Congenic Mapping and Candidate Gene Analysis for Streptozotocin-Induced Diabetes Susceptibility Locus on Mouse Chromosome 11

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Abstract

Streptozotocin (STZ) has been widely used to induce diabetes in rodents. Strain dependent variation in susceptibility to STZ has been reported; however, the gene(s) responsible for STZ susceptibility has not been identified. Here, we utilized the A/J-11^{SM} consomic strain and a set of chromosome 11 (Chr. 11) congenic strains developed from A/J-11^{SM} to identify a candidate STZ-induced diabetes susceptibility gene. The A/J strain exhibited significantly higher susceptibility to STZ-induced diabetes than the A/J-11^{SM} strain, confirming the existence of a susceptibility locus on Chr. 11. We named this locus *Stzds1* (STZ-induced diabetes susceptibility 1). Congenic mapping using the Chr. 11 congenic strains indicated that the *Stzds1* locus was located between *D11Mit163* (27.72Mb) and *D11Mit51* (36.39Mb). The *Mpg* gene, which encodes N-methylpurine DNA glycosylase (MPG), a ubiquitous DNA repair enzyme responsible for the removal of alkylated base lesions in DNA, is located within the *Stzds1* region. There is a close relationship between DNA alkylation at an early stage of STZ action and the function of MPG. A Sanger sequence analysis of the *Mpg* gene revealed five polymorphic sites in the A/J genome. One variant, p.Ala132Ser, was located in a highly conserved region among rodent species and in the minimal region for retained enzyme activity of MPG. It is likely that structural alteration of MPG caused by the p.Ala132Ser mutation elicits increased recognition and excision of alkylated base lesions in DNA by STZ.

Key Words: A/J mice, congenic mapping, consomic strain, N-methylpurine DNA glycosylase gene, streptozotocin-induced diabetes susceptibility locus

Introduction

Streptozotocin (STZ) is widely used to induce diabetes in experimental mammals by acting as a beta cell toxin (Lenzen 2008; Szkudelski 2012). Injection of a single high dose of STZ induces extensive DNA strand breaks in pancreatic ß-cells and subsequent cell death (Yamamoto et al. 1981). STZ is decomposed intracellularly and DNA damage is caused either by alkylation or by the generation of nitrous oxide (Lenzen 2008; Szkudelski 2012). It has been found that inbred strains of mice vary in their susceptibility to STZ-induced diabetes indicating the importance of genetic background on pancreatic ß-cell destruction by STZ (Rossini et al. 1977; Kaku et al. 1989). The NOD, NSY and C57BL/6J strains are susceptible to diabetes induction, whereas C3H is resistant to a single high dose of STZ (Rossini et al. 1977; Kaku et al. 1989; Gonzalez et al. 2003; Babaya et al. 2005). These susceptible strains are all considered as models for diabetes: the NOD strain in particular has been widely used for type 1 diabetes research (Kikutani and Makino 1992); the NSY strain is used as a model for type 2 diabetes with mild obesity (Ueda et al. 1995); and the C57BL/6J strain is used as a model with impaired glucose tolerance for type 2 diabetes (Kaku et al. 1988). Recently, pancreatic ß-cell fragility has been recognized as a common underlying risk factor in type 1 and type 2 diabetes (Dooley et al. 2016; Liston et al. 2017). We hypothesized that susceptibility to STZ-induced diabetes is closely related to pancreatic ß-cell fragility and that identification of the genetic basis of STZ sensitivity would help to determine the mechanism of this destructive process on pancreatic ß-cells. However, the genetic basis of the inter-strain difference in susceptibility to STZ-induced diabetes has not yet been identified.

Congenic strain mapping is a useful strategy for confirming and localizing loci of interest (Rogner and Avner 2003). This method has been successfully used for fine mapping and identification of causative genes for complex traits (Stylianou et al. 2005; Clee et al. 2006; Bhatnagar et al. 2011). Consomic strains provide a powerful resource for the analysis of complex genetic traits in mice (Nadeau et al. 2000; Singer et al. 2004; Takada et al. 2008) and offer a suitable starting point for the rapid development of congenic strains. We previously developed consomic strains from the A/J and SM/J strains (Ohno et al. 2012). A/J and SM/J mice possess many phenotypic trait differences that can be used for genetic mapping purposes (Nishimura et al. 1995; Festing 1996). Recently, we found that the A/J strain is highly susceptible to STZ-induced diabetes, whereas the SM/J strain is not susceptible.

Mouse chromosome 11 (Chr. 11) has been suggested as a candidate for location of the STZ-induced diabetes locus in NOD mice (Gonzalez et al. 2003) and NSY mice (Babaya et al. 2005). To determine the genetic basis of susceptibility to STZ-induced diabetes, we

focused on Chr. 11 in this study and utilized the A/J-11^{SM} consomic strain, which has introgression of the SM/J strain Chr. 11 into the A/J genetic background (Ohno et al. 2012), and Chr. 11 congenic strains that were subsequently developed from the A/J-11^{SM} strain.

