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GATA6 and its Roles in Human Pancreas Development

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Abstract

Understanding the regulation of pancreatic development is fundamental for the identification of novel therapeutic options for the highly prevalent diabetic patients all over the world. GATA6 mutations are associated with a broad pancreatic phenotype spectrum from severe pancreas agenesis and neonatal diabetes to the adult onset of diabetes. Many studies in mice verified the critical roles of GATA6 in pancreas development, however, phenotypes disparity is very large between human and mice. To understand the underlying mechanism of the human phenotypes, using human Pluripotent Stem Cells (hPSCs) to model the GATA6 deficiency in vitro becomes to be a very efficient research strategy. Deficient GATA6 gene dosage disrupts the differentiation of human pancreatic progenitors, leads to impaired formation of pancreatic β-like cells and the pancreas. Except for the gene dosage, GATA6 was identified activating transcription in its nearby genes temporally, which further activates cardiomyocyte and endoderm gene network through chromatin binding in intergenic regions without a GATA-binding motif. The elucidation of the GATA6 function in the pancreas can indicate a novel therapy strategy i.e. stem cell therapy.

Keywords: GATA6; Pancreas development; Genetics; Gene mutation

Introduction

The human pancreas functionally consists of both endocrine and exocrine tissues. The endocrine tissues are called as islets of Langerhans, which secrets five (at least) different hormones into the circulation for glucose regulation (α-cells, glucagon, β-cells, insulin, δ-cells, somatostatin, ε-cells, ghrelin, and γ-cells, [or PP]-Pancreatic Polypeptide) cells. The exocrine tissue is composed of clusters of the acinar cells, which produce and secrete digestive enzymes through a network of the ductal cells. Pancreas development begins with the formation of a dorsal and ventral pancreatic bud. The dorsal bud arises first and generates most of the pancreas. The ventral bud arises beside the bile duct and forms only part of the head and uncinate process of the pancreas [1].

GATA factors (GATA1-6) contain highly conserved zinc-finger factors, which recognize and bind the (A/T) GATA (A/G) regulatory motifs in enhancer and promoter regions to modulate their transcriptional output. They function therefore as pioneer factors during embryonic development [2,3]. Functionally, GATA1/2/3 was found to be critical for normal haematopoiesis and are important for the development of the brain, spinal cord, and inner ear [4]. GATA4/5/6 are important for the development of mesoderm and endoderm derived organs, including heart, liver, and pancreas [5]. The fundamental role of GATA factors involved in pancreas morphogenesis in mice has been widely illustrated [6]. In humans, how GATA6 plays role in the development of the pancreas and pancreas beta cells has not been as well studied as it is in mice. This review will summarize the association of human GATA6 mutations and their correlated pancreatic phenotypes, as well as the underlying molecular mechanism of these mutations.

Human gata6 mutations and the phenotype spectrum

The association between mutations in GATA factors and human abnormal development has been known for some time [4,7]. Only through the technology development in next-generation sequencing, the association of GATA6 mutations and pancreas pathology was started to be known. Alle and colleagues performed exome sequencing in 27 individuals with pancreatic agenesis and found de novo heterozygous inactivating mutations in GATA6 were the most frequent cause of pancreas agenesis [8]. Subsequently, many more reports confirmed this finding and revealed a much more complex pancreatic disease spectrum with GATA6 mutations [8-12]. The most common feature in pancreatic disorder is pancreas agenesis and diabetes, the majority of which are neonatal diabetes (98%), but also a small portion of adolescent or adult-onset diabetes [9]. Interestingly, congenital cardiac malformation was first revealed in many patients (with a penetrance of 87%) before the pancreas pancreatic phenotype (with a penetrance of 60%) [9,10,12,13]. Other extrapancreatic features like biliary tract defect, gut abnormalities, congenital diaphragmatic hernia, renal disorder, neurocognitive abnormalities, and many other developmental disorders are also present in the patients harboring GATA6 mutations [11,14-18] indicates the broad rules of GATA6 during embryonic development. Most of the identified GATA6 mutations involved pancreatic abnormalities are heterozygous de novo variants, which accounts for 58% of all the detected variants. The mechanism underlying the broad phenotype spectrum of the GATA6 mutations has not been well elucidated, even though the Loss of Function (LoF) due to haploinsufficiency can explain around 50% of described GATA6 mutations [15]. Very recently, Sharma and colleagues studied the Congenital Heart Disease cohort (CHD) by Whole-Exome Sequencing (WES) and identified nine heterozygous de novo variants (4 LoF and 5 missense damaging variants) in GATA6. Together with 61 previous published variants, all 70 variants caused CHD, pancreas agenesis/hypoplasia or congenital diagrammatic hernia occurred in 20/70 (28.5%) CHD patients [16].

