Observations

Absence of association between *SPINK1* trypsin inhibitor mutations and Type 1 or 2 diabetes mellitus in India and Germany

To the Editor: The serine protease inhibitor, Kazal type 1 (*SPINK1*), also known as pancreatic secretory trypsin inhibitor (*PSTI*), is a potent anti-protease that is thought to be a major inactivation factor of intra-pancreatic trypsin activity. The common N34S mutation of the *SPINK1* gene is strongly associated with idiopathic chronic pancreatitis [1, 2] and tropical pancreatitis [3, 4, 5, 6]. Recently, an increased frequency of the N34S mutation in Bangladeshi patients with an onset of diabetes mellitus before the age of 30 years was reported [4]. The patients in this study, although they were classified as having Type 2 diabetes, had a low BMI of around 18 kg/m^2. This could indicate that the study group was more heterogeneous in its aetiology of diabetes than initially anticipated. Interestingly, the investigators found no increase in the prevalence of *SPINK1* mutations in patients with adult-onset Type 2 diabetes from the same region. Other investigators [6] also reported that young Bangladeshi patients with Type 2 diabetes had an increased prevalence of the N34S mutation (odds ratio 11.9 vs control subjects). In both studies, the association of the N34S mutation with primary forms of diabetes was surprising because no pathophysiological explanation—as for the association between *SPINK1* mutations and pancreatitis—is readily available. We therefore proposed that an association between the N34S *SPINK1* mutation and diabetes mellitus might be accounted for by secondary diabetes due to sub-clinical or undiagnosed pancreatitis and we tested this hypothesis in a total of 631 patients with Type 1 and Type 2 diabetes from India and Germany.

We studied 85 north Indian patients with Type 1 diabetes attending the Diabetes clinic at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. The diagnosis was based on evidence of severe hyperglycaemia, ketosis (in 70%) and a requirement for continuous insulin therapy from the time of diagnosis. Patients had an age at diagnosis of 12.5±7.1 years (mean ± SD) and a BMI of 16.5±2.6 kg/m^2. From the same centre, we studied 58 patients with early-onset Type 2 diabetes (age at diagnosis <30 years, adequate glycaemic control for >2 years without insulin, absent GAD antibodies). These patients were 25.4±3.7 years of age at diagnosis and had a BMI of 24.0±5.0 kg/m^2. Forty-six of them (79%) had a family history of Type 2 diabetes. None of the patients with Type 1 or 2 diabetes gave a history of abdominal pain or steatorrhoea or changes in pancreatic morphology or calcification on abdominal ultrasound. Ninety-two healthy subjects from the same region served as the control subjects, whose data on N34S prevalence has been published [5]. To control for ethnic background and regional genetic variations, we used the same criteria to recruit patients in Germany. We recruited 438 children and adolescents (234 male, 204 female) with Type 1 diabetes (age at diabetes onset 11.2±4.6 years) at the Department of Paediatrics, Charité, Berlin. Diagnosis of Type 1 diabetes was based on the ADA classification criteria [7]. We also analysed 50 adult patients with Type 2 diabetes from the University Hospital Münster (age 62.4±9.7 years). No patient showed clinical signs of pancreatitis. The control group consisted of 480 healthy subjects from the Berlin area. Informed written consent was obtained from all subjects and the study was approved by the institutional ethics committees. The N34S mutation was detected by melting curve analysis using fluorescence resonance energy transfer probes, as described previously [1].

The prevalence of the N34S mutation in the various subgroups is shown in Table 1. Among patients from northern India with Type 1 diabetes, four (4.7%) carried an N34S mutation, a prevalence not statistically different from healthy control subjects (2.2%). All four patients were heterozygous for the N34S mutation and had clinical characteristics (age at onset 12.4±4.4 years, ketosis in three subjects) which were similar to those patients with wild-type *SPINK1*. We found GAD antibodies in two of the patients, confirming the autoimmune origin of diabetes. Among Indian patients with early onset Type 2 diabetes, two subjects (3.4%) carried the N34S mutation, a prevalence that was, again, not different from the control subjects. Similarly, the frequency of the N34S mutation did not differ among the German children with Type 1 diabetes (1.8%), the adults with Type 2 diabetes (2%) and the control subjects from Germany (1.3%). The age at diabetes onset was comparable between children with N34S mutation and those with wild type *SPINK1* (12.5±3.9 vs 11.2±4.6 years, p=0.53). In the only adult patient with Type 2 diabetes and an N34S mutation, diabetes was first diagnosed at age 54 years and was well controlled with glibenclamide and insulin (20 units/day).