Materials and methods

Animals

A/J, AKR/N, BALB/c, C3H/HeN, C57BL/6J, CBA/N, NSY and NZW/N mice were purchased from Japan SLC (Hamamatsu, Japan). DBA/2J, FVB, NOD and KK mice were purchased from CLEA Japan (Tokyo, Japan). A/J-11^{SM}, MSM/Ms, NC and SM/J mice are maintained in the Institute for Laboratory Animal Research, Graduate School of Medicine, Nagoya University. All mice were fed a commercial CE-2 diet (CREA Japan, Tokyo) and had *ad libitum* access to water. The mice were bred in a pathogen-free facility at the Institute for Laboratory Animal Research, Graduate School of Medicine, Nagoya University, and maintained under a controlled temperature of 23 ± 1°C, humidity of 55 ± 10%, and a light cycle of 12-hour light (from 09:00 to 21:00)/12-hour dark (from 21:00 to 09:00). Animal care and all experimental procedures were approved by the Animal Experiment Committee, Graduate School of Medicine, Nagoya University, and were conducted according to the Regulations on Animal Experiments of Nagoya University.

Development of Chr. 11 congenic strains

Male A/J-11^{SM} mice were mated to A/J females to produce F_1 mice. Male F_1 mice were backcrossed to A/J females to produce the N_2 mice. Twelve N_2 males that carried a fragmented Chr. 11 derived from SM/J were identified and used as founders of the Chr. 11 congenic strains; they were backcrossed to A/J females to produce N_3 mice. Finally, brother–sister mating of N_3 mice yielded homozygosity for the SM/J derived Chr. 11 fragment on an A/J genetic background. The set of Chr. 11 congenic strains was composed of 12 lines (A.SM-Chr.11A, 11B, 11C, 11D, 11E, 11F, 11G, 11H, 11I, 11J and 11K) (Supplemental Fig. 1). Genomic DNA was prepared from tail tissue by salt/ethanol precipitation. PCR genotyping of simple sequence length polymorphism (SSLP) markers was performed using standard methods. PCR products were separated by electrophoresis on a 4% NuSieve agarose gel (FMC, Rockland, ME, USA) and visualized by UV light after ethidium bromide staining. The 47 SSLP markers used in this study are listed in Supplemental Table 1.

STZ injection and blood glucose and insulin measurement

To induce diabetes, 8-week-old male mice were injected with a single dose of 175 mg/kg BW of STZ (Wako Pure Chemical Industries, Osaka, Japan). STZ was dissolved in sodium citrate buffer at pH 4.2 (Wako Pure Chemical Industries, Osaka, Japan) and immediately injected intraperitoneally under non-fasting conditions after measuring body

weight and blood glucose levels. Blood glucose level and body weight were measured every other day until 2 weeks after injection. To determine blood glucose levels, blood samples were taken from the tail tip and assayed with a Glutest analyzer (Sanwakagaku Kenkyusho, Nagoya, Japan). Mice with a blood glucose level higher than 250 mg/dl were considered diabetic. Blood samples were collected in heparinized tubes from the right atrium of the heart of deeply anesthetized mice at 2 weeks after STZ injection. Serum insulin concentrations were measured using a Mouse Insulin ELISA kit (Morinaga Institute of Biological Sciences, Yokohama, Japan).

Diabetes was also induced in mice using multiple low-dose injections; in this treatment group, 8-week-old male mice were injected with 40 mg/kg BW of STZ on 5 consecutive days. Blood glucose levels and body weights were measured at 5-day intervals until 25 days after the last injection; and blood samples were collected from the heart at 25 days after last injection of STZ.

Sanger sequence analysis

Primer sequences for the *Mpg* (N-methylpurine-DNA glycosylase) gene (Supplemental Table 2) were designed using genome assembly data (GRCm38.p4) as the reference sequence. PCR amplification was performed using a GoTaq Green Master mix (Promega, Madison, WI, USA), and amplification products were purified using an ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). PCR products were sequenced using the dideoxy chain-termination method with a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA), and then analyzed on an 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) automated DNA sequencer.

Statistical analysis

Results are expressed as means and standard errors. A one-way analysis of variance and subsequent Bonferroni’s test or Dunnett's test were used to determine the significance of differences for mean values in multi-group comparisons. Student’s t-test was used to compare two-group differences. Fisher’s exact test was used for testing proportions. Statistical significance was set as $P < 0.05$. Effect size (Cohen’s d) were calculated based on means and standard deviations.

Results

To confirm the existence of a susceptibility locus for STZ-induced diabetes on Chr. 11, we compared the effects of a single high-dose injection of STZ in the A/J, SM/J and A/J-11^{SM} strains. Blood glucose levels and incidence of diabetes in the A/J strain increased rapidly until 2 days after STZ injection (Fig. 1). By contrast, blood glucose levels and incidence of diabetes in the SM/J and A/J-11^{SM} strains increased gradually after the STZ

injection; at 14 days, SM/J and A/J-11^{SM} strains showed significantly lower effects than the A/J strain (Fig. 1). Blood insulin levels of SM/J and A/J-11^{SM} strains were significantly higher than those in the A/J strain at 14 days after STZ injection (Fig. 1). There were no significant differences between SM/J and A/J-11^{SM} strains with respect to incidence of diabetes and blood glucose levels (Fig. 1).

To map the STZ-induced diabetes susceptibility locus, we selected six Chr. 11 congenic strains (A.SM-Chr.11B, 11C, 11D, 11E, 11G and 11K) that covered the whole of Chr. 11, and compared STZ-induced diabetes susceptibility after a single high-dose injection. Four (11B, 11D, 11E and 11G) of the six congenic strains showed high susceptibility with hyperglycemia, hypoinsulinemia and high incidence of diabetes similar to the A/J strain (Fig. 2). By contrast, two congenic strains (11C and 11K), which carry the SM/J derived *D11Mit163* to *D11Mit51* region, exhibited resistance to STZ-induced diabetes and showed significantly lower blood glucose levels and incidence of diabetes than the other four congenic strains and A/J strain (Fig. 2). However, the reason why these two congenic strains showed greater resistance to induced diabetes (i.e., lower incidence of diabetes and lower blood glucose levels) than the SM/J strain is not known. The correlation between STZ-induced diabetes susceptibility and the presence of the SM/J derived Chr. 11 fragment in each congenic strain indicated that the STZ-induced diabetes susceptibility locus was located between *D11Mit163* (27.72Mb) and *D11Mit51* (36.39Mb) (Fig. 2). The existence and location of an STZ-induced diabetes susceptibility locus on Chr. 11 were clearly shown; we named this locus *Stzds1* (STZ-induced diabetes susceptibility 1).

