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Gene Expression Profiling of the Mouse Pancreas during the Secondary Transition in the Organogenesis of the Pancreatic Gland*

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Abstract

Diabetes mellitus is a chronic disease that impacts the homeostasis of blood sugar levels caused by loss or defect of insulin-producing β-cells in the Islets of Langerhans. *Type 1 diabetes* (*T1D*) is caused by auto-immune mediated destruction of β-cells, whereas in *T2D*, insulin is produced but used inefficiently. *T2D* accounts for 90% of people with diabetes worldwide (*WHO* 1999) and is the fastest increasing disease worldwide (https://diabetesatlas.org/en/). For an improved understanding of the pathomechanism of diabetes, profound knowledge of pancreas organogenesis and the associated gene regulatory networks is required. Therefore, we dissected and profiled the pancreatic endodermal and non-endodermal compartment between the embryonic stages (E) 12.5 and E 15.5 when progenitor cells commit to their different pancreatic lineages. Our associated study mined the global mRNA expression profile to increase the understanding of the secondary transition, endodermal-non-endodermal tissue interaction, and diabetic-related gene regulation. Furthermore, we validated 635 regulated pancreatic genes using the publicly available *GenePaint.org*, respective *gp3.mpg.de* to evaluate genes associated with genetic variants in Single-nucleotide polymorphism (SNP) related to *T2D*.

Keywords

Gene Expression Profile, Pancreas Organogenesis, Single-Nucleotide Polymorphism, Type 1 Diabetes, Type 2 Diabetes

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1. Experimental Design, Materials and Methods

1.1. Dissection, Isolation, and Processing

Pancreatic tissue was isolated from FVF+ embryos at E 12.5 to E 15.5 in triplicates [1]. A single-cell suspension was obtained by trypsinization with 0.25% Trypsin and the specific incubated pancreatic tissue resuspension. Fluorescent-Activated cell sorting (FACs) was used to separate the FVF+ endodermal and FVF− non-endodermal cells. Total RNA was isolated using Trizol (Qiagen) and purified by using the RNeasy Mini kit (Qiagen) according to the manufacturer's protocol. The Agilent 2100 Bioanalyzer had assessed RNA quality. The total RNA of three biological replicates for each embryonic stage was used to generate cRNA by two rounds of transcription. In general, a poly (dT) primer was utilized to synthesize cDNA from total RNA. In the next step, the cDNA amplified cRNA through T7 polymerase, using the T7 promoter sequence of the poly (dT) primer in the first round by random priming. Next, the cDNA was employed for the transcription into biotinylated cRNA, which hybridized to the 169 *Affymetrix*® array format according to the manufacturer protocol (*GeneChip*® Gene mouse 1.0 ST Array Card). Subsequently, processing of the Fluidics script FS450_0007 and scanning (*GeneChip*® Scanner 3000 7G Whole-Genome Association System) further lead to the probe set outcome.

The *Affymetrix*® Expression console normalized the robust multichip-analyses (RMA) on gene-level (ratio > 200 compared endodermal and non-endodermal). Comprehensive R based microarray analyses web frontend (CARMAweb) inducted gene-wise testing by Limma t-test and Benjamini-Hochberg multiple testing correction with a false discovery rate (FDR < 5%) offered statistically significant differential expression of 2921 probe sets [2]. Data were submitted to the Gene Expression Omnibus (GSE66856).

1.2. Gene ontology (GO) Term Analysis and Validation of Gene Expression by Gene Paint *in Silico in Situ*

Subsequently, GO term enrichment, annotated through association of InterPro records with GO term, grouped 635 genes in the following clusters: extracellular/located at plasma membrane (304 genes), signaling molecules (222 genes), transcription factors (TF; 178 genes), cilium related (40 genes) and previously not described (42 genes). Afterwards, pancreatic-specific gene expression and localization were analyzed using the database *GenePaint.org*, respective *gp3.mpg.de*, which offers *in silico in situs* of whole mount embryosat E1 4.5. Pancreatic genes had been arranged according to their expression pattern in mesenchyme (non-epithelial), tip (exocrine), trunk (endocrine) and epithelium (pancreatic multipotent cells).