On the Indian subcontinent, diabetes secondary to tropical pancreatitis (also known as fibro-calculous pancreatic diabetes, FCPD) is a common aetiology of the disease in adolescents and young adults [8, 9]. Such patients have a wide spectrum of beta-cell function and clinically have a variable requirement of insulin for glycaemic control [8, 9]. The N34S *SPINK1* mutation is strongly associated with FCPD [3, 4, 5, 6]. In contrast,

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Table 1. Prevalence of *N34S SPINK1* mutation in patients and control subjects

	India			Germany		
	Type 1 diabetes *n*=85	Type 2 diabetes *n*=58	Control subjects *n*=92	Type 1 diabetes *n*=438	Type 2 diabetes *n*=50	Control subjects *n*=480
N34S	4 (4.7)	2 (3.4)	2 (2.2)	8 (1.8)	1 (2.0)	6 (1.3)
N34S heterozygote	4 (4.7)	1 (1.7)	2 (2.2)	6 (1.4)	1 (2.0)	6 (1.3)
N34S homozygote	0	1 (1.7)	0	2 (0.4)	0	0

Figures in parentheses represent percentages

there is no suggested mechanism by which trypsin inhibitor gene mutations could lead to the primary forms of diabetes. In support of this, we found no association of the N34S mutation of the *SPINK1* gene, when compared to ethnically matched control subjects, in a large cohort of patients with Type 1 and Type 2 diabetes from both India and Germany. These results are in contrast to recent reports [4, 6] in young patients of Bangladeshi origin. A possible explanation for this discrepancy could be that there was some heterogeneity in the study populations from Bangladesh, with the possible inclusion of patients with FCPD in their cohorts. In contrast, pancreatitis was firmly ruled out as an underlying cause of diabetes in our patients with Type 1 and Type 2 diabetes.

In summary, our data indicate that *SPINK1* mutations do not represent a risk factor for Type 1 or Type 2 diabetes per se in either India or Germany.

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The diabetes prone BB rat model of IDDM shows duration of breastfeeding to influence Type 1 diabetes development later in life

To the Editor: Besides genetic background, environmental factors such as food ingredients and viral infections have been indicated as important and possibly decisive elements for the induction of insulin-dependent diabetes mellitus (IDDM). Our data show that diabetes development in diabetes-prone (DP)-BB rats can be delayed and prevented by prolonging the nursing period of these rats. These results provide experimental evidence strengthening human epidemiological data that the duration of exclusive breastfeeding influences diabetes development later in life.

Most human epidemiological data suggest that a short, as opposed to a long, duration of exclusive breastfeeding is related with an increased risk for IDDM development [1, 2, 3, 4, 5]. The introduction of cow's milk and wheat proteins at a young age has been suggested as potential diabetogenic risk factors for infants at risk of developing diabetes [1, 2, 3, 4, 5]. The importance of food ingredients in IDDM is further illustrated by the fact that the development of diabetes is reduced in DP-BB rats receiving a special diet in which hydrolysed casein is the sole source of protein [6]. Since, in epidemiological studies the period of nursing is established using questionnaires, the exact duration of breastfeeding remains unclear and duration of breastfeeding varies considerably between the siblings [1, 2, 3, 4, 5].

DP-BB rats normally spontaneously develop diabetes between 60 and 160 days of age and are used as an animal model for the study of Type 1 diabetes. In the DP-BB rat model for the study of diabetes, the period of suckling can be established precisely. To investigate whether a relation exists between the duration of exclusive breastfeeding and the onset of diabetes

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