

part of gestation.^{4,5} We recommend that AT1-receptor antagonists be avoided throughout pregnancy.

Jelena Martinovic, Alexandra Benachi,
Nicole Laurent, Farida Daïkha-Dahmane,
*Marie Claire Gubler

Departments of Histoembryology and Obstetrics, and *Institut National de la Santé et de la Recherche Médicale, Unité 423, Hospital Necker Enfants Malades, Université René, Descartes, 75743 Paris, France; Departments of Pathology and of Obstetrics, Hospital Bocage, Dijon; Department of Histoembryology, Hospital Robert Debré, Paris; and Department of Biochemistry, Hôpital Ambroise Paré, Paris

- 1 Saji H, Yamanaka M, Hagiwara A, Ijiri R. Losartan and fetal toxic effects. *Lancet* 2001; **357**: 363.
- 2 Hinchliffe SA, Sargent PH, Howard CV, Chan YF, Hutton JL, Van Velzen D. "Medullary ray glomerular counting" as a method of assessment of human nephrogenesis. *Pathol Res Pract* 1992; **188**: 775-82.
- 3 Pryde PG, Sedman AB, Nugent CE, Barr M. Angiotensin-converting enzyme inhibitor fetopathy. *J Am Soc Nephrol* 1993; **3**: 1575-82.
- 4 Spence SG, Allen HL, Cukierski MA, Manson JM, Robertson RT, Eydeloth RS. Defining the susceptible period of developmental toxicity for the AT1-selective angiotensin II receptor antagonist losartan in rats. *Teratology* 1995; **51**: 367-82.
- 5 Sorensen AM, Christensen S, Jonassen TE, Andersen D, Petersen JS. Teratogenic effects of ACE inhibitors and angiotensin II receptor antagonists. *Ugeskr Laeger* 1998; **160**: 1460-64.

Testicular cancer and syndrome X

Sir—Jourik A Gietema and colleagues (Jan 20, p 228)¹ report an interesting finding that long-term survivors of metastatic testicular cancer can develop an unfavourable profile of cardiovascular risk factors resembling syndrome X. They conclude that administration of cisplatin-based combination chemotherapy as well as subclinical hypogonadism seem to be important factors in pathogenesis.

Plasma concentrations of testosterone in the chemotherapy group were significantly lower than those in the stage 1 group. Moreover, in their chemotherapy-treated patients, insulin concentrations and insulin-to-glucose ratios correlated negatively with serum testosterone concentrations.

We agree that decreased circulating testosterone can cause insulin resistance. We have reported a small series in which we showed that decreased concentrations of testosterone after castration contributed to insulin resistance. We analysed events in six patients whose diabetic control was notably worsened by orchidectomy or administration of a gonadotropin-releasing hormone analogue to treat advanced prostate cancer, used in

addition to antiandrogenic drugs. Plasma concentrations of testosterone became undetectable in all patients, and all but one patient had increased insulin secretion. HbA_{1c} before and after castration was 6.3% (SD 0.6) and 10.2% (1.7), respectively.

Bilateral tumours are rare at diagnosis, but in 2% of patients with testicular germ-cell tumour, a meta-chronous new primary tumour will develop in the remaining testis. Even patients with testicular cancer in stage 1, whose concentrations of testosterone would decrease after bilateral orchidectomy, might result in insulin resistance.

The effect of cisplatin in the pathogenesis of insulin resistance remains to be elucidated, although plasma concentrations of testosterone in Gietema and colleagues' chemotherapy group were lower than in the stage 1 group. This difference probably reflects direct damage to the testis by cisplatin, which could be investigated in non-testicular cancer patients receiving cisplatin, such as those with lung cancer. Other effects of cancer upon development of insulin resistance, such as induction of various cytokines including interleukin 6, tumour necrosis factor α , and counter-regulatory hormone, might also be important. However, hypogonadism should not be overlooked as an important cause of insulin resistance in survivors of testicular cancer.

Michiaki Fukui

Department of Endocrinology and Haematology, Osaka General Hospital of West Japan Railway Company, 1-2-22 Matsuzaki-cho, Abeno-ku, Osaka 545-0053, Japan

- 1 Gietema JA, Meinardi MT, van der Graaf WTA, Sleijfer DT. Syndrome X in testicular cancer survivors. *Lancet* 2001; **357**: 228-29.

