Higher Maternal Than Paternal Inheritance of Diabetes in GK Rats

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Results from crosses between Goto-Kakizaki (GK) rats, which exhibit spontaneous non-insulin-dependent diabetes mellitus (NIDDM), and outbred nondiabetic Wistar rats have demonstrated an effect of maternal inheritance on diabetes in offspring of the first generation (F1). At 6 weeks of age, F1 offspring of sex-directed crosses exhibited plasma glucose values intermediate between GK and Wistar parents. Hyperglycemia in F1 rats born of female GK rats (F1GK) was more marked than in those born of female Wistar (F1W) rats. At 3 months of age, F1 rats showed a marked impairment of both glucose tolerance and insulin secretion, which was intermediate between GK and Wistar rats. Glucose intolerance was more pronounced in F1GK rats than in F1W. By contrast, insulin secretion in F1W rats was more deteriorated than in F1GK rats. No deletion in mitochondrial DNA was observed in the GK rats, which decreased the possibility of a mitochondrial inheritance effect as an explanation of our findings. These data support a polygenic model in diabetes inheritance of NIDDM and suggest that, in addition to genetic factors, a perturbed maternal metabolism can contribute to its inheritance.
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NIDDM, non-insulin-dependent diabetes mellitus; GK, Goto-Kakizaki; F1, first generation; F1GK, first generation offspring of female GK rats mated with male Wistar rats; F1W, first generation offspring of female Wistar rats mated with male GK rats; F0GK, GK rats used as genitors of the first generation rats; F0W, Wistar rats used as genitors of the first generation rats; RIA, radioimmunoassay; IRI, immunoreactive insulin; mtDNA, mitochondrial DNA; bp, base pair; STZ, streptozocin.

Non-insulin-dependent diabetes mellitus (NIDDM) is a heterogeneous disease with an etiology that involves both genetic susceptibility and environmental risk factors (1). Previous epidemiological studies have shown both an increased frequency of transmission of NIDDM to offspring from female than from male parents (2) and a much higher prevalence of diabetes in offspring of women with diabetes during pregnancy than offspring of nondiabetic or prediabetic women (3,4). Discrimination between gestational effects and genetic factors in maternal inheritance of diabetes is difficult in humans. Animal models of diabetes can help in research on this specific point.

For our study, we used the Goto-Kakizaki (GK) rat, which has been produced by Goto et al. (5) in Tohoku University, Sendaï, Japan, by selective breeding over many generations from a nondiabetic Wistar rat colony based on glucose intolerance and defective insulin secretion, as a model of spontaneous NIDDM without obesity. All offspring of GK rats showed glucose intolerance and impaired insulin secretion after the F10 generation, and the diabetic state becomes stable after the F30 generation (5). We used rats from a colony of GK rats initiated in 1988 with progenitors received from F35 of the Japanese colony.

In an attempt to study the importance of maternal inheritance of NIDDM, two crosses were performed: GK female rats with outbred Wistar male rats and outbred Wistar female rats with GK male rats. Glucose homeostasis in adult animals of first generations (F1) was investigated by performing glucose tolerance and insulin secretion tests.

RESEARCH DESIGN AND METHODS

GK rats were a gift from Bernard Portha (CNRS URA 307, Paris, France). Wistar rats, obtained from a commercial

supplier (Iffa-Credo, Orléans, France), were used as control nondiabetic rats. A genetic analysis of Wistar and GK rats using genetic markers confirmed that GK rats were inbred. However, Wistar rats appeared outbred because they were heterozygous at several loci. All rats had free access to water and standard laboratory chow pellets (Usine d'Alimentation Rationnelle 113, Villemoisson-sur-orge, France).

The crosses were performed by caging a female rat with a male for one night from 1700 to 0900. Pregnancy was detected by abdominal palpation 13 days later. Glucose tolerance and insulin secretion tests were performed in all rats 1 week before mating using the following protocol: Rats in the postabsorptive state were anesthetized by intraperitoneal injection of ketamine hydrochloride (95 mg/kg body weight) (Imalgène, Mérieux, France) and injected via the saphenous vein with 0.8 glucose/kg body weight. Then, blood samples were collected sequentially before glucose injection and 5, 10, 15, 20, and 30 min afterward. Samples were centrifuged, and the plasma was separated. Glycemia was immediately determined using a 10-μl plasma aliquot, and the remainder of the sample was stored at −20°C for radioimmunoassay (RIA) of insulin.

