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Glucose tolerance female-specific QTL mapped in collaborative cross mice

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Abstract Type-2 diabetes (T2D) is a complex metabolic disease characterized by impaired glucose tolerance. Despite environmental high risk factors, host genetic background is a strong component of T2D development. Herein, novel highly genetically diverse strains of collaborative cross (CC) lines from mice were assessed to map quantitative trait loci (QTL) associated with variations of glucose-tolerance response. In total, 501 mice of 58 CC lines were maintained on high-fat (42 % fat) diet for 12 weeks. Thereafter, an intraperitoneal glucose tolerance test (IPGTT) was performed for 180 min. Subsequently, the values of Area under curve for the glucose at zero and 180 min (AUC_{0-180}), were measured, and used for QTL mapping. Heritability and coefficient of variations in glucose tolerance (CVg) were calculated. One-way analysis of variation was significant ($P < 0.001$) for AUC_{0-180} between the CC lines as well between both sexes. Despite Significant variations for both sexes, QTL analysis was significant, only for females, reporting a significant female-sex-dependent QTL (~2.5 Mbp) associated with IPGTT AUC_{0-180} trait, located on Chromosome 8 (32–34.5 Mbp, containing 51 genes). Gene browse revealed QTL for body weight/size, genes involved in immune system, and two main protein-coding genes involved in the Glucose homeostasis, Mboat4 and Leprotl1. Heritability and coefficient of genetic variance (CVg) were 0.49 and 0.31 for females, while for males, these values 0.34 and 0.22, respectively. Our findings demonstrate the roles of genetic factors controlling glucose tolerance, which significantly differ between sexes requiring independent studies for females and males toward T2D prevention and therapy.

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Introduction

Type-2 diabetes (T2D) is one of the four leading causes of non-communicable diseases (NCDs) deaths in 2012, while, according to the World Health Organization (WHO) report for 2014, 1.5 million deaths of a total 38 million that occurred in 2012 were caused by diabetes (WHO 2014a, b). The annual number of deaths due to NCDs is steadily increasing (unlike infectious diseases) and projected to increase from 38 million in 2012 to 52 million by 2030, whereas Diabetes is predicted to be the seventh cause of death (with the projected increase of 2–3 places in rank from 2012 to 2030) (Mathers and Loncar 2006). Etiology of T2D is widely diverse involving multiple environmental (lifestyle, physical activity, stress, diet, age, etc.) and genetic factors and their complex interactions. T2D is one of the most ancient known human diseases (Ahmed 2002), characterized by complex pathogenesis and despite the

significant improvement through the years in T2D prevention and therapies via lifestyle and pharmacological intervention (Stevens et al. 2015), the genetic architecture of the disease remains still unexplained. Susceptibility toward development and progress of T2D differ between ethnic and ancestry (genomic heritage) groups in response to common environmental conditions. Individuals from different racial or ethnic groups experience the disease differently at the level of comorbidities, and at the level of Glycemic control despite socioeconomic status or health insurance availability (Saydah et al. 2007; Harris et al. 1999; Qi et al. 2008). Since the introduction of genome-wide association studies (GWAS), considerable efforts have been invested in exploring genes–environment interactions toward higher risk of T2D. Diet–gene interactions were one of the leading studies among populations, and several studies showed that Westernized dietary patterns had been associated with higher risk of impaired glucose tolerance and T2D (Van Dam et al. 2002; Gittelsohn et al. 1998; Qi and Liang 2010). To date, GWAS studies revealed approximately 88 genetic loci associated with T2D and 83 related with glycemic traits (glucose, HbA1c, insulin, and proinsulin) (Mohlke and Boehnke 2015). Dissecting the genetic architecture of T2D development and progress is crucial for the invention of personalized genetic risk-prediction tools and discovery of new therapeutic implementations among populations (Bao et al. 2013). Major limitations of the human GWAS is the lack of proper, controlled, environmental factors, which might generate biased results. Therefore, using the mouse model in the basal studies of complex diseases is widely accepted, where researchers can control the environmental factors while scanning mice from different genetic backgrounds. In this context, T2D is considered a complex trait disease, where the environmental challenge is the Western diet. The mouse model resource used in this study is the new CC, a unique model for dissecting QTL of host susceptibility toward the development of T2D in response to high-fat (42 % Fat) dietary challenge. The CC population was created by a community effort of the complex trait consortium (CTC, www.complextrait.org) (Churchill et al. 2004; Iraqi et al. 2012; Threadgill and Churchill 2012). This unique genetic resource comprises a set of approximately 300 recombinant inbred lines (RIL) that were created by full reciprocal 8-way matings of eight divergent strains of mice: A/J, C57BL/6 J, 129S1/SvImJ, NOD/LtJ, NZO/HiLtJ, CAST/Ei, PWK/PhJ, and WSB/EiJ. Controlled randomization and minimization of selection during the breeding process will recombine the natural genetic variation present in these inbred strains. The result is a unique collection of RIL exhibiting a large phenotypic and genetic diversity, and brings the tremendous genetic variation potential of the mouse inbred lines to phenotypic expression (Paterson 1995; Churchill et al. 2004). Full details of CC lines and their powers of dissecting and mapping QTL associated with host susceptibility to complex traits are presented elsewhere (Iraqi et al. 2008; Durrant et al. 2011; Aylor et al. 2011; Philip et al. 2011; Iraqi et al. 2012; Shusterman et al. 2013; Iraqi et al. 2014; Soller and Iraqi 2014; Vered et al. 2014; Welsh et al. 2012; Lore' et al. 2015; Levy et al. 2015; Abu Toamih-Atamni et al. 2016a, b; Dorman et al. 2016).

