

Reduced Pancreatic Volume in Hepatocyte Nuclear Factor 1A-Maturity-Onset Diabetes of the Young

Mette Vesterhus, Ingrid S. Haldorsen, Helge Ræder, Anders Molven, and Pål R. Njølstad

Departments of Pediatrics (M.V., H.R., P.R.N.), Radiology (I.S.H.), and Pathology (A.M.), Haukeland University Hospital, N-5021 Bergen, Norway; Department of Clinical Medicine (M.V., H.R., P.R.N.), Section for Radiology (I.S.H.), Department of Surgical Sciences, and The Gade Institute (A.M.), University of Bergen, N-5020 Bergen, Norway

Context: There are interplays between the endocrine and exocrine pancreas. We recently reported an increased frequency of exocrine dysfunction in *HNF1A*-maturity-onset diabetes of the young (MODY3) patients, compared with controls. Reduced pancreatic volume is seen in *HNF1B*-MODY (MODY5) and diabetes types 1 and 2.

Objective: The aim of this study was to investigate whether *HNF1A* mutation carriers have reduced pancreatic volume or abnormal pancreatic structure and whether any changes are associated with exocrine dysfunction.

Methods: Fifteen *HNF1A* mutation carriers recruited from the Norwegian MODY Registry, 31 subjects with type 1 diabetes, 10 subjects with type 2 diabetes, and 11 controls underwent computed tomography of the pancreas. We measured pancreatic volume and X-ray attenuation. Pancreatic volume index was defined as pancreatic volume divided by body surface area.

Results: Pancreatic volume index was reduced in subjects with *HNF1A*-MODY (34.5 ml/m²; $P < 0.02$) and type 1 diabetes (21.4 ml/m²; $P < 0.001$) as compared with nondiabetic controls (45.7 ml/m²), and was reduced in subjects with diabetes in combination with fecal elastase deficiency ($P = 0.03$). Subjects with type 1 diabetes had smaller pancreatic volume index, compared with *HNF1A* mutation carriers ($P < 0.001$). Reduced pancreatic volume index was associated with increasing duration of diabetes. Pancreatic X-ray attenuation in *HNF1A* mutation carriers was not significantly different from that of nondiabetic controls.

Conclusions: *HNF1A* mutation carriers have reduced pancreatic volume but less reduced than in patients with type 1 diabetes. Insulinopenia could explain both the pancreatic volume reduction and the associated pancreatic dysfunction. (*J Clin Endocrinol Metab* 93: 3505–3509, 2008)

There is an exocrine component in several forms of monogenic diabetes. In maturity-onset diabetes of the young (MODY) due to mutations in *HNF1B* (*HNF1B*-MODY, MODY5), pancreatic atrophy is a common feature (1, 2) and most likely caused by a developmental defect (3). Moreover, we have described pancreatic atrophy and lipomatosis in subjects with diabetes and pancreatic exocrine dysfunction caused by mutations in the carboxyl-ester lipase (*CEL*) gene (4, 5), suggesting that fatty replacement could reflect a process involved in pancreatic disease development in this MODY subtype. Interestingly, radiological

studies have reported reduced pancreatic volume in patients with type 1 diabetes and to some degree in patients with insulin-dependent type 2 diabetes (6–8). The pancreatic size reduction in type 1 and 2 diabetes may be secondary to a reduced insulinotropic effect on the acinar cells (9–11). A correlation between the pancreatic volume and pancreatic exocrine function measured by serum immunoreactive trypsin has also been reported (6).

We recently found that 13% of adult *HNF1A* mutation carriers have pancreatic exocrine dysfunction (12). *HNF1A* is closely related to *HNF1B*. Hence, morphological changes sim-

0021-972X/08/\$15.00/0

Printed in U.S.A.

Copyright © 2008 by The Endocrine Society

doi: 10.1210/jc.2008-0340 Received February 11, 2008. Accepted June 19, 2008.

First Published Online July 1, 2008

Abbreviations: BMI, Body mass index; *CEL*, carboxyl-ester lipase gene; CT, computed tomography; HU, Hounsfield Units; MODY, maturity-onset diabetes of the young.

ilar to those reported for HNF1B-MODY might be suspected. We therefore aimed to study pancreatic structure and its relation to exocrine function in HNF1A-MODY and to make a comparison with type 1 and type 2 diabetes subjects as well as normal controls.

