Identification of key genes associated with congenital heart defects in embryos of diabetic mice

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Abstract. Maternal diabetes has been reported to be a critical factor for congenital heart defects (CHD) in offspring. The present study aimed to screen the key genes that may be involved in CHD in offspring of diabetic mothers. The present study obtained the gene expression profile of GSE32078, including three embryonic heart tissue samples at embryonic day 13.5 (E13.5), three embryonic heart tissue samples at embryonic day 15.5 (E15.5) from diabetic mice and their respective controls from normal mice. The cut-off criterion of P<0.08 was set to screen differentially expressed genes (DEGs). Their enrichment functions were predicted by Gene Ontology. The enriched pathways were forecasted by Kyoto Encyclopedia of Genes and Genomes and Reactome analysis. Protein-protein interaction (PPI) networks for DEGs were constructed using Cytoscape. The present study identified 869 and 802 DEGs in E13.5 group and E15.5 group, respectively and 182 DEGs were shared by the two developmental stages. The pathway enrichment analysis results revealed that DEGs including intercellular adhesion molecule 1 (Icam1) and H2‑M9 were enriched in cell adhesion molecules; DEGs including bone morphogenetic protein receptor type 1A, transforming growth factor β receptor 1 and SMAD specific E3 ubiquitin protein ligase 1 were enriched in the tumor growth factor-β signaling pathway. In addition, DEGs including Icam1, C1s and Fc fragment of IgG receptor IIb were enriched in Staphylococcus aureus infection. Furthermore, the shared DEGs including Icam1, nuclear receptor corepressor 1 (Ncor1) and AKT serine/threonine kinase 3 (Akt3) had high connectivity degrees in the PPI network. The shared DEGs including Icam1, Ncor1 and Akt3 may be important in the cardiogenesis of embryos. These genes may be involved in the development of CHD in the offspring of diabetic mothers.

Introduction

Diabetes mellitus is a chronic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1). According to insulin-dependent and non-insulin-dependent, adult diabetes is classified: Type 1 and type 2 diabetes. A genetic defect on insulin was found in type 1 diabetes, whereas insulin resistance is the key metabolic abnormality in type 2 diabetes (2). In addition, paternal diabetes in pregnancy is defined as glucose intolerance or first recognition during pregnancy, who is easy to happen type 2 diabetes post-partum (3). Besides, maternal diabetes in pregnancy is related to an increased risk of congenital heart defects (CHD), macrosomia, miscarriage, and other birth defects in offspring (4,5). CHD was defined as deficits of the structure and function arising from cardiac embryogenesis stage (6). It has been reported that CHD is the major consequences in diabetic embryopathy (7).

An infant born to a diabetic mother has been shown to exhibit axial mesodermal dysplasia spectrum with atrioventricular septal defects (8). Many studies have reported that maternal diabetes altered expression of genes in developmental embryo (4,9,10). Bmp4, belongs to the TGF-β superfamily, is a myocardial signaling molecule which activated epithelial-mesenchymal transition (EMT) during cardiogenesis (11). The expression of Bmp4 has been reported to be down-regulated by the Msx1 that expressed in atrioventricular canal endocardial cells during EMT (12,13). Pax3 is also essential for heart formation and outflow tract development in the mouse embryo (14). Study showed that the downregulation of Bmp4, Msx1 and Pax3 could contribute to the pathogenesis of maternal diabetes-induced CHD (15). In addition, it has been reported that hyperglycemia altered the expression of eNOC and VEGF that are involved in the regulation of vasculogenesis (16). Furthermore, a recent study on microarray analysis showed that several genes were altered in embryonic heart tissues from diabetic mother and were closely associated with CHD, such as Smyd1, Tsc1 and Gja1 (4). However, the exact pathogenesis of CHD in offspring of diabetic mother is still unknown.