In our colony, birth occurs 22 days ± 12 h after mating both in GK and Wistar rats. Dams were allowed to be delivered, and pups were bred by their own mothers. However, the number in each litter was adjusted to between 8–10 pups, because of the important effect of litter size on growth (6) and insulin release (7). The pups that made up the F1 generation were weaned at 22 days of age.

In 6-week-old F1 rats, basal plasma glucose and HbA_{1C} concentrations were measured. Glucose tolerance and insulin secretion tests were performed in rats of the F1 generation at 3 months of age, as described above.

Plasma glucose was determined using a glucose analyzer (Beckman, Fullerton, CA). Plasma immunoreactive insulin (IRI) concentration was determined with an RIA kit (CEA, Gif-sur-Yvette, France). The lower limit of the assay was 15 pM with a coefficient of variation within and between an assay of 6%. Insulin secretion in response to glucose loading was calculated as the insulinogenic index (ΔIRI/ΔG), which is the ratio of incremental plasma insulin values above baseline, integrated over 30 min after glucose injection (ΔIRI expressed as pM/min) to the corresponding incremental plasma glucose values (ΔG expressed as mM/min) integrated over the same period.

The measure of HbA_{1C} was performed by affinity chromatography, according to the method developed by Bouriotis et al. (8). Results are expressed as means ± SE. The significance of differences between the groups was evaluated by a one- or two-way analysis of variance.

The possible presence of a deletion in the GK mitochondrial DNA was investigated by Southern blot analysis after *Bam*HI or *Pvu*II (Boehringer Mannheim, Mannheim, Germany) digestion of DNA extracted from skeletal muscle and brain of GK and Wistar rats (9). Rat mitochondrial DNA (mtDNA) is a circular 16,298 base pairs (bp) molecule. Its sequence contains two cleavage sites for the enzymes *Pvu*II (positions 6,273 and 7,198) and *Bam*HI (positions 9,361 and 14,433) (10). Total cellular DNA (500 ng) were loaded onto 1% SeaKem agarose gel (FMC Bioproducts, Rockland, ME), electrophoresed, blotted on nylon membrane (Hybond N+, Amersham, UK), and hybridized with a probe (DL) complementary to the D-loop of the mtDNA (10). This probe, produced by polymerase chain reaction amplification using the primers DL1 and DL2, was labeled with α32PdCTP by random priming.

DL1: 5′ CATCAACACCCAAAGCTGATA 3′
15359 15379
DL2: 5′ CGATGTGTAATCTTACCTCCA 3′
118 98

RESULTS

GK rats (F0GK); GK rats used as genitors of the first generation rats) exhibited basal hyperglycemia (time 0 before loading), and their plasma glucose concentrations were higher than those of control Wistar rats (F0W; Wistar rats used as genitors of the first generation rats) from 5 to 30 min after loading (Fig. 1). This was reflected in ΔG, which was significantly higher in F0GK than in F0W rats (Table 1). Basal plasma IRI concentration was at higher levels in F0GK rats compared with controls, but the insulin secretory response to glucose was lowered dramatically. As a consequence, ΔIRI and ΔIRI/ΔG were much lower in F0GK rats than in controls (Table 1).

In 6-week-old rats of the F1 generation, plasma glucose concentration (7.64 ± 0.12 mM; $n = 65$) was significantly higher ($P < 0.001$) than in F0W rats (5.7 ± 0.09 mM; $n = 12$). Moreover, a slight but significant ($P < 0.05$) difference was observed between rats born of female GK rats mated with male Wistar rats (F1GK; 7.91 ± 0.14 mM; $n = 40$) and rats born of female Wistar rats mated with male GK rats (F1W; 7.43 ± 0.12 mM, $n = 60$). HbA_{1C} concentration in 6-week-old animals (F1GK + F1W) was identical to controls (2.09 ± 0.07% in F1 vs. 2.10 ± 0.08% in controls).

Based on glucose tolerance tests performed in 3-month-old F1 rats, glycemic control was largely impaired in F0W rats, but was better than in the F0GK rats (Fig. 1). In general, glycemic control in F1 rats (F1GK + F1W) was closer to that of F0GK rats than that of F0W rats. We also observed a clear-cut difference in glucose intolerance between F1W and F1GK. Plasma glucose and ΔG were both significantly higher at each time point in F1GK rats than in F1W rats.