Subjects and Methods

Subjects

We invited all adult HNF1A mutation carriers in the Norwegian MODY Registry and subjects with types 1 and 2 diabetes recruited consecutively from an outpatient diabetes clinic to test for pancreatic exocrine dysfunction. The clinical characteristics of the study subjects are described in Table 1. Subjects diagnosed with exocrine dysfunction, were compared with subjects of the same diabetes subtype but with normal exocrine function. There was no significant difference in age, age at examination, body mass index (BMI) or body surface area between the groups. All subjects were investigated with computed tomography (CT) of the pancreas by a standardized protocol. From a CT archive, we included controls without known diabetes or pancreatic disease that were matched for age, sex, and body surface area.

The study was approved by the Regional Committee for Research Ethics and the Norwegian Data Inspectorate and performed according to the Helsinki Declaration. We obtained written informed consent from all participants.

Measurement of pancreatic volume and x-ray attenuation

For the radiological study, helical CT (Light Speed Ultra; GE, Milwaukee, WI) of the pancreas (5 mm collimation, 5 mm reconstruction increment) was performed with and without contrast. Some examinations performed at local hospitals had slightly modified scan parameters. A radiologist (I.S.H.) traced the contour of the pancreas on contrast series on every slice to estimate the pancreatic volume as previously described (4) Pancreatic attenuation on contrast series in the encircled areas were used to calculate average x-ray attenuation measured in Hounsfield Units (HU; values equaling –1000 in air, –100 in fat, 0 in water, and +1000 in bone). To control for the influence of body build on pancreatic volume, the pancreatic volume index was calculated by dividing pancreatic volume by body surface area (6) as calculated by the formula of DuBois (13).

Definition of pancreatic exocrine dysfunction

Fecal elastase-1 was determined by ELISA using a kit specific for human elastase-1 (ScheBo-Tech GmbH, Wettenberg-Giessen, Germany). Pancre-

atic exocrine dysfunction was defined as fecal elastase deficiency, *i.e.* fecal elastase levels less than 200 $\mu\text{g/g}$ in two consecutive tests. Seventy-two-hour stool samples were collected as previously described (12), and fecal fat was quantified according to van de Kamer *et al.* (14), considering 7 g or less of fat per 24 h normal.

Statistics

Results for continuous variables are given as mean (SD). We used one-way ANOVA for global assessment and two-tailed, unpaired Student's *t* tests in the subgroup analyses for comparison of clinical characteristics and for comparison of pancreatic volume index and x-ray attenuation between HNF1A mutation carriers and each control group. We chose a significance level of 5% but 1% in the case of multiple testing. Associations between pancreatic volume index and attenuation, respectively, with age, BMI, duration of diabetes, fecal elastase level (continuous), and fecal elastase deficiency status (defined as a discrete variable: fecal elastase more or less than 200 $\mu\text{g/g}$), respectively, were computed by univariate or multivariate linear regression (supplementary data, published as supplemental data on The Endocrine Society's Journals Online Web site at <http://jcem.endojournals.org>). In the multiple regression analyses, age and the significant variables from the univariate analyses were included. All data were analyzed using Stata 8.0 (Stata Statistical Software, Stata Corp., College Station, TX).

Results

By CT, none of the patients with exocrine dysfunction displayed dilatation of the pancreatic duct, calcifications, or pseudocysts, which are frequently identified in chronic pancreatitis. Clinical characteristics are given in Table 1.

Pancreatic volume

The global assessment by ANOVA showed significant variance for the pancreatic volume index ($P < 0.00001$; $F = 18.98$, 3 *df*). Mean pancreatic volume index was reduced in HNF1A mutation carriers ($34.5 \text{ ml/m}^2 \pm 9.9$) as well as type 1 diabetes patients ($21.4 \text{ ml/m}^2 \pm 7.4$), compared with nondiabetic controls ($45.7 \text{ ml/m}^2 \pm 11.8$; $P = 0.007$ and < 0.001 , respectively), whereas for type 2 diabetes patients, no significant reduction in pancreatic volume index was seen ($36.9 \text{ ml/m}^2 \pm 13.2$; Fig. 1A). Type 1 diabetes subjects had smaller pancreatic volume index, compared with HNF1A mutation carriers ($P < 0.001$). Further-