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In this study, we explored the gene expression profiles of embryonic heart tissue samples at embryonic day 13.5 (E13.5) and embryonic day 15.5 (E15.5) from diabetic mice, and their respective controls. The differentially expressed genes (DEGs) were screened in E13.5 and E15.5 groups. Gene Ontology (GO) functions, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome pathway enrichment analyses were then performed to identify the DEGs. We also constructed the protein-protein interaction (PPI) networks for the DEGs and analyzed several important shared genes that were associated with CHD. Our study aimed to identify the critical genes that might be involved in CHD in offspring of diabetic mother and to provide evidence to further clarify the relationship between CHD and diabetes.

Materials and methods

Affymetrix microarray data. The gene expression profile of GSE32078 was obtained from the study of Vijaya et al (4), which was deposited in Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) of National Center of Biotechnology Information (NCBI). A total of 12 samples were available, including three embryonic heart tissue samples at E13.5 and three embryonic heart tissue samples at E15.5 from streptozotocin-induced diabetic Swiss albino mice (8-10 weeks), as well as their respective controls from normal mice. The day when a copulation plug was observed was counted as E0.5. Raw data were collected with the Affymetrix Mouse Genome 430 2.0 Array (Affymetrix, CA, USA). All raw data files were pretreated by RMA method in Affy package (17).

Screening of DEGs. The Linear Models for Microarray Data (LIMMA) package (18) was used to identify DEGs. All genes were tested with F-test and the DEGs with the threshold P-value <0.08 were screened.

GO and KEGG pathway enrichment analysis. ClusterProfiler, a new ontology-based tool, offers three methods (group GO, enrich GO, enrich KEGG) for genes classification and enrichment analyses (19,20). ClusterProfiler package was used to identify the main biological processes and metabolic pathways in DEGs. Default parameters (organism, mouse; ont, BP; P-value cut-off, 0.05; P-adjust method, none; readable, T) were used as the cut-off criteria for GO function enrichment analysis. And the default parameters (organism, mouse; P-value cut-off, 1; q-value cut-off, 1; readable, 1) were used as the cut-off criteria for KEGG pathway enrichment analysis.

Reactome pathway enrichment analysis. ReactomePA package was used to identify the main biological processes and metabolic pathways in DEGs (21,22). Default parameters (organism, mouse; P-value cut-off, 1; q-value cut-off, 1; minGSSize, 1; readable, 1) were used as the cut-off criteria for Reactome pathway enrichment analysis.

PPI network construction. The Search Tool for the Retrieval of Interacting Genes (STRING) database was used to obtain PPI data. The PPI networks of up- and downregulated DEGs were visualized by Cytoscape (http://cytoscape.org/) that is an open source software for visualizing complex networks and integrating these networks with any type of attribute data (23). The Required Confidence score >0.4 was chosen as the threshold.

Results

Screening of DEGs. The microarray dataset GSE32078 from GEO database was obtained to identify the DEGs in E13.5 and E15.5 hearts of embryos from diabetic mice compared with their respective controls. Totally, 869 DEGs were obtained in E13.5 group when compared with the control group, including 411 up- and 458 downregulated DEGs. Meanwhile, 802 genes were upregulated and 1,295 genes were downregulated in E15.5 group when compared with the control group. Finally, a total of 182 DEGs were shared between E13.5 and E15.5 groups, including 63 up- and 119 downregulated DEGs.

GO function enrichment analysis of DEGs. According to the GO enrichment analysis, we found that the upregulated DEGs in E13.5 group were mainly enriched in developmental process (P=2.32E-08) and the downregulated DEGs were mainly enriched in organic substance metabolic process (P=2.93E-06). In the E15.5 group, the upregulated DEGs were mainly enriched in cellular process (P=2.64E-19) and the downregulated DEGs were mainly enriched in cellular process (P=2.01E-19). Besides, we also performed the GO function enrichment analysis for the genes that were shared by E13.5 group and E15.5 group. The results showed that the upregulated DEGs were mainly related to cellular process (P=0.00650) and the downregulated DEGs were mainly related to organic substance metabolic process (P=0.00031) (Fig. 1).