Herein we demonstrate the exclusive use of the CC unique mouse reference population to identify QTL and subsequently genes associated with host glucose tolerance in response to high-fat diet.

Materials and methods

Ethical statement

Mice were housed in the small animal facility of the Sackler faculty of medicine at the Tel-Aviv University (TAU) according to standard protocol approved by the animal use and care committee at TAU (approved experiment number M-12-025).

Housing and diet

Mice were housed, separately by sex and CC line on hardwood chip bedding in open-top cages, kept under 12-h light/dark cycle (6:00 am–6:00 pm) at 21–23 °C, and fed with tap water and standard rodent chow diet ad libitum (TD.2018SC, Teklad Global, Harlan Inc., Madison, WI, USA; containing %Kcal from Fat 18 %, Protein 24 %, Carbohydrates 58 %), since weaning at age of 3 weeks, until start of the experiment at the age of 8 weeks. To induce the development of diabetogenic response, the mice were fed by a purified experimental high-fat diet (TD 88137 Harlan Teklad, Madison, WI, USA; containing 42 % of calories from fat and 34.1 % from carbohydrate, primarily sucrose) with their ages ranging from 8 to 20 weeks.

CC lines

All the CC lines used in this study are being developed at TAU small animal facility. CC lines are developed by full-sib mating technique to exceed the currently inbreeding generation 16 (G16); further details of the TAU CC colony are available in previous reports (Iraqi et al. 2008, 2012). In the current study, we used 501 mice generated from 58 CC lines, with representation of both sexes. In detail, our study cohort consisted of 200 female mice from 44 CC lines and 301 male mice from 56 CC lines. Sex presentation was not equal for all CC lines due to breeding variations between lines.

Genotype

All CC lines were genotyped with high-density SNP markers, as previously described, and used (Yang et al. 2009; Iraqi et al. 2012; Vered et al. 2014; Levy et al. 2015; Abu Toamih-Atamni et al. 2016b). In brief, all SNPs were filtered out with heterozygous or missing genotypes in the 8 CC founders, or those that were not in common between the arrays. The SNPs were mapped onto build 37 of the mouse genomes. The HAPPY HMM computed a descent probability distribution for each of the used SNPs.

Phenotype

Diabetogenic effect in response to high-fat dietary challenge was measured by the intraperitoneally glucose tolerance test (IPGTT) (Montgomery et al. 2013; Leiter 2009) after 12 weeks of dietary challenge. For the IPGTT, mice were fasted for 6 h (6:00–12:00am), with free access to water. Subsequently, fasting blood glucose levels were determined at time 0, and then a solution of glucose (2.5gr glucose per Kg mouse) was administered by intraperitoneal (IP) injection, and blood glucose levels were monitored for 180 min (time 0, 15, 30, 60, 120, and 180 min). Blood glucose levels were measured from tail vein using U-RIGHT glucometer TD-4267 (TaiDoc Technology Corporation 3F, 5F, No. 127, Wungong 2nd Rd., 24888 Wugu Township, Taipei County, Taiwan). Quantitative glucose clearance ratio of the total 180 min IPGTT was calculated using area under curve (AUC) trapezoidal model from time zero (0) to time 180 min, as shown below:

$$AUC_{0-180} = 180\,minutes * (Glucose\ level\ at\ time\ 0 + Glucose\ level\ at\ time\ 180)/2$$

Full details of the recorded traits on these mice are presented in Abu Toamih-Atamni et al. (2016a).

Statistical analysis

Data analysis was performed using a statistical software package IBM SPSS statistics 23. One-Way ANOVA was carried out for testing the significance of the variations of the AUC_{0-180} means between the different CC lines, and a P value of 0.05 or less was considered significant.

Heritability and coefficient of genetic variation

Heritability (H^2) is a statistic that estimates the fraction of phenotypic variance that can be attributed to genetic variance among the population. Here, we used the ANOVA results of the AUC_{0-180} trait to calculate the broad sense heritability (including epistatic, but not dominance effects) using the H^2 formula as given below:

$$H^2 = V_g/(V_g + V_e)$$

where H^2 is the heritability, V_g is the genetic variance among CC lines (($MS_{between}–V_e$)/n), and V_e is the environment variance (MS_{within} = variance within CC line).

Following heritability statistic, we calculated the genetic coefficient of variation (CV_G), which indicates the absolute amount of genetic variation (between CC lines). CV_G was calculated using the results of standard deviation among CC lines (SD_G) and trait mean (AUC_{0-180}) overall CC lines, as below:

$$CV_G = SD_G/Mean$$

For further details on calculations, see Iraqi et al. (2014).

QTL mapping

The HAPPY package also tests for the existence of a QTL at each locus using the estimated probabilities of descent from the founder strains to estimate the phenotypic effect attributable to each founder strain. If these effects are significantly different, then there is evidence for a QTL. Thus, for the initial stage of linkage analysis based on single trait analysis, a standard polyallelic "marker" model will be used. This model tests to determine whether there is an overall association between the marker and the trait by comparing after fitting the complete model including the mean, any fixed effects such as sex and generation, and the marker alleles.