TABLE 1. Characteristics of the study subjects

	MODY3	Nondiabetic controls	P^1	Type 1 diabetes	P^2	Type 2 diabetes	P^3
n	15	11		31		10	
Males, n (%)	7 (47)	7 (64)		24 (77)		8 (80)	
Age at examination (yr)	51 ± 13	51 ± 10	ns	52 ± 12	ns	60 ± 11	ns
Age at onset of diabetes (yr)	24 ± 9	NA		22 ± 13	ns	48 ± 10	< 0.001
Duration of diabetes (yr)	28 ± 13	NA		31 ± 15	ns	12 ± 6	< 0.001
BMI (kg/m^2)	25 ± 3	26 ± 4	ns	26 ± 3	ns	27 ± 2	ns
Body surface area (m^2)	1.9 ± 0.1	1.9 ± 0.3	ns	2.0 ± 0.2	ns	2.0 ± 0.1	ns
Fecal elastase deficiency, n (%)	5 (33)	NI		15 (48)		5 (50)	
Fecal fat excretion (g/d) (< 7) ^a	14 \pm 7	NI		12 \pm 5	ns	9 \pm 6	ns
Fecal fat excretion greater than 7 g/d, n	6 of 6	NI		14 of 17		1 of 3	

Data are means \pm SD. The P values represent two-sample Student's *t* tests comparing MODY3 and P^1 (nondiabetic controls), P^2 (type 1 diabetes subjects), and P^3 (type 2 diabetes subjects), respectively. NA, Not applicable; NI, not investigated; ns, not significant.

^a Fecal fat excretion was measured in six MODY3, 17 type 1 diabetes, and three type 2 diabetes subjects.