KEGG and Reactome pathway analysis of DEGs. KEGG and Reactome enrichment pathway analysis were performed to explore the enriched pathways of the DEGs. The KEGG enrichment analysis results showed that the upregulated DEGs in E13.5 group were enriched in 64 pathways, such as Icam1, H2-M9, and 4930468A15Rik were enriched in cell adhesion molecules (CAMs) (P=1.56E-03). The downregulated DEGs in E13.5 group were enriched in 84 pathways, such as Bmpr1a, Tgfbr1 and Smurf1 were enriched in TGF-ß signaling pathway (P=4.03E-03). The upregulated DEGs in E15.5 group were enriched in 129 pathways, such as Icam1, C1s and Fcgr2b were enriched in Staphylococcus aureus infection (P=1.80E-04). Meanwhile, the downregulated DEGs in E15.5 group were enriched in 156 pathways, such as Erolib, Dnajc3 and Dnajb11 were enriched in protein processing in endoplasmic reticulum (P=1.27E-03). Moreover, the shared DEGs that were upregulated such as Cmah and Gmppa were enriched in nucleotide sugar metabolism (P=2.65E-03). And the shared DEGs that were downregulated such as Akt3, Pak3 and Hgf were enriched in renal cell carcinoma (P=5.00E-04); Akt3, Pak3 and Pak1 were enriched in T cell receptor signaling pathway (P=2.60E-03) (Table 1). Reactome enrichment analysis results showed that the upregulated DEGs in E13.5 group were enriched in 154 pathways and the downregulated DEGs in E13.5 group were enriched in 192 pathways. The upregulated DEGs in E15.5 group were enriched in 27 pathways.
and the downregulated DEGs in E15.5 group were enriched in 24 pathways. In addition, the shared DEGs that were upregulated such as Nr6a1 and Esrrg were enriched in nuclear receptor transcription pathway (P=5.09E-03) and the shared DEGs that were downregulated such as Pak3 and Pak1 were enriched in activation of Rac (P=1.85E-03) (Table II).

**PPI network construction.** STRING was used to construct the PPI networks for DEGs. The PPI networks for up- and downregulated DEGs in E13.5 group contained 114 and 112 nodes, respectively (Fig. 2); the PPI networks for up- and downregulated DEGs in E15.5 group contained 345 and 562 nodes, respectively (Fig. 3). The proteins such as Icam1, Akp3, Stat1 and Brca1 had high connectivity degrees in the PPI networks of E13.5 (Table III) and E15.5 (Table IV) groups. Additionally, the PPI network for shared DEGs contained 17 nodes and 12 PPI pairs (Fig. 4). The shared DEGs with top three connectivity degrees of the PPI network were Icam1, Ncor1 and Akt3 (Table V).