To evaluate the effect of the *Stzds1* locus on response to multiple low-dose (40mg/kg BW × 5) injection of STZ, we treated A/J and A.SM-Chr.11K congenic mice. No significant differences were observed between the strains for the incidence of diabetes or blood glucose levels at 25 days after last injection; however, a difference was present for insulin levels (Table 1). In a preliminary study, mice of both strains were injected with 50 mg/kg BW of STZ for 5 consecutive days; all of the mice died within a few days of the final injection (data not shown). These data indicate that the *Stzds1* locus could not protect against the effects of this low-dose treatment.

A number of studies have investigated STZ-induced diabetes susceptibility in genetically manipulated mice; these studies have identified 14 genes involved in susceptibility to STZ-induced diabetes on Chr. 11 (Supplemental Table 3). The N-methylpurine DNA glycosylase (*Mpg*) gene is the only one located within the *Stzds1* region (Supplemental Table 3). This suggests that *Mpg* may be a strong candidate for *Stzds1*. We analyzed the *Mpg* gene structure by amplifying the coding region (4 exons) and splice junctions using the primer pairs listed in Table S2. Four missense variants and one in-frame

variant were identified in the A/J genome (Table 2). In exon 1, nucleotide 40 of the coding region had a T to G alteration that resulted in the conversion of a serine to alanine at amino acid 14 (p.Ser14Ala); another change at nucleotide 139 of a G to C alteration resulted in conversion of a valine to leucine at amino acid 47 (p.Val47Leu) (Supplemental Figs. 2-3). In exon 2, nucleotide 257 had a C to T alteration that resulted in conversion of a serine to leucine at amino acid 86 (p.Ser86Leu) (Supplemental Fig. 4). In exon 4, nucleotides 826 to 828 showed a GTG deletion that resulted in an in-frame variant with a valine deletion at amino acid 276 (p.Val276del) (Supplemental Fig. 5). These three missense variants and the in-frame variant were all located in a region of the MPG protein that does not show conservation among rodent species (Supplemental Figs. 2-5). By contrast, the alteration of nucleotides 394 and 395 from GC to AG in exon 3 resulted in an alanine to serine conversion at amino acid 132 (p.Ala132Ser) in a highly conserved region of the MPG protein (Supplemental Fig. 6). However this variant was predicted to have less effect on MPG in a PROVEAN analysis (score = 0.29) that predicts the effects of amino acid substitutions (Choi and Chan 2015). We performed additional sequence analyses of the five polymorphic sites of the *Mpg* gene in 13 mouse strains (Supplemental Table 4). Three missense variants and one in-frame variant, but not the missense variant at amino acid 14 (p.Ser14Ala), completely classified two types (A/J or SM/J); this fact indicated that these variants lie within the same haplotype block.

It is likely that the p.Ala132Ser amino acid change is the causative variant of *Stzds1*. To confirm the effect of this variant on strain dependent variation in STZ-induced diabetes susceptibility, we tested 13 mouse strains after a single high dose of STZ. Blood glucose levels and incidence of diabetes in the “serine” type strains tended to be higher than those of the normal “alanine” type strains. Strains with strong resistance to induced diabetes, such as NC, CBA/N and C3H/HeN, were all alanine type at this amino acid. However, the differences between the two groups of strains were not significant (Table 3).

Discussion

For this study, we first developed a set of 12 Chr. 11 congenic strains from A/J-11^{SM} consomic mice and the A/J strain (Supplemental Fig. 1). Using these strains we were able to map the *Stzds1* locus. Our previous studies indicated the existence of various QTLs on Chr. 11 for pulmonary adenoma (Pataer et al. 1997), body weight (Ohno et al. 2012), adrenal and kidney weights (Tanaka et al. 2009), liver weight (Kobayashi et al. 2010), blood triglyceride levels (Ohno et al. 2012) and impaired glucose tolerance (Hada et al. 2008) using recombinant inbred (RI) strains, consomic strains, and the parental A/J and SM/J strains. The set of Chr. 11 congenic strains developed here provides a powerful tool for dissecting these QTLs. Along

with the previously established RI strains and consomic strains from A/J and SM/J mice, Chr. 11 congenic strains can also be a useful genetic resource for systematic genetic analysis. These congenic strains are available from RIKEN Bioresource Center (http://www.brc.riken.jp/lab/animal/en/).

