RESEARCH ARTICLE

Comparative Genome of GK and Wistar Rats Reveals Genetic Basis of Type 2 Diabetes

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Abstract

The Goto-Kakizaki (GK) rat, which has been developed by repeated inbreeding of glucose-intolerant Wistar rats, is the most widely studied rat model for Type 2 diabetes (T2D). However, the detailed genetic background of T2D phenotype in GK rats is still largely unknown. We report a survey of T2D susceptible variations based on high-quality whole genome sequencing of GK and Wistar rats, which have generated a list of GK-specific variations (228 structural variations, 2660 CNV amplification and 2834 CNV deletion, 1796 protein affecting SNVs or indels) by comparative genome analysis and identified 192 potential T2D-associated genes. The genes with variants are further refined with prior knowledge and public resource including variant polymorphism of rat strains, protein-protein interactions and differential gene expression. Finally we have identified 15 genetic mutant genes which include seven known T2D related genes (Tnfrsf1b, Scg5, Fgb, Sell, Dpp4, Icam1, and Pkd2l1) and eight high-confidence new candidate genes (Ldlr, Ccl2, Erbb3, Akr1b1, Pik3c2a, Cds5, Eef2k, and Cpd). Our result reveals that the T2D phenotype may be caused by the accumulation of multiple variations in GK rat, and that the mutated genes may affect biological functions including adipocytokine signaling, glycerolipid metabolism, PPAR signaling, T cell receptor signaling and insulin signaling pathways. We present the genomic difference between two closely related rat strains (GK and Wistar) and narrow down the scope of susceptible loci. It also requires further experimental study to understand and validate the relationship between our candidate variants and T2D phenotype. Our findings highlight the importance of sequenced-based comparative genomics for investigating disease susceptibility loci in inbreeding animal models.

Background

Type 2 diabetes (T2D), also known as non-insulin-dependent diabetes is characterized by defects in both insulin secretion and utilization and accounts for about 90% of the 346 million diabetic cases around the world [1]. Both environmental and genetic factors contribute to the etiology and progression of T2D [2, 3]. In the last two decades, significant efforts, ranging from
traditional candidate gene mapping to genome-wide association studies (GWAS), have identified nearly 120 T2D susceptibility genes in different human population [3–24]. However, the precise molecular pathogenesis of this heterogeneous disease remains poorly characterized. Therefore more T2D-related genes are expected to be uncovered.

The Goto-Kakizaki (GK) rat, a non-obese animal model of T2D, was developed by repeated inbreeding of glucose-intolerant Wistar rats [25]. GK rats suffer from reduced beta-cell mass with insulin resistance. Therefore, these model rats provide an ideal model system to search for T2D susceptible genes/loci to enhance our understanding of the etiology and pathogenesis of the disease [26, 27]. Several quantitative trait locus (QTL) analyses on this model have already identified a number of genomic loci harboring susceptible variants [28–30].

As large-scale changes in the genome such as copy number variations (CNVs) have been linked to dozens of human diseases [31–35], we previously conducted a genome-wide screen for CNVs between GK (T2D model) and Wistar rat (wild type) using comparative genomic hybridization (CGH) array. A set of T2D-associated CNV regions with the total length of about 36 Mb, including several novel T2D susceptibility loci which contain 16 protein-coding genes (Il18r1, Cyp4a3, Sul2a1, Sul2a2, Sul2a11, Nos2, Pstpip1, Ugt2b, Uxs1, RT1-A1, RT1-A3, RT1-Db1, RT1-N1, RT1-N3, RT1-O, and RT1-S2) and two microRNA genes (rno-mir-30b and rno-mir-30d), were identified [36].

The draft genome of the Brown Norway (BN) rat, covering around 92% of the genome, was released in 2004 [37], and was the third complete mammalian genome to be deciphered. Limitations in obtaining extensive genetic data have been largely overcome by the development and maturation of next-generation sequencing (NGS) technologies, which have significantly improved the throughput with reduced costs. Whole genome sequencing has become an alternative approach to identify genes involved in disease. For example, comparing the genomic sequence of spontaneously hypertensive rats (SHR) and the BN reference genome, Atanur et al., identified a number of candidate loci that may be involved in the development of hypertension [38].

We conducted whole genome sequencing and compared the genomic sequence differences between Wistar and GK rats (Fig 1). As a result we generated a list of GK-specific variations including 228 structural variations, 2660 CNV amplifications, 2834 CNV deletions, and 1796 protein affecting SNVs or indels. Comparing these variations with known rat genome variations and known human T2D-associated genes, we obtained 192 candidate genes including 15 with high confidence that may be associated with the T2D phenotype observed in GK rats. These genes are involved in multiple pathways, suggesting that multiple interacting biological networks may be involved in the GK T2D phenotype. We expected this work facilitates the understanding of the molecular processes involved in the development of T2D.

**Results**

**Sequencing and mapping**

We sequenced the genome of GK/Slac and Wistar/Slac using both Illumina Solexa and ABI SOLiD platforms. Sequencing reads from these two platforms were combined in the subsequent analyses to gain higher sequence coverage and depth. In total, we obtained 100.4 Gb for the GK/Slac and 85.6 Gb for the Wistar/Slac rat which represent an average sequencing depth of 35.9X for GK/Slac and 30.5X for Wistar/Slac (S1 File). About 50.45% (43.27%) SOLiD reads and 32.13% (18.68%) Solexa reads of GK/Slac (Wistar/Slac showed in parentheses) were evaluated as low quality by a strict quality control procedure (S2 File). These low quality reads were filtered before sequence mapping. The remained reads were mapped to BN reference genome, obtaining more than 99% coverage (99.40% and 99.35% genome were covered by at least one
read in GK/Slac and Wistar/Slac sequencing, respectively; 93.02% and 93.65% genome were covered by at least five reads).

In order to get accurate variant calls, we reassessed the quality of the mapping results through the GATK workflow [39]. After these steps, 0.42% (0.58%) of the Solexa reads, and 6.05% (4.87%) of the SOLiD reads were removed from GK/Slac (Wistar/Slac) mapping results, respectively (S3 File). In total we aligned 34.06 Gb (34.70 Gb) from GK/Slac (Wistar/Slac) sequencing data to the BN reference rat genome which corresponded to 13.27X (13.52X) coverage of high-quality reads for GK/Slac (Wistar/Slac) [38].

**Variant calling**

Sequence variants identified in GK/Slac and Wistar/Slac include single nucleotide variant (SNV), small insertion and deletion (indel), structural variation (SV), and copy number variation (CNV). In total, we identified 3,471,498 (3,194,675) raw SNVs and 492,731 (517,005) raw small indels for GK/Slac (Wistar/Slac). These variants were further filtered by sequence coverage, strand bias, and error-enriched regions (see Method for detailed description) which
resulted in 3,049,694 (2,727,649) high-quality SNVs and 476,841 (487,315) high-quality indels for GK/Slac (Wistar/Slac) (S4 File). Among them 2,927,627 (2,623,154) were homozygous SNVs in GK/Slac (Wistar/Slac). The percentage of homozygous SNVs was 95.99% (96.16%), consistent with the expected level for the inbred strain. There were 2,066,576 (1,843,133) transitions and 983,003 (884,403) transversion sequence changes in GK/Slac (Wistar/Slac). The ratio of transition to transversion ratio (Ti/Tv) is 2.10 (2.08), which is comparable to the Ti/Tv ratio of ~2.0–2.1 observed in human genomic sequence dataset [40].

Among all SNVs, 2,695,132 (66.8%) of them were present in the dbSNP database (http://rgd.mcw.edu/pub/data_release/GFF/SNPS/DbSNP/). Others may be strain-specific or private SNVs that were not covered by previous studies.

To evaluate the accuracy of variant calling, we compared the primary results to a public dataset from the STAR project [41]. This dataset includes genotypes for 20,238 SNVs across 167 distinct inbred rat strains including 10 GK and 6 Wistar strains. There were 7368 (4000) positions that were mutant in all GK (Wistar) strains, and 2491 (1372) positions that were not polymorphic in all GK (Wistar) strains. We checked our SNV calling results against these positions and calculated sensitivity and specificity. For GK/Slac strain, we called 6984 SNVs among the 7368 positions (94.79% sensitivity) and 2489 out of the 2491 non-polymorphic positions (99.9% specificity). For Wistar/Slac strain, we called 3319 SNVs among the 4000 positions (82.97% sensitivity) and 1104 out of the 1372 non-polymorphic positions (80.47% specificity).

