹⁵

Subjects and methods

Blood samples were collected within the framework of the Northern Sweden MONICA Project, a substudy in the WHO MONICA Project (Monitoring of Trends and Determinants in Cardiovascular Disease).¹⁶ The MONICA methodology and the Northern Sweden MONICA Project have been described in detail previously.¹⁷ Population screening of middle aged persons in the two northernmost counties of Sweden (Norrbotten and Västerbotten) was performed in January to April 1990. Within each age group (25-34, 35-44, 45-54, 55-64 years) 250 men and 250 women were randomly selected from continuously updated population registers. They were invited by letter to attend a medical examination including laboratory tests. If they did not attend, a reminder with a new appointment time was sent. People who still did not come were contacted by telephone to record reasons for failure to attend and basic information on social background and risk factors.

In all, 1583 25 to 64 year old persons participated in the study (79.2% of all invited). Table 1 shows the proportion of participants out of all invited in each age and sex category. Background data for participants and non-participants are also compared. In all age groups, participation rate was slightly higher among women than among men. It was lowest in the youngest age groups (25-34 years). The proportion of married or cohabitant people was significantly lower in non-participants than in participants ($p < 0.001$). Persons not born in the area of investigation were excluded (307).

The survey was performed by two mobile teams in local health centres (or corresponding) throughout the MONICA area and included collection of serum samples, which

Table 2 The frequency of TTR met³⁰ gene carriers in the 23 subpopulations in northern Sweden

Region	No examined	No met ³⁰ positive	% gene carriers
1	41	0	0
2	20	0	0
3	30	0	0
4	23	1	4.3
5	36	3 (1 homozygous)	8.3
6	39	0	0
7	40	1	2.5
8	143	1	0.7
9	23	1	4.3
10	30	0	0
11	36	0	0
12	97	1	1.0
13	32	0	0
14	33	0	0
15	53	1	1.9
16	52	0	0
17	67	0	0
18	125	8	6.4
19	115	1	0.9
20	46	0	0
21	77	0	0
22	69	1	1.4
23	49	0	0
Total	1276	19	1.5

were stored at -20°C , and used in the present study. The samples were decodified so that only sex, place of birth, and age group were known. The subjects were distributed according to their place of birth into 23 subpopulations (regions) consisting of one or a group of parishes¹⁵ (table 2). The 152 living FAP patients had an age of onset between 23 and 65 years. Standard statistical procedures were used to calculate proportions and heterogeneity between regions.

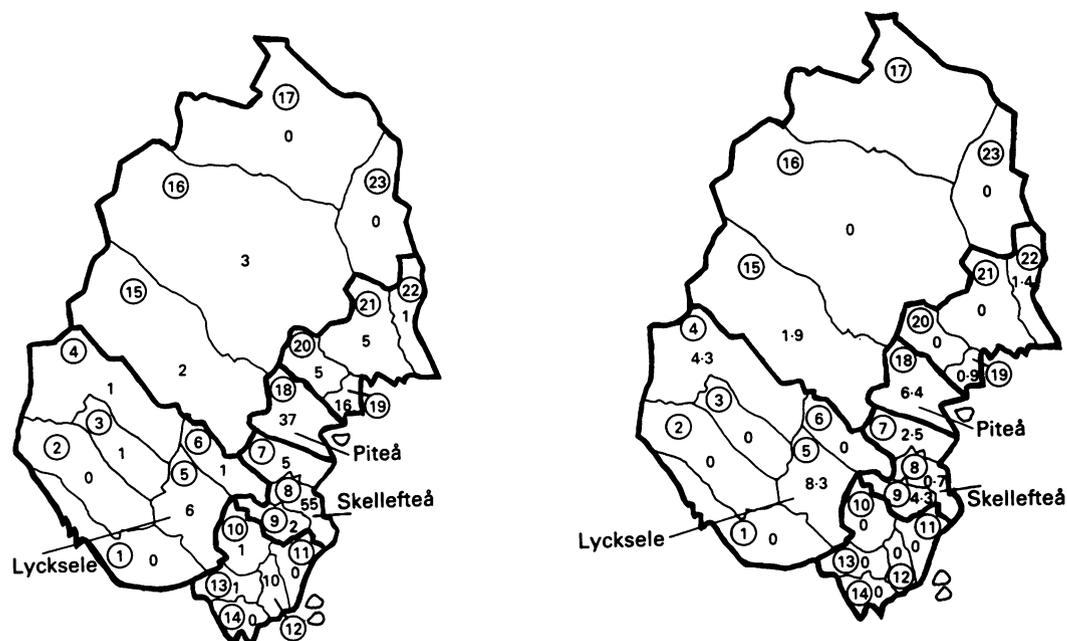
The study has been approved by the Research Ethical Committee of Umeå University and the data handling procedures by the National Computer Data Inspection Board.