The toxicity of STZ is dependent upon the DNA alkylating activity of its methylnitrosourea moiety. The transfer of the methyl group from STZ to the DNA molecule causes damage that eventually results in the fragmentation of the DNA. In an attempt to repair DNA, poly ADP-ribose polymerase (PARP) is upregulated in the base excision repair pathway. Upregulation of PARP diminishes cellular nicotinamide adenine dinucleotide (NAD) levels and, subsequently, ATP stores. The depletion of the cellular energy stores ultimately results in pancreatic ß-cell necrosis (Yamamoto et al 1981; Lenzen 2008; Szkudelski 2012). MPG, a ubiquitous DNA repair enzyme, is responsible for the removal of a wide variety of alkylated base lesions in DNA (Roy et al. 1996; Roy et al. 1998). Thus, there is a close relationship in DNA alkylation between the early stages of STZ action and the function of MPG. Therefore, *Mpg* is an extremely strong candidate gene for *Stzds1*. In a previous study, it was reported that *Mpg* null mice do not show pancreatic ß-cell damage after a single high dose of STZ (Cardinal et al. 2001). It has been suggested that PARP activity is not up-regulated in the islets of MPG deficient mice, preventing the depletion of NAD and ATP and therefore ß-cell necrosis (Wyatt et al. 1999; Cardinal et al. 2001). *Parp* null mice also show resistance to STZ, and thus do not show depletion of NAD and the consequent effects; this behavior is similar to that of *Mpg* null mice (Burkart et al. 1999; Masutani et al. 1999; Pieper et al. 1999). In contrast with *Mpg* null mice, A/J mice show high susceptibility to STZ. It has been presumed that the A/J type of the MPG variant is a gain-of-function variant, and induces excessive recognition and excision of alkylated base lesions in DNA, leading to a higher deficiency of NAD and ATP in the ß-cells.

Two methods can be used to induce diabetes in mice using STZ: a single high dose treatment, or multiple low dose injections over five consecutive days. The onset of diabetes is delayed using the latter method, which causes immune cell infiltration into islets followed by their autoimmune destruction (Kolb 1987; Burns and Gold 2007). The *Stzds1* locus had a large effect on the response of mice to a single high dose of STZ, but not on multiple low doses (Table 2). Interestingly, *Mpg* null mice showed resistance to a single high dose of STZ, but had similar susceptibilities to control mice after multiple low doses of STZ (Cardinal et al. 2001). Thus, there is a similarity in response to multiple low doses of STZ between *Stzds1* locus and *Mpg* null mice. This fact supports the interpretation that *Mpg* is the causative gene of *Stzds1*. There is no definite information on the involvement of DNA repair enzymes in the onset of diabetes, although an association between polymorphism of the *Ogg1* gene, which

encodes a DNA repair enzyme, with type 2 diabetes has been suggested (Daimon et al. 2009; Thameem et al. 2009). DNA lesions caused by reactive oxygen species and other free radicals have been implicated in the etiology of many diseases including diabetes (Hadjivassiliou et al. 1998). In view of the effect of stress on ß-cell fragility, it is likely that mutations of DNA repair enzymes may contribute to the susceptibility for diabetes.

Our sequence analysis of *Mpg* gene identified five polymorphic sites between the A/J and SM/J genomes; the positions of these are shown in Fig. 3. Our analysis suggested that the amino acid change p.Ala132Ser was the most influential of these variants, as the p.Ala132Ser variant was the only one located in the highly conserved region of MPG in rodent species (Supplemental Figs. 2-6) and in the minimal region for retained enzyme activity of MPG (Roy et al. 1998). We performed a preliminary real-time PCR analysis of *Mpg* expression in spleen tissue (Supplemental Table 5) as we have been unable to establish a technique for isolation of pancreatic islets. There was significant difference in *Mpg* expression between SM/J and A.SM-Chr.11C congenic strains, which are both resistant to STZ-induced diabetes, although the A/J strain showed significantly higher level of *Mpg* expression than the SM/J and A.SM-Chr.11C strains (Supplemental Fig. 7). There was no correlation between the level of *Mpg* expression and STZ susceptibility in five other inbred strains (Supplemental Fig. 7). It is likely that *Mpg* expression does not directly influence STZ susceptibility. Therefore, the structural alteration in MPG caused by the p.Ala132Ser variant might result in increased recognition and excision of alkylated base lesions in DNA by STZ; however, this speculation will be confirmed when information on the 3D structure of the MPG protein becomes available. We found no significant differences in diabetes susceptibility between the serine and alanine type strains at amino acid 132 of the MPG protein (Table 3). This indicates that strain dependent variation in susceptibility to STZ-induced diabetes is not simply due to this variant of *Mpg*. The NOD and NSY strains that suggested the existence of a locus on Chr. 11 for susceptibility to STZ-induced diabetes have alanine at this amino acid. Many loci are involved in strain dependent variation for susceptibility to STZ-induced diabetes and it is possible that the p.Ala132Ser variant is one of them.

In conclusion, the *Stzds1* locus was mapped within an 8.67 Mb region (*D11Mit163* - *D11Mit51*) on Chr. 11 by use of set of congenic strains developed from the A/J-11^{SM} strain. We identified the *Mpg* gene as a strong candidate for *Stzds1*. We also identified five variable sites in the *Mpg* gene in A/J genome, and suggested that the p.Ala132Ser amino acid change was the most effective with regard to alteration of susceptibility to STZ-induced diabetes. In future work, production of a knock-in mouse for this variant using the CRISPR/Cas9 system and/or comparison with enzymatic activity of MPG in pancreatic islets among MPG variant

types will help to identify the causative variant of *Stzds1*, and to reveal the molecular mechanisms regulating pancreatic ß-cell fragility.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (23500494 to T. Ohno).

Conflict of interest

The authors declare that they have no conflict of interest.

