ORIGINAL ARTICLE

Hereditary haemorrhagic telangiectasia: a questionnaire based study to delineate the different phenotypes caused by endoglin and *ALK1* mutations

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Background: Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant vascular dysplasia characterised by mucocutaneous telangiectasis, epistaxis, gastrointestinal haemorrhage, and arteriovenous malformations in the lung and brain. Causative mutations for HHT have been identified in two genes, endoglin and *ALK1*, which encode proteins involved in serine-threonine kinase signalling in the endothelial cell.
Methods: A number of people affected with HHT had completed a postal questionnaire as part of an international study to delineate the HHT phenotype. We identified questionnaires completed by subjects in whom we had identified a mutation in endoglin or *ALK1*. Further questionnaires were sent to families with known mutations. Data were only included from questionnaires returned by people known to carry disease causing mutations.
Results: Questionnaires were completed by 83 subjects with known mutations. Of these, 49 had endoglin mutations (HHT1) and 34 had *ALK1* mutations (HHT2). Subjects with HHT1 reported an earlier onset of epistaxis (p=0.01) and telangiectasis (p=0.0001) than those with HHT2. Pulmonary arteriovenous malformations were only reported in the endoglin mutation group in our study (p<0.001).
Conclusions: Our questionnaire based study provides evidence that the HHT phenotype caused by mutations in endoglin (HHT1) is distinct from, and more severe than, HHT caused by mutations in *ALK1* (HHT2). This has significant implications for diagnosis, screening, and treatment in the two different forms of HHT, as well as for understanding the pathogenesis of the disease.

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ereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant vascular dysplasia. Estimates of the incidence of HHT vary widely, but studies have shown that it affects between 1 in 8345 and 1 in 40 000 people, with even higher incidence in some isolated populations.[1–4] It is characterised by mucocutaneous telangiectases, most commonly affecting the tongue, lips, fingers, ears, and conjunctivae, in association with severe frequent epistaxis and gastrointestinal haemorrhage. A subset of subjects have pulmonary arteriovenous malformations (PAVMs) and cerebral arteriovenous malformations (CAVMs).[5–7] Screening for PAVMs, which allows early detection and treatment of lesions, is thought to reduce the incidence of associated complications, including stroke, cerebral abscess, cardiac failure, and pulmonary haemorrhage.[8] Complications of PAVMs are particularly likely to arise during pregnancy.[9] An increased incidence of classical migraine has also been reported in HHT.[10] Further complications of HHT include haemorrhage from gastrointestinal telangiectasis[11] and hepatic arteriovenous malformations.[12 13] Gastrointestinal haemorrhage most commonly occurs after the age of 60.[11]

Although HHT is highly penetrant, evidence of disease may not be present until after the age of 30.[2 3] Subjects at risk of inheriting a mutation may, therefore, require screening for PAVMs before definite assignment of affected status, and even after such screening may defy certain assignment of affected status.

Germline mutations which cause HHT have been identified in two genes, endoglin and *ALK1*.[14–18] Endoglin is a TGF-beta binding protein, expressed in endothelial cells.[19] Endoglin binds TGF-beta 1 and -beta 3, but not -beta 2. Endoglin also binds activin-A, BMP2, and BMP7.[20 21] Endoglin has been shown to influence TGF-beta

signalling in different in vitro assays.[22 23] Endoglin is also expressed, to some extent, in other cell types including syncytiotrophoblasts, activated monocytes, and other haematopoietic cells.[24–28] Endoglin expression in specific cell types is increased in a number of disease processes, including expression in fibroblasts in scleroderma[29] and in smooth muscle cells in atherosclerotic plaques.[30]

ALK1 is a type 1 serine-threonine kinase receptor, expressed predominantly in endothelial cells.[31 32] ALK1 has been shown to bind TGF-beta 1 and activin-A in vitro, and can show signalling in response to either TGF-beta 1 or -beta 3. There may be a further unidentified ligand.[31 33] ALK1 has been shown to signal via phosphorylation of SMAD1 and SMAD5.[34 35]

We have shown previously that subjects with known endoglin mutations have an incidence of pulmonary arteriovenous malformations of approximately 30%, but that the incidence is less than 5% in subjects in whom the endoglin locus had been excluded.[36] Two families that have been published with known *ALK1* mutations have shown PAVMs in some affected subjects, but in one family reported this was at a lower rate than in subjects with endoglin mutations.[37] In the other family reported, HHT was caused by a specific missense mutation in *ALK1*.[38] No previous study has attempted a more detailed comparison of different clinical features between subjects with endoglin mutations and those with *ALK1* mutations.

