The variable phenotype of the p.A16V mutation of cationic trypsinogen (*PRSS1*) in pancreatitis families

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ABSTRACT

Objective To characterise the phenotypes associated with the p.A16V mutation of *PRSS1*.

Design Clinical and epidemiological data were collected for any family in which a p.A16V mutation was identified, either referred directly to the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer or via a collaborator. DNA samples were tested for mutations in *PRSS1*, *SPINK1*, *CFTR* and *CTRC*.

Patients Participants were recruited on the basis of either family history of pancreatitis (acute or chronic) or the results of genetic testing. Families were categorised as having hereditary pancreatitis (HP), idiopathic disease or pancreatitis in a single generation. HP was defined as ≥2 cases in ≥2 generations.

Main outcome measures Onset of painful episodes of pancreatitis, death from pancreatic cancer, diagnosis of diabetes mellitus and exocrine pancreatic failure.

Results Ten families with p.A16V mutations were identified (22 affected individuals): six HP families, three with idiopathic disease and one with only a single generation affected. The median age of onset, ignoring non-penetrants, was 10 years (95% CI 5 to 25). There were eight confirmed cases of exocrine failure, four of whom also had diabetes mellitus. There were three pancreatic cancer cases. Two of these were confirmed as p.A16V carriers, only one of whom was affected by pancreatitis. Those with p.A16V pancreatitis were compared to affected individuals with p.R122H, p.N29I and no *PRSS1* mutation. No significant differences were proven using logrank or Mann—Whitney U tests.

Conclusions Penetrance of p.A16V is highly variable and family dependent, suggesting it contributes to multigenic inheritance of a predisposition to pancreatitis.

BACKGROUND

Hereditary pancreatitis (HP) is an autosomal dominant disease with penetrance that has been calculated to be as high as 93%.[1] HP is characterised by frequent attacks of epigastric pain, normally associated with nausea and vomiting. Symptoms may start shortly after birth, but onset varies greatly, with some individuals not exhibiting symptoms until adulthood.[2] There is usually progression to chronic pancreatitis with endocrine and exocrine failure and an increased risk of pancreatic cancer.[1–3] The natural history of HP follows a similar pattern to chronic pancreatitis associated with alcohol, but there are important differences: HP has an earlier age of onset of pancreatitis, but malabsorption and diabetes mellitus occur at a later stage in the disease.[2 4 5]

Causative mutations have been discovered in the cationic trypsinogen gene, *PRSS1* at bases 365 and 86 of the cDNA (c.365G→A[6] and c.86A→T[7]). These are now known as p.R122H[6] and p.N29I,[7] respectively, according to the amino acid substitution and position in the protein sequence. These mutations are rarely identified in general screens of patients with idiopathic disease.[8 9] The phenotype of p.R122H and p.N29I has been well characterised,[1 2] but there are many other rare mutations or polymorphisms of *PRSS1* that are less well understood.[10]

p.A16V is the third most common *PRSS1* mutation and is significantly associated with pancreatitis.[11] It was first identified in pancreatitics with no family history[11] and has subsequently been reported by other groups in apparently idiopathic patients.[8] It is relatively rare in families with multiple cases of pancreatitis.[2 8 11] In contrast, rare instances of p.R122H in individuals without a family history may be explained by either a limited pedigree or by spontaneous mutation.[12] This is the first study that attempts to characterise the clinical significance of p.A16V and is the largest series published to date.

A number of registries have been established to investigate both the phenotype and genotype of HP and the potential for new therapies. The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) was established in 1997 and has a network of collaborating clinicians and scientists. Families are defined as having HP if the inheritance is consistent with highly penetrant autosomal dominance. This usually requires at least two first degree relatives with pancreatitis in multiple generations, although in some cases three or more second degree relatives may be adequate evidence.[2] The *PRSS1* gene is tested in a consenting affected individual from each family. Due to the fact

that families are registered before full examination of the family tree, some individuals have been included who are subsequently shown to have no family history of pancreatitis. Other families are included on the registry where there are two or more cases, but where the pattern of inheritance is inconsistent with highly penetrant autosomal dominance. These families have previously been described as having familial idiopathic pancreatitis (FIP).[13] FIP families have a very high incidence of the p.N34S mutation in the *SPINK1* gene, this mutation corresponds to a change at position 101 of the cDNA (c.101A→G) and is in linkage disequilibrium with a series of other changes, none of which (including p.N34S) have a known functional consequence.[14] These FIP families do not have the p.R122H or p.N29I mutations of *PRSS1*.[13]

In 1999 Witt *et al*[11] published a paper detailing the results of genetic testing of children with chronic pancreatitis. Of the 44 children tested, 30 had apparent sporadic disease with a further 14 having a family history. Of the 30 sporadic cases, p.A16V was detected in three individuals. It was also detected in one individual with a family history. One p.R122H mutation was also detected in the family history group. Further individuals from families with p.A16V were tested for the mutation, with just one of seven carriers being affected. This suggested that p.A16V is a low penetrance mutation, although the numbers involved were too low for firm conclusions.

