The number of hospitalised infections in first year of life were also analysed for interaction with gender. This showed no difference between boys and girls (p=0.94).

To our knowledge this is the first study indicating a gender difference in the impact of neonatal infections on the risk of Type 1 diabetes in children. The results are not likely to be caused by recall bias since all information is based on register data collected prior to diabetes onset and less than 1% of the patients have missing information. The analysis is based only on hospital records and since boys are more often hospitalised in the first year of life there could be a selection bias towards higher registration rate of infections in boys. However, if we assume that this higher registration rate is the same in diabetic and non-diabetic cases, this should not influence the results.

The results might be explained by a gender difference in susceptibility to T cell-mediated autoimmune diabetes and gender difference in response to infections [5]. The observation of an interaction between gender and risk factors affecting the development of the immune regulatory system is of crucial relevance. Accordingly the effects of those risk factors should be taken into account in analyses where boys and girls are analysed together because of opposite effects in boys and girls. This result suggests that all studies on risk factors of autoimmune diabetes which possibly occur through an effect on the immune regulatory system have to be analysed separately for each gender or with interaction. This has implications for the evaluation of risk factors such as infections, vitamin D supplement and breastfeeding, especially when considering the impact of these risk factors in early childhood.

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References

1. Karvonen M, Pitkaniemi M, Pitkaniemi J, Kohtamaki K, Tajima N, Tuomilehto J (1997) Sex difference in the incidence of insulin-dependent diabetes mellitus: an analysis of the recent epidemiological data. World Health Organization DIAMOND Project Group. Diabetes Metab Rev 13:275–291
2. Szopa TM, Titchener PA, Portwood ND, Taylor KW (1994) Diabetes mellitus due to viruses – some recent developments see comments. COMMENT: Comment in: Diabetologia 37:222. Diabetologia 36:687–695
3. Morens DM, Pallansch MA (1995) Epidemiology. In: Rotbart H (ed.) Human enterovirus infections, pp 3–23
4. Aaby P, Samb B, Simondon F et al. (1994) Sex-specific differences in mortality after high-titre measles immunization in rural Senegal. Bull World Health Organ 72:761–770
5. Lisse IM, Aaby P, Whittle H, Jensen H, Engelmann M, Christensen LB (1997) T-lymphocyte subsets in West African children: impact of age, sex, and season. J Pediatr 130:77–85

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Does the −11377 promoter variant of *APM1* gene contribute to the genetic risk for Type 2 diabetes mellitus in Japanese families?

To the Editor: Although the physiopathological bases of Type 2 diabetes mellitus are well established, with an important role of generalized insulin resistance hitting key organs for glucose homeostasis – like muscle, liver and beta-cell – little is known about its molecular determinants and about the genetic factors involved in the transition between obesity and Type 2 diabetes. A considerable amount of evidence has suggested that a polymorphism in fat-expressed PPAR-γ, the major thiazolidinedione target, could contribute, even modestly, to the genetic risk for Type 2 diabetes [1]. In contrast, genome wide scans presented evidence of linkage between a region of chromosome 3q27, the metabolic syndrome and Type 2 diabetes in American and French Caucasian subjects [2], and also with coronary heart disease in Indo-Mauritians [3]. We recently replicated the linkage with diabetes in Japanese Type 2 diabetic families [4]. A strong positional candidate gene located on 3q27 is *APM1*, which encodes for the adipocyte complement related protein (Acrp30, also named adiponectin) which is specifically expressed in differentiated adipocytes. It was recently shown that its proteolytically-cleaved product (gAcrp30) lowers glucose concentrations through fatty acid oxidation without variations of insulin and glucagon concentrations, and prevents the development of high-fat diet induced obesity [5]. Furthermore, decreased expression of adiponectin correlates with insulin resistance in mouse models of obesity or lipoatrophy, and physiological doses of adiponectin decrease insulin resistance in these mice by lowering triglyceride content in muscle and liver [5]. In human, hypoadiponectinaemia was reported in obese and Type 2 diabetic subjects, and adiponectin concentrations are closely associated with insulin sensitivity in different ethnic groups [6]. We have recently shown that sequence variations in *APM1* gene are associated with circulating adiponectin concentrations and modulate the risk for insulin resistance and diabetes in French Caucasians [7]. A previous study has also suggested a large heritability for plasma adiponectin concentrations in humans [8].