Merge analysis

Testing of the sequence variations for segregation between the CC founders was performed, using merge analysis to fine-tune the AUC_{0-180} QTL and identify candidate genes. Analysis methodology will be merged (Yalcin et al. 2005) to test which variants under a QTL peak were compatible with the pattern of action at the QTL. We used the Perlegen SNP database to test sequence variants globally and the Sanger SNP database for individual genes. A successful use of this approach has recently been published (Durrant et al. 2011; Vered et al. 2014; Levy et al. 2015; Abu-Toamih Atamni et al. 2016b).

Genome browser

Using the mouse genome informatics (MGI) database resource (http://www.informatics.jax.org/), enabled us to examine the reported QTL in detail for genetic features located within the QTL genomic interval, related to glucose homeostasis/metabolism or the involved systems in

type-2 diabetes, such as immune/endocrine systems. The MGI search revealed known genes, mRNAs, QTLs, and many more. Each result observed at the MGI was further investigated in the literature for biological roles.

Results

Following 12 weeks of high-fat dietary (HFD) challenge, diabetogenic response of the CC lines was assessed by an Intraperitoneal Glucose Tolerance Test (IPGTT) for 180 min. Thereafter, the overall area under curve (AUC_{0-180}) of the glucose clearance was calculated and used as an indicator for diabetic status, where higher levels of AUC_{0-180} were associated with slower glucose clearance and even impaired glucose tolerance. Genotypic data of the CC lines was scanned using the HAPPY software for genetic linkage with the trait AUC_{0-180} , first for overall population and then for females and males separately. All results are reported in detail below, starting from phenotypic data, heritability and CV_g calculations, to QTL analysis and founders' effect analysis, ending with the identification of candidate genes.

Significant variations of glucose tolerance between CC lines

As shown in Fig. 1 (a–c), ANOVA revealed highly significant variations for AUC_{0-180} means between the overall CC lines population (Fig. 1a) (F (57,443) = 4.93, $P < 0.001$), as well as for females (Fig. 1b) (F (43,156) = 4.38, $P < 0.001$) and males (Fig. 1C) (F (55,245) = 3.23, $P < 0.001$), separately. The overall CC lines population ($n = 501/58$ CC lines) showed mean AUC_{0-180} of 45515.6 (±846.8) mg/dL*min, while the mean values ranged between IL-2146 representing the lowest value of 24,345 (±6345) mg/dL*min and IL-2452 representing the highest value of 82,575, and IL-2457 located at median point of 45,495 (± 8685) mg/dL*min.

Females AUC_{0-180} significantly lower than males

From the total assessed 58 CC lines, 42 lines had representation of both sexes (Females–$n = 194$/Males–$n = 245$). Two-way ANOVA was used to assess the significance of sex and line interaction (combined effects) affecting AUC_{0-180} means variations. ANOVA output revealed a highly significant independent sex and line effects ($P < 0.001$), and significant Sex * Line interactions ($P < 0.05$) influencing AUC_{0-180} variation of means. The reported AUC_{0-180} means were 35847.1 (±1285.6) mg/dL*min for females versus 48690.1 (±1209.1) mg/dL*min for males, ~1.4-fold difference in favor of females at 83 % (35 of 42 CC lines) of the cases and in favor of males only at 16 % (7 of 42 CC lines) of the cases (lower AUC_{0-180} indicates effective glucose clearance). The difference of AUC_{0-180} means between sexes within each individual CC line, varied in a wide range among the CC population as shown in Fig. 2.

Heritability and genetic coefficient variation

Heritability estimates have a value between 0 and 1. As shown in Table 1, heritability (H^2) and genetic coefficient variation (CV_G) calculations revealed values of 0.34 and 0.25, respectively, for the overall CC lines population. When data were classified by sex, H^2 and CV_G values were, respectively, 0.49 and 0.31 for females, while 0.34 and 0.22 for males. H^2 value of 0.5 and above can be considered as the high heritability of the trait; hence, the reported AUC_{0-180} H^2 value of the females (0.49) is high, indicating that 49 % of the observed variation of AUC_{0-180} between the CC lines is due to genetic factors. Consequently, CV_G value of the females reveals that 31 % of the genetic variations among the CC lines were generated by these genetic factors.

Female-dependent QTL associated with AUC_{0-180} trait and founder effect

Initially, we performed QTL mapping for AUC_{0-180} trait for the entire population of the 501 mice of the 58 CC lines, including males and females. However, no significant QTLs were detected. Subsequently, we performed QTL mapping for male and females, separately. Interestingly, data analysis for male only, did not show any significant QTL. However, the female data, indeed, showed significant QTL located on Chr8 with genomic interval of 2.5 Mbp (32.02–34.52 Mbp) (logP = 5.9) as presented in Fig. 3. The threshold based on permutation test is presented in figure and found to be −logP = 5.65.

Founder effect

We evaluated the effect of each founder genotype at the females AUC_{0-180} mapped QTL interval, and estimated to the assessed trait. Results of this analysis are presented for the QTL in Fig. 4. The locus showed complex pattern of haplotype effects of the founders, with the wild-derived strains (mainly PWK) playing a major role on increase AUC values, while NZO genotype reduces this trait, although other strains also contributed (positive or negative) to the overall QTL effect.