The association between the GATA6 mutations and the phenotype spectrum was described in detail in the reviewing paper (Figure 1) [15]. In 131 GATA6 mutation carriers with available cardiac assessment, eighty percent (n=105) of the carriers exhibited a structural cardiac

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abnormality (including cardiomyopathy). Besides, 16 mutation carriers (12%) present as arterial fibrillation without a cardiac structural abnormality (Figure 1a). For the 55 GATA6 mutation carriers with data on the presence and/or function of the pancreas, 84% (46/55) of them were abnormal (Figure 1b). Therefore, GATA6 mutations cause a wide range of phenotypic defects with variable clinical penetrance; the most frequent disorders are the heart and pancreatic malformations. Most of the GATA6 mutations locate at the functional domains of GATA6 (transcriptional activation domain and two Zinc finger domains) (Figure 1c) [15].

Gata6 plays critical roles in the definitive endoderm (de) formation and pancreas formation

The molecular networks in which GATA6 participates will help to elucidate the underlying mechanisms of how GATA6 mutations cause morphogenesis defects in the heart, pancreas, and other organs. Unlike many recessive loss of function gene mutations in key pancreatic developmental transcription factors (e.g. PDX1, PTF1A, RFX1, NEUROD2, NGN3, and NKX2.2), their pancreatic and extrapancreatic phenotypes are consistent in human and mouse, the phenotypes caused