**Discussion**

Maternal diabetes is a relatively common disease that results in an increased incidence of congenital malformations such as neural tube defects and heart defects (9). CHD are the most common type of birth defects and a main cause of
| DEGs Term          | Description                                      | P-value  | Gene ID         | Count |
|-------------------|--------------------------------------------------|----------|-----------------|-------|
| Upregulated DEGs in E13.5 | Cell adhesion molecules (CAMs)                   | 1.56 x 10^{-3} | Icam1, H2-M9, 4930468A15Rik, H2-D1, Itga4, H2-Ob, Ctnap2, Nrnx2 | 8     |
| mmu04672          | Intestinal immune network for IgA production    | 3.77 x 10^{-3} | Gm13306, Itga4, Il15, H2-Ob | 4     |
| mmu05330          | Allograft rejection                              | 8.24 x 10^{-3} | H2-M9, H2-D1, H2-Ob, Il12b | 4     |
| mmu04940          | Type 1 diabetes mellitus                         | 1.24 x 10^{-2} | H2-M9, H2-D1, H2-Ob, Il12b | 4     |
| mmu04146          | Peroxisome                                       | 2.74 x 10^{-2} | Apgs, Nudl19, Slc25a17, Acsbg2 | 4     |
| Downregulated DEGs in E13.5 | TGF-β signaling pathway                          | 4.03 x 10^{-3} | Bmpr1a, Tgfbr1, Smurf1, Ldbp1, Lefty2, Thbs2 | 6     |
| mmu04350          | Renal cell carcinoma                             | 9.14 x 10^{-3} | Akt3, Pak3, Hgf, Pak1, Pik3ca | 5     |
| mmu04080          | Neuroactive ligand-receptor interaction          | 1.05 x 10^{-2} | Prlr, Gabra1, Tshr, Gabra2, Gabrb2, Crrh2... | 11    |
| mmu05223          | Non-small cell lung cancer                       | 1.66 x 10^{-2} | Akt3, Pik3ca, Stk4, Rabr | 4     |
| mmu04210          | Apoptosis                                        | 1.87 x 10^{-2} | Akt3, Il3, Akt3, Endod1, Pik3ca | 5     |
| Upregulated DEGs in E15.5 | Staphylococcus aureus infection                  | 1.80 x 10^{-4} | Icam1, C1s, Fcgri2b, H2-Ab1, C2, Fpr2, Cfh, H2-Aa | 8     |
| mmu04514          | Cell adhesion molecules (CAMs)                   | 1.18 x 10^{-3} | Icam1, Vcan, H2-K1, Cd80, Ncam1... | 13    |
| mmu04145          | Phagosome                                        | 1.61 x 10^{-3} | Lamp2, H2-K1, Tap2, Tuba4a, Thbs2, Fcgr2b... | 14    |
| mmu05219          | Bladder cancer                                   | 2.20 x 10^{-3} | Rbl1, Mmp9, Myc, E2f3, Egfr, E2f2 | 6     |
| mmu05220          | Chronic myeloid leukemia                         | 2.27 x 10^{-3} | Bcl211, Rbl1, Myc, Chlb, E2f3, Sox2, E2f2, Ab1 | 8     |
| Downregulated DEGs in E15.5 | Protein processing in endoplasmic reticulum     | 1.27 x 10^{-3} | Erol1b, Dnajc3, Dnajb11, Sec62, Derl1, Mapk8... | 18    |
| mmu01100          | Metabolic pathways                               | 2.25 x 10^{-3} | Phgdh, B3gnt5, Sula2, Alad, B4galnt6, Mmbn... | 77    |
| mmu04977          | Vitamin digestion and absorption                 | 4.84 x 10^{-3} | Tcn2, Rhb2, Plb1, Lrat, Slc5a6 | 5     |
| mmu04010          | MAPK signaling pathway                           | 9.22 x 10^{-2} | Caeca1a, Pttn5, Mapk8, Caeca2d1, Akt3, Gna12... | 22    |
| mmu00512          | Mucin type O-Glycan biosynthesis                 | 9.57 x 10^{-3} | Gent3, C1galt1, Galt2, Galnt7, Galnt15 | 5     |
| Upregulated genes in shared DEGs | Amino sugar and nucleotide sugar metabolism    | 2.65 x 10^{-3} | Cmah, Gmppa | 2     |
| mmu01100          | Metabolic pathways                               | 6.08 x 10^{-3} | Lias, Gmppa | 2     |
| mmu05211          | Renal cell carcinoma                             | 5.00 x 10^{-4} | Akt3, Pak3, Hgf, Pak1, | 4     |
| mmu04660          | T cell receptor signaling pathway                | 2.60 x 10^{-3} | Akt3, Pak3, Pak1, Grap2 | 4     |
| mmu04730          | Long-term depression                             | 6.09 x 10^{-3} | Caeca1a, Gna12, Ppplr17 | 3     |
| mmu04210          | Apoptosis                                        | 9.95 x 10^{-3} | Akt3, Il3, Endod1 | 3     |
| mmu04012          | ErbB signaling pathway                           | 1.03 x 10^{-2} | Akt3, Pak3, Pak1 | 3     |
| mmu04510          | Focal adhesion                                   | 1.96 x 10^{-2} | Akt3, Pak3, Hgf, Pak1, | 4     |
| mmu04010          | MAPK signaling pathway                           | 5.02 x 10^{-2} | Akt3, Caeca1a, Gna12, Pak1 | 4     |
| mmu04080          | Neuroactive ligand-receptor interaction          | 5.48 x 10^{-2} | Prlr, Tshr, Gabrb2, Crrh2 | 4     |
| mmu04630          | Jak-STAT signaling pathway                       | 4.49 x 10^{-2} | Akt3, Prlr, Il3 | 3     |
| mmu00140          | Steroid hormone biosynthesis                     | 3.31 x 10^{-2} | Hsd17b7, Hsd11b2 | 2     |