In this paper a range of phenotypes will be described for the p.A16V mutation and we will discuss why p.A16V differs from the true HP mutations.

METHODS

Recruitment

Most kindreds were directly recruited and characterised by EUROPAC on the basis of presumed family history, but four families with known p.A16V mutations were referred as a result of this study being instigated. The first p.A16V family recruited by EUROPAC was identified as having an abnormal cluster of pancreatitis in 1998, but was only tested for p.A16V after the discovery of the mutation by Witt *et al* in 1999.[11] Five other families with a p.A16V mutation were recruited between 1999 and 2007. In addition, the registry became aware of one family with multiple cases, and two individuals with no known family history after clinicians contacted us for advice. No data from these families were included in any analysis as we were unable to confirm patient consent.

The registry obtained multi-centre research ethics committee approval to register participants and test their DNA for variations associated with pancreatitis. Recruitment was via the individual's specialist, family doctor or direct contact with the study co-ordinator. The first individual identified within a registered family was labelled as the proband. Affected probands or the closest relative to unaffected probands were taken as the index case. They were sent a patient information sheet and, following a suitable interval, gave written informed consent.

Data collection

Data were collected by a series of questionnaires supported by clinical consultations. DNA was collected and stored in compliance with the Human Tissue Act 2004 (England and Wales) under the care of the Mersey Regional Genetics Service. The data were used to build a family tree using Progeny software (v 7.01) and further members of the family were approached via the proband as appropriate, with data and DNA obtained from consenting individuals. The implications of testing were discussed with the participant before it was performed. Typically, all individuals had *PRSS1* analysis, which involved sequencing of exons 2 and 3 to identify common mutations and if none were detected, sequencing of all exons. In idiopathic cases and p.A16V carriers, *PRSS1* sequencing was supplemented by testing for mutations in the *SPINK1* (*PSTI*) and the cystic fibrosis transmembrane receptor (*CFTR*) genes. Exon 3 of *SPINK1* was sequenced to identify any possible p.N34S mutations and *CFTR* was tested in all cases for p.ΔF508, p.G542X, p.N1303K, p.R117H, 621+1 G-T, 1898+1GA, p.W1282X and p.G551D and in some cases with an additional 24 markers according to the recommendations of the American College of Medical Genetics (ACMG) and the American College of Obstetricians and Gynaecologists (ACOG).[15] In this study affected p.A16V carriers were also tested for mutations in *CTRC* exons 2, 3 and 7.

Mersey Regional Genetics Service used fluorescent sequence analysis to identify *PRSS1* mutations. This involved the use of nested PCR facilitated by uniseq tagged primers. Some of the testing performed in the families included in this paper was conducted at collaborating centres.

Categorisation of families

After families had been fully investigated, there was a multidisciplinary discussion by a group of clinicians and scientists. Families were designated as HP, single generation, or idiopathic. Idiopathic disease was defined as the presence of pancreatitis in the absence of a known aetiology or family history.

Statistical analysis

Data from the EUROPAC registry were analysed using StatView v 5.0. Endpoints including onset of pancreatitis and diagnosis of endocrine and exocrine pancreatic failure were analysed using the method of Kaplan–Meier and differences assessed using the Mantel–Cox logrank test. For patients where an end point was not reached, censor times were the age of last contact. Differences in median values for continuous data were tested using the Kruskal–Wallis (for comparison of multiple groups) and Mann–Whitney U (for comparison of two groups) tests.

RESULTS

Identification of p.A16V in EUROPAC families

A summary of families registered on the EUROPAC database in January 2009 is shown in table 1. This shows the prevalence of the p.A16V compared to the other mutation groups.

Nine of the ten p.A16V families originated from Western Europe (the UK and Ireland, Scandinavia, Belgium, France and Switzerland), with the final family being from the USA. A sample family from each group is shown in figure 1.