References

Babaya N, Ikegami H, Fujisawa T, Nojima K, Itoi-Babaya M, Inoue K, Ohno T, Shibata M, Ogihara T (2005) Susceptibility to streptozotocin-induced diabetes is mapped to mouse chromosome 11. Biochem Biophys Res Commun 328:158-164

Bhatnagar S, Oler AT, Rabaglia ME, Stapleton DS, Schueler KL, Truchan NA, Worzella SL, Stoehr JP, Clee SM, Yandell BS, Keller MP, Thurmond DC, Attie AD (2011) Positional cloning of a type 2 diabetes quantitative trait locus; tomosyn-2, a negative regulator of insulin secretion. PLoS Genet 7:e1002323

Burkart V, Wang ZQ, Radons J, Heller B, Herceg Z, Stingl L, Wagner EF, Kolb H (1999)Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozocin. Nat Med 5:314-319

Burns N, Gold B (2007) The effect of 3-methyladenine DNA glycosylase-mediated DNA repair on the induction of toxicity and diabetes by the beta-cell toxicant streptozotocin. Toxicol Sci 95:391-400

Cardinal JW, Margison GP, Mynett KJ, Yates AP, Cameron DP, Elder RH (2001)Increased susceptibility to streptozotocin-induced beta-cell apoptosis and delayed autoimmune diabetes in alkylpurine-DNA-N-glycosylase-deficient mice. Mol Cell Biol 21:5605-5613

Choi Y, Chan AP (2015) PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics 31:2745-2477

Clee SM, Yandell BS, Schueler KM, Rabaglia ME, Richards OC, Raines SM, Kabara EA, Klass DM, Mui ET, Stapleton DS, Gray-Keller MP, Young MB, Stoehr JP, Lan H, Boronenkov I, Raess PW, Flowers MT, Attie AD (2006) Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus. Nat Genet 38:688–693

Daimon M, Oizumi T, Toriyama S, Karasawa S, Jimbu Y, Wada K, Kameda W, Susa S, Muramatsu M, Kubota I, Kawata S, Kato T (2009) Association of the Ser326Cys polymorphism in the OGG1 gene with type 2 DM. Biochem Biophys Res Commun 386:26-9

Dooley J, Tian L, Schonefeldt S, Delghingaro-Augusto V, Garcia-Perez JE, Pasciuto E, Di Marino D, Carr EJ, Oskolkov N, Lyssenko V, Franckaert D, Lagou V, Overbergh L, Vandenbussche J, Allemeersch J, Chabot-Roy G, Dahlstrom JE, Laybutt DR, Petrovsky N, Socha L, Gevaert K, Jetten AM, Lambrechts D, Linterman MA, Goodnow CC, Nolan CJ, Lesage S, Schlenner SM, Liston A (2016) Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes. Nat Genet 48:519-27

Festing MF (1996) Origins and characteristics of inbred strains of mice. In: Lyon MF, Rasten S, Brown SDM (eds) Genetic variants and strains of the laboratory mouse. Oxford University Press, New York, pp 1537–1576

Gonzalez C, Cuvellier S, Hue-Beauvais C, Lévi-Strauss M (2003) Genetic control of non obese diabetic mice susceptibility to high-dose streptozotocin-induced diabetes. Diabetologia 46:1291-1295

Hada N, Kobayashi M, Fujiyoshi M, Ishikawa A, Kuga M, Nishimura M, Ebihara S, Ohno T, Horio F (2008) Quantitative trait loci for impaired glucose tolerance in nondiabetic SM/J and A/J mice. Physiol Genomics 35:65-74

Hadjivassiliou V, Green MH, James RF, Swift SM, Clayton HA, Green IC (1998) Insulin secretion, DNA damage, and apoptosis in human and rat islets of Langerhans following exposure to nitric oxide, peroxynitrite, and cytokines.Nitric Oxide 2:429-41

Kaku K, Fiedorek FT Jr, Province M, Permutt MA (1988) Genetic analysis of glucose tolerance in inbred mouse strains. Evidence for polygenic control. Diabetes 37:707-713

Kaku K, McGill J, Province M, Permutt MA (1989) A single major gene controls most of the difference in susceptibility to streptozotocin-induced diabetes between C57BL/6J and C3H/HeJ mice. Diabetologia 32:716-723

Kikutani H, Makino S (1992) The murine autoimmune diabetes model: NOD and related strains. Adv Immunol 51:285-322

Kobayashi M, Ohno T, Hada N, Fujiyoshi M, Kuga M, Nishimura M, Murai A, Horio F (2010) Genetic analysis of abdominal fat distribution in SM/J and A/J mice. J Lipid Res 51:3463-3469

Kolb H (1987) Mouse models of insulin dependent diabetes: low-dose streptozocin-induced diabetes and nonobese diabetic (NOD) mice. Diabetes Metab Rev 3:751-778

Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51:216-226

Liston A, Todd JA, Lagou V (2017) Beta-Cell Fragility As a Common Underlying Risk Factor in Type 1 and Type 2 Diabetes.Trends Mol Med 23:181-194

Masutani M, Suzuki H, Kamada N, Watanabe M, Ueda O, Nozaki T, Jishage K, Watanabe T, Sugimoto T, Nakagama H, Ochiya T, Sugimura T (1999) Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes. Proc Natl Acad Sci USA 96:2301-2304

Nadeau JH, Singer JB, Matin A, Lander ES (2000) Analysing complex genetic traits with chromosome substitution strains. Nat Genet 24:221–225

Nishimura M, Hirayama N, Serikawa T, Kanehira K, Matsushima Y, Katoh H, Wakana S, Kojima A, Hiai H (1995) The SMXA: a new set of recombinant inbred strain of mice consisting of 26 substrains and their genetic profile. Mamm Genome 6:850–857