1.3. Analysis of Mouse Orthologous Genes with Associated Single-Nucleotide Polymorphism (SNP) in T1D and T2D GWAS Hits

SNP of genes associated with *T1D* and *T2D* had been analyzed for their expres-

sion level during secondary transition and depicted in a heatmap (Figure 1). The heatmap represents the relative fold change in gene expression during the secondary transition stage between the non-endodermal and endodermal tissue compartments. The clustering of the genes was accomplished through extraction of gene symbols by Marullo *et al.* and Hakonarson *et al.* [3] [4]. Based on gene expression levels in the two tissue compartments we identified different clusters of the selected genes: transcriptionally regulated in both tissue populations or elevated either in the endodermal or the non-endodermal tissue compartment.

Heatmap shows clustering of 153 *T1D*- and *T2D*-associated genes according to their expression level during lineage commitment between E12.5 - 15.5. Predominantly, maturity-onset diabetes of the young (MODY) genes are upregulated in the endodermal compartment during pancreas organogenesis (*Gck*, *Pdx1*, *NeuroD*, *Hnf1α*, *Hnf1ß*). The black arrow marks the gene *Ins2*.

A subset of these *T2D*-associated genes which are regulated in our mRNA expression profile is illustrated in Table 1. Additional information of the mRNA localization (endodermal/non-endodermal), gene locus, chromosomal localization, the associated SNP and the disease as well as the related publications is provided as follows [4]-[12].

The GenePaint.org, respective *gp3.mpg.dein silico in situ* platform confirmed expression of *mouse orthologues* with associated SNPs in *T2D* shown in Table 1. Additional information about mRNA localization of the selected genes is obtained by inspection of the *in situ* whole mount embryos (see Figure 2).

Table 1. Genes associated with SNP in GWAS studies and related to T1D or T2D.

Genetic variants associated with T2D or glycemic traits					
Localization	Locus name	Chromosome	associated SNP	established loci	Literature
non- and endodermal	TCF7L2	10	rs4506565, rs7903146, rs12243326	T2DM	Dupuis *et al.*, 2012; Saxena *et al.*, 2010
endodermal	KCNQ1	11	rs2237892, rs231362	T2DM	Yasuda *et al.*, 2008; Voight *et al.*, 2010
endodermal	PROX1	1	rs340874	T2DM	Dupuis *et al.*, 2012
endodermal	HNF1β	17	rs757210, rs4430796, rs7501939	T2DM	Pearson *et al.*, 2007; Winckler *et al.*, 2007; Wang *et al.*, 2014
endodermal	KCNJ11	11	rs5219, rs757110	T2DM	Gloyn *et al.*, 2003
endodermal	GCK	7	rs1799881, rs6975024, rs4607517, rs3757840	T2DM	Dupuis *et al.*, 2012; Soranzo *et al.*, 2010; Scott *et al.*, 2012
endodermal	FOXA2	20	rs6113722, rs6048205	T2DM	Scott *et al.*, 2012; Manning *et al.*, 2012
endodermal	PDX1	13	rs11619319, rs2293941	T2DM	Scott *et al.*, 2012; Manning *et al.*, 2012