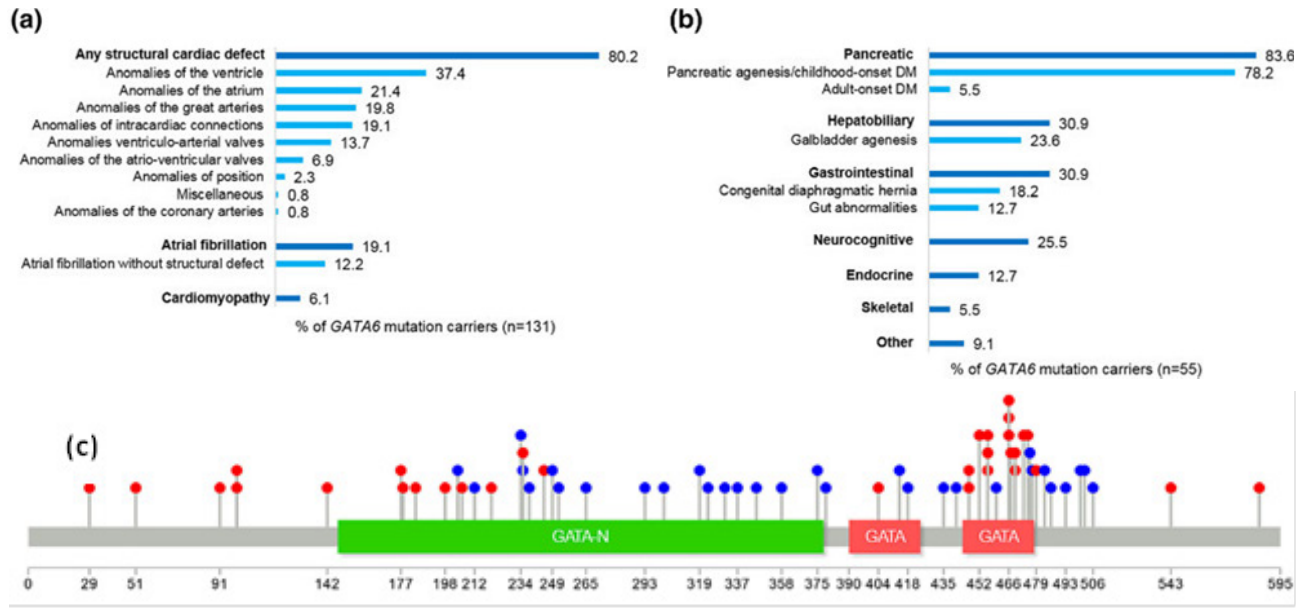

Figure 1: LPhenotypic spectrum of the patients with GATA6 mutations. a) Cardiac features among 131 GATA6 mutations carriers with available cardiac assessment. b) 55 GATA6 mutation carriers with available data on pancreas assessment. The main categories are cardiac and pancreatic malformation. c) Schematic diagram of the GATA6 protein and the distribution of the GATA6 mutations. Green: transcription activity domain, red: two zinc finger domains adapted from the review paper [15].

by GATA6 mutations is however very discrepant in human and mice. Therefore, using human Pluripotent Stem Cells (hPSCs) to model the GATA6 deficiency in vitro becomes one efficient research strategy to understand the underlying mechanism of the human phenotypes [17-22].

Villani and colleagues investigated the tip progenitor domain in the branched epithelium of the human fetal pancreas between 13.5 and 17.5 gestational weeks. They found that pancreatic multipotent progenitor cells (SOX9+/PTF1A+Cells) were enriched for MYC and GATA6 [23]. Through genome editing in hPSCs, Shi, et al. also revealed GATA6 haploinsufficiency especially affects the differentiation of human pancreatic progenitors, leading to impaired formation of pancreatic β-like cells in a dosage-sensitive requirement [24]. These findings were confirmed by an established CRISPR/Cas genome editing human Induced Pluripotent Stem (IPS) cell line derived from a patient heterozygous mutation. The 4 base pair (bp) duplication in exon 2 of GATA6 causes a frame-shift mutation resulting in truncating protein. The IPSinde/indel in both GATA6 alleles significantly decreased their Definitive Endoderm (DE) induction, underwent increased apoptosis, impaired its pancreatic specification, and reduced the differentiation efficiency from the definitive endoderm stage into β-like cells [25]. Chia, et al modelled GATA6 loss in vitro through combining both gene-edited and patient-derived hPSCs, then directed differentiation towards β-like cells. A modest reduction in the definitive endoderm formation was found in the GATA6 heterozygous hPSCs, while GATA6-null hPSCs failed to enter the DE lineage. The underlying mechanism was revealed through genome-wide studies that GATA6 governs definitive endoderm formation through cooperation with EOMES/SMAD2/3 to regulate the expression of cardinal endoderm genes [26].

Despite the different phenotypes associated with different GATA6 heterozygous variants at the definitive endoderm stage, complete loss of GATA6 was found in all-above mentioned studies impair definitive endoderm formation, which highlights that for robust definitive endoderm specification in human, the wide-type GATA6 gene dosage is unequivocally required through cooperation with different pathways. The gene-dose effect and variable disease penetrance indicate some gene modifiers to GATA6 contributing to the embryonic developmental process [16,25,26].

GATA6 affects heart and pancreas development under different regulatory ways

Except for the coding mutations in GATA6 cause embryonic development malformation, regulatory factors are also found to be involved in this procedure. GATA6-AS1, one long non-coding RNAs (lncRNA) divergently transcribed from GATA6 locus was found to be the highest expression in the human ESC lines (HUES8 and H9) during the definitive endoderm differentiation. Depletion of GATA6-AS resulted in definitive endoderm differentiation deficiency, and this defect could

be rescued by GATA6 over expression. GATA6 and GATA6-AS share downstream targets during the endoderm differentiation. GATA6-AS functions as regulating GATA6 expression by interacting with and mediating SMAD 2/3 to bind to the GATA6 promoter region [27]. Different phenotypes like pancreatic agenesis, congenital heart disease, or acute myocardial infarction were highly associated with variants locating in the locus GATA6/GATA6-AS1; these variants very likely act by affecting the function of the GATA6 protein, but how these variants locating in non-coding regions contribute to the broad disorders are so far poorly understood [8].