In the Japanese population, we have done a case control study and reported an association between two single nucleotide polymorphisms (SNP) in the *APM1* gene and Type 2 diabetes [9]. To confirm such relevant associations in another Japanese population, we studied 359 diabetic Japanese subjects from the 164 sib-ships where indication of linkage at 3q27 was found [4]. One affected subject without obesity was randomly chosen from each family (average BMI: 22.7±2.8 and 23.2±3.6 kg/m2 for men and women, respectively), and 183 non-diabetic Japanese subjects was the control group (average BMI: 22.3±3.1 and 22.5±3.4 kg/m2 for men and women, respectively). The 11 most frequent polymorphisms of the *APM1*

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Table 1. Distribution of genotypes for the eleven common SNP of *APM1* and results of the association study with Type 2 diabetes

SNPs Position*	Status	Number of subjects	Genotype Distribution, *n* (frequency)			Allele	Chi 2 *p* value
			11	12	22		
−11426	Controls	183	98 (0.53)	72 (0.40)	13 (0.07)	1-A	0, 4
	Type 2 diabetes	164	88 (0.54)	67 (0.41)	6 (0.05)	2-G	*p*=0.81
−11377	Controls	183	86 (0.47)	87 (0.47)	10 (0.06)	1-C	12, 2
	Type 2 diabetes	164	105 (0.63)	48 (0.30)	11 (0.07)	2-G	*p*=0.002
−11156	Controls	176	95 (0.54)	70 (0.40)	11 (0.06)	1-InsCA	0, 23
	Type 2 diabetes	163	92 (0.57)	62 (0.38)	9 (0.05)	2-DelCA	*p*=0.89
−11043	Controls	180	173 (0.96)	7 (0.04)	0	1-C	2, 39
	Type 2 diabetes	164	162 (0.98)	2 (0.02)	0	2-T	*p*=0.17
−4041	Controls	182	160 (0.88)	22 (0.22)	0	1-A	1, 42
	Type 2 diabetes	164	140 (0.85)	23 (0.14)	1 (0.01)	2-C	*p*=0.53
−3971	Controls	181	162 (0.90)	19 (0.10)	0	1-A	2, 99
	Type 2 diabetes	164	140 (0.86)	22 (0.13)	2 (0.01)	2-G	*p*=0.22
+45	Controls	179	90 (0.50)	74 (0.41)	15 (0.09)	1-T	1, 39
	Type 2 diabetes	164	78 (0.48)	66 (0.40)	20 (0.12)	2-G	*p*=0.50
+276	Controls	177	79 (0.44)	80 (0.45)	18 (0.11)	1-G	4, 84
	Type 2 diabetes	164	87 (0.54)	55 (0.33)	22 (0.13)	2-T	*p*=0.08
+349	Controls	177	90 (0.51)	73 (0.41)	14 (0.08)	1-A	1, 31
	Type 2 diabetes	164	80 (0.48)	65 (0.40)	19 (0.12)	2-G	*p*=0.51
+712	Controls	179	28 (0.15)	88 (0.50)	63 (0.35)	1-G	0, 15
	Type 2 diabetes	164	28 (0.17)	78 (0.48)	58 (0.35)	2-A	*p*=0.92
+2019	Controls	178	27 (0.15)	89 (0.50)	62 (0.35)	1-Ins A	0, 94
	Type 2 diabetes	164	31 (0.19)	76 (0.46)	57 (0.35)	2-Del A	*p*=0.62

* Numbers indicate the location of the SNP relative to the A of the ATG / initiator Met of the *APM1* gene.
Genotype Distribution, *n*, numbers of individuals tested by genotype (frequencies are shown in parentheses)

gene were studied by direct sequencing and Light Cycler assay (Roche, Manheim, Germany). The genotype distribution of the SNP and insertion or deletion variants is included in Table 1. No significant deviation of the Hardy-Weinberg equilibrium was observed for any of the SNPs studied.