AMPLIFICATION OF GENOMIC DNA

For the DNA analysis, total genomic DNA was extracted from supernatants as previously described.⁷ Approximately 1 μg of genomic DNA was amplified using a GeneAmp DNA amplification kit (Perkin Elmer Cetus) and a set of 20 and 21 base primers flanking the TTR gene region (Symbicon Co Umeå). A 217 bp segment of exon 2 of the transthyretin gene, containing the codon for amino acid 30, was amplified by PCR, and the amplification products cleaved with the restriction endonuclease *Nsi*I and analysed by gel electrophoresis.¹⁸

ELISA TEST FOR TTR MET³⁰ CARRIERS

An enzyme linked immunosorbent assay (ELISA) for the detection of TTR variants was used. It is based on a monoclonal antibody (FD6) which recognises distinct neuropathic TTR variants.¹⁹ Briefly, tests were carried out in 96 well microtitre plates (Nunc, Maxisorp grade), coated overnight with sera diluted 1/10 in 0.9% NaCl. The plates were then incubated with FD6 culture supernatant, followed by biotinylated antimouse IgG (Amersham) and then with preformed streptavidin biotinylated peroxidase complexes (Amersham). Colour was developed with ABTS substrate (Sigma), and end point readings done at



Numbers of patients alive (left) and the frequency of TTR met³⁰ carriers (right) in 23 regions in northern Sweden. Number of regions (within circles) correspond to those in table 2. Regions 1–14 = Västerbotten county; regions 15–23 = Norrbotten county. Heavy lines indicate aggregation of data into six areas used in heterogeneity analysis.

405 nm after 30 minutes. The test is semiquantitative and the positive samples show a strong green colour. All samples were tested in duplicate and positive and negative controls were included. Results were standardised on positive controls after subtraction of the negative control.

Results and discussion

In 19 subjects, 13 females and six males, a positive reaction was seen with the Elisa (table 2). DNA analysis using PCR and cleavage with *Nsi*I showed that 18 were heterozygous and one homozygous for the TTR met³⁰ mutation. Other mutations were not looked for in this study. The figure shows the regional distribution of living patients and TTR met³⁰ carriers in northern Sweden using a distribution into 23 regions which has been used in several previous population genetic and epidemiological studies. Northern Sweden shows marked ethnic heterogeneity with a strong Finnish influence in the northern and north eastern parts, whereas the strongest Saami (Lapp) influence has been found in the northern and north western parts. From the geographical pattern of variation of the TTR met³⁰ allele it appears that there is no evident correlation with Finnish or Saamish influence.

The mean frequency of TTR met³⁰ carriers in northern Sweden was 1.5% with a range of 0.0 to 8.3 between subpopulations. Using the average figure of 1.5% the number of carriers in the total population of about 500 000 inhabitants can be estimated to be approximately 7500.

Since the number of carriers was small, the data from the 23 regions were aggregated into six areas (figure), two inland and four in the coastal region, and subjected to analysis of heterogeneity. In this analysis the homozygote

was counted as one carrier. There was a significant heterogeneity between the six areas ($p < 0.001$). It is notable that the frequency of carriers was not particularly high in Skellefteå (region 8) where most FAP patients have been previously diagnosed. There was, for example, a significantly higher frequency of carriers in the Piteå region (region 18) compared to the Skellefteå region ($p < 0.025$) in spite of the fact that the prevalence of FAP has been found to be lower in the Piteå region.²⁰ A relatively low carrier frequency (2.0%) was found also in a previous investigation in the Skellefteå area.⁸ The high gene frequency in the Piteå region is in agreement with the fact that six homozygotes have previously been found.^{8,9,11} In the Skellefteå region one homozygote has been found.¹¹ Quite a high frequency (two heterozygotes and one homozygote out of 36 persons) was also found in the inland Lycksele region (region 5). The difference between the Lycksele and Skellefteå regions was statistically significant ($p < 0.05$). The number of diagnosed FAP patients in the Lycksele region is quite low, the age of onset is usually late, and the clinical course relatively mild.

It is obvious that in northern Sweden there is a discrepancy between the geographical distribution of the TTR met³⁰ allele predisposing to FAP and the numbers of FAP patients (figure). The number of patients alive in northern Sweden at the time of this study is 152 which gives a prevalence of 0.00031. With an overall carrier frequency of 0.015 the predictive (diagnostic) value can be estimated to be about 2%. However, earlier pedigree analyses have shown that the penetrance in some families may approach 50%.²¹ This strongly contrasts with the findings in the Portuguese FAP population where the gene penetrance is generally considered to be higher.

In the Swedish material there may be a

number of undiagnosed patients with very late onset and slight symptoms. Though increased awareness of FAP may influence the prevalence rate, it seems unlikely that the discrepancy between carrier frequencies and prevalence rates seen in this study can be explained by increased awareness only. It is more likely that some hitherto unknown factor in the Skellefteå subpopulation is responsible for the accumulation of FAP in this region.

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