Comparative genome analysis

Since the GK rat was obtained by selective inbreeding of Wistar rats, their specific genetic changes from Wistar should indicate the cause of type 2 diabetic phenotypes observed. Therefore, GK/Slac specific variants were determined by comparing variants in GK/Slac with Wistar/Slac. All GK/Slac specific variants were shown on a circular chromosome map (Fig 2).

There were 1,354,739 GK/Slac specific SNVs and 134,554 GK/Slac specific indels. The density of GK/Slac specific SNV/indel was calculated in each 1Mb segment, and their distribution was plotted in Fig 3A and 3B. Most genomic regions were relatively conserved with extremely low SNV density (0–0.0001) and regions with median SNV density (0.0001–0.001) were evenly distributed. When the SNV density increased, the frequency decreased smoothly (0.001–0.002). A long tail indicated the existence of extremely high SNV density (>0.002) regions.

The distribution of indel density was similar to the SNV distribution (Fig 3A and 3B). We calculated the Pearson correlation coefficient between SNV density and indel density in each 1Mb genomic segment. The result showed that the density frequency of GK/Slac specific SNVs and indels were positively correlated (R² = 0.959, Fig 3C). This indicated some SNVs and indels tend to co-localize at highly mutated regions of the genome (S5 File). As expected, there were regions in the genome that showed no or very few differences between GK/Slac and Wistar/Slac strain, termed variants deserts. Examples included chromosome 1 (20Mb-21Mb) and chromosome X (53Mb-54Mb).

Besides SNV and small indels, we also compared large SVs and CNVs between GK/Slac and Wistar/Slac. We identified 228 GK/Slac specific SVs, including 174 deletions, 12 inversions, 36 tandem duplications and 6 translocations. Based on sequence coverage, we predicted 2660 CNV amplified regions and 2834 CNV deleted regions between the GK/Slac and Wistar/Slac rat genomes. To validate our CNV calling, we compared the CNV candidates with a set of 116 CNVs identified by CGH array data from our previous work [36]. Out of 58 CNV gain regions identified by array, we successfully identified 38 in the sequencing results. The sequencing results also identified 31 out of the 58 CNV loss regions identified by array.
Identification of potential T2D candidate mutations specifically presented in the GK rat

We were interesting in the GK/Slac specific variants that might contribute to the development of type 2 diabetes phenotype in GK/Slac. We mapped GK/Slac specific SVs and CNVs to regions containing functional transcripts. For SVs, 26 genes were affected, including 18 olfactory receptor genes (ORs) (S7C File). 75 genes were associated with CNVs, among them 24
were OR genes (S7 File). The OR gene family is the largest superfamily in mammalian genome. There are 1,493 OR genes in the rat and 19.5% may be pseudo-genes [42]. OR genes are frequently clustered in regions with a large number of retrotransposons or around subtelomeric regions [43][44], which tend to exhibit a high rate of mutation. Therefore, we thought GK/Slac specific variants in ORs were background mutations rather than causal of the T2D phenotype. Besides the OR genes, other SV or CNV affected genes were randomly distributed with no enrichment in T2D related pathways and no literature evidence of either direct or indirect association between these genes and T2D.

Next we investigated potential T2D candidate variants from GK/Slac-specific SNVs and indels. We divided SNP/indels into five groups to illustrate their genotype patterns in GK/Slac and Wistar/Slac (Fig 4A). Group1 (0/1, 0/0) contained sites that were heterozygous variant in GK/Slac and homozygous reference in Wistar/Slac; Group2 (1/1, 0/0) contained sites that were homozygous variant in GK/Slac and homozygous reference in Wistar/Slac; Group3 (1/1, 0/1) contained sites that were homozygous variant in GK/Slac and heterozygous variant in Wistar/Slac; Group4 (1/2, 0/0) was similar with Group2 and Group5 (1/2, 0/1) was similar with Group3, which were rare sites with two mutant alleles. Among 1,354,739 GK/Slac specific SNVs, group 1 to 3 accounted for the majority of SNVs with 3.6%, 92.9% and 3.5%, respectively (Table 1). Like SNVs, among 134,554 GK/Slac specific indels, proportion of group1 to group3 were 5.0%, 81.9% and 13.1%, respectively. In summary, most SNV and indel variants belonged to groups 1, 2 and 3, and only few allele sites had the complicated allele composition in group 4 and 5 which was consistent with the low probability of de novo production of two rare alleles in
the lab inbreeding strain. Group 2 accounted for a large proportion that was concordant with the high homozygosity rate of inbred laboratory rat. Next we annotated the functional effect of GK/Slac specific SNVs/indels by ANNOVAR [45]. Table 2 showed the number of SNPs/indels in each genotype group and functional class. Variants had potential to interrupt the protein functions were called protein affecting variants (PAVs), including nonsynonymous, stopgain, stoploss, splicing, frameshift indels and exonic ncRNA. We detected 1796 PAVs, including 1762 SNVs and 34 indels (S7AB File).

To further refine the above PAVs, we compared our variants with the variants of public RGD datasets. Atanur et al. reported whole-genome sequencing results of 28 laboratory rat strains[46]. Depending on these variants and ours, we plotted a phylogenetic tree for these rats (Fig 5). As the phylogenetic relationship showed, GK/Slac was close to GK/Ox, and Wistar/Slac

**Table 1. Five different genotype of GK/Slac specific SNVs and indels.**

| GK/Slac specific | Total | Group1 (0/1, 0/0) | Group2 (1/1, 0/0) | Group3 (1/1, 0/1) | Group4 (1/2, 0/0) | Group5 (1/2, 0/1) |
|-----------------|-------|------------------|------------------|------------------|------------------|------------------|
| SNVs            | 1354739 | 48573            | 1258423          | 47735            | 2                | 6                |
| indels          | 134554  | 6731             | 110171           | 17647            | 1                | 4                |

Fig 4. Analysis of GK/Slac specific protein affecting SNVs. (A) Variants were classified into five groups based on their genotypes in GK/Slac and Wistar/Slac. As shown in the bottom legend, circles stand for the original reference allele whereas stars and triangles represent two different mutant alleles. Taken group 1 as an example, variants is heterozygous in GK/Slac that have one mutant allele and one reference allele, while it is homozygous-reference in Wistar/Slac. Almost all variants are in group 1, group 2, and group 3. (B) Genotype profiling for 1762 GK/Slac specific SNVs in 28 previous sequenced rat strains. GK/Ox and GK/Slac are GK strains which came from different geographical locations. BBDB is a type 1 diabetic model, another 11 Wistar derived rats are labeled by green. (C) T2D related prior genes. (D) Functional effect of nonsynonymous SNVs predicted by SIFT.
was close to Wistar derived strains in USA. Therefore, the genetic background of GK/Slac and Wistar/Slac were more similar with 12 Wistar derived strains (SHR/NHsd, SHRSP/Gla, SHR/OlaIpcv, WKY/NCrl, WKY/Gla, WKY/NHsd, LEW/Crl, LEW/NcrlBR, WAG/Rij, BBDP/Wor, MHS/Gib, MNS/Gib) than other rat strains, which convinced our samples and results were reliable.

In the light of the public resources of variants from different rat strains, we were able to further narrow down the mutant profile. Fig 4B, 4C and 4D showed the genotype profiles of 1762 GK/Slac specific PAVs in 28 rat strains, the overlap with T2D prior genes (S6 File), and the predicted functional effect of PAVs. To identify T2D phenotype-specific genetic changes, we further filtered the 1796 GK/Slac specific PAVs based on the genotype profile of 11 Wistar strains (except BBDP/Wor, which is a type 1 diabetic model) and 1 GK/Ox strain. Our GK specific variants, which had potential to contribute to T2D phenotype, were required to present in the GK/Ox strain but not the other 11 Wistar strains.

Considering the laboratory inbreeding process, we supposed homozygous variants in GK rat have a higher probability to account for the disease phenotype. Among the 1762 GK/Slac specific protein affecting SNVs, 300 were homozygous variants in both GK strains (GK/Slac in our report and GK/Ox strain studied by Atanur et al. [46]), but did not present in other 11 Wistar strains. These 300 SNVs were located in 252 genes including 60 OR genes and the other 192 genes were used for further analysis (S8A File). We also checked 34 protein affecting indels. Besides 7 indels were heterozygous in GK/Slac, one homozygous indel resided in the T2D prior gene Hif1a, but many other rat strains also had this homozygous indel; the other 26 homozygous indels were either located in OR genes or not reported to be associated with T2D.