E13.5, embryonic day 13.5; E15.5, embryonic day 15.5; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.
Table II. Enrichment analysis of top five Reactome pathways for DEGs.

| DEGs                  | Term                        | Description                                                                 | P-value          | Gene ID                                                                 | Count |
|----------------------|-----------------------------|-----------------------------------------------------------------------------|------------------|--------------------------------------------------------------------------|-------|
| Upregulated DEGs in E13.5 | 4809882                    | Neuronal system                                                             | 2.10x10⁻³       | Gjd2, Braf, Kcnb1, Chrna3, Gabrg2, Gls...                                | 10    |
|                      | 4810204                    | Neurotransmitter receptor binding and downstream transmission in the post-synaptic cell | 2.14x10⁻³       | Braf, Chrna3, Gabrg2, Arhgef9, Ap2a2, Epb4.111, Rps6ka2                  | 7     |
|                      | 4810917                    | Interferon γ signaling                                                      | 2.64x10⁻³       | Icam1, Oas1h, Irf7, Sp100, H2-D1                                         | 5     |
|                      | 4809881                    | Transmission across chemical synapses                                       | 3.10x10⁻³       | Braf, Chrna3, Gabrg2, Gls, Arhgef9, Ap2a2, Epb4.111, Rps6ka2            | 8     |
|                      | 4810848                    | Regulation of cytoskeletal remodeling and cell spreading by IPP complex components | 5.26x10⁻³       | Parvb, Pxn                                                               | 2     |

| Downregulated DEGs in E13.5 | 4810408                    | GABA receptor activation                                                   | 3.56x10⁻⁴       | Gabra1, Gabra2, Gabrb2, Gngt2, Kcnj3, Kcnj9                              | 6     |
|                      | 4809881                    | Transmission across chemical synapses                                       | 8.56x10⁻⁴       | Cacna1a, Gabra1, Gabra2, Gabrb2, Camk2b, Gngt2...                      | 10    |
|                      | 4809882                    | Neuronal system                                                             | 9.71x10⁻⁴       | Cacna1a, Gabra1, Gabra2, Gabrb2, Camk2b, Kcnq5...                      | 12    |
|                      | 4810957                    | GABA A receptor activation                                                  | 1.01x10⁻³       | Gabra1, Gabra2, Gabrb2                                                   | 3     |
|                      | 4810204                    | Neurotransmitter receptor binding and downstream transmission in the post-synaptic cell | 1.68x10⁻³       | Gabra1, Gabra2, Gabrb2, Camk2b, Gngt2, Kcnj3, Kcnj9, Chrnb3            | 8     |

| Upregulated DEGs in E15.5 | 4810016                    | Immune system                                                               | 1.13x10⁻⁷       | Ifitm3, Ifitm6, Lgals3, H2-K1, Tap2, Bcl2l1...                          | 64    |
|                      | 4810918                    | Interferon signaling                                                        | 5.69x10⁻⁵       | Icam1, Stat1, Irf1, Oas1h, Ncam1, Ifnar2...                            | 13    |
|                      | 4810008                    | EGFR interacts with phospholipase C-γ                                       | 5.88x10⁻⁵       | Adcy7, Plcg1, Adrbk1, Egfr, Creb1, Camk4, Adcy9                       | 7     |
|                      | 4810917                    | Interferon γ signaling                                                      | 6.00x10⁻⁵       | Icam1, Stat1, H2-K1, Irf1, Oas1h, Ncam1, Soesx3...                     | 10    |
|                      | 4810011                    | PLCG1 events in ERBB2 signaling                                             | 7.26x10⁻⁵       | Adcy7, Plcg1, Adrbk1, Egfr, Creb1, Camk4, Adcy9                       | 7     |