CD36 deficiency and insulin resistance

Sir—Koji Miyaoka and colleagues (March 3, p 686)¹ report phenotypic data on carbohydrate metabolism from their cohort of 26 Japanese patients with genetic mutations causing CD36 deficiency.

As Timothy Aitman points out in his March 3 commentary,² the question of whether or not insulin resistance is present in these patients is important, since data from one rodent model, the spontaneously hypertensive rat (SHR), has implicated a causal role for deficiency of the rodent homologue (*Cd36*) in impairment of insulin action.³ However, some uncertainty remains. We have reported that *Cd36* sequence

and expression were normal in another rodent model of hypertension with insulin resistance (the stroke-prone spontaneously hypertensive rat, SHRSP), which suggests the existence of other gene mutations leading to a similar phenotype.⁴

Unfortunately, Miyaoka and colleagues' data from patients with CD36 deficiency are inconclusive. First, few details are given of the matching procedures used and, in particular, whether participants with CD36 deficiency were individually matched with controls. The 26 cases were on average 4 years older than controls: since age is an important determinant of insulin sensitivity⁵ and serum lipids, inappropriate matching could have contributed to the apparent differences between the groups. Conversely, differences between the sexes (42% of cases *vs* 29% of controls were female) might have diminished the potential of the study to find differences in these variables.

Second, only five of 26 CD36-deficient participants underwent measurement of fasting insulin, oral glucose tolerance test, and hyperinsulinaemic clamp study. Fasting plasma insulin concentrations were all within the reference range derived from normal controls. As expected from a study with a small number of participants with a wide distribution of weight (body-mass index 17-33 kg/m²), the individual insulin profiles across the glucose tolerance tests were heterogeneous; results were equally compatible with a defect in insulin secretion as with the presence of insulin resistance.

Finally, the limited data reported from the clamp studies is difficult to compare with other published data without some indication of the rate of insulin infusion used. The argument seems to rest on the observation that all five cases individually had lower values for whole-body glucose uptake than the mean value for nine controls. Although this finding is in keeping with the hypothesis that insulin resistance was present, the separation between means (mean 5.1 [SD 1.59, five cases] *vs* 8.6 mg kg⁻¹ min⁻¹ [0.50 nine controls]) was not clear and may be too small to allow abandonment of the normal statistical requirement for formal rejection of the null hypothesis.

We agree with Miyaoka and co-workers that a case-control study with a larger number of people with CD36 deficiency is necessary. However, until such a study is done, putative direct or indirect effects of

CD36 deficiency on carbohydrate metabolism in human beings, although biologically plausibly and mechanistically appealing, must remain uncertain.

*John R Petrie, Mary Collison,
John M Connell, Gwyn W Gould,
Anna F Dominiczak

*Department of Medicine, Glasgow Royal Infirmary, Glasgow G31 2ER, UK; and MRC Cooperative Group on Cellular Signalling and Molecular Genetics in Metabolic and Cardiovascular Syndromes, University of Glasgow, Glasgow
(e-mail: jrp1s@clinmed.gla.ac.uk)

- Miyaoka K, Kuwasako T, Hirano K-I, Nozaki S, Yamashita S, Matsuzawa Y. CD36 deficiency associated with insulin resistance. *Lancet* 2001; **357**: 686–87.
- Aitman TJ. CD36, insulin resistance, and coronary heart disease. *Lancet* 2001; **357**: 651–52.
- Aitman TJ, Glazier AM, Wallace CA, et al. Identification of *Cd36* (*Fat*) as an insulin resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet* 1999; **21**: 76–83.
- Collison M, Glazier AM, Graham et al. *Cd36* and molecular mechanisms of insulin resistance in the stroke-prone spontaneously-hypertensive rat. *Diabetes* 2000; **49**: 2222–26.
- Ferrannini E, Natali A, Capaldo B, et al. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity: European Group for the Study of Insulin Resistance (EGIR). *Hypertension* 1997; **30**: 1144–49.