The variable phenotype of p.A16V families

Of the 10 different families with positive test results for a p.A16V mutation, six met the criteria for classification as having HP and three as having idiopathic disease, while one had multiple cases but only in a single generation. Of these families, three of the HP kindreds, two from the idiopathic group and the FIP family have been previously reported.[1 2]

Family A is an example of a kindred described as having HP (figure 1A); as with all such families the definition of HP was based on the phenotype and preceded genetic testing. All families in this group were defined as having HP regardless of whether mutations were detected. In this case the family was identified after the proband's children (individuals 3 and 5) were affected by pancreatitis. Both p.R122H and p.N29I were excluded and a p.A16V mutation in one copy of *PRSS1* was detected in the proband (individual 1). She was also tested for the p.N34S

Table 1 Summary of mutations present within EUROPAC pancreatitis families who have undergone genetic testing, January 2009

Mutation	Families	Affected	Total pancreatic cancer cases (in affected individuals)
Total HP	149	652	45 (37)
p.R122H	71	336	19 (16)
p.N29I	32	157	10 (10)
Neg All	37	134	13 (10)
Other mutation	3	10	1 (1)
p.A16V (HP)	6	15	2 (0)
Total idiopathic	142	142	16 (0)
No *PRSS1* mutation	139	139	16 (0)
p.A16V (single case)	3	3	0 (0)
Total single generation	40	89	7 (2)
No *PRSS1* mutation	39	85	6 (1)
p.A16V (single generation)	1	4	1 (1)
Overall total	331	883	68 (39)

HP, hereditary pancreatitis. 'Affected' refers to individuals with pancreatitis (acute or chronic). The total number of pancreatic cancer cases is given; values in brackets represent cancer cases in affected individuals. Values are broken down for each mutation. 'Neg All' means that the phenotype is consistent with HP but none of the known mutations have been identified in affected individuals. 'Other mutation' includes families with rare but known *PRSS1* mutations, eg p.R122C and p.V39A. 'Single generation' means there are multiple cases of pancreatitis within kindreds but no evidence of autosomal dominant inheritance. 'p.A16V (single generation)' refers to the one family with a p.A16V mutation that displays this phenotype. The idiopathic pancreatitis group includes three families who have a proven p.A16V mutation but only one case of pancreatitis.

mutation in *SPINK1*, 32 mutations in *CFTR* and mutations in exons 2, 3 and 7 of *CTRC*; none of these genes were mutated.

Mutations in modifier genes (*CTRC*, *SPINK1* and *CFTR*) were not detected in any of the HP families, although a previously reported polymorphism (c.180C→T, p.G60G)[16] was seen in two of six of these families (families C and E). This polymorphism has been previously reported in 21% of control samples[16] and is therefore clearly not over-represented in our cohort.

In this report 'affected' has been taken to mean that individuals have reported symptoms of pancreatitis, so the possibility of subclinical disease cannot be excluded in other family members. In family A one of the proband's siblings has diabetes (individual 8). This could be a manifestation of pancreatic inflammation in the absence of pain. This individual does not carry p.A16V. It is possible that, at least in this kindred, p.A16V is modifying the symptoms of an underlying inherited pancreatic disease, with the mutation increasing the chance of pain. However, as diabetes mellitus is not uncommon, the cases in such families may well be coincidental.

p.A16V could explain the pancreatitis in all the p.A16V HP kindreds, but even in these families, not all carriers had explicit symptoms. Unavoidably, most unaffected individuals in the families were not tested. Of 23 individuals who were confirmed to have the p.A16V mutation, only 15 had reported symptoms of pancreatitis. All of the proband's siblings in family A were tested for p.A16V; three (individuals 9–11) carried the mutation but none reported symptoms. The proband's mother was tested and no mutation was detected, indicating that the mutation was inherited from the father (individual 7). There were no cases of pancreatitis in any of his eight siblings, although three were affected by diabetes mellitus. No testing was conducted within this generation. Two of the siblings with diabetes were identical twins, consistent with a genetic predisposition to diabetes in this part of the kindred. Note however, that if the risk of diabetes is independent of the p.A16V mutation and the inherited risk accounts for the diabetes seen in individual 8, then the father of the proband (individual 7) must have carried both the p.A16V mutation and the predisposition to diabetes without developing symptoms.