In this study we compare clinical data from subjects with HHT with known endoglin mutations (HHT1) and subjects with HHT with known *ALK1* mutations (HHT2). Our cohort consists of those in whom we have identified mutations as a part of previous and ongoing studies. Clinical data were obtained by questionnaire, either sent to people in the United Kingdom and the USA as a part of a world wide postal survey

Table 1 The number of subjects and mutations identified in each family from which a person returned a questionnaire. Where a family has been published previously, the same family identification number is given as has been used in the previous article, and the source is given: a, McAllister et al,[14] b, McAllister et al,[15] c, Gallione et al,[18] d, Johnson et al,[16] e, Berg et al,[17] f, previously unpublished data

Family ID	Gene	Mutation identified	Mutation effect	No of subjects
1[c]	Endoglin	1311G>C	Splice disruption	11
8[c]	Endoglin	155G>T	G52V	1
32[b]	Endoglin	1414C>T	Q472X	3
49[a]	Endoglin	831C>G	Y277X	2
53[b]	Endoglin	1655delC	Frameshift	1
56[b]	Endoglin	1050T>A	C350X	4
58[c]	Endoglin	1432delAG	Frameshift	5
63[a]	Endoglin	1553delGC	Frameshift	3
73[c]	Endoglin	157T>C	C53R	6
93[c]	Endoglin	1078delCAGA	Frameshift	6
139[c]	Endoglin	2T>G	Abolish start	1
142[c]	Endoglin	447G>C	W149C	1
A[f]	Endoglin	511C>T	R171X	1
B[f]	Endoglin	245delT>C	Frameshift	1
C[f]	Endoglin	90T>A	C30X	1
D[f]	Endoglin	IVS5+1,gt	Splice disruption	1
E[f]	Endoglin	IVS3+1,gt	Splice disruption	1
17[e]	ALK1	865insT	Frameshift	1
20[e]	ALK1	998G>T	S333I	1
33[d]	ALK1	1127T>G	M376R	1
40[d]	ALK1	1232G>A	R411Q	7
67[d]	ALK1	694delTCC	Del Ser 232	15
92[e]	ALK1	924C>A	C308X	3
100[e]	ALK1	200G>A	R67Q	1
F[e]	ALK1	150G>T	W50C	2
G[e]	ALK1	1270C>A	P424T	3

Table 2 Proportion of males and females, with mean (SD) age, for the HHT1 and HHT2 groups

Gene	No of subjects	No of males	Mean age of males	No of females	Mean age of females
Endoglin	49	21 (43%)	48.0 (15.3)	28 (57%)	46.5 (11.9)
ALK1	34	14 (41%)	51.3 (24.1)	20 (59%)	65.3 (19.9)

to define the HHT phenotype, or distributed to members of families with HHT caused by known mutations.

METHODS
Subject selection and questionnaire administration
This study was conducted with IRB approval from the University of Vermont, protocol No 650, "Hereditary haemorrhagic telangiectasia: its natural history and genotype-phenotype correlations", as well as IRB approval from Duke University.

The majority of subjects included in this study had been sent a self-administered questionnaire as a part of an international survey to define the HHT phenotype better. The questionnaire was distributed to people with a clinical diagnosis of HHT without reference to the results of mutation analysis. From records held, we identified those subjects in whom a mutation had been identified and selected the questionnaires that had been returned by these people. The same questionnaire was subsequently sent to members of families in whom a mutation had been identified. Clinical data about any subject were only included in the study when a sample had been received from that subject and presence of a mutation confirmed. This methodology did not allow us to count how many questionnaires had been received by a person with a known mutation and not returned.

The questionnaire inquired about general health as well as specific features of HHT. For all symptoms, presence or absence of the symptom was noted. Information on frequency, age of onset, and severity of symptoms was requested.