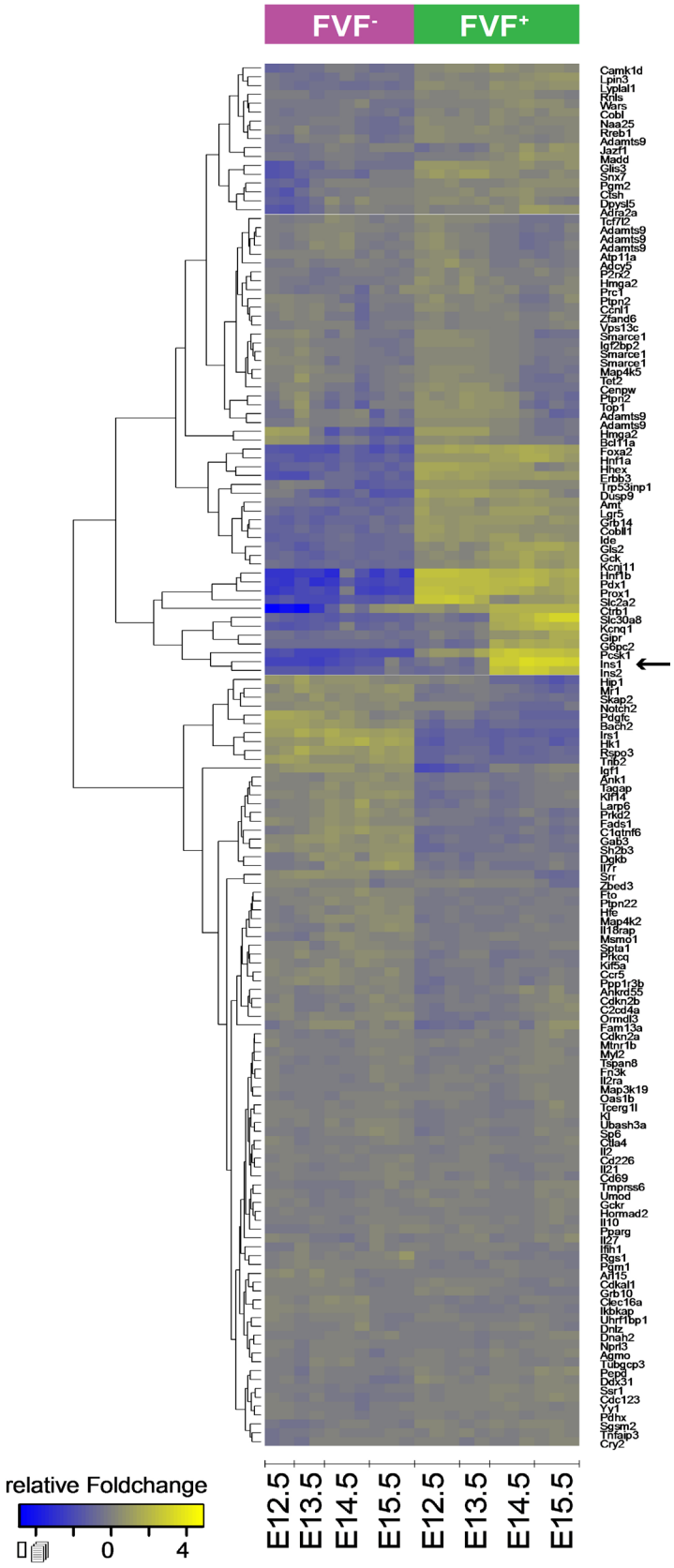

Figure 1. Heatmap depicting *mus musculus* orthologous genes with associated SNP in *T1D* and *T2D*GWAS hits.