The EUROPAC database has registered 142 families who have been classified as having idiopathic disease and three of these have a proven p.A16V mutation (2.1%). The families are named G (figure 1B), H and I (family trees not shown). In all cases, referral was atypical for EUROPAC, in that there was no family history. Unlike any of the HP families, genetic testing was performed before recruitment to the study. For example, family G (figure 1B), was referred after an 11-year-old boy (individual 1) was admitted to hospital with recurrent abdominal pain which had first started at the age of 5. An appendicectomy was performed, but histology showed no evidence of inflammation. The patient subsequently developed a pancreato-pleural fistula requiring a chest drain. Due to his personal history, his clinicians requested testing for a *PRSS1* mutation. Subsequent to discovery of a p.A16V mutation, his mother and brother (individuals 3 and 4) underwent testing and have been confirmed as unaffected p.A16V carriers. Neither of the proband's maternal grandparents (individuals 8 and 9) are affected and testing has not been performed in either case. Similarly in families H and I, there was childhood onset disease, consistent with the original identification of p.A16V in sporadic cases identified in paediatric units.[11] This may represent a referral bias rather than a feature of p.A16V per se. No modifier gene mutations were detected in any of these families, although the p.G60G polymorphism of *CTRC* was observed in the affected individual of family I.

HP is defined as an autosomal dominant disease and EUROPAC requires evidence for such transmission before classifying a family in this group. There are 40 pancreatitis families on the registry in whom despite thorough investigation, the disease appears to be limited to a single generation with no evidence for spontaneous mutation. This phenotype is consistent with a multigene or recessive syndrome. One of these 40 families, family J (figure 1C), carries an p.A16V mutation (2.5%). This family was referred after pancreatitis was diagnosed in four of eight siblings, one of whom had already died from a histologically proven adenocarcinoma of the pancreas at the age of 44. The other affected siblings all tested positive for the p.A16V mutation. Only one affected individual was tested in this family for modifier gene mutations. This individual was homozygous for the p.G60G polymorphism of *CTRC* but had no other variation. The remaining four siblings were unaffected and declined genetic testing. The individual with pancreatic cancer was not tested for p.A16V although she had been affected by pancreatitis prior to developing malignancy. Both the parents and offspring were asymptomatic and testing was not performed in either the preceding or subsequent generation.

Phenotype of p.A16V families compared to carriers of other *PRSS1* mutations

A visual comparison of pedigrees indicates that there are differences between the phenotype of p.A16V and the phenotype associated with other *PRSS1* mutations. However, the low incidence of p.A16V and the even smaller numbers of carriers affected by diabetes mellitus, malabsorption or pancreatic cancer means that there is insufficient power for a meaningful statistical analysis. Nevertheless, the data summarised in table 2 confirm that cancer and both endocrine and exocrine failure are features that occur within p.A16V families. To further illustrate the level of risk, survival curves were produced which are shown in figure 2. For all end points, Neg All HP is significantly different from the two groups with known HP mutations (p.R122H and p.N29I). These differences have already been described and discussed elsewhere.[1 2] There are too few data to prove differences between p.A16V and the other groups, but the survival curves illustrate