Sir—Koji Miyaoka and colleagues¹ and Timothy Aitman² argue for the possible association between CD36 and syndromes of insulin resistance based on findings in human beings and rodents with CD36 deficiency. We have several comments.

We have previously shown in the SHR model that there are two separate SHR strains: the original SHR strains with normal *Cd36* (rodent homologue of human CD36) and SHR with mutant *Cd36* caused by a spontaneous de novo mutation.³ The comparison of metabolic phenotypes observed in the *Cd36*-null SHR,³ as well as those reported in the *Cd36*-null mice⁴ and in human beings with CD36 deficiency^{1,5} is shown in the table. In the inbred rodent models,

increased non-esterified fatty acid concentrations and the reciprocally decreased glucose concentrations seem to be a common feature. However, the other phenotypes are virtually inconsistent between the four groups, which might be partly explained by the differences in the metabolic pathways among the species and in the genetic and environmental backgrounds between individuals.

With the relatively unequal distribution of the clinical phenotypes among the patients described by Miyaoka and colleagues,¹ as exemplified by their wide range of blood pressures, it seems difficult to ascribe any particular phenotype to the effects of CD36 deficiency but not to any other possible confounding factors. In this context, many Japanese patients with CD36 deficiency have been selected from groups who underwent cardiac scintigraphy on suspicion of the presence of coronary heart diseases or cardiomyopathy. If this is the case in Miyaoka and colleagues' patients, their data need to be interpreted cautiously because they must be affected by a substantial population stratification bias.

In view of the high prevalence of hypertension (>50%), hyperlipidaemia (>50%), and hyperglycaemia (>30%) among the general Japanese population in their 60s, according to the latest National Nutrition Survey of Japan, it may not be so surprising that 46% of Miyaoka and colleagues' patients (mean age 64 years) had at least two coronary risk factors.

Striking to us was their observation that all five patients who underwent glucose clamp studies showed decreases in the mean whole-body glucose uptake that should best reflect insulin sensitivity in skeletal muscles. Increase in blood non-esterified fatty acid concentrations and the resulting increase in the content of triglycerides in muscles cause insulin resistance in vivo. This effect, however, would not be the case in the deficiency of CD36, because the muscle triglyceride contents were not increased but were rather decreased, at least in the rodent

models, probably because of the impaired fatty acid uptake via CD36 in muscles. The metabolic sequel of this effect, which would possibly be the increased fatty acid uptake in the liver, remains unclear in the patients with CD36 deficiency and must be investigated carefully.

Yoko Iizuka, *Takanari Gotoda,
Shun Ishibashi, Nobuhiro Yamada

Department of Metabolic Diseases, University of Tokyo, Tokyo; *Diabetes Center, Tokyo Women's Medical University, Tokyo 162-8666, Japan; and Department of Internal Medicine, University of Tsukuba, Tsukuba
(e-mail: tkngtd-ky@umin.ac.jp)

- Miyaoka K, Kuwasako T, Hirano K-I, Nozaki S, Yamashita S, Matsuzawa Y. CD36 deficiency associated with insulin resistance. *Lancet* 2001; **357**: 686–87.
- Aitman TJ. CD36, insulin resistance, and coronary heart disease. *Lancet* 2001; **357**: 651–52.
- Gotoda T, Iizuka Y, Kato N, et al. Absence of *Cd36* mutation in the original spontaneously hypertensive rats with insulin resistance. *Nat Genet* 1999; **22**: 226–28.
- Febbraio M, Abumrad NA, Hajjar DP, et al. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem* 1999; **274**: 19055–62.
- Yanai H, Chiba H, Morimoto M, Jamieson GA, Matsuno K. Type I CD36 deficiency in humans is not associated with insulin resistance syndrome. *Thromb Haemost* 2000; **83**: 786.

Sir—Koji Miyaoka and colleagues' conclusions¹ are not supported by our published^{2,3} or unpublished results.