Insulin secretion in response to glucose loading remained profoundly impaired in F1 rats (F1GK + F1W) compared with F0W rats, although the situation was slightly improved compared with F0GK animals. F1GK rats showed higher basal plasma insulin concentration and glucose-induced insulin secretion than F1W rats. Therefore, ΔIRI/ΔG in F1GK rats was significantly higher than in F1W rats.

Southern blot analysis, using the DL probe, of both *Bam*HI and *Pvu*II digested mtDNA showed no polymorphism between GK and Wistar rats (Fig. 2). Because the

TABLE 1
Integrated incremental plasma glucose (ΔG), immunoreactive insulin (ΔIRI) concentrations, and insulinogenic index (ΔIRI/ΔG) in 3-month-old rats from F0 (GK and Wistar) and F1 generation (total F1, F1W, and F1G)

	n	ΔG (mM/min)	ΔI (pM/min)	ΔI/ΔG
Wistar	12	2.50 ± 0.05	264 ± 66	102.90 ± 21.60
GK	12	3.09 ± 0.3*	41.4 ± 14.4†	13.18 ± 2.38†
F1	100	2.44 ± 0.05‡	88.2 ± 9.6*	35.94 ± 3.99*
F1W	60	2.33 ± 0.05§	63 ± 9.6*	26.68 ± 4.32*
F1G	40	2.55 ± 0.09‖¶	126 ± 18†‖#	49.36 ± 7.13†‖**

Data are means ± SE.
*P < 0.001 vs. Wistar rats.
†P < 0.01 vs. Wistar rats.
‡P < 0.01 vs. GK rats.
§P < 0.001 vs. GK rats.
‖P < 0.05 vs. GK rats.
#P < 0.05 vs. F1W rats.
¶P < 0.001 vs. F1W rats.
**P < 0.01 vs. F1W rats.

probe DL is complementary to a 15,373 bp fragment of *Pvu*II digested mtDNA and a 11,226 bp fragment of *Bam*HI digested mtDNA, this result implies the absence of a large deletion in the GK mtDNA.

DISCUSSION

Diabetes, as developed in the GK rat, shows the main features of the metabolic and vascular disorders described in NIDDM. When compared with Wistar rats, adult GK rats show significantly higher basal plasma glucose and insulin levels, altered glucose tolerance, and impaired insulin secretory response to glucose in vivo and in vitro (5,11,13).

The principle for obtaining the spontaneous diabetic GK rat strain (5) implies that predisposing gene(s) to diabetes may be present in the nondiabetic Wistar rats