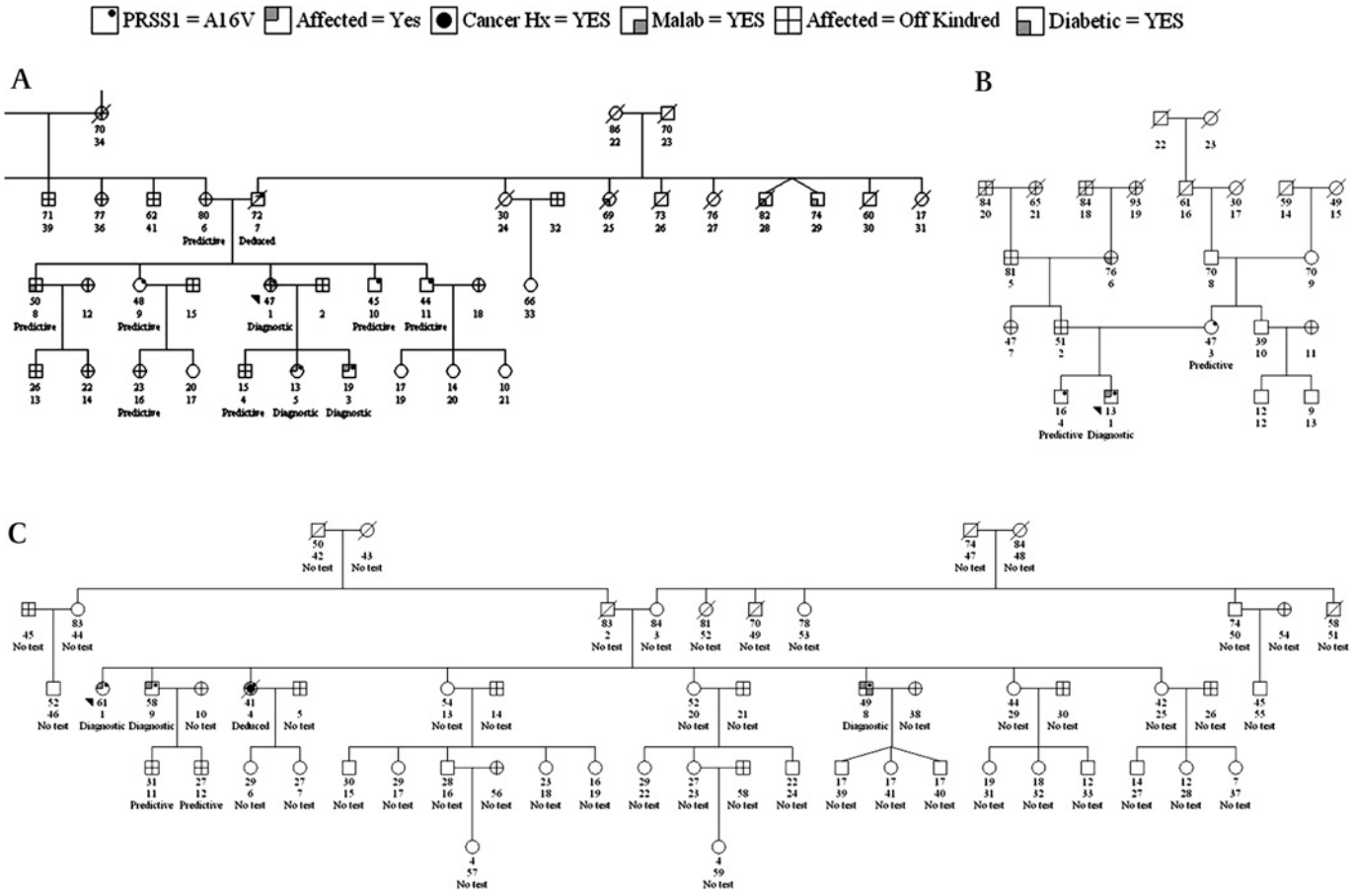
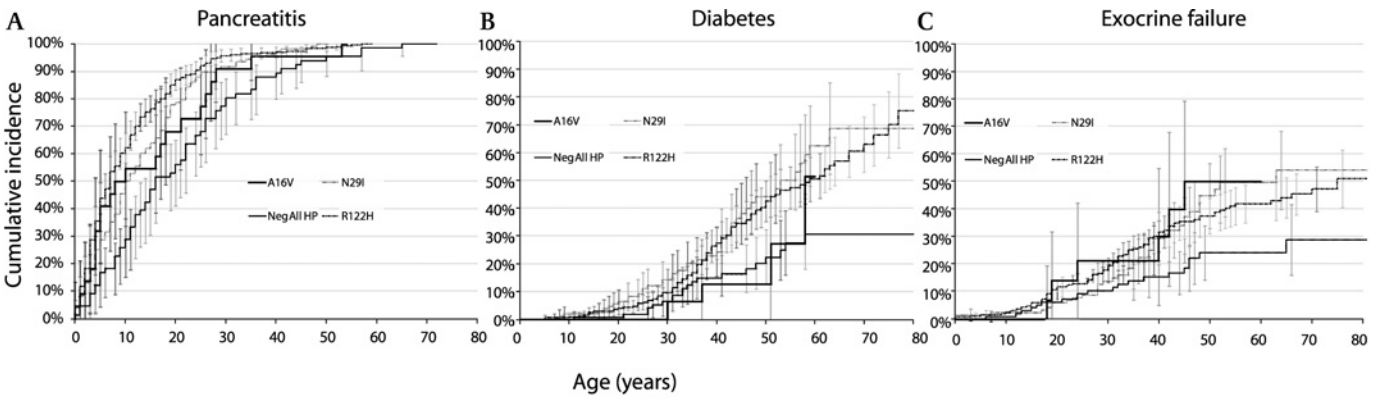