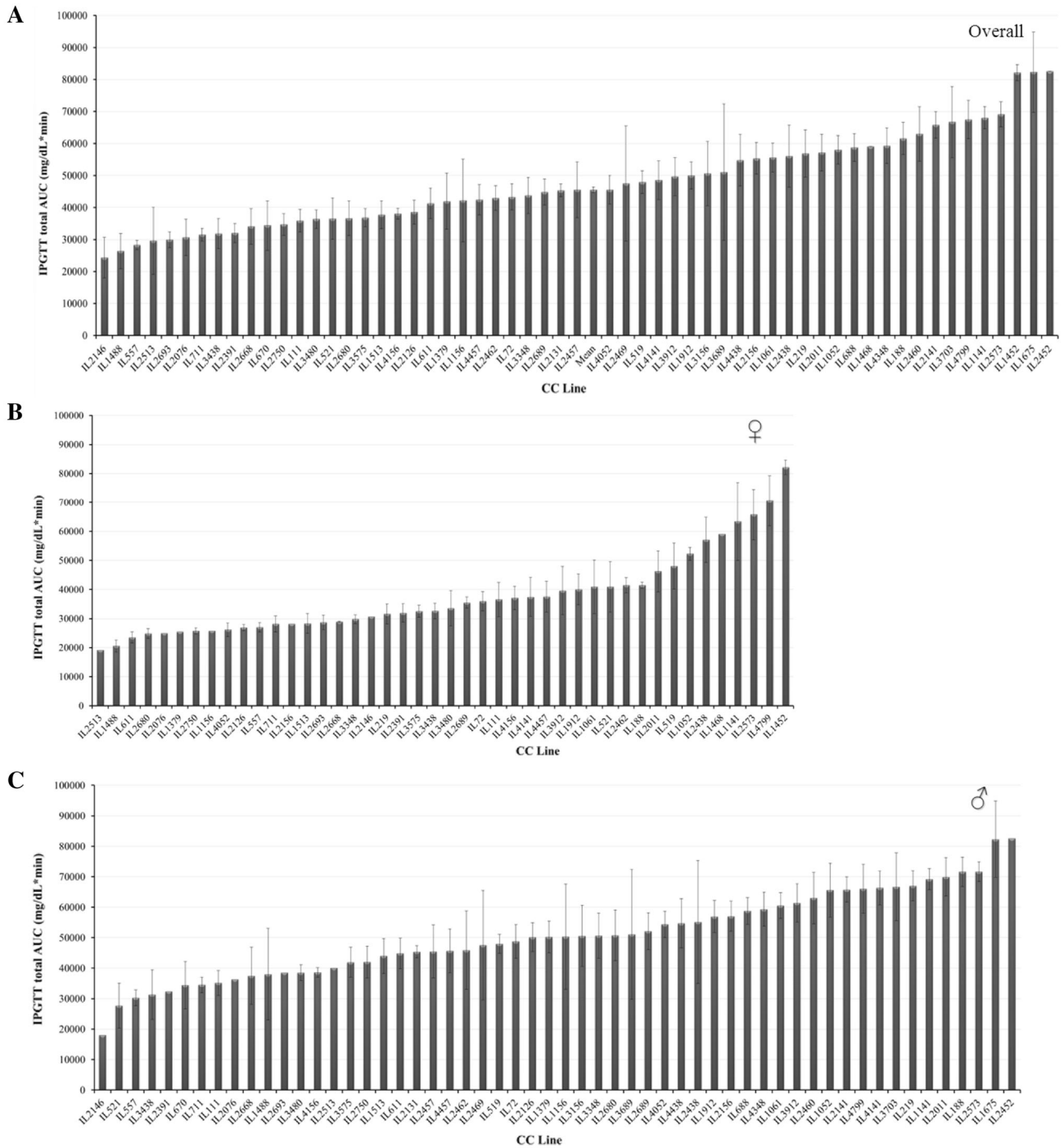

Fig. 1 Total area under curve (AUC_{0-180}) of glucose clearance (min*mg/dL) at initial (time 0) and 180 min of intraperitoneal glucose tolerance test (IPGTT) of different CC lines in response to HF (42 % Fat) dietary challenge. Bar graph **a** shows IPGTT total AUC of 58 CC lines of overall CC lines population. Bar graph **b** shows IPGTT total AUC of females from 44 CC lines. Bar graph **c** shows total AUC of males from 56 CC lines. *X-axis* line number, represents the different CC lines. *Y-axis* represents the total area under curve of glucose clearance (min*mg/dL) at initial (time 0) and 180 min of intraperitoneal glucose tolerance test (IPGTT). Significant variation was found between the different CC lines at $P < 0.001$

Merge analysis

During the testing sequence variations segregation between the CC founders, using merge analysis to fine-tune the AUC_{0-180} QTL and identify candidate genes. Figure 5 shows the merge analysis, and interestingly our results have shown that the majority of the SNPs within the haplotype QTL interval are highly significant and enabled

