

Murine model of obesity-induced type II diabetes by GADD34(PPP1R15A) and other phosphatase 1 regulatory subunits deficiency.Ken-ichi Isobe¹, Naomi Nishio^{1,2} and Tadao Hasegawa²¹Department of Food Science and Nutrition, Nagoya woman's university, 3-40 Shioji-cho, Mizuho-ku, Nagoya, Aichi, 467-8610, Japan²Department of Bacteriology, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi Mizuho-cho Mizuho-ku, Nagoya, 467-8601, Japan

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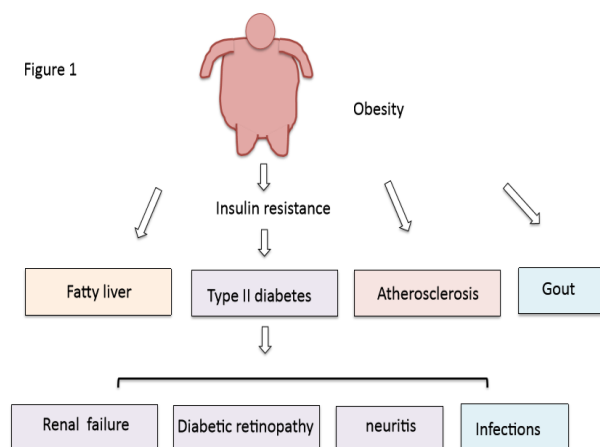
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Abstract: Obesity-derived metabolic syndrome is one of the most important medical problems in the world. Recently, we have developed murine model of age-dependent obesity and metabolic syndrome including Type II diabetes by using GADD34-deficient mice. GADD34 belongs one of phosphatase 1 regulatory subunits named PPP1R15A. Deficiency of glycogen-targeting regulatory subunits (G subunits) has been shown to develop metabolic diseases. Glycogen synthesis is mainly controlled by glycogen synthase (GS) and glycogen phosphorylase (GP). G subunits work to up-regulate glycogen synthesis by activating GS and suppressing GP. Seven G subunits (PPP1R3A to PPP1R3G) works to increase glycogen. PPP1R3A deficient mice became obese by age and showed insulin resistance. Because GADD34 binds several target proteins and PP1c catalytic subunit, GADD34 (PPP1R15A) has several functions in different stimulations by dephosphorylating target proteins. With age GADD34-deficient mice become obese, developing fatty liver followed by liver cirrhosis, hepatocellular carcinoma, and insulin resistance.

Key words: Pathogenic Escherichia coli, environmental stress, growth, cytotoxicity**1. Introduction**

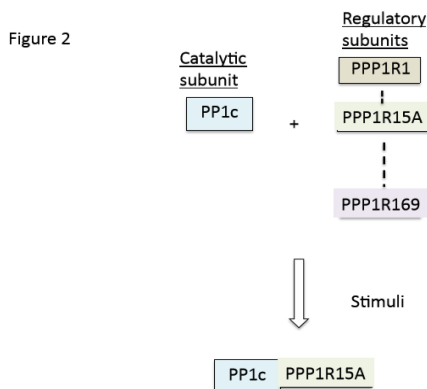
Metabolic syndrome is one of the most important medical problems in the world. Among them obesity induces several diseases including type II diabetes, fatty liver, atherosclerosis and gout. Especially type II diabetes has various complications including renal failure, neuritis, diabetic retinopathy and infections (Fig.1). It has been estimated that 415 million adults have diabetes and this will rise to 642 million by 2040 (1).

Protein phosphatase 1 belongs to phosphatase families known as serine/threonine phosphatases. Each PP1 enzyme contains both catalytic subunit and a regulatory subunit (2). PP1c (catalytic subunit) interacts with variety of regulatory subunit. Because PP1c isoforms have nearly 90% amino acids identity, regulatory subunit of PP1 predominantly control the specificity and diversity of PP1 function (Figure 2). Regulatory subunits of PP1 is written as PPP1R (protein



phosphatase 1 regulatory subunits), which binds to PPP1C/PP1c/PP1 (protein phosphatase 1 catalytic) subunit

(3,4). Protein phosphatase 1 regulatory subunits are also called PP1-interacting proteins (PIPs), which form stable complexes with PP1 via degenerate docking motif RVxF. Interestingly some PIPs are PP1 substrates, and their controlled dephosphorylation serves a regulatory function. A subset of PIPs (protein phosphatase 1 regulatory subunits) are both substrates and regulators for associated PP1 (5).