A significant association between the C/C genotype at position −11377 in the *APM1* promoter and Type 2 diabetes (p=0.002) was observed (Table 1). This promoter variant, under a recessive model of inheritance, increases the risk for Type 2 diabetes (OR=2, IC=1.30–3.08) in the population tested. A similar, although not significant, trend for association with Type 2 diabetes was reported in the previous Japanese case control study (p=0.25) [9]. A trend for association (p=0.08) was also found for the +276 G>T SNP in intron 2, which was shown to be associated with insulin sensitivity, adiponectin concentrations and Type 2 diabetes in the Japanese population [9]. The difference observed in the strength of the genetic association between the two populations can be explained by the fact that diabetic subjects of Hara's population were randomly recruited from patients attending the outpatient clinic, whereas in our study nuclear Japanese families with at least two Type 2 diabetic siblings were analyzed. However, clinical characteristics of these two populations seem to be comparable. In addition, we observed trends for association between Type 2 diabetes and the C>T SNP at −11043 in the promoter and A>G SNP at −3971 in intron 1 (Table 1).

We have calculated standardised linkage disequilibrium D' between each pair of SNPs using the PM+EH+ software. We observed a block of six closely associated variants showing D' values ranging from 0.9698 to 1.00 and comprising the −11377 promoter variant. Of note, this LD block includes four promoter and two nearby intron 1 variants, and that SNP +276 is not part of this group. The variant alleles of the other SNPs (−4041, −3971 and −11426) are very rare and not surprisingly fail to detect the same association as −11377 SNP.

An haplotype including two SNPs at positions −11391 and −11377, both located in the promoter sequence of *APM1*, was shown to be strongly associated with adiponectin concentrations and Type 2 diabetes in the French population [7]. Thus, we have tested if any haplotype prevalence might display a greater difference between cases and control subjects. No haplotype showed a better result than that obtained for the SNP at −11377 by itself. In the Japanese population, in contrast to French Caucasian subjects, the −11391 SNP is rather uncommon (frequency ≈0.02) [9], and we did not detect this variant in our study. Thus, even if −11391 SNP is a functional variant, because of its low frequency, it is unlikely to be involved in the genetic risk for Type 2 diabetes in Japan.

We also investigated the influence of the genotypes at the −11377 SNP on the modest linkage observed at the 3q27 locus in Japanese sib-pairs (maximum logarithm of odds score (MLS) =1.38, [4]). The pairs of sibs Identical by State (IBS) for genotypes 11, 12 and 22 were tested for linkage separately. Although the lod-score of pairs IBS for the 11 genotype reached a value of 2.0, it did not increase. Thus, we could not obtain evidence that the 11 genotype by itself contributes, even partially, to the observed linkage at the 3q27 locus.

Our results strengthen the contention that variations in the *APM1* gene contribute to the genetic risk for Type 2 diabetes in the Japanese population. As the C/G SNP at −11377 is frequent, we calculated an attributable risk of 31%, i.e. 1/3 of the genetic risk for diabetes in the Japanese subjects could be

related to this variant. All together, our data strongly support the hypothesis that adiponectin plays a pivotal role in the pathogenesis of Type 2 diabetes. Finally, adiponectin might be considered as a molecular link between the metabolic syndrome, obesity and Type 2 diabetes.

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References

1. Altshuler D, Hirschhorn JN, Klannemark M et al. (2000) The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 26:76–80
2. Vionnet N, Hani EH, Dupont S et al. (2000) Genomewide search for type 2 diabetes-susceptibility genes in french whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet 67:1470–1480
3. Francke S, Manraj M, Lacquemant C et al. (2001) A genome-wide scan for coronary heart disease suggests in Indo-Mauritians a susceptibility locus on chromosome 16p13 and replicates linkage with the metabolic syndrome on 3q27. Hum Mol Genet 10:2751–2765
4. Mori Y, Otabe S, Dina C et al. (2002) Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. Diabetes 51:1247–1255
5. Fruebis J, Tsao T, Javorschi S et al. (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 98:2005–2010
6. Weyer C, Funahashi T, Tanaka S et al. (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86:1930–1935
7. Froguel P, Dina C, Mori Y et al. (2002) A SNP in ACRP30/Adiponectin is associated with insulin resitance and type 2 diabetes. Diabetes 51 [Suppl 2]:A39 (Abstract)
8. Comuzzie AG, Funahashi T, Sonnenberg G et al. (2001) The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. J Clin Endocrinol Metab 86:4321–4325
9. Hara K, Boutin P, Mori Y et al. (2002) Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 51:536–540

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