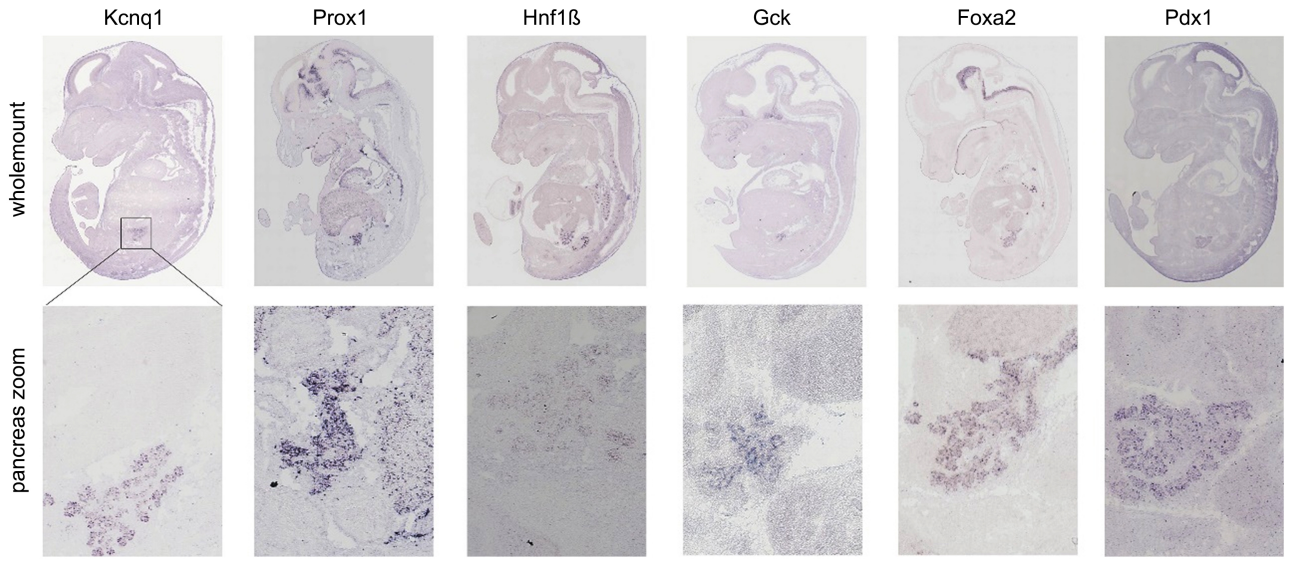

Figure 2. GenePaint *in silico in situ* of the genes represented in **Table 1** at E14.5.

Sagittal sections of whole mount embryos and magnification of the pancreatic region are shown. The genes associated with SNP in *T2D* as *Kcnq1*, *Prox1*, *Hnf1ß*, *Gck*, *Foxa2* and *Pdx1* exhibit epithelial *in situ* expression pattern.

2. Conclusion

The study comprehensively underpins the association of de-/regulation of developmental genes in the mouse embryo with distinct mRNA expression, validated by *genepaint*, respective *gp3.mpg.de* and genetic variants in SNP connected to the disease *diabetes mellitus*. Thus, the database may serve as a resource for further evaluation of mouse genes and their respective human orthologues in developmental stages and in a next step in different diseases. Further genes in the context of major health diseases as described in this study on *Diabetes mellitus*, *Diabetes mellitus* related *corornary artery disease* (*CAD*), *cancers* and *respiratory diseases* might shed light on disease mechanism and will improve therapeutic approaches for a better health outcome of the patients' need.

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Direct Link to the Deposited Data

Data is available through the NCBI database under the following link http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=gnubwesiphanjiv&acc=GSE66856.

Statistical analyses of the dataset were accomplished by using the GEO2R platform.

Conflict of Interest

The author declares that there is no conflict of interest on the work presented in the paper.

References

[1] Burtscher, I., Barkey, W. and Lickert, H. (2013) Foxa2-Venus Fusion Reporter Mouse Line Allows Live-Cell Analysis of Endoderm-Derived Organ Formation. *Genesis*, **51**, 596-604. https://doi.org/10.1002/dvg.22404

[2] Rainer, J., Sanchez-Cabo, F., Stocker, G., Sturn, A. and Trajanoski, Z. (2006) CARMAweb: Comprehensive R- and Bioconductor-Based Web Service for Microarray Data Analysis. *Nucleic Acids Research*, **34**, 498-503. https://doi.org/10.1093/nar/gkl038

[3] Marullo, L., El-Sayed Moustafa, J.S. and Prokopenko, I. (2014) Insights into the Genetic Susceptibility to Type 2 Diabetes from Genome-Wide Association Studies of Glycaemic Traits. *Current Diabetes Reports*, **14**, Article Number: 551. https://doi.org/10.1007/s11892-014-0551-8

[4] Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A.U., *et al.* (2010) New Genetic Loci Implicated in Fasting Glucose Homeostasis and Their Impact on Type 2 Diabetes Risk. *Nature Genetics*, **42**, 105-116. https://doi.org/10.1038/ng.520

[5] Saxena, R., Hivert, M.-F., Langenberg, C., Tanaka, T., Pankow, J.S., Vollenweider, P., *et al.* (2010) Genetic Variation in GIPR Influences the Glucose and Insulin Responses to an Oral Glucose Challenge. *Nature Genetics*, **42**, 142-148. https://doi.org/10.1038/ng.521

[6] Yasuda, K., Miyake, K., Horikawa, Y., Hara, K., Osawa, H., Furuta, H., *et al.* (2008) Variants in KCNQ1 Are Associated with Susceptibility to Type 2 Diabetes Mellitus. *Nature Genetics*, **40**, 1092-1097. https://doi.org/10.1038/ng.207