Ohno T, Hata K, Baba T, Io F, Kobayashi M, Horio F, Nishimura M (2012) Establishment of consomic strains derived from A/J and SM/J mice for genetic analysis of complex traits. Mamm Genome 23:764-769

Pataer A, Nishimura M, Kamoto T, Ichioka K, Sato M, Hiai H (1997) Genetic resistance to urethan-induced pulmonary adenomas in SMXA recombinant inbred mouse strains. Cancer Res 57:2904-2908

Pieper AA, Brat DJ, Krug DK, Watkins CC, Gupta A, Blackshaw S, Verma A, Wang ZQ, Snyder SH (1999) Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. Proc Natl Acad Sci USA 96:3059-3064

Rogner UC, Avner P (2003) Congenic mice: cutting tools for complex immune disorders. Nat Rev Immunol 3:243-252

Rossini AA, Appel MC, Williams RM, Like AA (1977) Genetic influence of the streptozotocin-induced insulitis and hyperglycemia. Diabetes 26:916-920

Roy R, Kumar A, Lee JC, Mitra S (1996) The domains of mammalian base excision repair enzyme N-methylpurine-DNA glycosylase. Interaction, conformational change, and role in DNA binding and damage recognition. J Biol Chem 271:23690-23697

Roy R, Biswas T, Hazra TK, Roy G, Grabowski DT, Izumi T, Srinivasan G, Mitra S (1998) Specific interaction of wild-type and truncated mouse N-methylpurine-DNA glycosylase with ethenoadenine-containing DNA. Biochemistry 37:580-589

Singer JB, Hill AE, Burrage LC, Olszens KR, Song J, Justice M, O'Brien WE, Conti DV, Witte JS, Lander ES, Nadeau JH (2004) Genetic dissection of complex traits with chromosome substitution strains of mice. Science 304:445–448

Stylianou IM, Clinton M, Keightley PD, Pritchard C, Tymowska-Lalanne Z, Bunger L,

Horvat S (2005) Microarray gene expression analysis of the Fob3b obesity QTL identifies positional candidate gene Sqle and perturbed cholesterol and glycolysis pathways. Physiol Genomics 20:224–232

Szkudelski T (2012) Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med 237:481-490

Takada T, Mita A, Maeno A, Sakai T, Shitara H, Kikkawa Y, Moriwaki K, Yonekawa H, Shiroishi T (2008) Mouse inter-subspecific consomic strains for genetic dissection of quantitative complex traits. Genome Res 18:500–508

Tanaka S, Mizorogi T, Nishijima K, Kuwahara S, Tsujio M, Aoyama H, Taguchi C, Kobayashi M, Horio F, Ohno T (2009) Body and major organ weights of A/J-Chr11^{SM} consomic mice. Exp Anim 58:357-361

Thameem F, Puppala S, Lehman DM, Stern MP, Blangero J, Abboud HE, Duggirala R, Habib SL (2009) The Ser(326)Cys polymorphism of 8-oxoguanine glycosylase 1 (OGG1) is associated with type 2 diabetes in Mexican Americans. Hum Hered 70:97-101

Ueda H, Ikegami H, Yamato E, Fu J, Fukuda M, Shen G, Kawaguchi Y, Takekawa K, Fujioka Y, Fujisawa T, Nakagawa Y, Hamada Y, Shibata M, Ogihara T (1995) The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. Diabetologia 38:503-508

Wyatt MD, Allan JM, Lau AY, Ellenberger TE, Samson LD (1999) 3-methyladenine DNA glycosylases: structure, function, and biological importance.Bioessays 21:668-676

Yamamoto H, Uchigata Y, Okamoto H (1981) Streptozotocin and alloxan induce DNA strand breaks and poly (ADP-ribose)synthetase in pancreatic islets. Nature 294:284–286

Figure legends

Fig. 1 Time-dependent changes in the incidence of diabetes (A), blood glucose level (B) and blood insulin level (C) at 14 days after injection of 175 mg/kg BW STZ in A/J, SM/J and A/J-11^{SM} strains. * $P < 0.05$ vs A/J (incidence of diabetes by Fisher's exact test, and for blood glucose and insulin by Bonferroni's test).

Fig. 2 Congenic mapping of *Stzds1* (Streptozotocin susceptible locus 1) region. White areas are A/J derived genomic regions, black areas are SM/J derived genomic regions, and gray areas are regions not definitively identified as A/J or SM/J genome. * $P < 0.05$ vs A/J (Fisher's exact test), $^{\#}$ $P < 0.05$ vs A/J (Dunnett's test) with effect size (Cohen's d) in parentheses.

Fig. 3 Schematic diagram of the mouse MPG protein including the five identified variants in the A/J strain. Numbers correspond to the amino acid sequence.

Table 1 Incidence of diabetes, blood glucose and blood insulin level in A/J and A.SM-Chr.11K male mice at 25 days after 5×40mg/kg BW injection of STZ

Strain	Number	Incidence of Diabetes (%)	Blood Glucose (mg/dL)	Blood Insulin (ng/mL)
A/J	12	17	169±27	0.127±0.008
A.SM-Chr.11K	10	0	140±9	0.164±0.010* (1.24)

* P < 0.05 vs A/J（t-test）with effect size (Cohen's d) in parentheses.

