# Detection of a variant protein in hair: new diagnostic method in Portuguese type familial amyloid polyneuropathy

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Portuguese type familial amyloid polyneuropathy is a hereditary amyloidosis caused by a variant form of transthyretin in which valine is replaced by methionine at position 30.[1 2] Transthyretin is produced predominantly in the liver and circulates in the blood as a carrier protein for thyroid hormones and retinol binding protein.[1 2] All carriers of this transthyretin variant have large amounts of the variant protein in their blood, so it may be present in the hair just as A and B blood group antigens are. To test this possibility, we examined the immunoreactivity of hair from carriers and control subjects.

## Subjects, methods, and results

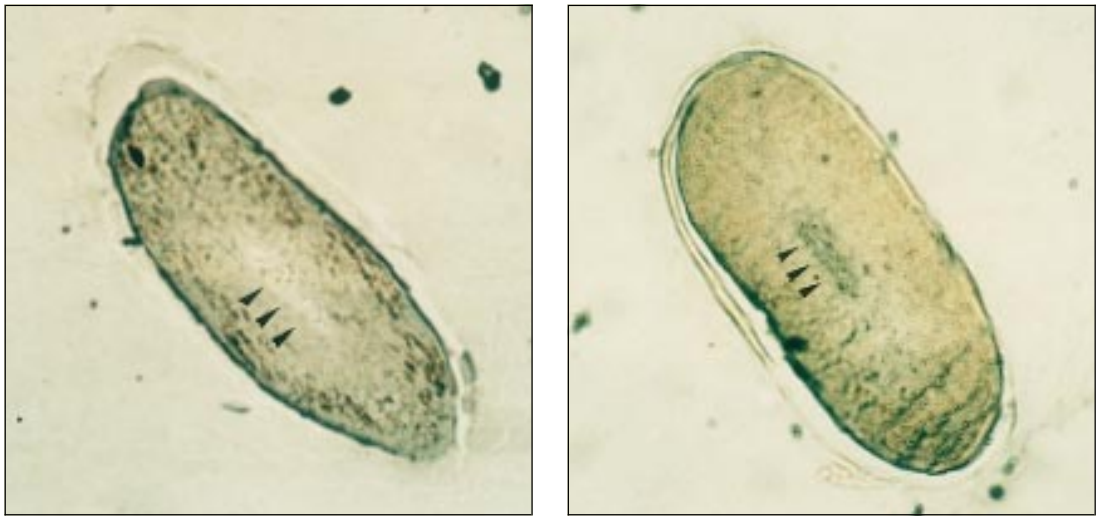
We tested the immunoreactivity of hair using a monoclonal antibody specific to the methionine variant of transthyretin that does not react with normal transthyretin.[3 4] We investigated hair from five Japanese patients with familial amyloid polyneuropathy (age range 28-51 years; all black hair), one carrier of the gene for familial amyloid polyneuropathy who was symptom free (61 year old Swedish woman; grey hair), and eight healthy controls (age range 25-48; five were Japanese (black hair), two were Swedish (one blond, one brown hair), and one was Iranian (black hair)). Hair was embedded directly in epoxy resin without being treated, and sections were cut at 3 µm. Sections were immersed in 0.01% hydrogen TRIS buffer (pH 7.4) for 10 minutes to inhibit endogenous peroxidase and then treated with 1% bovine serum albumin to occupy nonspecific binding sites. The sections were incubated overnight at room temperature with the monoclonal transthyretin antibody. Immunoreactivity to the antibody was detected by the avidin-biotin complex method (Dakopatts, Glostrup, Denmark). Finally, hairs were stained with True Blue peroxidase substrate (Novakemi, Stockholm, Sweden).

Immunoreactivity was found in the medulla of hairs from the patients with familial amyloid polyneuropathy (figure (right)) and the carrier of the gene for familial amyloid polyneuropathy, but no immunoreactivity was observed in the medulla of hairs from the controls (figure (left)). The reactivity was found even in the tip of the hair, but it was more obvious in the root.

## Comment

With this method we were able to detect the presence of a variant form of a protein in hair samples. DNA diagnosis, radioimmunoassay, and high performance liquid chromatography have been used to detect transthyretin variants in blood samples. These methods are, however, time consuming, and obtaining a blood sample may be difficult. In contrast, this immunocytochemical method is simple and samples are easy to obtain. Hair that has fallen out may be tested, which means that the method could be applied in forensic medicine to detect variant proteins other than transthyretin mutations.

