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Report

SPINK1 Is a Susceptibility Gene for Fibrocalculous Pancreatic Diabetes in Subjects from the Indian Subcontinent

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Fibrocalculous pancreatic diabetes (FCPD) is a secondary cause of diabetes due to chronic pancreatitis. Since the *N34S* variant of the *SPINK1* trypsin inhibitor gene has been found to partially account for genetic susceptibility to chronic pancreatitis, we used a family-based and case-control approach in two separate ethnic groups from the Indian subcontinent, to determine whether *N34S* was associated with susceptibility to FCPD. Clear excess transmission of *SPINK1 N34S* to the probands with FCPD in 69 Bangladeshi families was observed ($P < .0001$; 20 transmissions and 2 nontransmissions). In the total study group (Bangladeshi and southern Indian) the *N34S* variant was present in 33% of 180 subjects with FCPD, 4.4% of 861 nondiabetic subjects (odds ratio 10.8; $P < .0001$ compared with FCPD), 3.7% of 219 subjects with type 2 diabetes, and 10.6% of 354 subjects with early-onset diabetes (aged <30 years) ($P = .02$ compared with the ethnically matched control group). These results suggest that the *N34S* variant of *SPINK1* is a susceptibility gene for FCPD in the Indian subcontinent, although, by itself, it is not sufficient to cause disease.

Fibrocalculous pancreatic diabetes (FCPD) has been classified by the World Health Organization as a secondary cause of diabetes due to disease of the exocrine pancreas (Alberti and Zimmet 1998). It is a condition in which, in addition to diabetes being present, there is also evidence of chronic pancreatitis of unknown origin with large intraductal pancreatic stones (Mohan et al. 1998). Patients frequently have a low body mass and a history of chronic abdominal pain and require insulin treatment, although, unlike in type 1 diabetes (T1D), they are not prone to ketosis. We have hypothesized that FCPD is likely to be a multifactorial disease, with genetic and environmental components to both the diabetes and chronic pancreatitis. In support of a genetic background to FCPD, we have demonstrated familial clustering of the disease (Mohan et al. 1989). More specifically, we have demonstrated associations between FCPD and human leukocyte antigen (HLA) genotype in subjects from southern India and Bangladesh, which revealed both similarities to and differences from T1D (Kambo et al. 1989; Chowdhury et al. 2002). Other groups have also demonstrated HLA associations with FCPD, although the disease-associated alleles are not always consistent between studies (Sanjeevi et al. 1999). We have also found an association between the insulin gene hypervariable region and FCPD in southern Indian but not in Bangladeshi subjects (Kambo et al. 1989; Chowdhury et al. 2002).

Recently, there has been rapid progress in understanding the genetic basis of hereditary and idiopathic pancreatitis (Whitcomb 1999, 2000, 2002; Truninger et al. 2001) with the identification of mutations in the cationic trypsinogen gene (Whitcomb et al. 1996; Gorry et al. 1997; Teich et al. 2002), the cystic fibrosis transmembrane conductance regulator (CFTR) (Cohn et al. 1998; Sharer et al. 1998; Ockenga et al. 2000; Noone et al. 2001), and the serine protease inhibitor, Kazal type 1 (*SPINK1* [MIM

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Table 1

Frequency of the *SPINK1 N34S* Variant in the Various Study Groups

Group (*n*)	*SPINK1* Genotype Frequency (*n*)			*P* Value[a]
	Homozygote Wild-Type	Heterozygote *N34S*	Homozygote *N34S*	
Bangladeshi:				
Probands with FCPD (69)	.739 (51)	.217 (15)	.043 (3)	<.0001
Other FCPD (43)	.581 (25)	.279 (12)	.140 (6)	<.0001
Nondiabetic[b] (393)	.944 (371)	.053 (21)	.003 (1)	
Under-30 diabetes (354)	.893 (316)	.099 (35)	.008 (3)	.02
Sylheti Nondiabetic[c] (156)	.968 (151)	.032 (5)	0	
Sylheti T2D (142)	.965 (137)	.035 (5)	0	NS
South Indian:				
FCPD (68)	.647 (44)	.265 (18)	.088 (6)	<.0001
Nondiabetic (312)[b]	.965 (301)	.032 (10)	.003 (1)	
Impaired fasting glucose/IGT (56)	.964 (54)	.036 (2)	0	NS
T2D (77)	.961 (74)	.039 (3)	0	NS

[a] For comparison with nondiabetic group. NS = not significant.
[b] Defined by fasting blood glucose <6 mmol/liter.
[c] Defined by either a random or fasting blood glucose <6 mmol/liter.