Figure 1 Sample pedigrees indicating the variable phenotype of p.A16V. Ages and unique personal identification numbers are shown below each family member followed by a description of the type of p.A16V test. Arrows indicate index cases. A shaded left upper quadrant indicates that the individual is affected by pancreatitis, shading of the left lower quadrant indicates diabetes mellitus, and a shaded right lower quadrant indicates exocrine pancreatic failure. The presence of a dot in the right upper quadrant signifies the presence of a p.A16V mutation, while individuals assumed not to have a p.A16V mutation are described as off kindred and are marked with a cross. The central black circle indicates the presence of a confirmed pancreatic adenocarcinoma. (A) Displays autosomal dominant inheritance. (B) Includes an idiopathic case of pancreatitis. (C) Defined as having familial idiopathic pancreatitis as cases of pancreatitis, diabetes and pancreatic cancer are restricted to a single generation.

Table 2 Summary of EUROPAC p.A16V families, January 2009

Family	Classification	Number testing positive for p.A16V* (with pancreatitis)	Median age of onset (IQR) (years) (all patients testing positive for p.A16V*)	Median age of onset (IQR) (years) (patients with pancreatitis)	Endocrine pancreatic failure (with pancreatitis)	Exocrine pancreatic failure (with pancreatitis)	Pancreatic cancer cases (with pancreatitis)
A	HP	7 (3)	18 (7—53)	10 (5—21)	4 (0)†	1 (1)	0
B	HP	2 (2)			2 (1)	2 (2)	0
C	HP	2 (2)			0	0	0
D	HP	4 (4)			0	0	0
E	HP	5 (2)			0	0	1 (0)
F	HP	3 (2)			2 (2)	2 (2)	1 (0)
G	Idiopathic	3 (1)	Not reached (5—not reached)	2 (1—5)	1 (0)†	0	0
H	Idiopathic	3 (1)			1 (1)	1 (1)	0
I	Idiopathic	4 (1)			0	0	0
J	Single generation	4 (4)	27 (25—28)	27 (25—28)	0	2 (2)	1 (1)
Totals		37 (22)	26 (7—54)	10 (5—26)	10 (4)	8 (8)	3 (1)

HP, hereditary pancreatitis; IQR, interquartile range.
*Including deduced carriers.
†Including one case confirmed not to have the p.A16V mutation.
Each p.A16V family is described including the number of individuals with symptomatic pancreatitis. The median age of onset of pancreatitis is derived using the method of Kaplan—Meier. Median age of onset is given for all patients with pancreatitis or for all patients with p.A16V mutations (censoring at age of last contact for unaffected individuals). IQR is the age of 25% incidence to the age of 75% incidence. The numbers of individuals diagnosed with diabetes, malabsorption or pancreatic cancer are also given. Of the HP families without p.A16V, 58 p.R122H, 24 p.N29I, 21 Neg All and 2 with other mutations have been previously reported. In addition, 115 idiopathic cases have been reported previously. Of the p.A16V families, three of the HP kindreds, two from the idiopathic group and the family with multiple cases in a single generation have been previously reported.[1 2 13]



At Risk												
0	20	40	60	0	20	40	60	0	20	40	60	Age
22	8	3	0	22	18	14	1	19	13	9	2	p.A16V
108	28	4	0	140	110	54	12	134	109	53	16	p.N29I
66	31	10	3	113	97	58	21	109	90	61	23	Neg All
301	54	10	0	336	290	145	46	336	263	136	62	p.R122H

Figure 2 Kaplan–Meier curves comparing p.A16V to other mutation groups. Inverted Kaplan–Meier curves are shown for three different endpoints: (A) onset of pancreatitis symptoms; (B) diagnosis of diabetes mellitus; and (C) diagnosis with exocrine failure. Confidence intervals are shown in grey, with those for the p.A16V group in a darker shade. The wide CIs in the p.A16V group are due to the low data volume, which is also indicated by the number of at risk individuals shown below each graph.

possible trends. To simplify analysis and increase power, the two mutation groups (p.R122H and p.N29I) were combined and will be described as the 'mutation group' in contrast to the Neg All HP group.

The age of diagnosis of diabetes appears similar in the p.A16V and Neg All HP groups (logrank p value for p.A16V compared to the combined mutation group was 0.076, with a p value for p.A16V compared to the Neg All HP group of 0.83). In contrast, when looking at age of diagnosis of exocrine pancreatic failure, the p.A16V is more like the mutation group than the Neg All HP group (logrank p value for p.A16V compared to the Neg All HP group is 0.087 with a p value for p.A16V compared to the combined mutation group of 0.88). There is the suggestion that the age of onset of pancreatitis in p.A16V appears later than with the mutation group and is more like the Neg All HP group than either p.R122H or p.N29I (logrank p value for p.A16V compared to the combined mutation group is 0.080, $\chi^2_1=3.06$, contrasting with a p value for p.A16V compared to the Neg All HP group of 0.18).