Mutation analysis and assignment of affected status
Subjects were only included if a mutation had been identified in previous studies or as a part of ongoing research. Mutations were identified by sequencing of endoglin and ALK1 as previously described.[14–18]

Data analysis
Questionnaire data were collected on the following features: presence and age of onset of epistaxis, presence and age of onset of telangiectasis, symptoms suggestive of gastrointestinal haemorrhage, presence of PAVMs (diagnosed by a physician), presence of CAVMs (diagnosed by a physician), history of miscarriage, and symptoms of classical migraine. Symptoms of classical migraine were taken to be present when a person described symptoms of visual disturbance followed by headache. For each person with an identified mutation who returned a questionnaire, clinical data were entered on a Microsoft Access 97™ database. Mutation data were entered into the same database for each person. The proportion of subjects in each mutation group with each complication was calculated. Groups were compared using 2×2 table analysis with either the chi-square test or Fisher's exact test. The p values for individual tests were multiplied by the number of comparisons made, to compensate for multiple testing (Bonferroni method).

To compare age of onset of epistaxis and age of first appearance of telangiectases in subjects with endoglin and ALK1 mutations, Kaplan-Meier survival curves were plotted, and the logrank test used to compare subjects in the two groups.

RESULTS
Questionnaires were completed by 83 subjects with known mutations. Of these, 49 had endoglin mutations (HHT1) and 34 had ALK1 mutations (HHT2). This sample set is only a small proportion of the people affected with HHT in the UK and USA, because we chose only to include those patients in whom a confirmed pathogenic mutation had already been identified. Details of the type of mutation identified, the number of subjects with each mutation, and source of the original mutation data, are given in table 1. Numbers, percentages, and mean (SD) ages of males and females within each group are given in table 2. There was no significant difference between the proportion of males and females in the HHT1 and HHT2 groups. The mean age of females with HHT2 who returned questionnaires was significantly (p=0.001) higher than the mean age of the females in the other group. There was no significant difference between the mean age of males in the two groups.

The proportion of subjects affected with each feature of HHT is given in table 3. In this analysis we have found a reported incidence of PAVMs, in subjects with HHT1, of 35%. This agrees with previous estimates. We found a much lower incidence of PAVM in those with HHT2 (0% in this study, p<0.001).

No significant differences were seen between the HHT1 and HHT2 groups for the proportion of subjects affected with CAVMs, gastrointestinal haemorrhage, or history of miscarriage in women aged over 16. Only two subjects in each group reported hepatic involvement.

More people with HHT1 reported a CAVM (4/49) than those with HHT2 (1/34), but the numbers in both groups were small. Symptoms suggestive of migraine were also reported more frequently in HHT1 (20/49) than HHT2 (6/34); however, this difference was not significant after correction for multiple testing. Gastrointestinal bleeding after the age of 60 was

Table 3 A comparison of the HHT1 and HHT2 groups looking at the presence or absence of disease features

Feature	Endoglin mutation	ALK1 mutation	Significance
Epistaxis	48/49	34/34	NS
Telangiectasis	47/49	28/34	NS
Pulmonary arteriovenous malformation (PAVM)	17/49	0/34	p<0.001
Cerebral arteriovenous malformation (CAVM)	4/49	1/34	NS
Gastrointestinal haemorrhage	6/49	9/34	NS
Gastrointestinal haemorrhage (age 60+)	1/9	7/19	NS
Miscarriage in affected female (age 16+)	8/28	8/20	NS
Symptoms suggestive of classical migraine	20/49	6/34	NS

reported less frequently in HHT1 (affecting 1/9 subjects) than in HHT2 (affecting 7/19 subjects), but this finding did not achieve significance. The average age of females in the HHT2 group was greater than the average age in the HHT1 group. As

gastrointestinal bleeding is more common in the elderly, this may account for the greater number of subjects affected with gastrointestinal bleeding in the HHT2 group.