Table 2 List of variants in *Mpg* gene between A/J and SM/J strains

Location (bp)	Nucleotide Position	Exon	Amino Acid (AA) Position	SM/J		A/J		dbSNP
				Allele	AA	Allele	AA	
32,226,580*	c.40	1	14	T	Ser	G	Ala	rs13465944
32,226,679**	c.139	1	47	G	Val	C	Leu	rs29471099
32,227,831**	c.257	2	86	C	Ser	T	Leu	rs26841239
32,229,867 - 32,229,868**	c.394 - c.395	3	132	GC	Ala	AG	Ser	rs4228650, rs4228649
32,231,819 - 32,231,821**	c.826 - c.828	4	276	GTG	Val	—	—	rs234739634

* A/J allele is consistent with the reference genome sequence（GRCm38）

** SM/J allele is consistent with the reference genome sequence（GRCm38）

Table 3 STZ-induced diabetes susceptibility of 15 inbred mouse strains that are classified according to amino acid 132 of the MPG protein.

Amino Acid 132	Strain	Number	Blood Glucose (mg/dl)	Incidence (%)
Serine	MSM/Ms	11	523±43	82
	NZW/N	12	501±36	92
	A/J	20	462±9	100
	DBA/2J	11	265±30	55
	AKR/N	18	201±20	17
	Average	5	390±66	69±15
Alanine	NSY	14	460±42	92
	FVB	10	430±36	90
	NOD	9	436±76	67
	C57BL/6J	12	408±49	75
	SM/J	20	314±37	50
	KK	10	301±33	70
	BALB/c	12	188±32	33
	NC	16	153±3	0
	CBA/N	11	149±14	0
	C3H/HeN	12	145±4	0
	Average	10	299±42	47±12

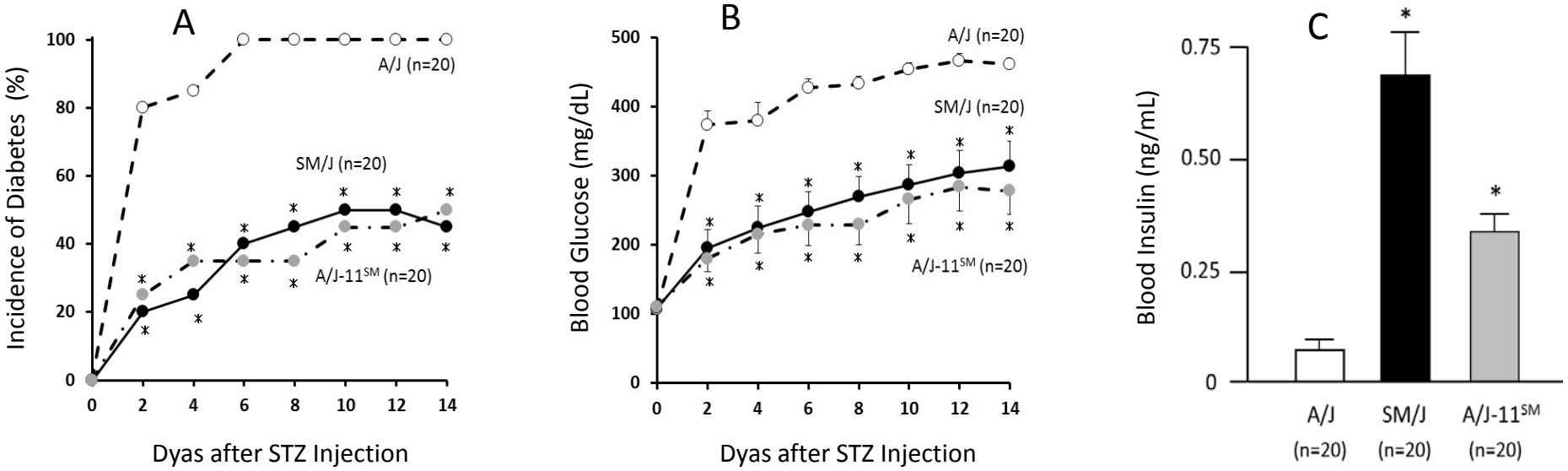
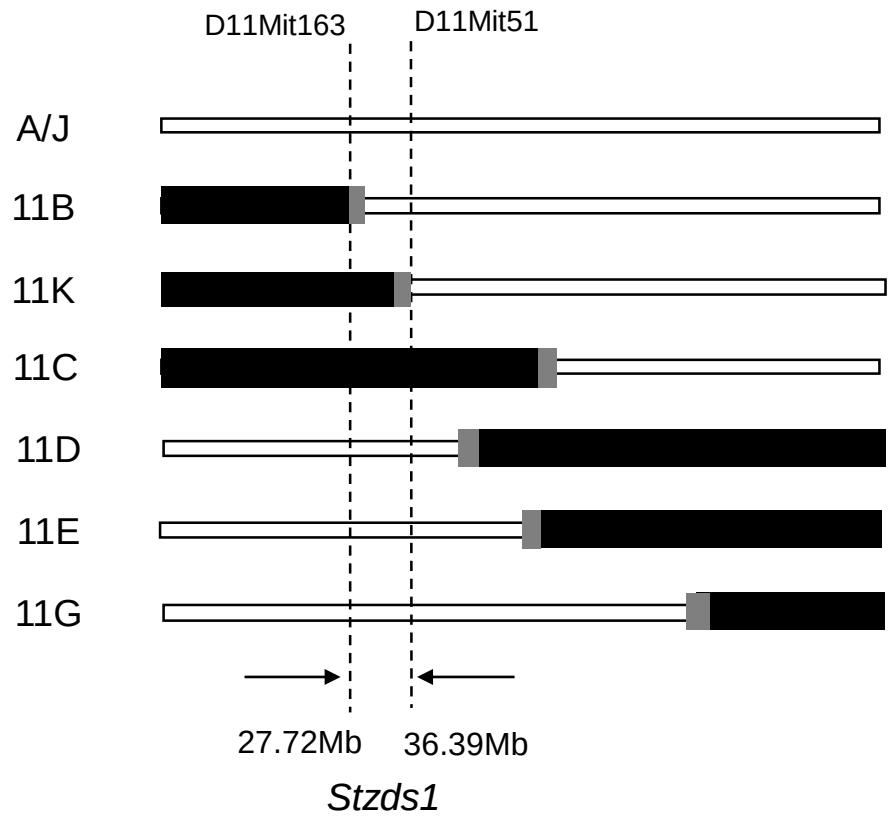