Gadue's group reported that even after the coding mutation was corrected, a lower expression of GATA6 in Pancreas Agenesis (PA) patient-derived pancreatic progenitor cells were still detected compared to the wide type pluripotent stem cells lines, which led to the screening of the non-coding variants in the regulatory region of GATA6. The minor allele of SNP rs12953985 (A) in Trans was found more frequent in the PA patients than non-PA cohort, which suggests this allele may strongly increase the risk of PA (OR=7.78, 95% CI=0.8-76.1, p=0.0779). Using genome-edited isogenic human patient-derived PSC lines, this non-coding SNP rs12953985 reduced the efficiency of generating pancreatic progenitor cells, in conjunction with a heterozygous GATA6 mutation in vitro [28]. The minor allele variant of rs12953985 lowers GATA6 expression during pancreas specification through disruption of the RORα binding site, which was implicated as one of the key regulating pancreatic transcription factors [29].

Sharma and colleagues created GATA6 LoF (1 bp insertion on exon2) and GATA6R456G/R456G hiPSCs using CRISPR/Cas9 technology. In this study, GATA6 was found acting as a pioneer factor, altering network-level transcriptional pathways that are critically involved in the development of the heart (GATA4 and HAND2), endodermal lineages (HNF, FOXA1, and FOXA2), pancreas (PDX1, SOX6), and diaphragm (NR2F2, STRA6, ZFPM2) (Figure 2). The GATA6 gene dosage from LoF and missense variants in the second zinc-finger domain that alter RA signalling affects the development of the related organs [16]. This pioneer function of GATA6 was identified by Chromatin Immuno Precipitation-sequencing (ChIP-seq), Transposase-Accessible Chromatin sequencing (ATAC-seq), and Hypergeometric Optimization of Motif EnRichment (HOMER) analyses of WT and GATA6 mutant hiPSCs. GATA6 was identified activating transcription in its nearby genes temporally, which further activates cardiomyocyte and endoderm gene network through chromatin binding in intergenic regions without a GATA-binding motif. Nonsense-mediated decay of GATA6+/- transcripts reduced GATA6 protein levels, altered chromatin accessibility, and decreased gene transcription, implying that intergenic sites are sensitive to GATA6 dosage (Figure 2).