Most subjects had a history of epistaxis and telangiectasia at the time of completion of the questionnaire. We therefore compared age of onset of these features between the HHT1 and HHT2 groups. Kaplan-Meier survival curves are shown in fig 1 for age of onset of epistaxes and of first appearance of telangiectases. For both of these clinical features the logrank test showed a significant difference between the survival curves for the two mutation groups: epistaxes (p=0.01) and telangiectases (p=0.0001). In both cases subjects with HHT1 had earlier onset of symptoms. Further logrank tests were carried out where the survival distributions were adjusted for sex differences, and the significance levels remained the same. The proportion of subjects developing these symptoms by a given age, estimated from the curves, is shown in table 4.
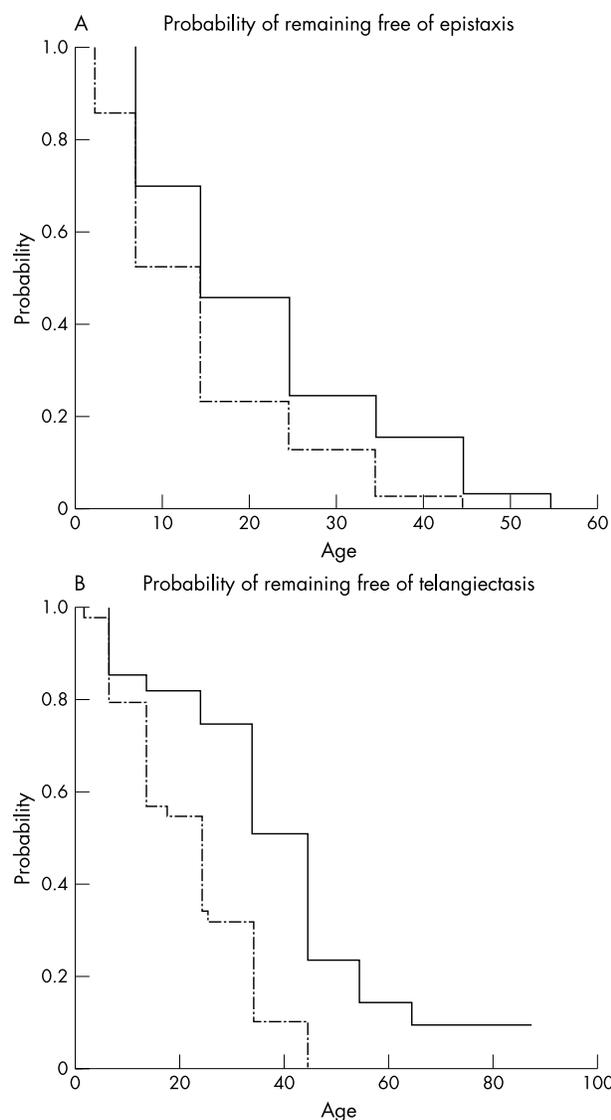
DISCUSSION

This is the first study of HHT to compare subjects with known endoglin mutations and those with known *ALK1* mutations for a range of clinical features. This study identifies a distinct difference in phenotype between people with endoglin mutations (HHT1) and those with *ALK1* mutations (HHT2). Subjects with HHT1 and those with HHT2 both have epistaxis, telangiectasis, and gastrointestinal haemorrhage. However, subjects with HHT1 have significantly earlier onset of epistaxis and telangiectasis. Overall, HHT1 has a more severe phenotype than HHT2.

As HHT is a comparatively rare disease, the use of a questionnaire is an efficient and practical method for obtaining data from a cohort of subjects across a large geographical area. However, a significant limitation of this study is that, for practical reasons, clinical data were collected solely by questionnaire, without confirmation of events from medical records. As a number of subjects were not formally screened for internal vascular malformations, especially PAVMs or

Figure 1 Kaplan-Meier survival curves showing probability of remaining symptom free for (A) epistaxis and (B) telangiectasis. The curves for subjects with HHT1 are given as the interrupted line and for subjects with HHT2 as the continuous line. For both epistaxis (p=0.01) and telangiectasis (p=0.0001), the age of onset of symptoms is significantly younger in subjects with HHT1.

Table 4 Proportion of subjects showing epistaxis or telangiectasis by a given age

| Age | Endoglin mutation | | ALK1 mutation | |
	Epistaxis	Telangiectasis	Epistaxis	Telangiectasis
4	0.15	0.02	0.0	0.0
9	0.48	0.21	0.30	0.14
19	0.77	0.46	0.55	0.18
29	0.88	0.68	0.76	0.26
39	0.98	0.90	0.85	0.49
49	1.0	1.0	0.97	0.77
59	1.0	1.0	1.0	0.86
69	1.0	1.0	1.0	0.91

CAVMs, there will be significant under-reporting of these lesions in both the HHT1 and HHT2 groups. As patients who present to medical services are likely to be more severely affected, frequency and severity of epistaxis and telangiectasis are probably over-reported in both groups. However, we feel that these factors are likely to have affected both cohorts fairly equally, and that comparison between the groups remains valid.

There will be differences in screening protocols, and these differences are likely to be particularly marked between subjects from the USA and those from other areas. Screening protocols, particularly those for PAVMs, do vary in their sensitivity and specificity, and the questionnaire was not able to identify which protocol was used to screen each subject. However, the majority of people from both the HHT1 and HHT2 cohorts were from the USA, so we do not feel that difference in screening protocols is a significant source of bias in determining the difference between HHT1 and HHT2 groups. The variation in quality of screening offered will, however, reduce the number of lesions reported in both groups.