A
Incidence of Diabetes (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Dyas after STZ Injection
A/J (n=20)
SM/J (n=20)
A/J-11SM (n=20)
B
Blood Glucose (mg/dL)
500
400
300
200
100
0
0
2
4
6
8
10
12
14
Dyas after STZ Injection
A/J (n=20)
SM/J (n=20)
A/J-11SM (n=20)
C
Blood Insulin (ng/mL)
0.75
0.50
0.25
0
A/J
(n=20)
SM/J
(n=20)
A/J-11SM
(n=20)

Fig. 1



Strain	n	Incidence of diabetes (%)	Blood glucose (mg/dl)	Blood insulin (ng/ml)
A/J	20	100	462±9	0.06±0.01
11B	12	100	494±10	0.06±0.01
11K	17	6*	130±14# (6.75)	0.13±0.01
11C	23	13*	183±20# (3.66)	0.18±0.04# (0.80)
11D	14	86	404±33	0.09±0.03
11E	18	83	394±29	0.08±0.01
11G	14	93	411±22	0.07±0.01

Fig. 2

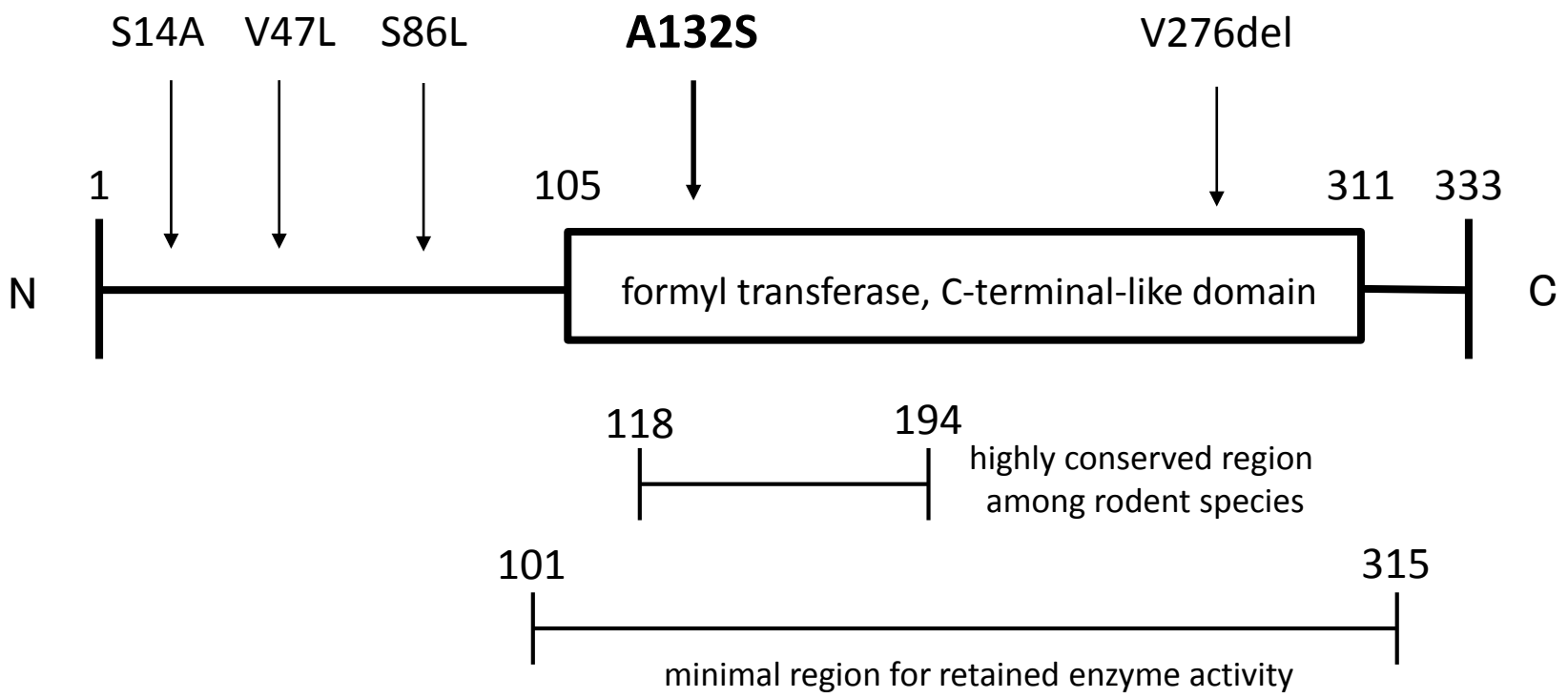

S14A
V47L
S86L
A132S
V276del
1
105
311
333
N
formyl transferase, C-terminal-like domain
C
118
194
highly conserved region
among rodent species
101
315
minimal region for retained enzyme activity

Fig. 3