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STUDY DESIGN AND METHODS

Description of the outpatient cohort and selection criteria for *ABCC8* gene sequencing

The patients included in the outpatient cohort study are followed at the Endocrinology-Diabetology Department of the Corbeil-Essonnes Hospital (France). Clinical and biological information of the patients at each consultation are recorded in a database that is the property of this department. From this database, we selected: 1/ the patients treated with a SU drug (alone or associated with other oral hypoglycemic agent(s)) (n=2,016), 2/ among them, those with an HbA1c value at last examination of ≤7.5% (n=966) and a history of type 2 diabetes in at least one first degree relative (n=432), 3/ those diagnosed with diabetes for over 5 years (n=318), and 4/ a BMI <35 kg/m² at diagnosis (n=262). Among these 262 patients, 139 individuals with a DNA sample available were sequenced for *ABCC8*.

The study was conducted according to the principles of the Declaration of Helsinki. A written informed consent was obtained from all patients.

Metabolic studies

Oral and intravenous glucose tolerance test

An oral glucose tolerance test (OGTT) was performed after a 12-h overnight fast. Blood samples were collected before (T0) and 30 (T30) and 120 min (T120) after a 75-g oral glucose load, for determination of plasma glucose and insulin concentrations. Intravenous glucose tolerance test (IVGTT) was done to determine the acute insulin response (AIR). An intravenous catheter was placed in the antecubital vein for the infusion of glucose. Another cannula for blood sampling was inserted. A bolus of glucose (20g of 50% solution) was given (within 30 seconds) into the antecubital vein to rapidly increase the blood glucose concentration. Samples for the measurement of blood glucose and plasma insulin were drawn at ±5, 0, 2, 4, 6, 8 and 10 min.

Euglycemic hyperinsulinemic clamp

Euglycemic hyperinsulinemic clamp was performed as previously reported (1). Briefly, after a 12h fast, using a continuous intravenous (IV) administration of insulin was performed at the rate of 80 mIU/m^2 of body surface area per minute. Blood glucose was clamped at 5.5 mmol/l for 100 min IV infusion 20% glucose at varying rates according to blood glucose measurements performed at 5 min intervals.

Graded glucose infusion

Graded glucose infusion consisted of consecutive 40 min IV infusion periods of 20% glucose at 2, 4, 8, and if needed 12 and 16 mg/kg/min, respectively, in order to reach mean blood glucose levels around 20 mmol/l during the last period (1).

Arginine test

An IV bolus of 5 g arginine was administered at the end of the graded glucose infusion test, while blood glucose concentration was stabilized at 20 mmo/l. Arterialised blood samples were obtained at baseline and every 10 minutes during each glucose infusion step, and were immediately centrifuged and stored at –80°C.

Body composition

Body composition was measured by Dual Energy X-ray Absorptiometry (DEXA).

Metabolic parameters

Early insulin response (EIR) in response to OGTT was calculated as the ratio [insulinemia at 30 min (I30) - fasting insulinemia (I0)]/[blood glucose at 30 min (G30) – fasting blood glucose (G0)]. Acute insulin response to IV glucose during IVGTT (AIR-Gluc) was calculated as the sum of insulin plasma concentrations at time 1 and 3 min. Glucose disposal rate (M Value) was calculated from the glucose

infusion rate during the last 20 min of the glucose clamp after accounting for inter-individual differences in glucose space, and was expressed in mg/kg of fat-free body mass (FFM). Peripheral *Insulin sensitivity* (IS) was calculated by dividing the M value by the steadystate clamp insulin level and expressed per FFM (Mvalue/moy insulinemia, mg/kg free mass/min/μIU/ml). Insulin secretion in response to the graded glucose infusion was evaluated from the changes in C-peptide concentration and the pre-hepatic insulin secretion rate (ISR). ISR was derived by deconvolution assuming a two-compartment model of C-peptide clearance kinetics using version 3·4a of the ISEC software as described (2). Acute insulin response to arginine during hyperglycemia (AIR-Arg) was estimated using the area under the curve AUC of insulin plasma concentrations measured each minute within the 5 min following arginine injection. A disposition index, indicating the beta-cell function relative to insulin sensitivity, was calculated as the product of IS by EIR.

The protocol was approved by the Ethics Committee of Paris Saint-Louis hospital. All participating patients gave a written informed consent.