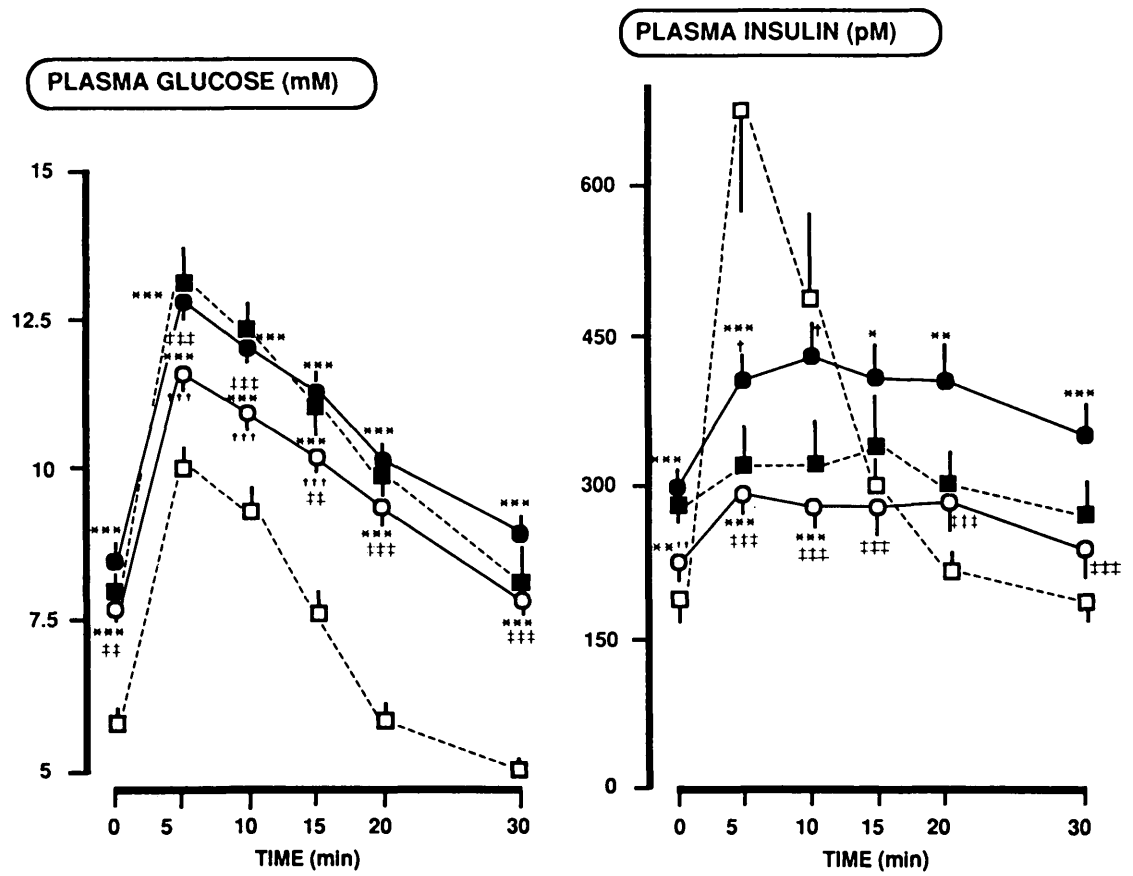

FIG. 1. Effects of intravenous glucose loading on plasma glucose and IRI concentrations in 3-month-old Wistar (□; *n* = 12) and GK (■; *n* = 12) rats from F0 generation and in 3-month-old F1GK (●; *n* = 40) and F1W rats (○; *n* = 60). Values are means ± SE. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs. Wistar rats. †*P* < 0.05; ††*P* < 0.01; †††*P* < 0.001 vs. GK rats. ‡‡*P* < 0.01; ‡‡‡*P* < 0.001 vs. F1G rats.

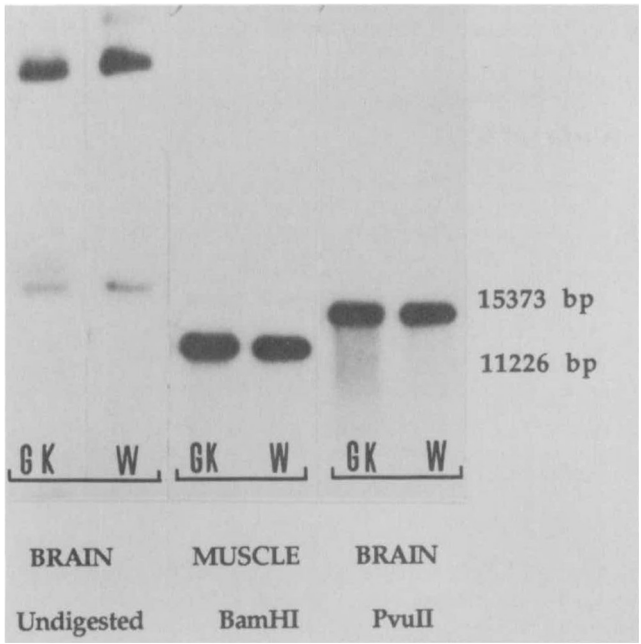

FIG. 2. Southern-blot analysis of DNA isolated from brain and skeletal muscle of Wistar and GK rats. DNA, intact or digested by *Bam*HI or *Pvu*II, were electrophoresed onto a 1% agarose gel, blotted onto nylon membrane, and hybridized with the α32PdCTP-labeled oligonucleotide probe DL complementary of the D-loop of the mtDNA.

and that they might be concentrated through repeated selective breedings, when glucose intolerance is used as a selection index.

Our results demonstrate that both glucose intolerance and impaired insulin secretion are transmitted from the GK rat to offspring in an F1 generation. Although both these phenotypes are improved in F1 rats, compared with GK rats, they remained significantly diminished compared with controls. In F1 animals, ΔIRI and ΔIRI/ΔG were roughly intermediate between GK and Wistar rats, whereas ΔG were closer to the situation of Wistar than that of GK. However, data concerning ΔG are to be interpreted with caution because of the important influence of basal glycemia on this parameter. These results are consistent with a polygenic model of diabetes inheritance. They are also consistent with previous data from oral glucose tolerance tests performed in F1 rats (GK × Wistar)(12) and with genetic analysis of the difference in glucose tolerance between inbred strains of mice (C3H/HeJ and C57BL/6J) (14).