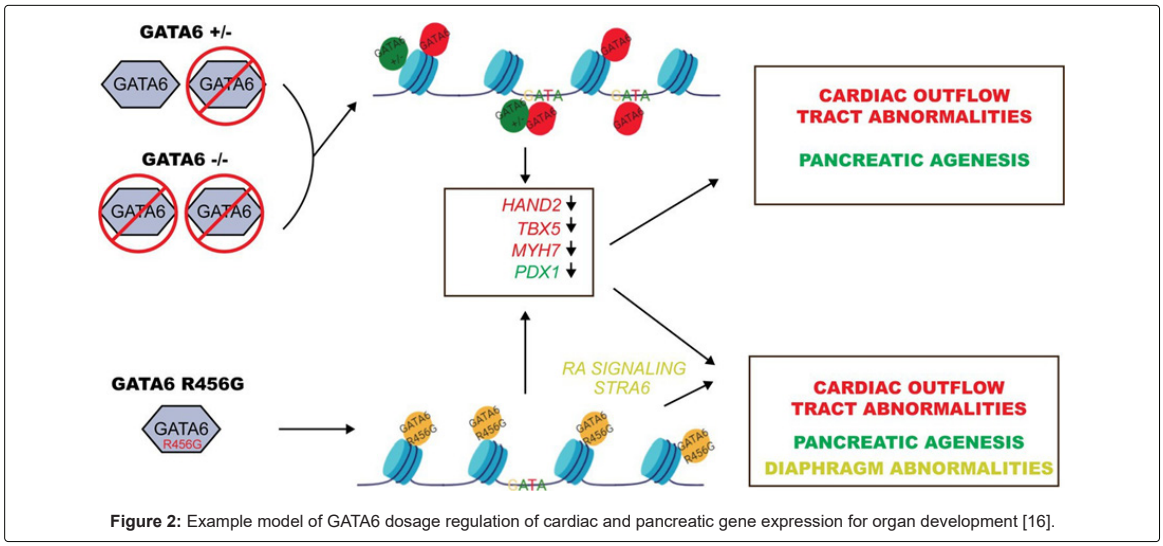

Figure 2: Example model of GATA6 dosage regulation of cardiac and pancreatic gene expression for organ development [16].

Conclusion and Future Perspective

Due to the different development processes in mice and humans, the use of hPSC may help to validate the detected genome variants contributing to a disease penetrance in heterogeneous genetic disorders and reveal the potential molecular mechanism. Through analyzing the regulatory regions of the GATA6 gene from patients with these disorders by the hPSC methods can confirm and reveal the functional consequence of the coding and non-coding regions variants to the wide variable phenotypes. The elucidation of the molecular mechanism of the malformation would lead to better early diagnosis and genetic consulting, further for stem cell therapy soon.

Conflict of Interest Statement

The author has no interest conflicts.

References

1. Jennings RE, Berry AA, Kirkwood-Wilson R, Roberts NA, Hearn T, et al. (2013) Development of the human pancreas from foregut to endocrine commitment. Diabetes 62: 3514-3522.
2. Bossard P, Zaret KS (1998) GATA transcription factors as potentiators of gut endoderm differentiation. Development 125: 4909-4917.
3. Tremblay M, Sanchez-Ferras O, Bouchard M (2018) GATA transcription factors in development and disease. Development 145: 164-384.
4. Viger RS, Guittot SM, Anttonen M, Wilson DB, Heikinheimo M (2008) Role of the GATA family of transcription factors in endocrine development, function, and disease. Mol Endocrinol 22: 781-798.
5. Lentjes MHFM, Niessen HEC, Akiyama Y, de Bruine, Melotte V, et al. (2016) The emerging role of GATA transcription factors in development and disease. Expert Rev Mol Med 18: e3.
6. Villamayor L, Cano DA, Rojas A (2020) GATA factors in pancreas development and disease. IUBMB Life 72: 80-88.
7. Laforest B, Nemer M (2011) GATA5 interacts with GATA4 and GATA6 in outflow tract development. Dev Biol 358: 368-378.

8. Allen HL, Flanagan SE, Shaw-Smith C, Franco ED, Akerman I, et al. (2011) GATA6 haploinsufficiency causes pancreatic agenesis in humans. Nat Genet 44: 20-22.

9. Sanchez-Lechuga B, Saqlain M, Ng N, Colclough K, Woods C, et al. (2020) Case report: Adult onset diabetes with partial pancreatic agenesis and congenital heart disease due to a de novo GATA6 mutation. BMC Med Genet 21: 70.

10. Gong M, Simaite D, Kuhnen P, Heldmann M, Spagnoli F, et al. (2013) Two novel GATA6 mutations cause childhood-onset diabetes mellitus, pancreas malformation and congenital heart disease. Horm Res Paediatr 79: 250-256.

11. De Franco E, Shaw-Smith C, Flanagan SE, Shepherd MH, Hattersley AT, et al. (2013) GATA6 mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. Diabetes 62: 993-997.

12. Chao CS, McKnight KD, Cox KL, Chang AL, Kim SK, et al. (2015) Novel GATA6 mutations in patients with pancreatic agenesis and congenital heart malformations. PLoS One 10: e0118449.