Genetic study

Genotyping method

High Resolution Melting (HRM) curve analysis was used to genotype two *ABCC8* mutations (C418R and R620C) in 4,446 samples of the DESIR study for estimating their frequency at the population level. Annealing temperature, pooling of three DNA samples and reaction conditions ($MgCl_2$ concentration, use of a GC-rich reaction buffer) were optimized on the Roche LightCycler 480 system following the manufacture's instructions (Roche Diagnostics GmbH, Mannheim). Thanks to a well-optimized assay for detecting each mutation (amplified fragments of 163 bp and 140 bp), specific melting curve shapes were obtained allowing us to identify a single heterozygote genotype with two homozygotes in a DNA sample pool (2). A genotype call rate of 95.6% and 94.5% was obtained for C418R and R620C, respectively, in the tested population.

REFERENCES

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2. Sobngwi E, Boudou P, Mauvais-Jarvis F, Leblanc H, Velho G, Vexiau P, Porcher R, Hadjadj S, Pratley R, Tataranni PA, Calvo F, Gautier JF 2003 Effect of a diabetic environment in utero on predisposition to type 2 diabetes. Lancet 361:1861-1865
3. Becsagh P, Varga K, Szakacs O, Kopper L, Orosz Z 2010 High resolution melting curve analysis of DNA sequence alterations of various sizes. Pathol Oncol Res 16:421-426

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Supplementary Table 1. Insulin secretion and insulin sensitivity data in four untreated subjects from two families compared to normoglycemic controls

			Controls* (n=29)	**Subject 3-II.1**	**Subject 3-II.2**	**Subject 4-III.1**	**Subject 4-III.2**
	Gender, n (%) of females		15 (52)	M	M	F	M
	Age (years)		25 (21-30)	38	32	36	34
	Body-mass index (kg/m²)		21.5 (20.8-24.4)	21.0	37.0	20.3	23.0
Insulin secretion	**Methods**	**Index**					
	OGTT	EIR (mIU/mmol)	11.3 (6.4-17.1)	nd	nd	2.58	7.95
	IVGTT[§]	AIR-Gluc [1+3] (mIU/l)	114 (92-154)	21.6	15.5	nd	nd
	Arginine test	AIR-Arg (mIU/l)	181 (110-253)	173	343	91	135
Insulin sensitivity	Euglycemic hyperinsulinemic clamp	M value (mg/kg free mass/min)	11.6 (10.6-13.0)	16.0	4.8	12.3	6.7
		IS (mg/kg free mass/mIU/l)	0.11 (0.09-0.12)	0.08	0.04	0.18	0.11
Disposition Index after OGTT		IS x EIR	1.20 (0.56-2.05)	nd	nd	0.46	0.87

OGTT, Oral glycemic tolerance test; IVGTT, Intra-venous glycemic tolerance test; EIR, Early insulin response to oral glucose; AIR-Gluc [1+3], Acute insulin reponse to IV glucose index 1+3; AIR-Arg, Acute insulin response to arginine (calculated as mean insulinemia [measured at T_{2min} -T_{5min}] - insulinemia measured at T_0); IS, Insulin sensitivity (calculated as M value divided by the average of insulin levels measured during the last 20 minutes of the euglycemic clamp); nd, not determined.

*in the control group, the values are median with interquartile (Q1-Q3) range for insulin secretion and insulin sensitivity data.

[§]another group of eight control subjects had IVGTT (age 26.5±3.0 years, 6 males and 2 females, BMI 22.0±1.6 kg/m²).