Refinement of the genes with homozygous mutation based on PPI network and gene expression identify prior T2D genes and new candidates

Among 192 potential T2D associated genes, seven of them (Tnfrsf1b, Sgc5, Fgb, Sell, Dpp4, Icam1, and Pkd2l1) were clearly reported to be T2D prior genes (see Methods for detailed description, S6 File). As T2D phenotype was correlated with protein network dysregulation, we hypothesized T2D candidate genes were more likely to have interactions with reported T2D prior genes. We used Fisher’s test to evaluate whether their interaction partners were enriched.

Table 2. Functional annotation of GK specific variants. There are 1796 GK/Slac specific protein affecting variants, including 1762 SNVs and 34 indels. Values in table are the number of SNVs in each function class and values in parentheses are the number of indels.

| Annotation               | Total   | Group1 (0/1, 0/0) | Group2 (1/1, 0/0) | Group3 (1/1, 0/1) |
|--------------------------|---------|-------------------|-------------------|-------------------|
| Protein affecting variants |         |                   |                   |                   |
| Nonsynonymous            | 1721    | 106               | 1526              | 89                |
| Stopgain                 | 14      | 2                 | 12                | 0                 |
| Stoploss                 | 1       | 1                 | 0                 | 0                 |
| RNA splicing             | 8 (5)   | 1 (1)             | 7 (2)             | 0 (2)             |
| ncRNA_exonic             | 18 (1)  | 1                 | 17 (1)            | 0                 |
| Frameshift deletion      | (18)    | (4)               | (13)              | (1)               |
| Frameshift insertion     | (10)    | (2)               | (8)               | (0)               |
| Synonymous               | 3485    | 154               | 3173              | 158               |
| UTR                      | 4484 (467) | 214 (23)       | 4076 (393)        | 194 (51)          |
| Intronic                 | 303876 (32349) | 10279 (1523) | 283225 (26523)    | 10372 (4303)      |
| Up/Down-stream           | 13145 (1456) | 503 (68)        | 12084 (1207)      | 558 (181)         |
| Intergenic               | 1027253 (100220) | 37282 (5109)  | 953648 (82009)    | 36323 (13102)     |

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with T2D prior genes (S8B File). There were 16 genes whose interaction partners were enriched with prior genes (adjusted p-value < 0.05), among of which six genes (Tnfrsf1b, Scg5, Fgb, Sell, Dpp4, and Icam1) were known T2D prior genes. Fig 6A shows the protein-protein interaction (PPI) network between these six genes and other T2D prior genes. PPIs contained validated and predicted protein associations from STRING database and genes were annotated by KEGG pathway database. Five T2D related pathways were labeled by different colored boxes, including Adipocytokine signaling pathway, Glycerolipid metabolism, PPAR signaling pathway, T cell receptor signaling pathway and Insulin signaling pathway.

The six T2D prior genes were closely correlated with T2D phenotype according to previous investigations. Genetic variation in or near Tnfrsf1b might predispose clinical neuropathy,
reduced glycosylated hemoglobin, and increased HDL cholesterol in type 2 diabetes patients. The latter could be part of a protective response [47]. *Tnfrsf1b* and its interacting proteins were involved in the adipocytokine signaling pathway and increased TNF-alpha action would protect the organism from the damage by increasing HDL cholesterol in T2D patients [47, 48].

**Fig 6.** Protein-protein interaction (PPI) network for T2D candidate genes identified in GK rats. (A) PPI network for six T2D prior genes that have GK/Slac specific PAVs. Edges indicate PPI got from STRING database (only considering interaction with other T2D prior genes). Genes involved in important KEGG pathways were shown by colored boxes. (B) Relationship network among fifteen T2D candidate genes. Seven genes were T2D prior genes and have GK/Slac specific PAVs; another eight genes were enriched with T2D prior genes as PPI partners. Widths of edges were proportional to the number of shared PPI patterns.

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The nonsynonymous SNV in Scg5 (chr3: 99641204:G->C) was predicted to be deleterious (Fig 4D) by SIFT [49]. Its homologous site in mouse is annotated as “type 2 diabetes mellitus 2 in SMXA RI mice” based on QTL data in UCSC genome browser. Also Scg5 (SGNE1) might impair glucose intolerance and insulin resistance [50], which was consistent with the insulin resistant phenotype of GK strain. Fibrinogen (Fgb) was described as one of the cardiovascular risk factors [51] and Fgb concentration was correlated with fasting insulin concentration [52]. Fgb was also involved in T2D related PPARγ signaling pathways [53]. Sell was a cell surface adhesion/homing receptor that played important roles in leukocyte-endothelial cell interactions. Although its interaction partners did not show enrichment in any T2D related pathway, previous literature had reported that Sell was associated with T2D-associated pathologies, such as diabetic microangiopathy [54], nephropathy [55] and diabetic retinopathy [56]. Dpp4 was a famous drug target of T2D [57], and Dpp4 inhibitors could ameliorate many symptom of T2D [58, 59]. PPI showed that Dpp4 was involved in a number of biological functions (Fig 6A) [57]. Dpp4 played a critical role in both the adipocytokine signaling pathway and insulin signaling pathway [60]. Inhibiting Dpp4 activity increased glucose-dependent insulinoetric polypeptide and glucagon like peptide 1 induced insulin secretion [61]. T2D patients showed increased ICAM-1 and VCAM-1 plasma concentrations, which was thought to be related to hyperglycaemia [62]. Pkd2I1 had been associated with T2D by GWAS study [63] and Mancusi S et al. demonstrated that the expression change of PKD2, which was responsible for the formation of the renal cysts and associated with early diabetes onset [64].

Some of the mutant genes were supposed to show expression changes between GK and Wistar strain. We compared the expression profile of 192 potential T2D genes in GK and Wistar rats by analyzing a public microarray dataset (GSE13271). There were 32 differentially expressed genes and 38 differential co-expressed genes in at least one tissue between GK and Wistar rat (S8B File). Among above 16 candidate genes, seven of them (Tnfrsf1b, Ldlr, Pik3c2a, Sell, Icam1, Ef2k, Cpd) also had significant expression changes (differentially expression or differential co-expression) between GK and Wistar. (Table 3)

Integrating prior knowledge, PPI network and differential gene expression, we finally selected 15 higher confidence T2D candidate genes with homozygous variants in GK strains (Table 3). These 15 genes were involved in multiple T2D related pathways. We counted the number of shared interaction partners between any two genes, and constructed a relationship network for 14 genes out of the 15 high-confidence T2D candidate genes (Fig 6B). Fig 6B illustrates the close relationship between 8 new genes (Ldlr, Ccl2, Erbb3, Akr1b1, Pik3c2a, Cd5, Ef2k, Cpd) and known T2D prior genes, indicating these 8 genes have strong relationships with T2D associated pathways. We manually checked their function and possible relationship to T2D in published literature. For instance, Ldlr had previously been shown to be associated with diabetes mellitus and its lipid phenotype [65]. A GWAS study of French population also identified Ldlr as a T2D risk locus [66]. Akt2/Ldlr double knockout mice displayed impaired glucose tolerance [67], and increased inflammation response [68]. CCL2/C-C chemokine receptor 2 (CCR2) signaling was suggested to play a significant role in diabetic nephropathy and in adipose tissue inflammation during insulin resistant. Blocking CCL2/CCR2 signaling not only improved blood glucose levels but also altered renal nephrin and VEGF expressions in type 2 diabetic mouse model [69]. Butcher et.al. showed that type 2 diabetic islets displayed higher CCL2 expression than healthy islets [70]. Polymorphism of Akr1b1 was associated with diabetic nephropathy and type 2 diabetes mellitus by two GWAS studies [71, 72]. Pik3c2a played a critical role in insulin secretion in ß cells and down-regulation of PI3K-C2a might be a feature of type 2 diabetes [73]. Ef2k was a kinase of Ef2 and renal cortical homogenates from db/db mice in early stage of type 2 diabetes showed decrease in Ef2 phosphorylation and increment in Ef2 kinase phosphorylation [74]. Carboxypeptidase D (Cpd) and carboxypeptidase E (Cpe)
belonged to same family of enzymes and defects in Cpe could lead to β-cell stress and hyperproinsulinemia, both of which were features of type 2 diabetes in GK rat [75]. Chu KY et al. also found that Cpd was significantly up-regulated by elevated glucose or low doses of insulin [76].

**Conclusion**

We presented a comprehensive analysis pipeline of re-sequencing study with general case-control study design. Besides identifying some prior T2D genes with mutations, we found 8 new candidate genes which required further wet-lab experimental evaluation and validation. As a bioinformatic analysis of NGS data, our workflow could be adopted in other re-sequencing study of organism with disease model.