The proportions of males and females in the HHT1 and HHT2 groups were the same, which is important, as oestrogen levels can affect endothelial cell function. Oestrogens are also used to treat gastrointestinal bleeding in HHT. The higher average age of females in the HHT2 group is likely, if it has any effect, to lead to understatement of the difference between the HHT1 and HHT2 groups, as older subjects would have had longer to manifest signs of disease or suffer complications from PAVMs or CAVMs. We do not think, therefore, that this age difference will have led to a significant bias in our results.

In this study, PAVMs were identified only in subjects with HHT1; 35% of those with HHT1 who returned a questionnaire reported a PAVM. The cohort in this study overlaps with that previously reported.[36] The collection of clinical data for this study was, however, independent of the previous survey.

While the difference in frequency of gastrointestinal (GI) haemorrhage between the groups did not achieve statistical significance, a larger proportion of subjects with HHT2 over the age of 60 had symptoms of GI haemorrhage (7/19) compared to those with HHT1 (1/9). While this may also be caused by earlier mortality of HHT1 patients who have GI haemorrhage, there may be a genuine difference in phenotype, with subjects with HHT2 being more prone to GI telangiectasis.

Do phenotypic differences provide information about the signalling pathways for ALK1 and endoglin in the endothelial cell?

The fact that HHT1 and HHT2 share most of their phenotypic features suggests that endoglin and ALK1 share a common signalling pathway in the endothelial cell. They may interact directly at the cell surface, or their signalling pathways may converge within the cell. Interaction of endoglin with ALK1 has been shown in vitro.[33 39] Ligand binding assays in COS cells showed a TGF-beta 1 and -beta 3 dependent endoglin/ALK1/TGF-beta receptor II complex.[39]

Greater severity of HHT1 may suggest that endoglin has an extra signalling role in addition to the pathway it shares with ALK1. Evidence for this comes from Barbara et al,[21] who have shown that endoglin can bind activin-A, BMP2, and BMP7 when co-expressed in vitro with different serine threonine kinase receptors. In addition, although endoglin and ALK1 knockout mice show similar phenotypes and are embryo lethal around E10.5 owing to defects in angiogenesis, there is a major difference in the vascular morphology of the embryo proper at E9.5.[40] Zwijsen et al[40] suggest that endoglin plays a role in both the ALK1 and the TGF-beta receptor type I (ALK5) pathway. Endoglin has also been shown to interact directly with the TGF-beta receptor type 1.[41] Therefore, mutations in endoglin would disrupt both ALK1 and ALK5 signalling,

whereas mutations in ALK1 would lead to a dominance of ALK5 mediated effects.

It is possible that the presence of other type I serine threonine kinase receptors in the endothelial cell that also signal through SMAD1/SMAD5 (including the bone morphogenic protein receptors) may also compensate for ALK1 deficiency, leading to a less severe phenotype in HHT2.

Clinical implications for the difference between HHT1 and HHT2

Clinical differences between HHT1 and HHT2 have important implications for the diagnosis and management of HHT. Although data collected by questionnaire should be used with caution, these clinical data will be useful in genetic counselling of subjects, providing a clearer picture of disease, both for those affected and for those considering presymptomatic testing. Although no PAVM was reported in a subject with an ALK1 mutation in our study, it is important to note that two papers have reported PAVMs in HHT2 families, although usually at lower frequency than in HHT1 families.[36 38] Therefore, all subjects with HHT should, at present, be offered pulmonary screening for PAVMs. Subjects with HHT1 represent a particularly high risk group, although further investigation is required to identify whether people with HHT1 or HHT2 merit different screening protocols. In the absence of a known mutation in a family with HHT, a family history of PAVM is suggestive of HHT1 rather than HHT2, and this information can be used to target mutation analysis more efficiently.

Our study is not large enough to identify differences in clinical features caused by different types of mutation at one locus. For example, do missense mutations have a more severe phenotype than null mutations? Only a study of a larger number of subjects with known mutations can answer such questions.