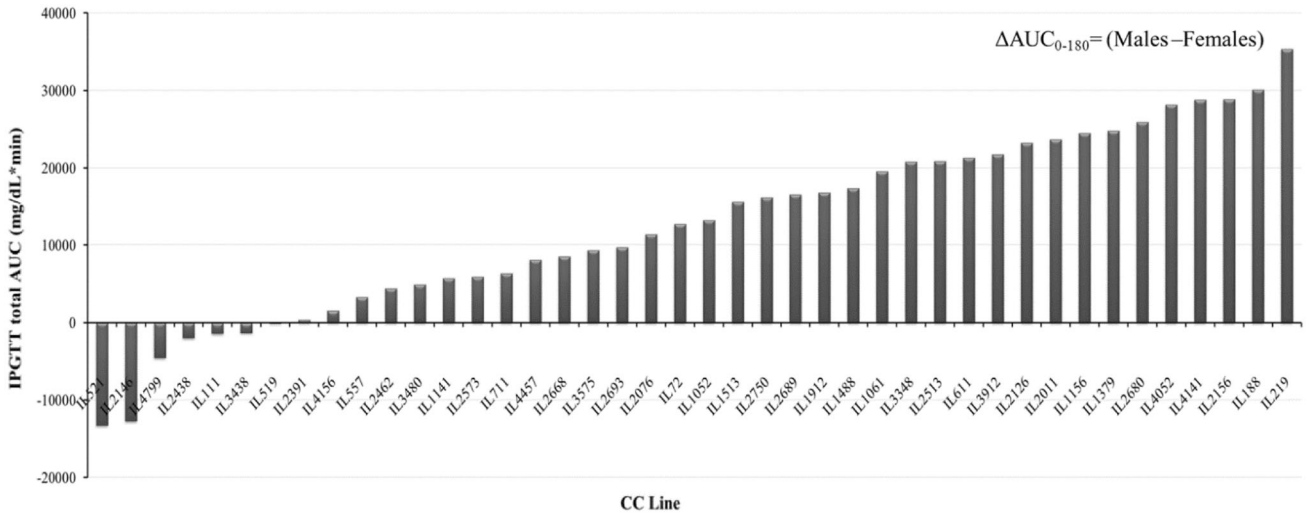

Fig. 2 Difference between sexes of AUC_{0-180} (mg/dL*min) means within each CC line (Total 42 CC lines) in response to HF (42 % Fat) dietary challenge. The difference calculated between means of AUC_{0-180} of males and females from the same CC line (ΔAUC_{0-180} = Males–Females). *X-axis* represents the different CC lines. *Y-axis* represents the total area under curve of glucose clearance (min*mg/dL) at initial (time 0) and 180 min of intraperitoneal glucose tolerance test (IPGTT)

Table 1 Positions of the QTL underlying the total area under curve of glucose clearance trait (AUC_{0-180} QTL) associated with impaired glucose tolerance in response to 12 weeks HF (42 % Fat) dietary challenge in Collaborative Cross (CC) lines

Sex	QTL	Chr	logP	Sig. level	Location (Mb)	Width (Mb)	No. of genes	H^2	CVg
♀	AUC_{0-180}	8	5.65	0.05	32.02–34.52	2.50	13	0.49	0.31
♂	–	–	–	–	–	–	–	0.34	0.22
Both	–	–	–	–	–	–	–	0.34	0.25

QTL quantitative trait loci, *Chr* chromosome, $-logP$ negative logarithm of significance value, H^2 estimated broad-sense heritability, *CVg* coefficient of genetic variation

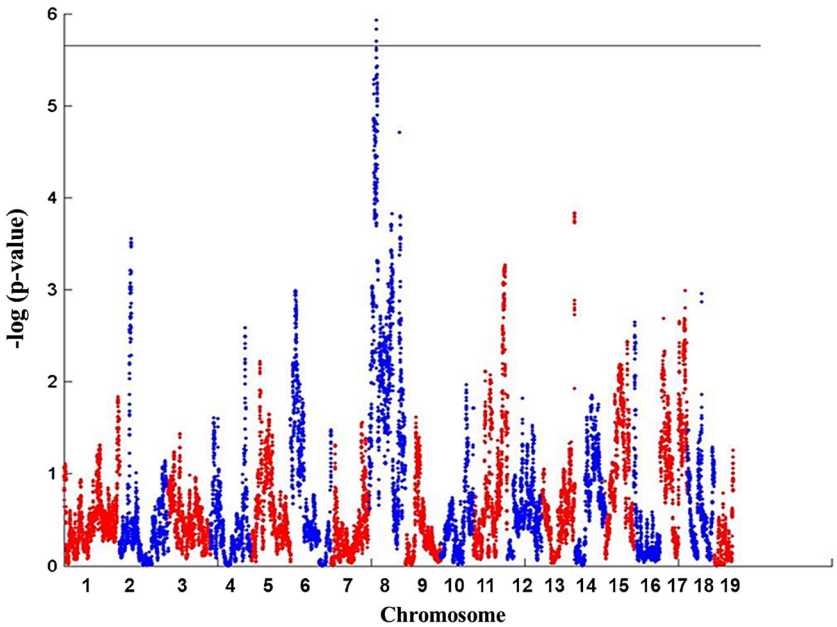

Fig. 3 Genome scan of Quantitative Trait Loci (QTL) associated with total area under curve of glucose clearance (AUC_{0-180} (mg/dL*min)) trait in female mice, in a population of 44 Collaborative Cross (CC) lines after 12 weeks on high-fat (42 % Fat) dietary challenge. *X-axis* represents the 19 mouse Chromosomes and the position of mapped QTL on Chromosome 8 (Chr8). *Y-axis* represents the logP of the test of association between locus and AUC_{0-180} trait. QTL with logP exceeded 5.65 threshold, based on permutation genome-wide test, at significant level of $P < 0.05$, was identified

higher resolution of the QTL peak location (logP score = 5.9, threshold 5.65 at P value < 0.05).