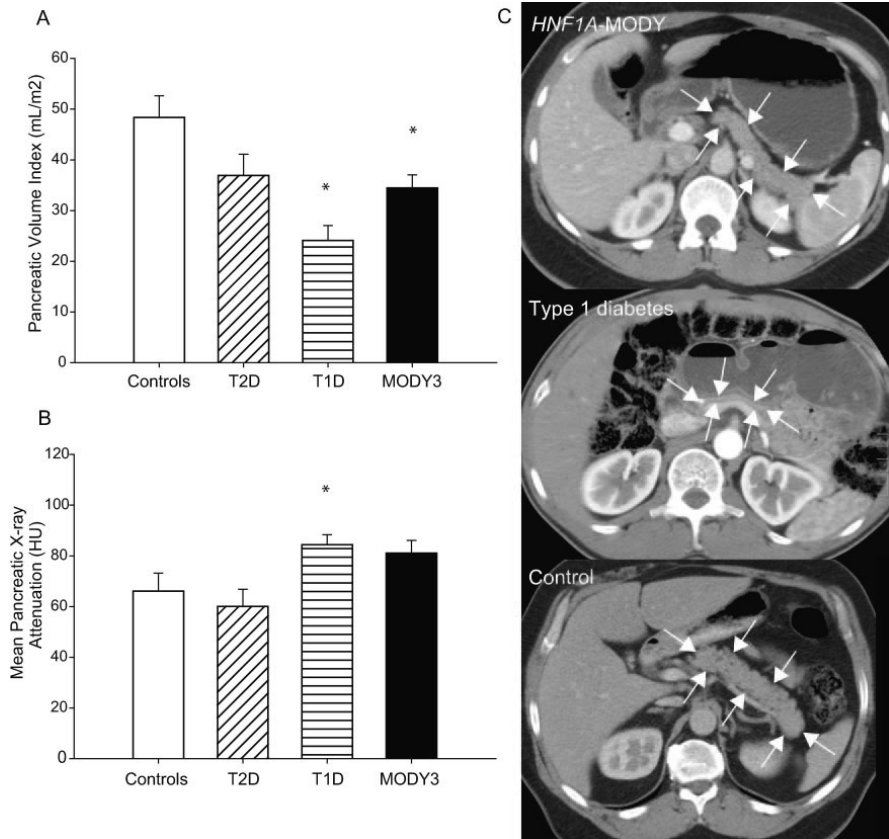


FIG. 1. Pancreatic volume and x-ray attenuation. Helical CT was performed as described in *Subjects and Methods*. The pancreatic volume index was calculated by dividing pancreatic volume by body surface area to control for the influence of body build on pancreatic volume. *, Statistical difference ($P < 0.05$). A, Pancreatic volume index was reduced in *HNF1A* mutation carriers, compared with nondiabetic controls ($P = 0.02$), whereas type 1 diabetes (T1D) subjects had reduced pancreatic volume index compared with *HNF1A* mutation carriers, type 2 diabetes (T2D) patients, and nondiabetic controls (all $P < 0.001$). B, For *HNF1A* mutation carriers, the mean pancreatic attenuation did not differ from nondiabetic controls or type 1 diabetes patients. The mean pancreatic attenuation was increased in type 1 diabetes patients, compared with nondiabetic controls ($P = 0.04$). C, CT at the level of the pancreatic body and tail in an *HNF1A* mutation carrier, a patient with type 1 diabetes and fecal elastase deficiency, and a nondiabetic control. The pancreas of the type 1 diabetes subject demonstrates reduced volume and increased attenuation. No striking differences are apparent between the *HNF1A* mutation carrier and the control subjects. When analyzed at the group level, *HNF1A* mutation carriers have significantly smaller pancreatic volume and a trend toward higher mean x-ray attenuation than nondiabetic subjects. The arrows mark the position of the pancreas.

more, diabetes duration was inversely associated with pancreatic volume index in all diabetes patients [$P = 0.001$, $\beta = -0.37$ ($-0.58, -0.17$), $R^2 = 0.2$], and this was also found in a subgroup analysis of *HNF1A* mutation carriers [$P = 0.004$, $\beta = -0.53$ ($-0.86, -0.20$), $R^2 = 0.48$], whereas there was no association between pancreatic volume index and current age or BMI. Pancreatic volume index was associated with fecal elastase deficiency in subjects with diabetes of any type [$P = 0.03$; $\beta = -6.86$ ($-13.0, -0.72$), $R^2 = 0.09$]; however, no such association was observed within any diabetes subgroup. In the multivariate analysis, pancreatic volume index was significantly higher in *HNF1A* mutation carriers than in type 1 diabetes subjects after adjusting for age, diabetes duration, and fecal elastase deficiency [$P < 0.001$, $\beta = -11.86$ ($-16.91, -6.81$), $R^2 = 0.49$].

Pancreatic x-ray attenuation

The global assessment by ANOVA showed significant variance for the mean pancreatic x-ray attenuation ($P = 0.007$; $F = 4.37$, 3 *df*). The mean pancreatic x-ray attenuation in *HNF1A* mutation carriers (81.1 ± 19.4 HU) was not statistically different from nondiabetic controls (66.0 ± 23.6 HU, $P = 0.09$; Fig. 1, B and C). Interestingly, the attenuation was significantly increased in type 1 diabetes patients (84.4 ± 21.9 HU), compared with nondiabetic controls ($P = 0.02$), whereas subjects with type 2 diabetes had attenuation similar to nondiabetic controls (60.1 ± 21.4 HU). Fecal elastase deficiency was not associated with pancreatic attenuation. Pancreatic attenuation was significantly and inversely associated with age [$P = 0.04$, $\beta = -0.50$ ($-0.97, 0.03$), $R^2 = 0.06$] and BMI [$P < 0.001$, $\beta = -3.27$ ($-5.20, -1.19$), $R^2 = 0.20$] in the uni-

variate linear regression analyses. Adjusting for age and BMI in a multivariate regression analysis only slightly modified the association of pancreatic attenuation with diabetes status in the comparison of nondiabetic controls and *HNF1A* mutation carriers [$P = 0.11$, $\beta = 12.99$ ($-2.94, 28.93$), $R^2 = 0.41$] or type 1 diabetes [$P = 0.009$, $\beta = 18.42$ ($4.84, 31.99$), $R^2 = 0.45$], respectively.

Discussion

To our knowledge, this is the first study on pancreatic size and structure in *HNF1A*-MODY. We found that *HNF1A* as well as type 1 diabetes patients had significantly reduced pancreatic volume index, compared with nondiabetic controls. Our results for types 1 and 2 diabetes are consistent with previous reports (6, 15, 16). Subjects with type 1 diabetes had smaller pancreatic volume index than *HNF1A* mutation carriers, and this reduction remained significant after adjusting for age, diabetes duration, and fecal elastase deficiency. Reduced pancreatic volume index was associated with diabetes duration in agreement with some (8) but not all (6, 7) previous studies. Reduced insulinotropic effects on the acinar cells is a possible mechanism for the reduction in pancreatic volume (10, 11), and reports show association of small pancreatic volume with insulin use (8) or reduced insulin secretion (7). This is in line with our data because type 1 diabetes patients generally have the most pronounced endogenous insulinopenia.