**Discussion**

Rodents have been used to model human diseases because of their similarity in genome and physiology. GK is a classic rat T2D model, which is obtained by selective inbreeding of Wistar rats. GK/Slac and Wistar/Slac rats have been bought from a commercial company (www.slaccas.com), which import rat strains from the place of production and then bred locally in China. These two strains have been widely used by Chinese investigators [77–82]. Our work provides the whole-genome sequences of GK/Slac strain and Wistar/Slac strain for the first time.

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**Table 3. High-confident T2D candidate genes and their homozygous SNVs in GK rat.**

| Gene  | Chromosome location | Nucleotide change | Protein change | SIFT prediction | Evidence of associationa | Previous publication | Adjusted P-valueb | Differential (co-) expression |
|-------|--------------------|------------------|---------------|----------------|--------------------------|---------------------|------------------|-------------------------------|
| Tnfrsf1b | chr5: 163680822    | C→T              | p.A51T        | tolerated      | 1), 2), 3)               | [47]                | 4.49E-2          | adiposec                    |
| Scg5   | chr3: 99641204     | G→C              | p.P156R       | deleterious    | 1), 2)                   | [50]                | 1.56E-2          |
| Fgb    | chr2: 174775040    | G→T              | p.L8I         | tolerated      | 1)                       | [51, 52]            | 5.16E-3          |
| Sell   | chr13: 79831304    | C→G              | p.R167G       | tolerated      | 1), 3)                   | [60, 73]            | 5.39E-3          | musclec                    |
| Dpp4   | chr3: 44359652     | T→C              | p.I20V        | tolerated      | 1)                       | [57, 59, 60, 94]    | 2.28E-13         |
| Ldr    | chr8: 20844228     | A→G              | p.K742E       | tolerated      | 2), 3)                   | [65–68]             | 2.73E-33         | liverd                     |
| Ccl2   | chr10: 70257779    | A→C              | p.E141D       | tolerated      | 2)                       | [69, 70]            | 4.91E-22         |
| Erbb3  | chr7: 1859081      | C→T              | p.R1072H      | tolerated      | 2)                       | -                   | 1.80E-18         |
| Akr1b1 | chr4: 61654486     | T→C              | p.S77G        | tolerated      | 2)                       | [71, 72]            | 8.97E-7          |
| Pik3c2a| chr1: 174467615    | C→T              | p.V598I       | deleterious    | 2), 3)                   | [73]                | 1.14E-4          | adiposed                    |
| Pik3c2a| chr1: 174436078    | T→C              | p.T1550A      | tolerated      | 2), 3)                   | -                   | 1.14E-4          | adiposed                    |
| Cd5    | chr1: 213215518    | C→T              | p.V445M       | tolerated      | 2)                       | -                   | 2.33E-3          |
| Icam1  | chr6: 20050122     | A→G              | p.I314V       | tolerated      | 1), 2), 3)               | [62]                | 1.05E-23         | adiposed                    |
| Pkd2l1 | chr1: 249075571    | A→T              | p.T169S       | -              | 1)                       | [63, 64]            | 1                |
| Pkd2l1 | chr1: 249075657    | G→T              | p.L197F       | deleterious    | 1)                       | [63, 64]            | 1                |
| Eef2k  | chr1: 179746185    | A→G              | p.Q547R       | tolerated      | 2), 3)                   | [74]                | 2.26E-4          | muscled                    |
| Cpd    | chr10: 67359919    | C→T              | p.P490S       | tolerated      | 2), 3)                   | [75]                | 2.23E-2          | musclec, adiposec, liverc    |

a. Additional evidence of association with T2D. 1) T2D prior genes curated from literatures. 2) Protein-protein interaction partners are enriched with T2D prior genes. 3) Differential expression or differential co-expression in GSE13271 dataset.

b. P-value for enrichment of T2D prior genes in the interaction partners. P-value was calculated by fisher test, and was adjusted by p.adjust in R.

c. Differential expression between GK and Wistar rats.
d. Differential co-expression between GK and Wistar rats.

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time. This sequencing dataset will be very useful for the researchers who use these two strains as study objects. It is also an important complement to the Rat Genome Database (RGD) [83], which include the international sequencing resources of different rat strains.

Comparing the whole genome sequence of T2D phenotypic GK rats with the corresponding Wistar rats provides insights into the genomic evolution of GK during the laboratory selective inbreeding for developing the insulin resistant T2D phenotype. In the light of sequencing technology, the genetic difference between T2D GK and control Wistar rats is easy to identify. Many years of selective inbreeding in the laboratory makes these genetic variants are correlated to a consistent phenotype. Such advantages make the GK rat an ideal model to discover T2D causative genes. Here we studied the genomes of GK and Wistar rats by both sequencing and computational strategies. We have combined two sequencing platforms with different read lengths and insert-sizes. The reads are processed with stringent quality control to obtain accurate high-quality variants. In order to identify the causal variants of T2D phenotype, we used Wistar strains as background to screen GK specific variants, which not only are required to present consistently homozygous in both our and public GK rat samples, but also are absent in either our Wistar sample or any Wistar derived samples from the public databases. Then we selected high-confidence affected genes by integrating T2D prior knowledge, protein interaction and gene expression data. Finally we got fifteen genes with homozygous SNVs in GK rats and their functions are related with T2D phenotype. The integration of public resources and prior knowledge can increase the power of detection and narrow down the scale of candidates. Our data mining approach would inspire similar bioinformatic studies for disease animal model.

Our analysis focus on variants that affect protein sequences so that variants in the intergenic or intronic regions are ignored due to the lack of function annotations for these regions in rat genome. The understanding of noncoding regions may be improved by more studies on translational regulation and evolutionary conserved regions. We also observed that GK strains and Wistar strains coming from different laboratories have slightly genetic difference (Fig 4B, Fig 5), showing it is important to use biological repeats even for inbreed organisms. With decreased sequencing cost and improved computational ability, it is possible to sequence more samples to increase analysis power. The whole-genome sequencing-based disease study will be extended to other disease models and our approach can be used as an example to study these disease model organisms.

Methods
Sample preparation
One male GK/Slac rat and one male Wistar/Slac rat were obtained from SHANGHAI SLAC LABORATORY ANIMAL CO. LTD (www.slaccas.com). The rats were anesthetized by formalin at the age of 8 weeks, and the blood was taken from the pericardia with anticoagulant. Genomic DNA was then isolated using DNeasy Blood & Tissue Kit (Qiagen, p/n69504). All animal experiments were approved by the Biomedical Research Ethics Committee of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (IRB00005813).

Whole genome sequencing and data preprocessing
GK/Slac and Wistar/Slac rat DNA samples were sequenced by ABI SOLiD and Illumina Solexa paired-end sequencing technologies. To increase the coverage of genome, three SOLiD sequencing libraries and one Solexa library were constructed with different read length and insert length (S1 File). All sequence reads were deposited in the European Nucleotide Archive under accession number PRJEB6678.
Read quality was assessed by per base sequence quality, per sequence quality score, per base N content and overrepresented sequences using software FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Low quality reads were filtered by stringent criteria: 1) removing overrepresented adaptor found by FastQC using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), 2) removing N base and low quality base (Phred quality score was below 20), 3) removing reads that’s shorter than 15 bp or paired read was filtered.

Mapping to reference genome
In order to combine data from two different sequencing platforms, we transferred ABI SOLiD color space encoding data and their quality file to Solexa base space encoding format (Fastq file). Then we aligned the high-quality reads to the BN rat reference genome (UCSC rn4 / Baylor HGSC Build 3.4) by Bowtie 2 with default parameters [84]. The coverage proportion of reference genome and estimated genome were calculated by the following formula:

\[
\text{reference coverage proportion} = \frac{\text{number of bases that/s covered by at least 1 read}}{\text{total bases in reference genome}}
\]

\[
\text{genome coverage proportion} = \frac{\text{number of bases that/s covered by at least 1 read}}{\text{estimated number of bases in the genome}}
\]

We further filtered the mapping results to increase the accuracy of variant calling. Firstly, we did local realignment around known indels using Smith-Waterman algorithm. Then we removed duplicate reads to reduce amplification bias. Lastly we recalibrated base quality depending on the reference genome and dbSNP information. These three main processes were done by GATK (The Genome Analysis Toolkit) [39], and PICARD TOOLS and SAMTOOLS [85] were used to sort the bam file, fix mate pair information and do format transformation, which facilitated the GATK running process.

Variants calling and comparison
After pre-mapping and post-mapping quality control, remained bam files were used to call variants: single nucleotide variant (SNVs), small insertion and deletion (indels), structural variation (SVs), and copy number variations (CNVs).