167790]) genes (Chen et al. 2000; Pfützer et al. 2000, 2002; Witt et al. 2000; Threadgold et al. 2002), all associated with disease. We have already excluded common mutations of the cationic trypsinogen gene in FCPD (Rossi et al. 1998; Hassan et al. 2000). In contrast, recently published data have suggested that the *SPINK1 N34S* variant may be a cause of FCPD (Rossi et al. 2001; Chandak et al. 2002). The *N34S* variant was found in 7 of 24 patients with FCPD and in 16 of 44 patients with tropical pancreatitis without diabetes (TCP) living in India (Chandak et al. 2002). In a pilot study of Bangladeshi subjects, the *N34S* variant was present in five of eight patients with FCPD but was absent in four patients with TCP and in four control individuals (Rossi et al. 2001). More recent data analyzing a further 140 Bangladeshi subjects (14 patients with FCPD, 11 patients with TCP, 43 young patients with T2D, and 72 control individuals) support the pilot data for FCPD (Schneider et al., in press).

The purpose of the present study was multifold. First, we used a family-based study of subjects from Bangladesh to test for an association between the *SPINK1 N34S* variant and either FCPD or early-onset diabetes. Second, we tested for an interaction between *SPINK1* and either HLA-DQB genotype or the insulin-gene hypervariable region, in the family resources. Since we found an association between FCPD susceptibility and the *SPINK1 N34S* variant, we then proceeded to determine the frequency of this variant in two further ethnic groups from the Indian subcontinent, in order to replicate the original study and to investigate a possible association with type 2 diabetes (T2D). No patients are duplicated between this study and the other pilot data (Rossi et al. 1998; Schneider et al., in press).

We used a PCR-RFLP assay (endonuclease *Taa*I) to identify *SPINK1 N34S* (Plendl et al. 2001) in 69 families from Bangladesh, which were included in the study if they met the following two criteria: (1) an index case individual with FCPD was present and (2) both parents of the index case individual were available for study. Twenty-six percent of the probands with FCPD (mean ± SD age at onset 18.8 ± 4.7 years; mean ± SD BMI 15.9 ± 2.83 kg/m^2) possessed the variant. There was clear excess transmission of the variant from the parents to the index case individual in the trios ($P < .0001$); 20 transmissions and 2 nontransmissions. The multifactorial nature of FCPD is supported by the observation that, although parents carried the *SPINK1 N34S* variant, none had FCPD on the basis of clinical criteria.

In our previous study of the same families (Chowdhury et al. 2002), there was a significantly decreased transmission of HLA-DQB1*0202 from the parents to the index case individual. It could therefore be postulated that HLA-DQB1*0202 might protect a subject from FCPD in the presence of the disease-associated *N34S* variant. To address this question, we compared phenotype frequencies in the parents according to the presence or absence of *N34S* and DQB1*0202; no difference in distribution was found ($P = .86$). We have also observed increased transmission of the HLA marker TNFc and HLA-DQB1*0302 (Chowdhury et al. 2002) to the probands with FCPD. No gene-to-gene interaction in the index case individuals with FCPD was found between *SPINK1* and either of two major histocompatibility complex (MHC) markers on chromosome 6 (TNFc, $P = .70$; and HLA-DQB1*0302, $P = 1.0$). Furthermore, no interaction between *SPINK1* and alleles defined by *Hph*I of the insulin gene ($P = .68$) was found. This would suggest that, in an individual with FCPD, the known genetic factors predisposing to either chronic pancreatitis or diabetes are

acting independently of each other, rather than in a synergistic manner.

The frequencies of the *N34S* variant in the other study groups are presented in table 1. This variant was present in 41.9% of the additional unrelated Bangladeshi subjects with FCPD (mean age at onset of diabetes 20.4 ± 5.7 years; mean BMI 17.0 ± 3.33 kg/m^2), compared with 5.6% of control individuals (mean age 22.8 ± 4.8 years; mean BMI 20.0 ± 3.4 kg/m^2) ($P < .0001$ for genotype differences). In southern Indian subjects with FCPD (mean age at onset of diabetes 28.0 ± 7.9 years; mean BMI 19.4 ± 3.7 kg/m^2), the variant was present in 35.3% of patients, compared with 3.5% of control individuals (mean age 42.8 ± 12.7 years; mean BMI 22.0 ± 4.3 kg/m^2) ($P < .0001$), suggesting the *SPINK1 N34S* predisposes to FCPD in subjects from both southern India and Bangladesh. Five southern Indian families were also screened (three families had more than one member with FCPD, and two had one member with FCPD and at least one other with T2D). The variant was present in only one family. In this consanguineous family (fig. 1) the father had FCPD and the mother had idiopathic TCP; both were homozygous for the *N34S* variant, as were all six children (two with FCPD, one with TCP, one with impaired glucose tolerance [IGT], and two without diabetes).

To further investigate a possible association between *N34S* and diabetes, we investigated several additional resources (table 1). The first group ($n = 354$) consisted of Bangladeshi subjects presenting with diabetes before the age of 30 years (subsequently referred to as "under-30 diabetes" [mean age at onset 18.7 ± 6.2 years; mean BMI 18.3 ± 5.1 kg/m^2]) at the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) clinic, none of whom had either FCPD (normal abdominal x-ray and no history of severe abdominal pain) or T1D (defined by an acute onset of disease with ketosis or insulin dependency). The *N34S* variant was present in 10.9% of subjects in the under-30 diabetes group ($P = .02$ compared with the control group). The increased frequency of the variant in this group is likely to reflect the presence of subjects with diabetes and subclinical chronic pancreatitis. A different study design and further genetic and immunological investigations are required to investigate this further.