Identification of candidate genes within the mapped QTL interval

The reported QTL contains 13 protein-coding genes, 10 QTLs and other genetic features (non-coding RNA/heritable phenotypic markers/gene segments/pseudogenes). The protein coding genes with most relevance to glucose tolerance were related to immune system, endocrine system and regulation of glucose homeostasis.

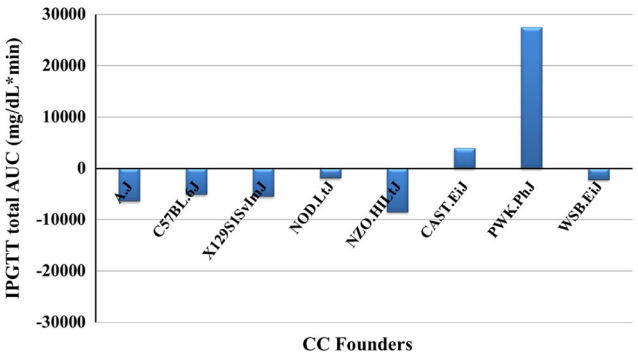

Fig. 4 Estimated effect size on total area under curve of glucose clearance AUC_{0-180} (mg/dL*min)) for the 8 CC founder strains for Chromosome 8 QTL. *X-axis* represents eight founder strains of the CC mice. *Y-axis* represents haplotype effect size of the CC founder at the AUC_{0-180} QTL

With most relevance to the glucose homeostasis traits, we may suggest genes involved in regulation glucose homeostasis, such as Mboat4 (MGI:2685017), named membrane-bound *O*-acyltransferase domain containing 4, which encodes enzymes that transfer organic acids, typically fatty acids, onto hydroxyl groups of membrane-embedded targets (Hofmann 2000). MBOAT4 mediates the octanoylation of the stomach hormone *ghrelin* (GOAT—Ghrelin *O*-Acyltransferase), which plays endocrine role in the regulation of glucose homeostasis (Yang et al. 2008; Kirchner et al. 2013).

Another major candidate gene is Leprotl1 (MGI:1915442), named leptin receptor overlapping transcript-like 1, plays a role in the control of hepatic growth hormone (GH) resistance to involved between nutritional signals and GH actions on body growth and metabolism. LEPROTL1 expression is regulated in the mouse liver by physiologic and pathologic changes in glucose homeostasis (Touvier et al. 2009). As well, we observed the Wrn gene (MGI:109635), named Werner syndrome homolog