Penetrance

An exact calculation of penetrance is difficult to perform as individuals who are not affected are often reluctant to agree to genetic testing. Taking family A (figure 1A) as an example, the oldest age of onset was 16 and four out of the seven known carriers were unaffected (at least until the age of 40). On this basis, penetrance can be estimated at 43% in this family. However, half of the proband's paternal antecedents would be expected to be carriers. As none were reported to be affected, the actual penetrance of p.A16V in this kindred is probably far lower.

An alternative approach is to take a family member affected by pancreatitis and see how many first degree relatives they have who are also affected. Assuming an autosomal dominant pattern of inheritance, each first degree relative of an affected individual has a 50% chance of being a mutation carrier. The index case in each family can be identified and the proportion of affected first degree relatives calculated; doubling this figure gives an estimate of penetrance. This was performed blind to any mutation analysis using all families on the EUROPAC registry with the results shown in table 3.

The analysis is subject to ascertainment bias. Families with greater numbers of cases are more likely to be recruited. Furthermore, in families with variable penetrance, an index case is more likely to be in a section of the family with the greatest number of affected individuals. On the other hand, this approach also means that one affected carrier is always omitted from the calculation (the index case). In figure 3, the penetrance for individual families is represented in a box plot. Kruskal–Wallis testing of the medians showed a significant difference across the five groups with a p value of 0.02, although p.A16V

Table 3 Estimate of penetrance in pancreatitis families

Mutation	First degree relatives of the index case	First degree relatives affected by pancreatitis	Estimated penetrance (if 50% of first degree relatives are carriers)
p.R122H	448	157 (35.0%)	70%
p.N29I	189	57 (30.2%)	60.4%
Neg All HP	210	66 (31.4%)	62.8%
Single generation (excluding p.A16V)	218	43 (19.7%)	39.4%
p.A16V (all groups)	62	14 (21.9%)	43.8%

A summary of the total number of first degree relatives of the index case in each mutation or phenotype group and the number that are affected by pancreatitis. Assuming that 50% of all first degree relatives are mutation carriers, the result can be doubled to give an estimate of penetrance in each group.

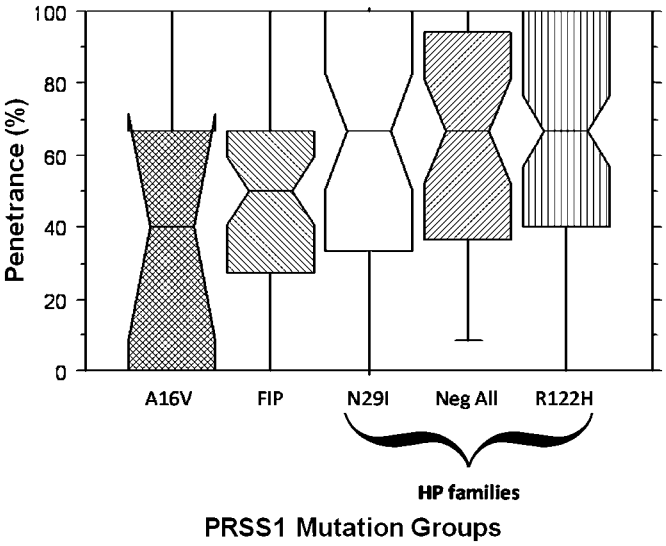


Figure 3 An estimate of penetrance in different mutation groups. Penetrance for individual families is estimated by doubling the proportion of affected first degree relatives of index cases. This assumes that 50% of first degree relatives of the index case are mutation carriers. Results of actual mutation analysis and knowledge of off kindred relationships were ignored in this calculation. The box plot shows the inter-quartile range (box) and the range (whiskers). The indented region indicates the 95% CI for the median (marked with a horizontal line). Kruskal–Wallis testing of the medians showed a significant difference across the five groups with a p value of 0.02. HP, hereditary pancreatitis.

was not significantly different from any of the other four groups if tested with the Mann–Whitney U test (p=0.06 to 0.8, going from right to left in figure 3).