Small indels and SNVs were called by the UnifiedGenotyper module in GATK software and filtered by following filtering criteria: minimum number of consensus is 5, minimum base quality required to consider a base for calling is 17. Furthmore, we filtered the candidate small indels by criteria: minimum depth (DP) is 8 and allele number (AN) is 4. We filtered SNVs by criteria: minimum depth (DP) for each allele in per sample is 10, allele number (AN) is 4, minimum base quality is 30, minimum qual by depth (QD) is 5, maximum mapping quality zero (MQ0) is 4, removing SNVs that were located on indel regions or in SNV cluster regions (defined by 3 SNV calling in a 10 bp window).

DELLY was used to detect structural variants from discordantly mapped read pairs [86]. The predicted SVs were classified as four groups: deletions, Inversions, tandem duplications and translocations. To avoid false-positive SVs in GK, DELLY was run with “-p” option that combined discordant alignment with split-read to get higher confident SVs. Then GK SVs were compared with Wistar SVs to get GK/Slac specific SVs.

The software BIC-seq [87] was used to detect copy number alterations between GK strain and Wistar strain. Differential CNVs were selected by two criteria: a) ratio of mapped reads number between GK and Wistar is greater than 2; b) Bofferoni adjusted p-value is smaller than 0.01.
Variant density and function annotation

To illustrate the genome-scale difference between GK/Slac and Wistar/Slac, we analyzed the density and distribution of GK/Slac specific variants. Reference genome were segmented into 1Mb bins and variant density was defined as the number of variants in each bin divided by the number of nucleotide bases in the same bin that were covered by at least three reads in GK sequencing.

ANNOVAR was used to annotate SNVs/indels to gene region (exonic, splicing, ncRNA, UTR, intronic, up/down-stream, and intergenic). Functional impacts of exonic SNVs/indels were further classified as synonymous, nonsynonymous, stopgain, stoploss, and frameshift indels. SIFT was used to predict whether a nonsynonymous SNV affects protein function. SVs and CNVs were compared with gene annotation to get effected genes.

Identification of potential T2D candidate genes

Genotype was encoded as allele values separated by “/”. The allele values are 0 for the reference allele (what is in BN rat), 1 for the first variant allele, 2 for the second variant allele and so on. We compared SNVs and indels called from GK/Slac and Wistar/Slac, chose GK/Slac strain specific variants that are not presented or differently presented in Wistar/Slac strain. These GK/Slac specific variants were classified into five groups based on their genotypes in GK/Slac and Wistar/Slac: 1) 0/1, 0/0; 2)1/1, 0/0; 3)1/1, 0/1; 4)1/2, 0/0; 5)1/2, 0/1.

Further analysis focused on protein affecting variants (PAV): nonsynonymous, stopgain, stoploss, frameshift indels, splicing, and exonic ncRNA. We investigated their genotype profiles in 28 sequenced rat strains (including 1 GK strain and multiple Wistar strains arising from the different geographical locations), whose genomes were sequenced by Atanur et.al. [46] and downloaded from RGD database. Whole-genome SNPs in GK/Slac, Wistar/Slac and 28 sequenced rat strains were compared. Distance between all possible pairs of strains were measured by net nucleotide substitutions [88]. The phylogenetic tree was constructed using UPGMA (unweighted pair-group method with arithmetic means) method in MEGA 6.06 package [89]. T2D related candidate PAVs were selected if they were homozygous-variant in two GK strains (GK/Slac sequenced in our experiment, GK sequenced by Atanur.et.al.) and was not homozygous-variant in eleven Wistar-derived strains (SHR/NHsd, SHRSP/Gla, SHR/OlaIpcv, WKY/NCrl, WKY/Gla, WKY/NHsd, LEW/Crl, LEW/NcrlBR, WAG/Rij, MHS/Gib, MNS/Gib).

Functional analysis of potential candidate genes

Candidate gene lists were further filtered by integrating other information: T2D prior genes, protein-protein interaction, and differential gene expression.

We manually curated T2D related genes from published literatures and human GWAS catalog [http://www.genome.gov/admin/gwascatalog.txt] [90]. Totally, 506 T2D related genes were collected as prior genes (S6 File), including T2D susceptible genes, genes involved in important T2D pathways (such as insulin signaling pathway, adipocytokine signaling pathway), and genes associated with T2D related traits. Genes with GK/Slac specific variants were compared with T2D prior genes to narrow down the candidate gene list.

Known and predicted protein-protein interaction were obtained from STRING database [91], which quantitatively integrates interaction data from previous knowledge, genomic context, high-throughput experiments and conserved gene co-expression. We only used interaction pairs whose score is higher than 0.4. For T2D candidate gene list, we counted the number of their interaction partners in rat genome and in 506 T2D prior genes. Then we used Fisher test to calculate P-value, and adjusted it by multiple test. Genes with P-value $< 0.05$ were regarded as better candidate genes.
Gene expression data was downloaded from a GEO dataset GSE13271 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13271), which measured the expression of GK and Wistar in three tissues (liver, muscle and adipose) at five time points during T2D development [92]. To compare GK and Wistar, T-test and fold-change threshold was used to get significantly differentially expressed genes (P-value < 0.01 and fold-change > 2); R package DCGL 2.0 [93] was used to mine differentially co-expressed genes (P-value < 0.05 after Bonferroni correction). Results of each time point were combined to get the final differential gene list.

Supporting Information

S1 File. Detail of four libraries for whole genome sequencing of GK/Slac and Wistar/Slac rats. A total of 100.4 Gb (35.9X) and 85.6 Gb (30.5X) of short reads were generated for GK/Slac (Wistar/Slac) rat, respectively.

S2 File. Statistics for quality control and genome mapping results.

S3 File. Statistics for post-mapping quality control. Number of filtered reads were shown in the table. Values in parentheses are the percentage of filtered reads in total mapping results.

S4 File. Summary of variants in GK and Wistar rat, with BN rat as reference genome.

S5 File. Chromosome segments which have higher (top 5%) or lower (bottom 5%) SNV density and indel density. Density was defined as the number of SNV/indel in each 1Mb bin divided by the number of nucleotide bases in the same bin that were covered by at least three reads in GK sequencing. Low-density regions overlapped with gap were removed.

S6 File. A list of T2D prior genes that were collected from literatures or human GWAS catalog.

S7 File. GK/Slac specific variants and functional effect on genes. (A) Protein affecting SNV (B) Protein affecting indels. (C) Structural variations that overlapped with gene. (D) Copy number gain or loss that overlapped with gene.

S8 File. Progress for selecting potential T2D candidate genes. (A) 300 GK/Slac specific PAVs in 252 genes, which are homozygous mutant locus in GK/Slac strain but not in Wistar derived strains [46]. (B) After removing 60 ORs genes, there are 228 GK/Slac specific PAVs in 192 genes. These genes are analyzed by the following steps: 1) comparison with T2D prior genes; 2) differential (co-)expression between GK and Wistar rats in liver, muscle or adipose; 3) protein-protein interaction with T2D prior genes.

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Author Contributions
Conceived and designed the experiments: LL YYL YXL. Analyzed the data: TCL HL. Wrote the paper: TCL HL. Performed phylogenetic analysis: GHD ZW. Performed data preprocessing: YQC.