Transthyretin has up to 60 known mutations.[5] Two mutations (in which serine replaces glycine at position 6 and methionine replaces threonine at position 119) are known benign polymorphisms that are present in 10-15% of the population.[2] Thus, detection of transthyretin variants can be used to identify a particular person. Other variant proteins could also be detected in hair by this method if suitable antibodies were available or could be produced. This may prove an valuable additional tool to DNA diagnostic procedures in identifying particular people because routine DNA analysis is not always specific.



Immunoreactivity for variant transthyretin in healthy male volunteer aged 43 (left) and male patient with familial amyloid polyneuropathy aged 36 (right). Arrows indicate medulla of hair

Contributors: YA initated the study, participated in the collection of samples, analysed and interpreted the data, and wrote the paper. IA developed and performed the histotechnical analysis of the hair, participated in the interpretation of the data, and contributed to the paper. OS collected samples, participated in the interpretation of the data and in editing the paper. GH participated in the interpretation of the data and in editing the paper. PMPC provided the antibody and contributed to the paper.

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Conflict of interest: PMPC developed the antibody, which may become commercially available.

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# Commentary: A new hair test for rare antigens

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Hair analysis has a respectable history in detecting heavy metals[1]—for example, arsenic in Napoleon's hair in Elba—and, more recently, in detecting drug ingestion of both drugs of abuse[2] and therapeutic drugs.[3] Hair can even be used to assess diabetic control by measuring its glycation index.[4] If the claims made for hair analysis in the less scientific branches of alternative medicine are discounted there is still a sound body of knowledge about this accessible universal mammalian attribute.

Much of the interest has been in forensic analysis. Hair may simply be plucked, with acceptable minor trauma, from competitive athletes or criminal suspects. Hairs may be shed at the scene of a crime. Such hair samples have some limitations because the length of the hair represents the limited length of time it has grown in equilibrium with body tissue concentrations of the substance in question. Short hairs give a short history—a shaven head may mean that (unshaven) armpit or pubic hair may need to be sought. Like fingernails, growing hair shafts show change along their length such that one recent episode of drug ingestion gives one blip while chronic or multiple ingestion affects many sections along the shaft.

In all the analyses so far described, which may be high performance liquid chromatography, immunoassay, or gas chromatography-mass spectroscopy the hair is destroyed before analysis by being dissolved or digested. Passive contamination[5] without any true ingestion is a major problem: the outside of the hair, like banknotes with cocaine, can easily become passively coated with drugs from the environment.

The technique described by Ando et al ingeniously gets round this problem by simply localising and detecting the substance, a genetic variant of transthyretin, in the inner medulla of the transected hair by using the immunoperoxidase technique. Contamination from the hair surface should be visually obvious. Although transthyretin is present (as prealbumin) in large amounts in the circulation and secretions (so hair might be passively coated, particularly in animals that groom themselves by licking their hair), the fact that the substance is inside the hair establishes its source as the hair itself.

Ando et al recognise that this technique has considerable potential in forensic and genetic medicine. Any protein to which a specific antibody can be raised might be detectable in hair in this way. The assay is qualitative, and it is hard to see how it could be made accurately quantitative. However, as a method of reliably detecting rare mutations in proteins, and hence in identifying a particular person, this seems to be a powerful new tool.

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*One hundred years ago*

## Deathbed scenes

Sir,—In reading the newspaper accounts of the illness and death of Mr Gladstone, it strikes me as being a great pity that medical men of high standing, on being interviewed by representatives of the press, should enter into such unnecessary details. How many of the general public, for instance, are likely to know the meaning or significance of "Cheyne-Stokes breathing?" If such graphic descriptions of a deathbed must appear to satisfy the cravings of morbid minds, let them come from other than members of the medical profession. We have no wish to surround the practice of our noble profession with mystery, but I do think the way in which medical men enter into details of diseases and the action of drugs with their patients and the public is both unnecessary and absurd. But here, again, those high in the profession are often the greatest offenders. Is it that they are so learned they cannot keep it to themselves, or is it that they know so little they wish to make the most of it? To me the whole thing seems very akin to advertising, but this is only the opinion of A Country Practitioner. (*BMJ* 1898;i:1425)