| Downregulated DEGs in E15.5 | 4809896                    | Metabolism of water-soluble vitamins and cofactors                          | 2.69x10⁻³       | Amn, Mmaa, Mmbb, Tcn2, Slc23a2, Mthfr, Rfk, Slc5a6, Mocs1, Ppcs        | 10    |
|                      | 4809897                    | Metabolism of vitamins and cofactors                                       | 2.69x10⁻³       | Amn, Mmaa, Mmbb, Tcn2, Slc23a2, Mthfr, Rfk, Slc5a6, Mocs1, Ppcs        | 10    |
|                      | 4809898                    | Defective TCN2 causes hereditary megaloblastic anemia                       | 2.69x10⁻³       | Amn, Mmaa, Mmbb, Tcn2, Slc23a2, Mthfr, Rfk, Slc5a6, Mocs1, Ppcs        | 10    |
|                      | 4809899                    | Defects in cobalamin (B12) metabolism                                      | 2.69x10⁻³       | Amn, Mmaa, Mmbb, Tcn2, Slc23a2, Mthfr, Rfk, Slc5a6, Mocs1, Ppcs        | 10    |
|                      | 4809900                    | Defects in vitamin and cofactor metabolism                                  | 2.69x10⁻³       | Amn, Mmaa, Mmbb, Tcn2, Slc23a2, Mthfr, Rfk, Slc5a6, Mocs1, Ppcs        | 10    |

| Upregulated genes in shared DEGs | 4810720                    | Nuclear receptor transcription pathway                                       | 5.09x10⁻³       | Nr6a1, Esrrg                                                            | 2     |
|                                 | 4810917                    | Interferon γ signaling                                                      | 9.26x10⁻³       | Icam1, Oas1h                                                            | 2     |
|                                 | 4810918                    | Interferon signaling                                                        | 2.35x10⁻²       | Icam1, Oas1h                                                            | 2     |
|                                 | 4810429                    | Generic transcription pathway                                               | 3.07x10⁻²       | Nr6a1, Esrrg                                                            | 2     |
|                                 | 4810016                    | Immune system                                                               | 6.30x10⁻²       | Icam1, Oas1h, C6, Fbxw11, Kif2a                                         | 5     |
Table II. Continued.

| DEGs                | Term                        | Description                        | P-value          | Gene ID     | Count |
|---------------------|-----------------------------|------------------------------------|------------------|-------------|-------|
| Downregulated genes in shared DEGs | 4810839 | Activation of Rac | $1.85 \times 10^{-03}$ | Pak3, Pak1 | 2     |
|                     | 4810573 | CD28 co-stimulation | $5.02 \times 10^{-03}$ | Pak1, Grap2  | 2     |
|                     | 4810613 | Generation of second messenger molecules | $5.02 \times 10^{-03}$ | Pak1, Grap2  | 2     |
|                     | 4810625 | Signaling by Robo receptor | $9.02 \times 10^{-03}$ | Pak3, Pak1   | 2     |
|                     | 4810145 | FCERI mediated MAPK activation | $1.55 \times 10^{-02}$ | Pak1, Grap2  | 2     |

E13.5, embryonic day 13.5; E15.5, embryonic day 15.5; DEGs, differentially expressed genes.

Table III. DEGs with the top 10% connectivity degree in the PPI network in E13.5 group.

| DEGs       | ID | Degree | ID | Degree | ID | Degree | ID | Degree |
|------------|----|--------|----|--------|----|--------|----|--------|
| Upregulated DEGs |     |         |    |         |    |         |    |         |
| Icam1      | 48 | 9      | H2-M9 | 5     | H2-L | 8      | Gabrg2 | 5   |
|            |    |         |      |        |     |         |      |        |
| Irf7       | 48 | 7      | Pxn  | 5     |      |         |      |        |
|            |    |         |      |        |     |         |      |        |
| Oas1h      | 48 | 5      | II12b| 4     |      |         |      |        |
| Downregulated DEGs |    |         |    |         |    |         |    |         |
| Akp3       | 48 | 13     | Ncor1| 7     |      |         |      |        |
|            |    |         |      |        |     |         |      |        |
| Nr3c1      | 48 | 10     | Stat2| 6     |      |         |      |        |
|            |    |         |      |        |     |         |      |        |
| Ar         | 48 | 9      | II3  | 6     |      |         |      |        |

DEGs, differentially expressed genes; PPI, protein-protein interaction; E13.5, embryonic day 13.5.