13. Maitra M, Koenig SN, Srivastava D, Garg V (2010) Identification of GATA6 sequence variants in patients with congenital heart defects. Pediatr Res 68: 281-285.

14. Yau D, De Franco E, Flanagan SE, Ellard S, Blumenkrantz, et al. (2017) Case report: Maternal mosaicism resulting in inheritance of a novel GATA6 mutation causing pancreatic agenesis and neonatal diabetes mellitus. Diagn Pathol 12:1.

15. Škorić-Milosavljević D, Tjong FVY, Barc J, Backx APCM, Clur SAB, et al. (2019) GATA6 mutations: Characterization of two novel patients and a comprehensive overview of the GATA6 genotypic and phenotypic spectrum. Am J Med Genet A 179: 1836-1845.

16. Sharma A, Wasson LK, Willcox JA, Morton SU, Gorham JM, et al. (2020) GATA6 mutations in hiPSCs inform mechanisms for maldevelopment of the heart, pancreas, and diaphragm. Elife 9: e53278.

17. Flanagan SE, De Franco E, Allen HL, Zerah M,Abdul-Rasoul MM, et al. (2014) Analysis of transcription factors key for mouse pancreatic development establishes NKX2-2 and MNX1 mutations as causes of neonatal diabetes in man. Cell Metab 19: 146-154.

18. Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371: 606-609.

19. Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, et al. (1996) PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122: 983-995.

20. Rubio-Cabezas O, Minton JAL, Kantor I, Williams D, Ellard S, et al. (2010) Homozygous mutations in NEUROD1 are responsible for a novel syndrome of permanent neonatal diabetes and neurological abnormalities. Diabetes 59: 2326-2331.

21. Rubio-Cabezas O, Jensen JN, Hodgson MI, Codner E, Ellard S, et al. (2011) Permanent neonatal diabetes and Enteric anendocrinosis associated with biallelic mutations in NEUROG3. Diabetes 60: 1349-1353.

22. Rubio-Cabezas O, Klupa T, Malecki MT (2011) Permanent neonatal diabetes mellitus:The importance of diabetes differential diagnosis in neonates and infants. Eur J Clin Invest 41: 323-333.

23. Villani V, Thornton ME, Zook HN, Crook CJ, Grubbs BH, et al. (2019) SOX9+/PTF1A+ cells define the tip progenitor cells of the human fetal pancreas of the second trimester. Stem Cells Transl Med 8: 1249-1264.

24. Shi ZD, Lee K, Yang D, Amin S, Verma N, et al. (2017) Genome editing in hPSCs reveals GATA6 haploinsufficiency and a genetic interaction with GATA4 in human pancreatic development. Cell Stem Cell 20: 675-688.

25. Tiyaboonchai A, Cardenas-Diaz FL, Ying L, Maguire JA, Sim X, et al. (2017) GATA6 plays an important role in the induction of human definitive endoderm, development of the pancreas, and functionality of pancreatic beta cells. Stem Cell Reports 8: 589-604.

26. Chia CY, Madrigal P, Denil SLIJ, Martinez I, Garcia-Bernardo J, et al. (2019) GATA6 cooperates with EOMES/SMAD2/3 to deploy the gene regulatory network governing human definitive endoderm and pancreas formation. Stem Cell Reports 12: 57-70.

27. Yang J, Lu P, Li M, Yan C, Zhang T, et al. (2020) GATA6-AS1 regulates GATA6 expression to modulate human endoderm differentiation. Stem Cell Reports 15: 694-705.

28. Kishore S, De Franco E, Cardenas-Diaz FL, Letourneau-Freiberg LR, Sanyoura M, et al. (2020) A non-coding disease modifier of pancreatic agenesis identified by genetic correction in a patient-derived iPSC line. Cell Stem Cell 27: 137-146.

29. Jennings RE, Berry AA, Gerrard DT, Wearne SJ, Strutt J, et al. (2017) Laser capture and deep sequencing reveals the transcriptomic programmes regulating the onset of pancreas and liver differentiation in human embryos. Stem Cell Reports 9: 1387-1394.