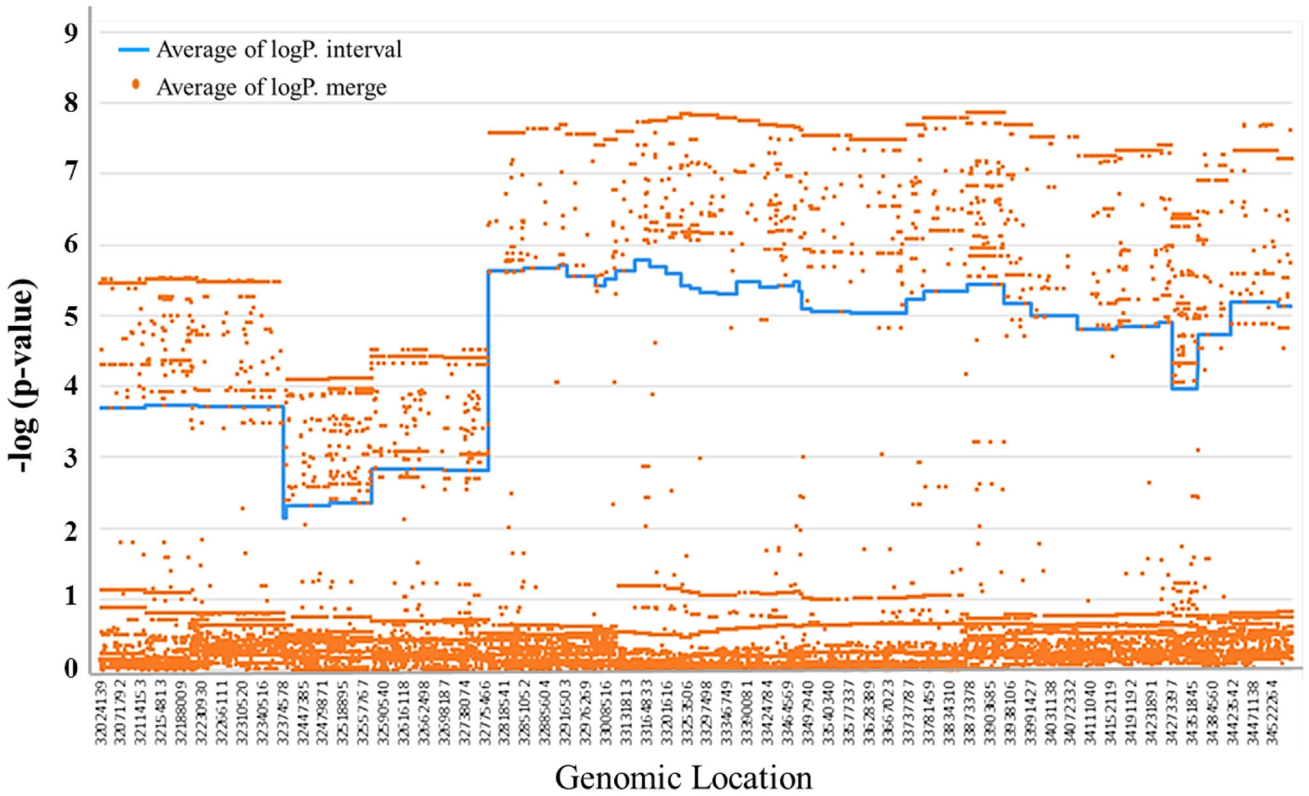

Fig. 5 Merge analysis of sequence variants around the AUC_{0-180} QTL on Chromosome 8 in the Collaborative Cross (CC) lines. *X-axis* represents the genomic location. *Y-axis* represents the logP of the linkage analysis between AUC_{0-180} and the locus. The *orange dots* resemble the results of merge analysis. The *blue* continuous lines resemble the haplotype QTL mapping scan on Chromosome 8

(human) and involved in age-related osteoporosis, adipose tissues/cardiovascular system/digestive/growth/size/body/immune system/mortality/aging (Brennan et al. 2014).

Furthermore, the MGI search revealed candidate genes were involved in the immune system, and might be indirectly related to the T2D progress as an inflammatory disease. One of the observed genes was the Gsr gene, named glutathione reductase (MGI:95804), and which had been shown to be involved in immune response against bacterial challenge (phagocytes), catalyzed the reduction of glutathione disulfide to glutathione (Yan et al. 2012), alongside additional functionaries in Cardiovascular System/vision eye (Blackshaw et al. 2004), also crucial for limiting tissue damage (Rogers et al. 2006) which are highly engaged to T2D metabolic syndrome health complication.

Saraf gene (MGI:1915137), named store-operated calcium entry-associated regulatory factor, was involved inter alia in the Endocrine system, rather in the Pancreas development (Hoffman et al. 2008), the major organ of the body that responds to glucose levels and plays main role in glucose homeostasis.

In addition to protein-coding genes, ten QTL feature types were exposed, located within our QTL genomic interval; eight of the ten QTLs were with relevance to our phenotypes and therefore find mention here. Three named Egq12 (early growth QTL 12), W10q14 (weight 10 weeks QTL 14), and W6q9 (weight 6 weeks QTL 9), were related to Growth and body size (Rocha et al. 2004). Another three QTLs named Eae36 (experimental allergic encephalomyelitis susceptibility 36) (MazónPeláez et al. 2005), Hbnr12 (Heligmosomoides bakeri nematode resistance 12) (Behnke et al. 2010), and Pgia4 (proteoglycan induced arthritis 4) (Otto et al. 1999), were related to different immune system responses. Following the previously mentioned order of the immune system QTLs, the first was related to Inflammatory demyelinating disease of the nervous system, the second related to Immune response to infection (Heligmosomoides bakeri), and the third associated with autoantibody production in the context of rheumatoid arthritis study in the murine model, PGIA. Two additional QTLs were related to known T2D high-risk comorbidities, and one associated with bone mineral density, Bmd39 (sex-specific), named bone mineral density 39, and linked to bone geometry and strength (Robling et al. 2007). Previous studies reported osteopenia and osteoporosis complications of T2D diabetes (Mathen et al. 2015), even though several studies have shown that BMD levels were not affected by T2D (Caglayan et al. 2015)—the incidence of bone fracture is increased among the T2D patients—suggesting that the quality of the bones might still be affected by the disease (Carnevale et al. 2014). The second comorbidity was associated with tumor genesis of colon cancer, Scc8, named colon tumor susceptibility 8 (vanWezel et al. 1999). According to previous studies and the most recent meta-analysis, diabetic patients have an increased risk of colon cancer compared with non-diabetic (Guraya 2015).

Finally, Human–Mouse: Disease Connection search for the given QTL genomic location revealed about 18 gene homologs from 28 phenotypes/diseases. Out of this list, directly relevant to glucose-tolerance trait, six genes (GSR/Leprotl1/Mboat4/Nrg1/Tex15 and WRN) _ with homologs in human are suggested to be playing important roles in the homeostasis/metabolism system.

Discussion

In this paper, we demonstrate, for the first time, the implementation of the unique CC mouse model to study the complex genetic architecture of T2D in response to environmental dietary challenge of high-fat diet (42 %). The accumulated data of T2D studies prove repeatedly the complexity of the disease etiology, once attributed mainly to environmental risk factors, and now is known to be controlled by numerous genetic–environmental interactions. Therefore, massive efforts are invested for dissecting the genetic architecture of T2D, which will restrain the alarming predictions for T2D epidemic, first at the level of quick diagnosis using genetic biomarker and second at the level of personalized medicine. Achieving this challenge requires great efforts, collaboration of scientists and clinicians, and the realization that T2D complications differ among populations and should be defined into categories based on genetic and environmental factors.