In our original study,² four healthy young volunteers with CD36 deficiency (aged 21 years [SD 0.5]) were tested by oral glucose loading and had normal insulin responses and normal fasting glucose and lipid concentrations. The CD36-deficient patients identified by Miyaoka and colleagues were elderly (mean 64 years), and 12 (46%) of 26 had at least two coronary risk factors, including impaired glucose metabolism and hyperlipidaemia. We question whether their 26 patients were sampled without bias. If the patients were detected among clinical patients who underwent cardiac imaging with radiolabelled fatty acid analogue, they would generally have ischaemic heart disease and coronary risk factors.

Second, the mean lipid profiles for their 26 patients were characterised by increased triglyceride and decreased HDL-cholesterol, which is certainly typical for the insulin resistance syndrome, and four of the five patients subjected to a glucose clamp study also had

	Rodent models		Human being	
	Cd36-null SHI ³	Cd36-null mice ⁴	Miyaoka et al ¹	Yanai et al ⁵
NEFA	↑	↑	?	?
FPG	↓	↓	↑	↔
Glucose tolerance	↑	?	↓	↔
Triglycerides	↓	↑	↑	↔
HDL cholesterol	↓	↑	↓	↔
Blood pressure	↔	?	↑	?

↑ ↓ ↔ denote increased, decreased, and unchanged phenotype compared with controls; ?=unknown.
NEFA=non-esterified fatty acids; FPG=fasting plasma glucose.

Comparison of metabolic phenotypes in rodents and human beings with CD36 deficiency

hypertriglyceridaemia. However, in our study on apparently healthy elderly volunteers, CD36 deficiency in four was associated with decreased triglyceride and increased HDL-cholesterol, although not significantly so.³ Thus, the lipid profiles seen in Miyaoka and colleagues' patients cannot be automatically attributed to CD36 deficiency. The type IV (their case 5) and the type IIb (cases 1–3) hyperlipoproteinaemias are frequent phenotypes of familial combined hyperlipoproteinaemia (FCH), which is known to associate with insulin resistance.⁴

Finally, we have done oral glucose loading studies in three matched groups of healthy young volunteers (aged 20·7 years [1·7]) composed of normal donors (n=16; CD36 present in platelets and monocytes), type I CD36 deficiency (CD36 absent on both platelets and monocytes; n=5) and type II CD36 deficiency (CD36 absent from platelets but present on monocytes; n=16). We confirmed that there were no significant differences between the groups in serum insulin concentrations at any time point nor in major indices for insulin sensitivity, such as integrated area under the curve for insulin, fasting glucose to insulin ratios, and the homeostasis model assessment (submitted for publication). Nor were differences detectable in their clinical features (age, sex, body-mass index, and blood pressures), haemoglobin A_{1c}, fasting glucose, and lipid concentrations. Hence, we believe that CD36 deficiency cannot be a proximate cause of human insulin resistance.

However, we noted also that plasma fatty acid clearance after glucose loading is significantly retarded in both types of CD36 deficiency. This effect could be explained by impaired fatty acid uptake by muscle, and might result in increased hepatic fatty acid entry. The latter, in turn, could increase hepatic gluconeogenesis.⁵

*Hitoshi Chiba, Hidekatsu Yanai, Hironobu Fujiwara, Kazuhiko Matsuno, Kunihiro Kobayashi

Departments of *Laboratory Medicine and Paediatrics, Hokkaido University School of Medicine, Sapporo 060-8648, Japan; and College of Medical Technology, Hokkaido University, Sapporo (e-mail: chibahit@med.hokudai.ac.jp)

- 1 Miyaoka K, Kuwasako T, Hirano K, Nozaki S, Yamashita S, Matsuzawa Y. CD36 deficiency associated with insulin resistance. *Lancet* 2001; **357**: 686–87.
- 2 Yanai H, Chiba H, Morimoto M, Jamieson GA, Matsuno K. Type I CD36

deficiency in humans is not associated with insulin resistance syndrome. *Thromb Haemost* 2000; **83**: 786.

- 3 Yanai H, Chiba H, Morimoto M, et al. Human CD36 deficiency is associated with elevation in low-density lipoprotein-cholesterol. *Am J Med Genet* 2000; **93**: 299–304.
- 4 Ascaso JF, Real JT, Merchante A, Rodrigo A, Carmena R. Lipoprotein phenotype and insulin resistance in familial combined hyperlipidemia. *Metabolism* 2000; **49**: 1627–31.
- 5 Kruszynska YT, Mulford MI, Yu JG, Armstrong DA, Olefsky JM. Effects of nonesterified fatty acids on glucose metabolism after glucose ingestion. *Diabetes* 1997; **46**: 1586–93.