The second cohort we studied were Bangladeshi subjects from Sylhet, ascertained either from a diabetes clinic at the Royal London Hospital, London, or from a coronary heart disease study in East London. In subjects from Sylhet with T2D (mean age at onset of diabetes 44.7 ± 10.0 years; mean BMI 26.5 ± 3.4 kg/m^2), the frequency of *N34S* (3.5%) was no different than in ethnically matched control individuals (mean age 41.5 ± 10.4 years; mean BMI 26.9 ± 9.7 kg/m^2) ($P = 1$). Similarly,

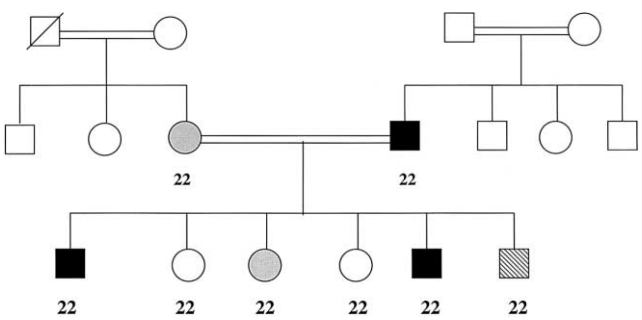

Figure 1 Southern Indian family in which each individual genotyped is homozygous for the *SPINK1* N34S variant. A symbol with a line through it denotes a deceased individual, an unshaded symbol denotes an unaffected individual, a black symbol denotes an individual with FCPD, a gray symbol denotes an individual with TCP, and a diagonally striped symbol denotes an individual with IGT. *SPINK1* genotypes are given under the symbols; 2 = variant. The wild-type allele was not detected in genotyped individuals from this family.

in the third cohort of southern Indian subjects (Ramachandran et al. 1992), the frequencies of either IGT (mean age 47.9 ± 12.6 years; mean BMI 23.4 ± 4.2 kg/m^2) (3.6%) or T2D (mean age 53.6 ± 10.7 years; BMI 23.8 ± 3.2 kg/m^2) (3.9%) was no different than that in control individuals (mean age 42.8 ± 12.7 years; mean BMI 23.4 ± 4.1 kg/m^2) (3.5%) ($P = .94$). It is, therefore, highly unlikely that an association exists between the *SPINK1 N34S* variant and the more common forms of T2D or IGT.

There is overwhelming evidence that the *SPINK1 N34S* variant predisposes to chronic pancreatitis, since it is present in 13%–40% of patients with idiopathic chronic pancreatitis (Truninger et al. 2001; Witt 2002). *SPINK1* is a pancreatic secretory trypsin inhibitor, secreted from the pancreatic acinar cells into the pancreatic juice, that prevents premature activation of zymogens within the pancreas and pancreatic duct. Functional studies on the *N34S* variant have not yet been published, but it is likely to be of functional significance because of its location near the reactive lysine-isoleucine site of *SPINK1* (Threadgold et al. 2002); furthermore, structural modeling has revealed several possible pathological mechanisms for the *N34S* mutation (Pfützer et al. 2000). In one of the earliest publications to indicate the importance of *SPINK1* in chronic pancreatitis, a clear excess transmission of *SPINK1* variants to affected subjects ($P < .0001$ in 29 informative transmissions), similar to what we have observed for FCPD (Witt et al. 2000), was found.

In many studies of non-Asian subjects, the frequency of *N34S* has been ⩽1% in control subjects (Chen et al. 2000; Pfützer et al. 2000; Witt et al. 2000; Threadgold et al. 2002). In people without FCPD from the Indian subcontinent, we have found the variant in 38 of 861

(4.4%; range 3.2%–5.6%) nondiabetic subjects, with no heterogeneity between ethnic groups ($P = .54$). Furthermore, a prevalence of 3% has been reported in a control group from Hyderabad, India (Chandak et al. 2002). Although the frequency appears higher than in non-Asian subjects, in a recently reported study in subjects from Leeds, United Kingdom, the frequency was 4% (4/100) (Threadgold et al. 2002). However, these control subjects were ascertained as healthy blood donors and the ethnic group was not reported, and it is known that Leeds has a large South Asian community. It therefore remains to be determined whether the frequency of *N34S* is higher in South Asians and whether this may, in part, account for the comparatively higher frequency of FCPD in this ethnic group.

In conclusion, we found a striking association, using family-based and case-control methods, between *N34S* and FCPD, with the variant being present in 33% of 180 subjects with FCPD, compared with 4.4% of 861 subjects without diabetes (odds ratio 10.8; 95% CI 6.9–17.0). These studies demonstrate that *SPINK1* is an important gene for FCPD susceptibility, although, by itself, it is not sufficient for full expression of the disease. This finding is very similar to that observed in idiopathic pancreatitis. Our data also emphasize the importance of other environmental and genetic factors that are required for the full expression of FCPD.

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Electronic-Database Information

The accession number and URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *SPINK1* [MIM 167790])

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