Human GWAS survey so far revealed that at least 88 genetic loci associated with T2D and 83 related to glycemic traits (Mohlke and Boehnke 2015) require further study to understand their biological roles and mechanisms involved in T2D. Interestingly, none of the genes, which were mapped within our QTL interval, were identified in the human-mapped genes. Therefore, we considered our results to be novel by identifying new genes associated with the tested trait. Human GWAS methodologies ameliorate in recent years with evident success to unravel phenotype–genotype associations; nevertheless, this powerful tool brings out several limitations mainly due to lack of proper control and constraints of further trials in human population under controlled environments for investigation of candidate genes' biological roles/functioning. Hence, using an animal model for human diseases study was preferred with the aim to overcome these limitations. Particularly for GWAS studies, the mouse model is widely used with notable potential due to many advantages, including the availability of mouse genomic data resources, the availability of genetic experimental tools, the ease of

breeding, and its ability to provide strictly controlled environmental conditions (Cox and Church 2011). For this purpose, the CC mouse population was designated, representing a model for heterogeneous human population for dissecting phenotypic–genotypic association underlining the differences among population toward common environmental exposure. In the present study, the CC lines correspond to heterogeneous population, and high-fat diet (HFD—42 % fat) corresponds to T2D risk of environmental challenge, whereas QTL mapping analyzes the linkage between diabetogenic phenotypes (impaired glucose tolerance measured as AUC_{0-180}) and genotypic components. Once a QTL is mapped, the next level will be the search for candidate genes, validation of candidate gene, and functioning pathways, toward translation to advanced level of human genetics, and numerous efforts invested by extensive collaborations of basic and clinical researchers .

In the current study, QTL mapping revealed a new sex-specific loci standing for total Area Under Curve in response to HFD, calculated for the area under curve between the starting time point to the ending time point of the 180 min' duration of the IPGTT. Eventually, data processing exposed various patterns of glucose clearance process, which differ significantly between the CC lines in response to the same environmental conditions. In addition, a significant sex-related effect was observed for the overall CC lines population, in which AUC_{0-180} of males was higher than that of females, indicating greater glucose clearance impairment in response to HFD for males, i.e., higher sensitivity to HFD-induced glucose-impaired tolerance than that in females. Sex difference was significant as an overall trend but varied between the CC lines, when examined within each CC line.

The reported QTL is a female-dependent one, named AUC_{0-180} QTL ($\sim$2.5 Mb) and mapped at genomic location of Chr8:32.02–34.52 Mb. Merge analysis enabled higher resolution of the QTL peak location (logP = 5.9, threshold P value < 0.05); thereafter, we scanned the observed interval for candidate genes using the international database resource for the laboratory mouse, Mouse Genome Informatics (MGI; http://www.informatics.jax.org). The scanned interval included 10 QTL and 50 genes, of which 13 genes were protein-coding genes, studied with known functions. Among the 13 protein-coding genes, Mboat4 (MGI: 2685017) and Leprotl1 (MGI: 1915442) were reported to be highly involved in the regulation of glucose homeostasis, an evidence that supported the significance of the AUC_{0-180} association with glucose-tolerant phenotype. As well, 8 out of the mentioned 10 QTL were related to phenotypes of high relevance to impaired glucose tolerance and T2D comorbidities, such as body weight and size, immune response, bone mineral density, and colon cancer. Recently, we have published results of mapping QTL for females, only with non-alcoholic fat liver accumulations (Abu Toamih-Atamni et al. 2016b). It is believed that there are different mechanisms that control obesity and related diseases including NAFLD and blood glucose in males and females.

Furthermore, we searched the AUC_{0-180} interval for Human–Mouse Disease Connection (HMDC; http://www.informatics.jax.org/humanDisease.shtml) to identify possible connections of our findings in the mouse genome to corresponding genetic findings reported in the human disease. Search results revealed five gene homologs in humans (Wrn/Tex15/Ppp2cb/Gsr/Mboat), where the genes Wrn and Mboat were associated with phenotypes of growth/size/-body and homeostasis/metabolism pathways. Two of the above-mentioned five homologous genes, Wrn and Gsr, were associated with known human diseases—Werner Syndrome and Glutathione Reductase disease, respectively. Werner Syndrome, attributed to Wrn protein-coding gene, is characterized by wide range of metabolic/growth complications, including development of T2D. Glutathione Reductase disease, attributed to Gsr protein-coding gene deficiency, leads to multiple complications including Cardiovascular system and cataract development, which are known as the main complications also in T2D. Altogether, genes search of AUC_{0-180} interval corroborates the significance of our findings to be relevant to T2D direct/indirect pathways, along with the suitability of using AUC_{0-180} as diabetogenic phenotype.

Finally, we find that in previous studies, researchers usually focused more on impaired glucose clearance, but less on QTL associated with improved glucose clearance. This report, however, is one of the few studies, which addresses both, impaired glucose clearance phenomenon and QTL associated with this trait.

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Compliance with ethical standards

Conflict of Interest The authors declare no competing financial interests or other associations that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy).

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