Table IV. DEGs with the top 10% connectivity degree in the PPI network in E15.5 group.

| DEGs       | ID | Degree | ID | Degree | ID | Degree | ID | Degree |
|------------|----|--------|----|--------|----|--------|----|--------|
| Upregulated DEGs |     |         |    |         |    |         |    |         |
| Stat1      | 48 | 38     | Mmp9 | 19     | Iffi1 | 16     | Ezr  | 13     |
| Egfr       | 48 | 37     | Irf1 | 18     | Cxcl1 | 15     | Mbp  | 13     |
| Icam1      | 48 | 27     | Ccr2 | 18     | Ifil35 | 14   | Hrrma1 | 13   |
| Myc        | 48 | 27     | Fpr2 | 18     | Actb  | 14     | Rmcs2 | 13     |
| Pten       | 48 | 25     | Ncam1| 17     | Ccl6  | 14     | Cpsf6 | 13     |
| Stat2      | 48 | 21     | Plcg1| 17     | Cblb  | 14     | Adbk1| 13     |
| Yes1       | 48 | 20     | Creb1| 16     | H2-K1 | 14     | Rsad2| 12     |
| Downregulated DEGs |    |         |    |         |    |         |    |         |
| Brca1      | 48 | 33     | Dlgap5| 20   | Tmem48 | 17   | Rpn1 | 12     |
| Chek1      | 48 | 30     | Creb1| 20     | Hspa5 | 16     | Kcnj11| 12    |
| Bub1       | 48 | 29     | Aspm | 20     | Lsm4  | 14     | Med1 | 11     |
| Rrm2       | 48 | 26     | Zwint| 20     | Act3  | 14     | Calr | 11     |
| Sgol2      | 48 | 25     | Cenpm| 19     | Sec61a| 13   | Kalm | 11     |
| Cep55      | 48 | 25     | Spc24| 19     | Lpar3 | 13     | Scl10a1| 11   |
| Plk4       | 48 | 24     | Eme1 | 19     | Hjurnp| 13    | Rgn  | 10     |
| Tpx2       | 48 | 24     | Foxm1| 19     | Nasp  | 13     | Cfi  | 10     |
| Kif23      | 48 | 23     | Xpo1 | 18     | Igtf1r| 13    | Ddost| 10     |
| Ska1       | 48 | 23     | Troap| 18     | Gucy1b3| 13   | Prkx | 10     |
| Prc1       | 48 | 23     | Op5  | 17     | Ncor1 | 12    | Rae1 | 10     |

DEGs, differentially expressed genes; PPI, protein-protein interaction; E15.5, embryonic day 15.5.
Figure 2. PPI networks for upregulated (A) and downregulated (B) DEGs in E13.5 hearts of embryos from diabetic mice. The size of node indicates the degree of DEG. DEGs, differentially expressed genes; PPI, protein-protein interaction; E13.5, embryonic day 13.5.
Figure 3. PPI networks for upregulated (A) and downregulated (B) DEGs in E15.5 hearts of embryos from diabetic mice. The size of node indicates the degree of DEG. DEGs, differentially expressed genes; PPI, protein-protein interaction; E15.5, embryonic day 15.5.
birth defects-related mortality and morbidity (24,25). It has been reported that CHD are closely associated with maternal diabetes (26). Previous study had screened and analyzed several DEGs in embryonic heart tissues from diabetic mother (4). However, the molecular mechanism between CHD and diabetes remains largely unknown. In this study, we have screened 869 and 1,295 in E13.5 and E15.5 groups, respectively and 182 DEGs were shared by two groups. Moreover, the DEGs such as Icam1 and H2-M9 were significantly enriched in cell adhesion molecules (CAMs); DEGs such as Bmpr1a, Tgfbr1 and Smurf1 were enriched in TGF-β signaling pathway; DEGs such as Icam1, C1s and Fcgr2b were enriched in Staphylococcus aureus infection. Finally, several key shared DEGs that were the genes with the top three node degrees in the network were analyzed, including Icam1, Ncor1 and Akt3.