Authors' reply

Sir—One of the major issues for medical science is to clarify the molecular and cellular mechanism for development of multiple risk factor clustering (MRF) syndrome. In many cases people develop MRF syndrome in middle age and subsequently have atherosclerotic cardiovascular diseases, whereas such clinical signs are not always manifest at a younger age. In these cases, the combination of genetic factors and environmental and acquired ones, as well as the impact of each factor, is important. Genetic factors include mutations or polymorphisms in some genes, in addition to sex and race. Environmental factors include age and lifestyle, such as loss of physical activities and high calorie intake. The genetic background, as well as food content, might affect the results of the experiments, as John Petrie and Yoko Iizuka and their colleagues note.

So far, we have seen 28 patients with type I CD36 deficiency.¹ Nine patients were identified by presence of isoantibodies to CD36. The remaining patients were identified by the negative uptake of radiolabelled long-chain fatty acid analogues.² Even when separated by sex, or when the patients identified on scintigraphy are excluded, our CD36-deficient patients have higher concentrations of plasma lipids, fasting plasma glucose, and blood pressure than normal controls (unpublished data). The difference of clinical phenotype between Hitoshi Chiba and colleagues' study and ours might be partly explained by the difference of age and sex of the participants. The young and healthy women with CD36 deficiency they are studying would fill in the blank of our study. They seem to have noted subclinical abnormalities and delayed and smaller decay of plasma free fatty acid concentrations after oral glucose loading, even in these participants. Fatty acid response after oral glucose

loading is thought to result from the inhibition of lipolysis in adipocytes by insulin;³ therefore, we think that the observed abnormal free fatty acid reaction could be related to possible insulin resistance.

There is no definite way to measure insulin resistance in daily clinics. We use fasting concentrations of glucose and insulin, which is easy, but the index is not a direct parameter for insulin resistance. We have used the euglycaemic hyperinsulinaemic clamp,⁴ a more direct way, but the method is complicated and time consuming for patients and doctors. We have also used the clamp test in a young CD36 deficient person, and noted lower glucose uptake, which suggests insulin resistance (unpublished observation). We hope to have some better way to measure insulin resistance in the near future.

Finally, the CD36-deficiency phenotype we report is milder than other known phenotypes except rare genetic insulin resistance syndromes such as mutants of insulin receptor and peroxisome proliferator activated receptor γ gene.⁵ CD36 deficiency could be an important genetic factor for MRF syndrome, which we see frequently. Investigation is needed of the pathophysiology of CD36 deficiency, especially the mechanism of insulin resistance, to understand the molecular mechanism for development of MRF syndromes and how to assess insulin resistance in the clinics.

*Ken-ichi Hirano, Takahiro Kuwasako, Hirokazu Kashiwagi, Shizuya Yamashita, Yuji Matsuzawa

*Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan (e-mail: khirano@kb3.so-net.ne.jp)

- 1 Kashiwagi H, Tomiyama Y, Nozaki S, et al. Analysis of genetic abnormalities in type I CD36 deficiency in Japan: Identification and cell biological characterization of two novel mutations that cause CD36 deficiency in man. *Hum Genet* (in press).
- 2 Yoshizumi T, Nozaki S, Fukuchi K, et al. Pharmacokinetics and metabolism of iodine-123-BMIPP fatty acid analogue in normal and CD36 deficient subjects. *J Nucl Med* 2000; **41**: 1134–38.
- 3 Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's biochemistry, 24th edn. Connecticut: Appleton and Lance, 1996.
- 4 Miyaoka K, Kuwasako T, Hirano K, et al. CD36 deficiency associated with insulin resistance. *Lancet* 2001; **357**: 686–87.
- 5 Barroso I, Crowley VEF, Agostin I, et al. Dominant negative mutations in human PPAR γ associated with severe insulin resistance, diabetes mellitus, and hypertension. *Nature* 1999; **402**: 880–83.