References
1. WHO. WHO Diabetes Fact Sheet N°312 2008 [cited 2009]. Available: http://www.who.int/mediacentre/factsheets/fs312/en/print.html.
2. Stumvoll M, Goldstein BJ, van Haften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005; 365(9467):1333–46. Epub 2005/04/13. S0140-6736(05)61032-X [pii]. doi:10.1016/S0140-6736(05)61032-X PMID: 15823385.
3. Prokopenko I, McCarthy MI, Lindgren CM. Type 2 diabetes: new genes, new understanding. Trends Genet. 2008; 24(12):613–21. Epub 2008/10/28. S0168-9525(08)00257-6 [pii]. doi:10.1016/j.tig.2008.09.004 PMID: 18952314.
4. Frayling TM. Genome-wide association studies provide new insights into type 2 diabetes aetiology. Nat Rev Genet. 2007; 8(9):657–62. Epub 2007/08/19. nrg2178 [pii]. doi:10.1038/nrg2178 PMID: 17703236.
5. McCarthy MI, Zeggini E. Genome-wide association studies in type 2 diabetes. Curr Diab Rep. 2009; 9(2):164–71. Epub 2009/03/03. PMID: 19329962.
6. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science. 2007; 316(5829):1336–41. Epub 2007/04/28. 1142364 [pii]. doi:10.1126/science.1142364 PMID: 17463249.
7. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007; 316(5829):1331–6. Epub 2007/04/28. 1142358 [pii]. doi:10.1126/science.1142358 PMID: 17463246.
8. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007; 316(5829):1341–5. Epub 2007/04/28. 1142382 [pii]. doi:10.1126/science.1142382 PMID: 17463248.
9. Unoki H, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, Andersen G, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet. 2008; 40(9):1098–102. Epub 2008/08/20. doi:10.1038/ng.208 PMID: 18711366.
10. Timpson NJ, Lindgren CM, Weedon MN, Randall J, Ouwehand WH, Strachan DP, et al. Adiposity-related heterogeneity in patterns of type 2 diabetes susceptibility observed in genome-wide association data. Diabetes. 2009; 58(2):505–10. Epub 2008/12/06. doi:10.2337/db08-0906 PMID: 19056611; PubMed Central PMCID: PMC2628627.
11. Takeuchi F, Serizawa M, Yamamoto K, Fujisawa T, Nakashima E, Ohnaka K, et al. Confirmation of multiple risk loci and genetic impacts by a genome-wide association study of type 2 diabetes in the Japanese population. Diabetes. 2009; 58(7):1690–9. Epub 2009/04/30. doi:10.2337/db08-1494 PMID: 19401414; PubMed Central PMCID: PMC2649880.
12. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, Chang CT, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. PLoS Genet. 2010; 6(2):e1000847. Epub 2010/02/23. doi:10.1371/journal.pgen.1000847 PMID: 20745558; PubMed Central PMCID: PMC2824763.
13. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nature Genetics. 2010; 42(7):579–U155. doi:10.1038/ng.609 PMID: 20027924 PMID: 20027924; Epub 2010/09/24. doi:10.1371/journal.pgen.1001127 [pii] PMID: 20862305; PubMed Central PMCID: PMC2940731.
14. Shu XO, Long J, Cai Q, Qi L, Xiang YB, Cho YS, et al. Identification of new genetic risk variants for type 2 diabetes. PLoS Genet. 2010; 6(9):e1001127. Epub 2010/09/24. doi:10.1371/journal.pgen.1001127 PMID: 20862305; PubMed Central PMCID: PMC2940731.
15. Sim X, Ong RT, Suo C, Tay WT, Liu J, Ng DP, et al. Transferability of type 2 diabetes implicated loci in multi-ethnic cohorts from Southeast Asia. PLoS Genet. 2011; 7(4):e1001363. Epub 2011/04/15. doi: 10.1371/journal.pgen.1001363 PMID: 21490949; PubMed Central PMCID: PMC3072366.
16. Parra EJ, Below JE, Krithika S, Valladares A, Barta JL, Cox NJ, et al. Genome-wide association study of type 2 diabetes in a sample from Mexico City and a meta-analysis of a Mexican-American sample from Starr County, Texas. Diabetologia. 2011; 54(8):2038–46. Epub 2011/05/17. doi: 10.1007/s00125-011-2172-y PMID: 21573907; PubMed Central PMCID: PMC3818640.

17. Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W, Frossard P, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet. 2011; 43(10):984–9. Epub 2011/08/30. doi: 10.1038/ng.921 PMID: 21574001; PubMed Central PMCID: PMC3773920.

18. Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet. 2012; 44(1):67–72. Epub 2011/12/14. doi: 10.1038/ng.1019 PMID: 22158593; PubMed Central PMCID: PMC3582398.

19. Palmer ND, McDonough CW, Hicks PJ, Roh BH, Wing MR, An SS, et al. A genome-wide association study identifies three novel loci for type 2 diabetes genes in African Americans. PLoS One. 2012; 7(1):e29202. Epub 2012/01/13. doi: 10.1371/journal.pone.0029202 PMID: 22235693; PubMed Central PMCID: PMC3251563.

20. Perry JR, Voight BF, Yengo L, Amin N, Dupuis J, Ganser M, et al. Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. PLoS Genet. 2012; 8(5):e1002741. Epub 2012/06/14. doi: 10.1371/journal.pgen.1002741 PMID: 22693455; PubMed Central PMCID: PMC3364960.

21. Li HX, Gan W, Lu L, Dong X, Han XY, Hu C, et al. A Genome-Wide Association Study Identifies GRK5 and RASGRP1 as Type 2 Diabetes Loci in Chinese Hans. Diabetes. 2013; 62(1):291–8. doi: 10.2337/db12-0454 PMID: 23900306; PubMed Central PMCID: PMC3773920.

22. Hara K, Fujita H, Johnson TA, Yamauchi T, Yasuda K, Horikoshi M, et al. Genome-wide association study identifies three novel loci for type 2 diabetes. Hum Mol Genet. 2014; 23(1):239–46. Epub 2013/08/16. doi: 10.1093/hmg/dd399 PMID: 23945395.

23. Han JS, Muller YL, Kobes S, Guo TW, Bian L, Ossowski V, et al. A Genome-Wide Association Study in American Indians Implicates DNER as a Susceptibility Locus for Type 2 Diabetes. Diabetes. 2014; 63(1):369–76. doi: 10.2377/diabetes.2013-0416 PMID: 24390345; PubMed Central PMCID: PMC4127086.

24. Williams AL, Jacobs SB, Moreno-Macias H, Huerta-Chagoya A, Churchhouse C, Marquez-Luna C, et al. Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. Nature. 2014; 506(7486):97–101. Epub 2014/01/07. doi: 10.1038/nature12828 PMID: 24390345; PubMed Central PMCID: PMC4127086.

25. Goto Y, Kakizaki M, Masaki N. Spontaneous diabetes produced by selective breeding of normal Wistar rats. Proc Jpn Acad. 1975; 51(1):80–5.

26. Portha B, Lacraz G, Kergoat M, Homo-Delarche F, Portha B, et al. The GK rat beta-cell: a prototype for the diseased human beta-cell in type 2 diabetes? Mol Cell Endocrinol. 2009; 297(1–2):73–85. Epub 2008/07/22. S0303-7207(08)00272-4 [pii]. doi: 10.1016/j.mce.2008.06.013 PMID: 18640239.

27. Movassat J, Calderani S, Fernandez E, Martin MA, Escriva F, Plachot P, et al. Type 2 diabetes—a matter of failing beta-cell neogenesis? Clues from the GK rat model. Diabetes Obes Metab. 2007; 9 Suppl 2:187–95. Epub 2007/10/09. DOM786 [pii]. doi: 10.1111/j.1463-1326.2007.00786.x PMID: 17919193.

28. Gauguier D, Froguel P, Parent V, Bernard C, Bhoreau MT, Portha B, et al. Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. Nat Genet. 1996; 12(1):31–8. Epub 1996/01/01. doi: 10.1038/ng0196-31 PMID: 8528247.

29. Galli J, Li LS, Glaser A, Ostenson CG, Jiao H, Fakhrai-Rad H, et al. Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. Nat Genet. 1996; 12(1):38–43. Epub 1996/01/01. doi: 10.1038/ng0196-38 PMID: 8528248.

30. Granhall C, Park HB, Fakhrai-Rad H, Luthman H. High-resolution quantitative trait locus analysis reveals multiple diabetes susceptibility loci mapped to intervals<800 kb in the species-conserved Niddm1i of the GK rat. Genetics. 2006; 174(3):1565–72. Epub 2006/09/05. genetics.106.062208 [pii]. doi: 10.1534/genetics.106.062208 PMID: 16951059; PubMed Central PMCID: PMC1667097.

31. Wain LV, Armour JA, Tobin MD. Genomic copy number variation, human health, and disease. Lancet. 2009; 374(9686):340–50. Epub 2009/06/19. S0140-6736(09)60249-X [pii]. doi: 10.1016/S0140-6736(09)60249-X PMID: 19535135.

32. Cook EH Jr, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. Nature. 2008; 455(7215):919–23. Epub 2008/10/17. nature07458 [pii]. doi: 10.1038/nature07458 PMID: 18923514.
33. Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS, et al. Identifying autism loci and genes by tracing recent shared ancestry. Science. 2008; 321(5886):218–23. Epub 2008/07/16. 321/5886/218 [pii]. doi: 10.1126/science.1157657 PMID: 18621663; PubMed Central PMCID: PMC2586171.

34. Schaschl H, Aitman TJ, Vysy TJ. Copy number variation in the human genome and its implication in autoimmunity. Clin Exp Immunol. 2009; 156(1):12–6. Epub 2009/02/18. CEI3865 [pii]. doi: 10.1111/j.1365-2249.2008.03865.x PMID: 19220326; PubMed Central PMCID: PMC2673736.

35. McCarroll SA, Altshuler DM. Copy-number variation and association studies of human disease. Nat Genet. 2007; 39(7 Suppl):S37–42. Epub 2007/09/05. ng2080 [pii]. doi: 10.1038/ng2080 PMID: 17597780.