DISCUSSION

p.A16V has mainly been described in idiopathic pancreatitis,[8 11 17] but this study shows that it can also have a phenotype consistent with both HP and a compound recessive disease. EUROPAC has traditionally recruited individuals with a family history indicating a genetic predisposition to pancreatitis, prior to any genetic testing. This approach did not prove adequate to characterise p.A16V. To produce this publication, active recruitment was required from Europe and elsewhere, to identify and recruit any individual with a p.A16V mutation. This in itself indicates the distinct nature of this particular *PRSS1* mutation. As stated previously, p.A16V was initially reported in children with idiopathic pancreatitis. The low rate of genetic testing in idiopathic pancreatitis makes the exact incidence in this group difficult to fully characterise. There is inherent age related bias as genetic testing is more likely to be indicated in younger patients. Overall, it is unlikely that clinical testing would be cost effective, even in the young.

The low number of p.A16V families, combined with the different referral pattern for this mutation, makes comparison of disease severity between p.A16V and the other mutations difficult. In previous papers, hierarchical analysis has been used to allow for family structure[2]; this is clearly not possible with the small number of p.A16V families. Relatively simplistic statistical approaches have therefore been adopted and this must be taken into account when considering the results. Nevertheless, the great variability in penetrance for this mutation is self evident. This is suggestive of a multi-gene effect, whereas there is no evidence against simple autosomal dominant aetiology with either p.R122H or p.N29I. Such multigene dependence may explain the trends seen for both later onset of pancreatitis and diabetes.

Mechanistically, p.R122H and p.N29I have both been linked to either increased auto-activation of cationic trypsinogen or reduced deactivation.[18 19] Phenotypically, p.R122H and p.N29I are very similar, although p.R122H results in a slightly earlier age of onset as described here and as reported previously.[2] p.A16V affects the very first amino acid of mature trypsinogen, lying at the edge of the signal peptide, although not forming a part of it. It has therefore previously been considered to influence secretion.[11]

It is tempting to assume that a secretion defect is inadequate to cause pancreatitis without some other factor (genetic or environmental), hence the difference in phenotype. However, secretion failure is considered to explain the link between the p.R116C mutation of *PRSS1* and pancreatitis and this mutation has thus far only been linked to autosomal dominant disease,[20] and furthermore the latest data indicate that the p.A16V mutant is actually secreted normally.[20] Other work has established that p.A16V increases the rate of chymotrypsinogen C (*CTRC*) activation of trypsinogen by approximately fourfold. This results in accelerated trypsinogen activation in vitro, possibly explaining the link with pancreatitis.[21] How this would explain the features of p.A16V penetrance is unclear.

Some families do appear to have a phenotype consistent with HP. This suggests relatively common polymorphisms in modifier genes within the populations that contribute to these families, although it is also possible that this is explained by a shared environment. One family has highly aggressive disease restricted to a single generation, suggesting a 'jackpot' combination of the p.A16V mutation from one side of the family and polymorphisms in modifier genes from the other. Relatively few families were identified with single cases of pancreatitis, but it is likely that a higher proportion of such families go unreported. Penetrance in such families may require specific environmental exposures.

These data suggest that the clinical significance of identifying a p.A16V mutation for the patient and their family is context dependent. A mutation in a family with a history of pancreatitis must be considered as an indication that other members of the family are at risk and that further testing within the family will identify individuals who will benefit from clinical vigilance. This may be refined by characterisation of the inheritance pattern and concentrating mutation screening on those most likely to be affected, siblings if all cases to date have been in a single generation or children if there is apparent autosomal dominant inheritance. In contrast, a mutation in an individual with apparently sporadic disease will tell us very little about the risk to other members of the family and at best this will be a partial explanation of the patient's condition.

There is obvious bias in our cohort of idiopathic patients, but even so only two out of 141 apparently idiopathic patients registered with EUROPAC had a p.A16V mutation, so testing of idiopathic patients will have little chance of identifying a mutation and will have only marginal benefit for the patient. At its most extreme, clinical vigilance may extend beyond pancreatitis and its immediate complications to concern about pancreatic cancer risk. It remains very difficult to quantify this risk. The best advice at present would be to consider individuals on the basis of their family history regardless of the p.A16V mutation status; if the family appears to have HP, the family should be considered as having the cancer risk associated with HP and if the pancreatitis appears to be sporadic, then the cancer risk should be considered equivalent to any other idiopathic case.

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The variable phenotype of the p.A16V mutation of cationic trypsinogen (*PRSS1*) in pancreatitis families

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