[7] Voight, B.F., Scott, L.J., Steinthorsdottir, V., Morris, A.P., Dina, C., Welch, R.P., *et al.* (2010) Twelve Type 2 Diabetes Susceptibility Loci Identified through Large-Scale Association Analysis. *Nature Genetics*, **42**, 579-589. https://doi.org/10.1038/ng.609

[8] Pearson, E.R., Donnelly, L.A., Kimber, C., Whitley, A., Doney, A.S.F., Mccarthy, M.I., *et al.* (2007) Variation in *TCF7L2* Influences Therapeutic Response to Sulfonylureas. *Diabetes*, **56**, 2178-2182. https://doi.org/10.2337/db07-0440

[9] Winckler, W., Weedon, M.N., Graham, R.R., McCarroll, S.A., Purcell, S., Almgren, P., *et al.* (2007) Evaluation of Common Variants in the Six Known Maturity-Onset Diabetes of the Young (MODY) Genes for Association with Type 2 Diabetes. *Diabetes*, **56**, 685-693. https://doi.org/10.2337/db06-0202

[10] Gloyn, A.L. (2003) The Search for Type 2 Diabetes Genes. *Ageing Research Reviews*, **2**, 111-127. https://doi.org/10.1016/S1568-1637(02)00061-2

[11] Scott, R.A., Lagou, V., Welch, R.P., Wheeler, E., Montasser, M.E., Luan, J., *et al.* (2012) Large-Scale Association Analyses Identify New Loci Influencing Glycemic Traits and Provide Insight into the Underlying Biological Pathways. *Nature Genetics*, **44**, 991-1005. https://doi.org/10.1038/ng.2385

[12] Manning, M.-F., Hivert, Scott, R.A., Grimsby, J.L., Bouatia-Naji, N., Chen, H., *et al.* (2012) A Genome-Wide Approach Accounting for Body Mass Index Identifies Genetic Variants Influencing Fasting Glycemic Traits and Insulin Resistance. *Nature Genetics*, **44**, 659-669. https://doi.org/10.1038/ng.2274

Abbreviations

T1D—type 1 diabetes
T2D—type 2 diabetes
WHO—world health organisation
E—embryonic stage
mRNA—messenger ribonucleic acid
SNP—single-nucleotide polymorphism
GWAS—genome-wide association study
FACs—fluorescent-activated cell sorting
FVF—Foxa2-Venus-Fusion
cDNA—complementary deoxynucleic acid
GRN—gene regulatory network
RNA—ribonucleic acid
cRNA—complementary ribonucleic acid
RMA—robust multichip-analyses
CARMA—Comprehensive R based microarray analyses web frontend
GO—gene ontology
TF—transcription factor

Specifications Table

Subject area	Biology
More specific subject area	Developmental biology
Type of data	Figure, Table
How data was acquired	Affymetrix® mouse gene 1.0 ST array card and Affymetrix® Expression console.
Data format	CARMAweb based Limma t-test and Benjamini Hochberg multiple testing corrections.
Experimental factors	Comparison of the mouse endodermal and non-endodermal pancreatic compartment during the secondary transition between E12.5 and E15.5. Bioinformatic analysis identified differentially regulated genes and additionally offered the narration of Genome-Wide Association Study (GWAS) annotated diabetes-related genes.
Experimental features	Fluorescent-activated cell sorting (FACs) and RNA extraction of Foxa2VenusFusion (FVF)+ endodermal and FVF– non-endodermal cells. Conversion to cDNA and Hybridization to the Affymetrix®array.
Data source location	Garching 85748, Germany, Am Parkring 11, Institute of Diabetes Research (IDR)
Data accessibility	Data available at the Gene Expression Omnibus, GSE66856. http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=gnubwesiphanjiv&acc=GSE66856

Value of the Data

- Demonstrates the value of the Foxa2-Venus fusion reporter mouse line for diabetic related research [1].
- Provides a rich data set of differentially regulated genes within the endodermal and non-endodermal tissue compartments during the secondary transition of the pancreas organogenesis.
- Offers temporal and spatial progression of mouse orthologues *T1D* and *T2D* associated genes.
- Evaluates database-dependent gene regulatory network (GRN) of genome-wide association studies (GWAS)-annotated diabetes genes.