Our results showed that Icam1 had the highest connectivity degree not only in the PPI network of the upregulated DEGs in E13.5 group but also in the PPI network of the shared DEGs. ICAMI, also called CD54, is a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system (27) and the protein is considered to participate in atherogenesis by promoting monocyte accumulation in the arterial intima (28). An earlier study has found that the expression of ICAMI on endothelial cells and circulating monocytes may be critical for the adhesion of the cells on the vascular endothelium (29). And the expression of ICAMI has been also detected on cardiac myocytes both in adult humans with unexplained cardiac dysfunction (30) and animals with myocarditis (31,32). According to the pathway enrichment results in our study, we found that Icam1 were enriched in Staphylococcus aureus infection, CAMs and interferon γ signaling. Inflammation is showed to participate in the pathogenesis of type 2 diabetes (33). Upregulation of adhesion molecules such as ICAM1 is pivotal in the development of inflammatory responses (34). Besides, autoimmune-associated congenital heart block (CHB) may result from pathogenic cross-talk between inflammatory and profibrosing pathways (35). Therefore, Icam1 might be involved in the development of heart in mouse embryos.

Ncor1 also had a high node degree in the PPI network for the shared DEGs. It is well known that Ncor1 is a transcriptional coregulator that controls the activity of many transcription factors (such as MEF2, ERRs) and has wide-ranging effects on gene expression patterns (36). MEF2 family has been associated with regulation of myocardially-expressed genes within the heart, such as cardiac α-actin (37). MEF2 is critical for normal heart development and mitochondrial integrity (38). There is a strict relationship between oxygen consumption and cardiac work (39). Oxidative stress is thought to play a particularly critical role in the development of cardiovascular pathology (40). SIRT1 that is part of Ncor1/SMRT complex (36) could retard aging and confer oxidative stress resistance to the heart in vivo (41,42). SMRT (Ncor2), the homolog of Ncor1, is also considered to take part in heart formation (43). Thus, Ncor1, as a transcriptional coregulator, might regulate several genes that are related to the heart development through several different signaling pathways.

Akt3 was interacted with Ncor1 in the PPI network for the shared DEGs. Study has showed that Akt3 is a member of the
Akt subfamily that comprises three closely related isoforms Akt1, Akt2 and Akt3. Akt regulates several cellular processes including metabolism, cell growth, proliferation, survival and angiogenesis (44). Dysregulation of Akt leads to many diseases such as cancer, diabetes, cardiovascular and neurological diseases (44). Akt has an important role in the functional behavior in the cardiovascular system such as cardiomycocytes, thrombocytes and endothelial cells (44). Akt1 is demonstrated to be essential for heart development and function (45). In addition, Akt3 was found to be enriched in most of the KEGG pathways in shared DEGs, especially renal cell carcinoma. Renal insufficiency in patients with acquired heart failure and ischemic heart disease is related to higher mortality and morbidity (46). Therefore, Akt3 might be related to the heart development during embryogenesis. Additionally, there are some limitations in the present study. For example, much more samples should be used to clarify the finding; the expression level of Icam1, Ncorl, and Akt3 should be verified by RT-PCR in the maternal diabetes associated with CHD. However, the present study may provide a scientific guidance for future study to clarify the relationship between CHD and diabetes. It is helpful to explain the molecular mechanism of the CHD development in offspring of diabetic pregnancies. These DEGs may be the therapeutic target in the offspring of diabetic pregnancies with CHD.

In conclusion, we have identified many DEGs in embryonic tissue samples at E13.5 and E15.5 from diabetic mice using bioinformatics analysis. And we found that the shared DEGs such as Icam1, Ncorl, and Akt3 had high connectivity degrees in the network. Our study implied that maternal diabetes could affect Icam1, Ncorl, and Akt3 which are critical in the heart development during embryogenesis and might result in CHD. However, more research is needed to confirm these results and further explore the complex molecular mechanism.

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