36. Ye ZQ, Ni S, Yu Y, Yu H, Liu BH, Li RX, et al. Analyses of copy number variation of GK rat reveal new putative type 2 diabetes susceptibility loci. PLoS One. 2010; 5(11):e14077. Epub 2010/12/03. doi: 10.1371/journal.pone.0014077 PMID: 21124896; PubMed Central PMCID: PMC2999013.

37. Gabb RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature. 2004; 428(6982):493–521. Epub 2004/04/02. doi: 10.1038/nature02426 nature02426 [pii]. PMID:15057822.

38. Atanur SS, Birol I, Guryev V, Hummel O, Morrissey C, et al. The genome sequence of the spongilla Eudiadophora spongiosa. Genet. 2007; 39(7 Suppl):S45–61. Epub 2007/09/05. ng2080 [pii]. doi: 10.1038/ng2080 PMID: 17597780.

39. Atanur SS, Diaz AG, Maratou K, Sarkis A, Rotival M, Game L, et al. Genome sequencing reveals loci under artificial selection that underlie disease phenotypes in the laboratory rat. Cell. 2013; 154(3):691–703. Epub 2013/07/31. doi:10.1016/j.cell.2013.06.040 S0092-8674(13)00779-4 [pii]. PMID:23890820.

40. Quignon P, Giraud M, Rimbault M, Lavigne P, Tacher S, Morin E, et al. The dog and rat olfactory receptor repertoire. Genome Biol. 2005; 6(10):R83. Epub 2005/10/07. gb-2005-6-10-r83 [pii]. doi:10.1186/gb-2005-6-10-r83 PMID:16207354; PubMed Central PMCID: PMC1245004.

41. Riethman H, Ambrosini A, Castaneda C, Finklestein J, Hu XL, Mudunuri U, et al. Mapping and initial analysis of human subtelomeric sequence assemblies. Genome Res. 2004; 14(1):18–28. Epub 2004/01/07. doi:10.1101/gr.1299703 PMID:14707167; PubMed Central PMCID: PMC314271.

42. Saar K, Beck A, Bihoreau MT, Birney E, Brocklebank D, Chen Y, et al. SNP and haplotype mapping for genetic analysis in the rat. Nat Genet. 2008; 40(5):560–6. Epub 2008/04/30. doi: 10.1038/ng.124 ng.124 [pii]. PMID:18443594.

43. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010; 38(16):e164. Epub 2010/07/06. doi:10.1093/nar/gkp206 [pii]. PMID:20601685; PubMed Central PMCID: PMC2938201.

44. Bo et al. Sequencing of GK Rat Model with Type 2 Diabetes

45. Kvasnicka J, Skrha J, Perusicova J, Maslowska H, Pochopova L. Levels of tissue-type plasminogen activator (T-PA), its inhibitor (PAI-1) and fibrinogen in the blood of patients with type 1 and 2 diabetes mellitus. Cas Lek Cesk. 1996; 135(6):174–7. Epub 1996/03/20. 8681360. PMID: 8681360.
52. Maumus S, Marie B, Vincent-Viry M, Siest G, Visvikis-Siest S. Analysis of the effect of multiple genetic variants of cardiovascular disease risk on insulin concentration variability in healthy adults of the STAINSLAS cohort. The role of FGB-G455 G/A polymorphism. Atherosclerosis. 2007; 191(2):369–76. Epub 2006/05/16. S0021-9150(06)01990-0 [pii]. doi: 10.1016/j.atherosclerosis.2006.04.003 PMID: 16997386.

53. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, et al. PPARgamma signaling and metabolism: the good, the bad and the future. Nat Med. 2013; 19(5):557–66. Epub 2013/05/09. doi: 10.1038/nm.3159 nm.3159 [pii]. PMID: 23625116; PubMed Central PMCID: PMC3870016.

54. Mastej K, Adamiec R. Neutrophil surface expression of CD11b and CD62L in diabetic microangiopathy. J Diabetes Complications. 2002; 16(5):333–7. Epub 2002/08/30. S1056872710002264 [pii]. PMID: 12200076.

55. Kamiuchi K, Hasegawa G, Obayashi H, Kitamura A, Ishii M, Yano M, et al. Leukocyte-endothelial cell adhesion molecule 1 (LECAM-1) polymorphism is associated with diabetic nephropathy in type 2 diabetes mellitus. J Diabetes Complications. 2002; 16(5):333–7. Epub 2002/08/30. S1056872710002264 [pii]. PMID: 12200076.

56. Karadayi K, Top C, Gulecek O. The relationship between soluble L-selectin and the development of diabetic retinopathy. Ocul Immunol Inflamm. 2003; 11(2):123–9. doi:10.1080/10787220310001342718.

57. Paal L, Rotella CM. The role of DPP4 activity in cardiovascular districts: in vivo and in vitro evidence. J Diabetes Res. 2013; 2013:590456. Epub 2013/07/16. doi:10.1155/2013/590456 PMID: 23853775; ISI:000185483000006.

58. Mancusi S, La Manna A, Bellini G, Scianguetta S, Roberti D, Casale M, et al. HNF-1beta mutation sequencing of GK Rat Model with Type 2 Diabetes. Cardiopulmonary Medicine. 2013; 2013:44. doi:10.2337/db07-1731 PMID: 18678618; PubMed Central PMCID: PMC2570412.

59. Sell H, Bluhmer M, Kloeting N, Schlich R, Willems M, Ruppe F, et al. Adipose Dipeptidase-4 and Obesity Correlation with insulin resistance and depot-specific release from adipose tissue in vivo and in vitro. Diabetes Care. 2013; 36(12):4083–90. doi:10.23736/S0149-1992-13-36-12. PMID:24615699.

60. Cauchi S, Proenca C, Choquet H, Gaget S, De Graeve F, Marre M, et al. Analysis of novel risk loci for type 2 diabetes mellitus. Cardiovasc Diabetol. 2013; 12. Artn 143. doi:10.1186/1475-2840-12-143 PMID: IS000327605400001.

61. Shah P, Ardestani A, Dharmadhikari G, Laue S, Schumann DM, Kerr-Conte J, et al. The DPP-4 inhibitor linagliptin restores beta-cell function and survival in human isolated islets through GLP-1 stabilization. J Clin Endocrinol Metab. 2013; 98(7):E1163–72. Epub 2013/05/02. doi:10.1210/jc.2013-1029 jc.2013-1029 [pii]. PMID: 23633194.

62. Bluher M, Unger R, Sassoul F, Richter V, Paschke R. Relation between glycaemic control, hyperinsulinaemia and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or Type II diabetes. Diabetologia. 2002; 45(2):210–6. doi:10.1007/s00125-001-0723-3 PMID: 11935152.

63. Gaulton KJ, Willer CJ, Li Y, Scott LJ, Conneely KN, Jackson AU, et al. Comprehensive association study of type 2 diabetes and related quantitative traits with 222 candidate genes. Diabetes. 2008; 57 (11):3136–44. doi: 10.23736/S0012-5661-08-14316-8 PMID: 18678618; PubMed Central PMCID: PMC2570412.

64. Vanpoucke A, Bellini G, Scianguetta S, Roberti D, Casale M, et al. HNF-1beta mutation affects PKD2 and SOCS3 expression causing renal cysts and diabetes in MODY5 kindred. Journal of nephrology. 2013; 26(1):207–12. doi: 10.5301/jn.5000126 PMID: 22641569.

65. Wu SH, Wang YQ, Sun DQ. The association of HincII/low density lipoprotein receptor (LDLR) restriction fragment length polymorphism (RFLP) with diabetes mellitus and its lipid phenotype with PCR gene amplification. Zhonghua Yi Xue Za Zhi. 1993; 73(1):10–3. Epub 1993/01/01. PMID: 8099307.

66. Cauchi S, Proenca C, Choquet H, Gaget S, De Graeve F, Marre M, et al. Analysis of novel risk loci for type 2 diabetes in a general French population: the D.E.S.I.R. study. J Mol Med (Berl). 2008; 86 (3):341–8. Epub 2008/01/23. doi: 10.1007/s00109-007-0295-x PMID: 18210030.

67. Rensing KL, de Jager SC, Stroes ES, Vos M, Twickler MT, Dallinga-Thie GM, et al. Akt2/GER double knockout mice display impaired glucose tolerance and develop more complex atherosclerotic plaques than LDLr knockout mice. Cardiovasc Res. 2014; 101(2):277–87. Epub 2013/11/14. doi: 10.1093/cvr/cvt252 cvt252 [pii]. PMID: 24220638.