Subjects with fecal elastase deficiency had a significant reduction in pancreatic volume index, in accordance with a recent report showing an association between pancreatic volume index and the exocrine pancreatic function marker serum immunoreactive trypsin (6). Both the reduced pancreatic volume and reduced pancreatic exocrine function observed in our patients may be due to local paracrine effects of insulinopenia (10). The more severe insulin deficiency observed in type 1 diabetes probably explains the greater pancreatic volume reduction compared with *HNF1A*-MODY. We cannot, however, rule out the possibility that primary exocrine disease is involved in the pancreatic volume reduction.

A recent study reported higher pancreatic fat content in type 2 diabetic compared with nondiabetic men and a negative correlation of pancreatic fat with β -cell function (17), whereas others found no increase in pancreatic fat in type 2 diabetes (16). Interestingly, we experienced that pancreatic x-ray attenuation adjusted for age and BMI was significantly increased in subjects with type 1 diabetes and observed the same tendency in *HNF1A* mutation carriers. In contrast, type 2 diabetes patients had pancreatic x-ray attenuation similar to controls. We further found that pancreatic x-ray attenuation decreased with increasing BMI, confirming previous findings of strong correlation between BMI and fat content in the pancreas (16, 18). The observed differences between diabetes subtypes may be due to differences in insulin secretion, which has been reported to be directly associated with pancreatic fat content (18).

Pancreatic lipomatosis is an early structural marker of pancreatic exocrine disease in *CEL* mutation carriers preceding

development of diabetes (5). Fatty replacement of the pancreas has also been observed in cystic fibrosis and Johansson-Blizzard syndrome, two monogenic conditions with primary affection of the exocrine pancreas and frequent development of secondary diabetes (19, 20). We did not find any association of pancreatic x-ray attenuation with fecal elastase in *HNF1A* mutation carriers. This suggests a different pathophysiological mechanism for the development of pancreatic changes in *HNF1A*-MODY.

In conclusion, the pancreatic volume index was reduced in *HNF1A*-MODY, compared with nondiabetic controls, but less than in type 1 diabetes. A striking pancreatic atrophy such as in *HNF1B* MODY was not observed. Although *HNF1A* and *HNF1B* are closely related and interact in the same transcriptional network, *HNF1A* clearly has less pronounced effect on pancreas volume and structure than *HNF1B* (1, 2, 21). Our data support the notion that insulinopenia is the main factor determining the reduction in pancreatic volume in *HNF1A*-MODY as well as in type 1 diabetes, whereas in *HNF1B*-MODY a developmental effect is likely to be involved. Insulinopenia probably also explains the pancreatic exocrine dysfunction associated with pancreatic volume reduction, but further studies, particularly of nondiabetic *HNF1A* mutation carriers, would be helpful to evaluate a possible primary role of the exocrine pancreas.

Acknowledgments

Address all correspondence and requests for reprints to: Professor Pål Rasmus Njolstad, M.D., Ph.D., Department of Pediatrics, Haukeland University Hospital, N-5021 Bergen, Norway. E-mail: pal.njolstad@uib.no.

This work was supported in part by grants from Haukeland University Hospital, Innovest, University of Bergen, Translational Research Grant Consortium, and the Norwegian Research Council (FUGE Program).

Disclosure Statement: M.V., I.S.H., H.R., A.M., and P.R.N. have nothing to declare.