The importance of maternal factors implicated in NIDDM has been suggested previously in large human family studies that demonstrate an increased maternal transmission of diabetes (2–4). The involvement of maternal factors in the GK rat offspring is indicated by the differences observed in glucose tolerance and insulin secretion between F1GK and F1W rats.

These differences between F1GK rats and F1W rats may be explained by the expression of defect(s) in mtDNA in the GK rat, because in mammals mtDNA is transmitted exclusively through maternal lineage (15). Studies of diseases caused by mutations in mtDNA suggest that a variety of degenerative processes may be associated with defects in oxydative phosphorylation (15). Ballinger et al. (16) recently reported a heteroplasmic 10.4-kilobase mtDNA deletion in members of a family with maternally inherited NIDDM and deafness. A role for the effects of mtDNA damages in animal models of diabetes has been suggested by studies of rats injected neonatally with streptozocin (STZ) (17). STZ selectively damages rat pancreatic islet mtDNA and decreases it in ratio to nuclear DNA, which consequently lowers mitochondrial gene expression (18).

Southern-blot analysis of mtDNA showed no evidence of a large deletion in either muscle or in brain mtDNA of GK rats. Muscle and brain tissues, which have a low replication level, were used because the distribution of deleted mtDNAs seems to be determined by replicative segregation (15). In addition, cells can harbor mixtures of mutant and normal mtDNAs (heteroplasmy) and deleted molecules accumulate in localized regions of muscle fibers (19). Because the D-loop is required for the replication of the mtDNA, the use of a probe complementary to this region ensures detection of only those forms of the molecule that are able to replicate.

The absence of large deletion in mtDNA from GK rat does not rule out the hypothesis that point or other mutations may exist. Recently, Reardon et al. (20) reported an association between diabetes and a pathogenic point mutation in the mitochondrial tRNA leucine gene observed in a large human pedigree. However, because the frequency of mutation is higher in mtDNA than in the nuclear genome (15) and because the GK rat has been selected over >35 generations from Wistar rats, any differences detected in GK and Wistar rat mtDNA sequence might reflect strain polymorphism unrelated to diabetes. The involvement of mtDNA point mutations in diabetes of the GK rat needs to be correlated with defects in mitochondrial genes expression.

Alternatively, although the GK rat is primarily a genetic model of NIDDM, environmental factors, such as gestational hyperglycemia, may contribute to the transmission of diabetes by the mother. Studies in the lineage of pregnant rats injected with STZ or infused with glucose have demonstrated that mild hyperglycemia induced in the mother during the pregnancy leads to persistent impairment of glucose tolerance and insulin secretion in the adult progeny regardless of genetic interference (21–23). This impairment was transmitted to the subsequent generation by the mother via a presumably nongenetic process (24). Our data concerning glucose tolerance are consistent with these studies. However, we also observed a reduced glucose-induced insulin secretion in F1W rats, compared with F1GK rats. The concomitant deterioration of glucose tolerance in F1 rats and the increase of insulin secretory response to glucose in F1GK rats compared with F1W rats suggests a more marked insulin resistance in F1GK than in F1W rats. This increase of insulin secretion may be a response to this possibly aggravated insulin resistance rather than an improved pancreatic β-cell function. Nevertheless, the use of euglycemic-hyperinsulinemic clamp technique is required to explore insulin sensitivity in F1 rats. Taken together, although a genetic heterogeneity may exist in

Wistar rats from our colony, these results support the concept of fuel-mediated teratogenesis, which has been proposed by Freinkel (25) in an attempt to explain both short- and long-term effects of maternal diabetes on human offspring.

The results reported herein suggest that, in addition to genetic factors, a deteriorated maternal metabolism contributes to the inheritance of diabetes. However, to explain the differences between animals born of female GK rats and those born of female Wistar rats, we can exclude neither an effect of mutations in the chromosome X of GK rats nor a contribution of loci subject to parental imprinting, as has been demonstrated for the IGF-II locus in mice (26). Linkage analysis on further breedings with the GK strain (F2 and back-cross populations) using genetic markers of the rat genome (27) are required to test these hypotheses.

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