Seventy-seven percent of those with HHT1 reported onset of nosebleeds before the age of 20, and 88% before the age of 30, so in this group at risk relatives without this symptom after the age of 30 are less likely to be affected. The later onset of classical symptoms in HHT2, with only 55% showing epistaxis before the age of 20 and 76% before the age of 30, suggests that some subjects with HHT2 will not manifest sufficient clinical features for a diagnosis of HHT until their third or fourth decade of life. In the absence of mutation testing, it is therefore important to maintain clinical surveillance of those at risk of inheriting a HHT mutation, at least until the age of 40, if not longer. Genetic testing for HHT, where available, will be essential to allow targeting of surveillance and screening to only those who have a mutation.

We conclude that knowing which locus is mutated is important for the accurate genetic counselling of subjects with HHT. The difference in frequency and age of onset of different complications of HHT suggests that future clinical studies of the disease will only be valid if they take into account the locus involved. Large genotype-phenotype studies, including confirmation of details from medical records or a detailed, consistent investigation protocol for each patient, are required to investigate whether there are genuine differences in the frequency of GI haemorrhage, cerebral arteriovenous malformations, and migraine between HHT1 and HHT2. Such investigations will also be able to confirm and quantify more accurately the significant differences that we have observed in our study for epistaxis, telangiectasis, and pulmonary arteriovenous malformations.

The differences between HHT1 and HHT2 patients will also be an important consideration in therapeutic trials for patients with HHT, as the locus involved may have a profound influence on response to treatments targeted to specific signalling pathways.

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ECHO ....

Doctors get closer to deciding the true birth prevalence of Prader-Willi syndrome

Please visit the Journal of Medical Genetics website [www.jmedgenet. com] for link to this full article.

A more reliable estimate of the prevalence of Prader-Willi syndrome in Australian babies is now available, following the first population study to assess genetically proven cases.

The birth prevalence of 4/100 000 live births (95% confidence interval 2.7 to 5.7) or ~1/25 000 live births /year is based on 30 notified cases between January 1998 and December 2000 in children aged under 1 year. It is a minimum estimate because of the possibility of missing data for the target age and failure to recognise cases clinically.

The 30 cases (71%) were among 42 cases in children aged under 15 years, all confirmed by DNA methylation, fluorescent in situ hybridisation, or DNA polymorphisms. A paternal deletion on chromosome 15q(11–13) was the cause in 21 (70%) of the 30 cases and disomy in one parent in three (10%) but was unknown in six.

The results were based on notifications to the Australian Paediatric Surveillance Unit. Paediatricians and child health specialists were asked to report newly diagnosed cases, whether on clinical or genetic criteria, in the past month in children aged under 15. They were sent a copy of clinical diagnostic criteria and asked for demographic data, growth and genetic test results, perinatal and neonatal histories, and clinical details. Data on children aged under 1 year were used. The Australian Bureau of Statistics provided data on live births.

Prader-Willi syndrome is not always recognised, so is underestimated among babies and overestimated among obese retarded teenagers, and all previous estimates of prevalence have been retrospective.

▲ *Archives of Disease in Childhood* 2003;**88**:263–264.

ECHO ....

SPINK1 mutations predispose to chronic pancreatitis

Please visit the Journal of Medical Genetics website [www. jmedgenet.com] for link to this full article.

There is a high prevalence of serine protease inhibitor Kazal type 1 (SPINK1) mutations, not only in chronic idiopathic, but also alcoholic pancreatitis, with around one in eight patients affected, finds Dutch research. The findings are based on mutational analysis of 115 adult patients with alcoholic (n=72), hereditary (n=10), idiopathic (n=24) and miscellaneous origin (n=9) chronic pancreatitis.

The research team looked for two mutations in the PRSSI gene—R122H and N291— and four specific mutations in the SPINK1 gene—M1T, L14P, N34S and P55S—and compared the results with those from a group of 120 healthy adults.

The prevalence of SPINK1 mutations was 12.2% among patients with alcoholic and idiopathic pancreatitis, and seven times higher than among the comparison group. In 13 out of the 14 cases, the most common variant was the N34S mutation, but two patients carried the P55S allele, one as a compound heterozygote with N34S. Just two people in the comparison group carried the N34S mutation.

PRSS1 mutations were found in six out of 10 patients with hereditary pancreatitis and were associated with earlier disease onset than the N34S allele.

There are around 50 to 75 cases of chronic pancreatitis per 100 000 of the population, with one in two people dying from their disease 20 years after diagnosis. Alcohol misuse accounts for up to 70% of cases in the West, but the authors conclude that SPINK1 mutations are an important predisposing factor in the development of the disease.

▲ *Gut* 2002;**50**:687–692.