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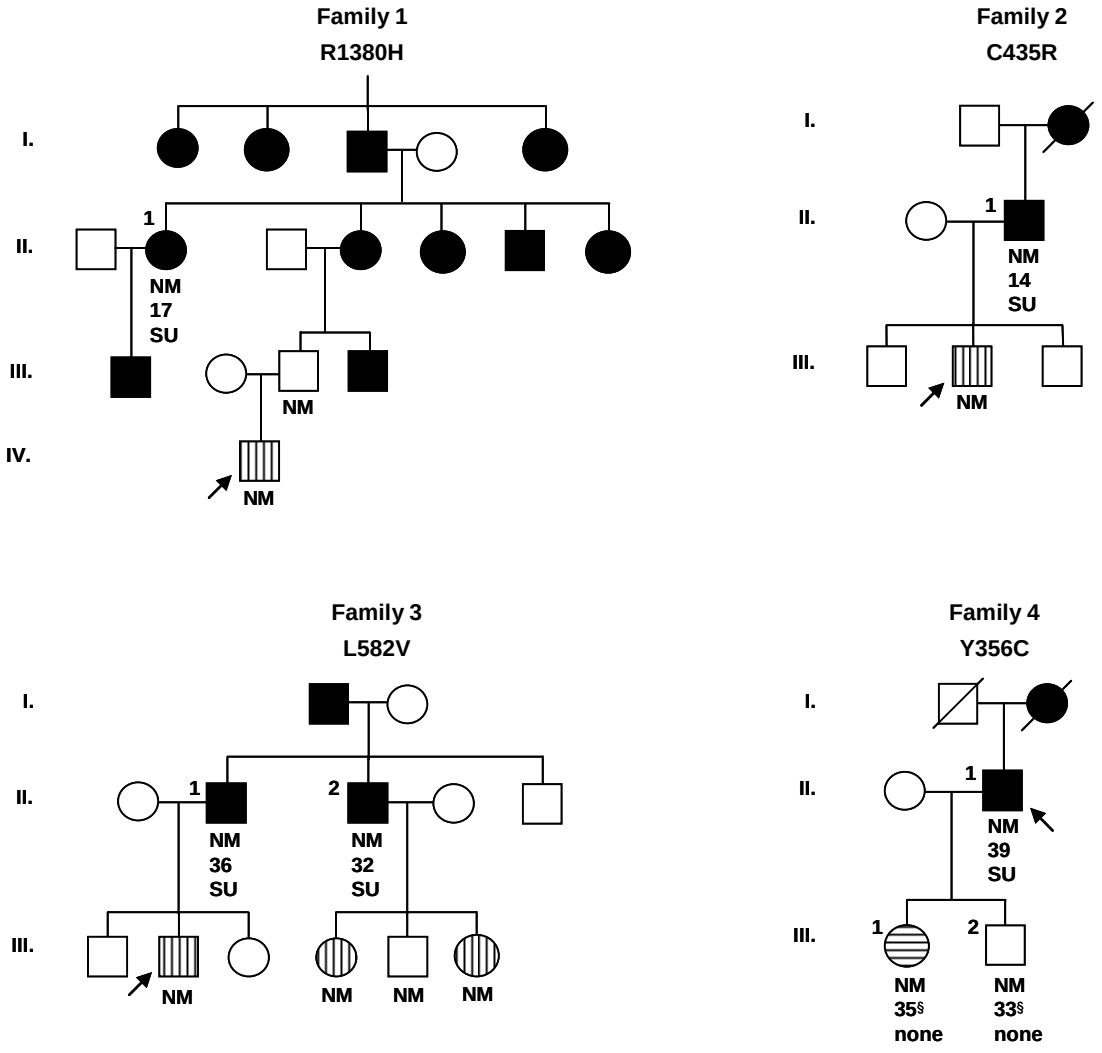
Supplementary Figure 1. Pedigrees of four families showing genetic status for *ABCC8* Mutations, affected members and treatment

Squares represent male and circles represent female subjects. Adult-onset diabetes patients are in black, those with neonatal diabetes mellitus are the vertically hatched symbols, one with impaired glucose tolerance the horizontically hatched symbol and the subjects with normal glucose tolerance are in white. The arrows indicate the proband in each family. NM denotes a heterozygous mutation. Below the genotype are indicated: age at diagnosis/or at ascertainment[§], and treatment.



SUPPLEMENTARY DATA

Supplementary Figure 2. Evaluation of insulin secretion in patients with adult-onset diabetes who are carriers of an *ABCC8* mutation

A. Graded glucose infusion for measurement of insulin secretory response: Insulin secretion in response to the graded glucose infusion was evaluated in four patients from the changes in C-peptide concentration and the pre-hepatic insulin secretion rate (ISR). ISR was derived by deconvolution assuming a two-compartment model of C-peptide clearance kinetics using version 3·4a of the ISEC software. Average ISR were plotted against different mean glucose levels.
Black circles denote patient 3II.1, white circles patient 3II.2, black squares patient 4 III.1 and white squares patient 4 III.2, black diamonds are controls for +1 standard deviation (SD) and white diamonds controls for -1 SD.

B. Acute insulin response to IV glucose at time 1 and 3 min (AIR-gluc 1+3) before (in white) and after a 4-week trial with oral sulfonylurea (in black) in patients 3 II.1 (glibenclamide 7.5 mg/day) and 3 II.2 (glibenclamide 15 mg/day). A fully normal response to IV glucose was restored in both patients as shown by AIR-gluc 1+3 raising to 55 µUI/ml (2,5 fold) and 345 µUI/ml (22 fold), respectively.