68. Engelbertsen D, To F, Duner P, Kotova O, Soderberg I, Alm R, et al. Increased inflammation in atherosclerotic lesions of diabetic Akita-LDLr(-/-) mice compared to nondiabetic LDLr(-/-) mice. Exp Diabetes Res. 2012; 2012:176162. Epub 2012/12/18. doi: 10.1155/2012/176162 PMID: 23243415; PubMed Central PMCID: PMC3515907.
69. Seok SJ, Lee ES, Kim GT, Hyun M, Lee JH, Chen S, et al. Blockade of CCL2/CCR2 signalling ameliorates diabetic nephropathy in db/db mice. Nephrol Dial Transpl. 2013; 28(7):1700–10. doi: 10.1093/ndt/gst558 PMID: ISI:000321821900014.

70. Butcher MJ, Hallinger D, Garcia E, Machida Y, Chakrabarti S, Nadler J, et al. Association of proinflammatory cytokines and islet resident leucocytes with islet dysfunction in type 2 diabetes. Diabetologia. 2014; 57(3):491–501. doi: 10.1007/s00125-013-3116-5 PMID: 24429578.

71. Makishii T, Araki S, Koya D, Maeda S, Kashiwagi A, Haneda M. C-106T polymorphism of AKR1B1 is associated with diabetic nephropathy and erythrocyte aldose reductase content in Japanese subjects with type 2 diabetes mellitus. Am J Kidney Dis. 2003; 42(5):943–51. doi: 10.1053/s0272-6386(03)01010-2 PMID: ISI:000186422000009.

72. Prasad P, Tiwari AK, Kumar KMP, Ammini AC, Gupta A, Gupta R, et al. Association analysis of ADPRT1, AKR1B1, RAGE, GFPT2 and PAI-1 gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes. BMC Medical Genetics. 2010; 11. Art n 52. doi: 10.1186/1471-2350-11-52 PMID: ISI:000276848800001.

73. Dominguez V, Raimondi C, Somanath S, Bugliani M, Loder ME, Edling CE, et al. Class II Phosphoinositide 3-Kinase Regulates Exocytosis of Insulin Granules in Pancreatic beta Cells. Journal of Biological Chemistry. 2011; 286(6):4216–25. doi: 10.1074/jbc.M110.200295 PMID: ISI:000286975700020.

74. Satarranatarajan K, Mariappan MM, Lee MJ, Felliars D, Coudhury GG, Barnes JL, et al. Regulation of elongation phase of mRNA translation in diabetic nephropathy: amelioration by ramiprilum. The American journal of pathology. 2007; 171(6):1733–42. doi: 10.2333/ajpath.2007.070412 PMID: 17992118; PubMed Central PMCID: PMC2111098.

75. Guest PC, Abdel-Halim SM, Gross DJ, Clark A, Poitout V, Amaria R, et al. Proinsulin processing in the diabetic Goto-Kakizaki rat. The Journal of endocrinology. 2002; 175(3):637–47. PMID: 12475375.

76. Chu KY, Briggs MJ, Albrecht T, Drain PF, Johnson JD. Differential regulation and localization of carboxypeptidase D and carboxypeptidase E in human and mouse beta-cells. Islets. 2011; 3(4):155–65. Epub 2011/06/02. 15767 [pii]. PMID:21628999.

77. Deng WJ, Nie S, Dai J, Wu JR, Zeng R. Proteome, phosphoproteome, and hydroxyproteome of liver mitochondria in diabetic rats at early pathogenic stages. Mol Cell Proteomics. 2010; 9(1):100–16. Epub 2009/08/25. doi: 10.1074/mcp.M900020-MCP200 M900020-MCP200 [pii]. PMID:19700791; PubMed Central PMCID: PMC2808256.

78. Jiang XS, Dai J, Sheng QH, Zhang L, Xia QC, Wu JR, et al. A comparative proteomic strategy for subcellular proteome research: ICAT approach coupled with bioinformatics prediction to ascertain rat liver mitochondrial proteins and indication of mitochondrial localization for catalase. Mol Cell Proteomics. 2005; 4(1):12–34. Epub 2004/10/28. M400079-MCP200 [pii]. doi: 10.1074/mcp.M400079-MCP200 PMID:15507458.

79. Zheng L, Feng Y, Shi Y, Zhang J, Mu Q, Qin L, et al. Intralipid decreases apolipoprotein M levels and insulin sensitivity in rats. PLoS One. 2014; 9(8):e105681. Epub 2014/08/22. doi: 10.1371/journal.pone.0105681 PONE-D-14-01693 [pii]. PMID:25120793; PubMed Central PMCID:PMC4140822.

80. Sun X, Zheng M, Song M, Bai R, Cheng S, Xing Y, et al. Ileal interposition reduces blood glucose levels and decreases insulin resistance in a type 2 diabetes mellitus animal model by up-regulating glucagon-like peptide-1 and its receptor. Int J Clin Exp Pathol. 2014; 7(7):4136–40. doi: 10.2333/ajpath.2014.070412 PMID: 25083789; PubMed Central PMCID:PMC4118962.

81. Zhou D, Jiang X, Ding W, Zhang D, Yang L, Zhen C, et al. Impact of bariatric surgery on ghrelin and obestatin levels in obesity or type 2 diabetes mellitus rat model. J Diabetes Res. 2014; 2014:569435. Epub 2014/03/29. doi: 10.1155/2014/569435 PMID: 24672803; PubMed Central PMCID:PMC3941146.

82. Twigger S, Lu J, Shimoyama M, Chen D, Pasko D, Long H, et al. Rat Genome Database (RGD): mapping disease onto the genome. Nucleic acids research. 2002; 30(1):125–8. PMID: 11752273; PubMed Central PMCID: PMC99132.

83. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012; 9(4):357–9. Epub 2012/03/06. doi: 10.1038/nmeth.1923 nmeth.1923 [pii]. PMID: 22388286; PubMed Central PMCID:PMC3232381.

84. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009; 25(16):2078–9. Epub 2009/06/10. doi: 10.1093/bioinformatics/btp352 btp352 [pii]. PMID:19505943; PubMed Central PMCID:PMC2723002.
86. Rausch T, Zichner T, Schlattl A, Stutz AM, Benes V, Korbel JO. DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinformatics. 2012; 28(18):i333–i9. Epub 2012/09/11. doi: 10.1093/bioinformatics/bts378 [pii]. PMID:22962449; PubMed Central PMCID: PMC3436805.

87. Xi RB, Luquette J, Hadjianpanayis A, Kim TM, Park PJ. BIC-seq: a fast algorithm for detection of copy number alterations based on high-throughput sequencing data. Genome Biology. 2010; 11. Artn O10. doi: 10.1186/Gb-2010-11-S1-O10 PMID: ISI:000283779100021.

88. Nei M, Kumar S. Molecular evolution and phylogenetics. New York: Oxford University Press; 2000.

89. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular biology and evolution. 2013; 30(12):2725–9. doi: 10.1093/molbev/mst197 PMID: 24132122; PubMed Central PMCID: PMC3840312.

90. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic acids research. 2014; 42(Database issue):D1001–6. doi: 10.1093/nar/gkt1229 PMID: 24316577; PubMed Central PMCID: PMC3965119.

91. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013; 41(Database issue):D808–15. Epub 2012/12/04. doi: 10.1093/nar/gks1094 gks1094 [pii]. PMID: 23203871; PubMed Central PMCID: PMC3531103.

92. Almon RR, DuBois DC, Lai W, Xue B, Nie J, Jusko WJ. Gene expression analysis of hepatic roles in cause and development of diabetes in Goto-Kakizaki rats. J Endocrinol. 2009; 200(3):331–46. Epub 2008/12/17. doi: 10.1677/JOE-08-0404 JOE-08-0404 [pii]. PMID: 19074471.

93. Yang J, Yu H, Liu BH, Zhao Z, Liu L, Ma LX, et al. DCGL v2.0: An R Package for Unveiling Differential Regulation from Differential Co-expression. PLoS One. 2013; 8(11):e79729. Epub 2013/11/28. doi: 10.1371/journal.pone.0079729 PONE-D-13-20749 [pii]. PMID: 24278166; PubMed Central PMCID: PMC3835854.

94. Lee SA, Kim YR, Yang EJ, Kwon EJ, Kim SH, Kang SH, et al. CD26/DPP4 Levels in Peripheral Blood and T Cells in Patients With Type 2 Diabetes Mellitus. J Clin Endocr Metab. 2013; 98(6):2553–61. doi: 10.1210/Jc.2012-4288 PMID: ISI:000319736500064.