References

- Bellanne-Chantelot C, Chauveau D, Gautier JF, Dubois-Laforgue D, Clauin S, Beaufils S, Wilhelm JM, Boitard C, Noel LH, Velho G, Timsit J 2004 Clinical spectrum associated with hepatocyte nuclear factor-1 β mutations. *Ann Intern Med* 140:510–517
- Edghill EL, Bingham C, Ellard S, Hattersley AT 2006 Mutations in hepatocyte nuclear factor-1 β and their related phenotypes. *J Med Genet* 43:84–90
- Haumaitre C, Barbacci E, Jenny M, Ott MO, Gradwohl G, Cereghini S 2005 Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. *Proc Natl Acad Sci USA* 102:1490–1495
- Raeder H, Johansson S, Holm PI, Haldorsen IS, Mas E, Sbarra V, Nermoen I, Eide SA, Grevle L, Bjorkhaug L, Sagen JV, Aksnes L, Sovik O, Lombardo D, Molven A, Njolstad PR 2006 Mutations in the *CEL* VNTR cause a syndrome of diabetes and pancreatic exocrine dysfunction. *Nat Genet* 38:54–62
- Raeder H, Haldorsen IS, Erslund L, Gruner R, Taxt T, Sovik O, Molven A, Njolstad PR 2007 Pancreatic lipomatosis is a structural marker in nondiabetic children with mutations in carboxyl-ester lipase. *Diabetes* 56:444–449
- Goda K, Sasaki E, Nagata K, Fukui M, Ohsawa N, Hahafusa T 2001 Pancreatic volume in type 1 and type 2 diabetes mellitus. *Acta Diabetol* 38:145–149
- Alzaid A, Aideyan O, Nawaz S 1993 The size of the pancreas in diabetes mellitus. *Diabet Med* 10:759–763

8. Gilbeau JP, Poncelet V, Libon E, Derue G, Heller FR 1992 The density, contour, and thickness of the pancreas in diabetics: CT findings in 57 patients. *AJR Am J Roentgenol* 159:527–531
9. Henderson JR 1969 Why are the islets of Langerhans? *Lancet* 2:469–470
10. Williams JA, Goldfine ID 1985 The insulin-pancreatic acinar axis. *Diabetes* 34:980–986
11. Korc M, Owerbach D, Quinto C, Rutter WJ 1981 Pancreatic islet-acinar cell interaction: amylase messenger RNA levels are determined by insulin. *Science* 213:351–353
12. Vesterhus M, Ræder H, Johansson S, Molven A, Njølstad PR 2008 Pancreatic exocrine dysfunction in maturity-onset diabetes of the young type 3. *Diabetes Care* 31:306–310
13. DuBois D, DuBois EF 1916 A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med* 17:863–871
14. van de Kamer JH, ten Bokkel Huinink H, Weyers HA 1949 Rapid method for the determination of fat in feces. *J Biol Chem* 177:347–355
15. Williams AJ, Chau W, Callaway MP, Dayan CM 2007 Magnetic resonance imaging: a reliable method for measuring pancreatic volume in type 1 diabetes. *Diabet Med* 24:35–40
16. Saisho Y, Butler AE, Meier JJ, Monchamp T, Allen-Auerbach M, Rizza RA, Butler PC 2007 Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. *Clin Anat* 20:933–942
17. Tushuizen ME, Bunck MC, Pouwels PJ, Bontemps S, van Waesberghe JH, Schindhelm RK, Mari A, Heine RJ, Diamant M 2007 Pancreatic fat content and β -cell function in men with and without type 2 diabetes. *Diabetes Care* 30:2916–2921
18. Kovanlikaya A, Mittelman SD, Ward A, Geffner ME, Dorey F, Gilsanz V 2005 Obesity and fat quantification in lean tissues using three-point Dixon MR imaging. *Pediatr Radiol* 35:601–607
19. Zenker M, Mayerle J, Lerch MM, Tagariello A, Zerres K, Durie PR, Beier M, Hulskamp G, Guzman C, Rehder H, Beemer FA, Hamel B, Vanlieferinghen P, Gershoni-Baruch R, Vieira MW, Dumic M, Auslender R, Gil-da-Silva-Lopes VL, Steinlicht S, Rauh M, Shalev SA, Thiel C, Winterpacht A, Kwon YT, Varshavsky A, Reis A 2005 Deficiency of UBR1, a ubiquitin ligase of the N-end rule pathway, causes pancreatic dysfunction, malformations and mental retardation (Johanson-Blizzard syndrome). *Nat Genet* 37:1345–1350
20. Sodhi KS, Thapa BR, Khandelwal S, Suri S 2005 Pancreatic lipomatosis in an infant with cystic fibrosis. *Pediatr Radiol* 35:1157–1158
21. Haldorsen IS, Vesterhus M, Ræder H, Jensen DK, Søvik O, Molven A, Njølstad PR 2007 Lack of pancreatic body and tail in *HNFI1B* mutation carriers. *Diabet Med*, in press