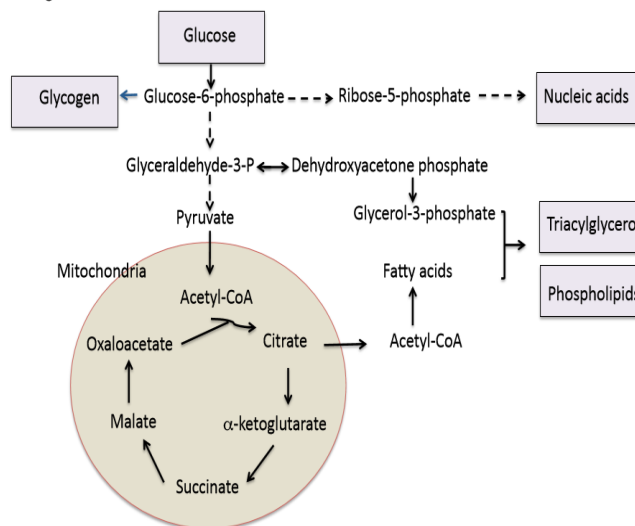


One group of PPP1Rs, which related to glucose metabolism have been extensively studied. Especially gene knockout mice have revealed the function of these genes (6). First, we review PPP1Rs, which target PP1 to glycogen and regulate glucose metabolism. Because free fatty acids are made from Acetyl-CoA, which is derived from glucose, these protein phosphatase 1 regulatory subunits also works in lipid metabolism. Then we introduce our recent findings of PPP1R15A known as GADD34. We found that GADD34 knockout mice develop obesity in advanced age and high fat diet (HFD), which induce type II diabetes, fatty liver, liver cirrhosis and hepatic carcinoma.

1. Glycogen target protein phosphatase 1regulatory subunits.

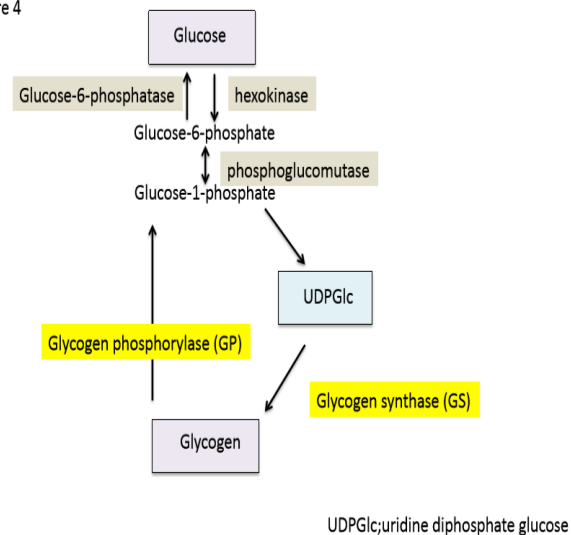
We as human being absorb monosaccharide such as glucose, fructose and galactose. Each cell in the body has to produce ATP as energy source. We can make ATP from glucose metabolism. After meal such as starch, blood glucose level increases and insulin induces glucose uptake into cells and glycolysis occurs. Pyruvate from glycolysis of glucose enter mitochondria and produces Acetyl-CoA and oxaloacetate, which produces citrate following TCA cycle. NADH and FADH2 produced by TCA cycle are oxydized in the electron transport chain to produce ATP. After meal excess food is stored for use in fasted stage. We store energy as glycogen 4 (mainly liver and muscle) and lipid (adipose tissues), which are stimulated by insulin (Fig.3).

Figure 3



Glycogen synthesis is mainly controlled by glycogen synthase (GS) and glycogen phosphorylase (GP) (7) GS catalyzes the addition of glucose to the glycogen chain, and GP catalyzes the breakdown of glycogen to release glucose-1-phosphate (Fig.4).

Figure 4



The activity of GS is stimulated by dephosphorylation via glycogen synthase phosphatase. Glycogen-targeting regulatory subunits (G subunits) localize PP1c to glycogen and dephosphorylate and activate GS to glycogen synthesis. G subunits work to up-regulate glycogen synthesis by activating GS and suppressing GP. Newgard CB et al., grouped 4 glycogen targeting subunits of protein phosphatase-1. GM/RGI (PPP1R3A), GL (PPP1R3B), PTG/PPP1R5 (PPP1R3C) and PPP1R6 (PPP1R3D) have glycogen targeting sequences at C terminal and RRVSF, KRVSF, KRVSF or LRVRF sequences at N terminal (8). Now, there are seven G subunits from PPP1R3A to PPP1R3G (Table 1).

Table 1

	expression	function	Ref.
PPP1R3A (G _M)	skeletal muscle, heart	Deletion; insulin resistance	9
PPP1R3B (G _L)	liver	Overexpression; improved glucose tolerance	10
PPP1R3C (PPP1R5,PTG)	Many tissues	Heterozygous deletion; insulin resistance with aging	11,18
PPP1R3D (PPP1R6)	brain	glycogen accumulation in neurons	12
PPP1R3E	Liver,heart		13
PPP1R3F	membrane-associated G subunit	regulate GS in astrocytoma cells	14
PPP1R3G	liver	hepatic glycogen accumulation and reduced hepatic triglyceride concentration	15

(a) Model of obesity induced type II diabetes in PPP1R3A (GM) deficient mice.

Cohen T.W's group established PPP1R3A (GM/RGL; glycogen targeting subunit) deficient mice (GM^{-/-}). Homozygous GM^{-/-} mice show increased weight gain after 3 months of age and become obese. They found that obesity of GM^{-/-} mice at 12 months old age developed insulin resistance in skeletal muscle (9).

(b) PPP1R3C (PTG) prevent steatosis.

PPP1R3C was cloned as PTG (16). In liver PTG and GL are expressed and PTG induces PP1 and GS to glycogen particles and marked increase in GS activity and glycogen synthesis (17). Deletion of the PTG gene in mice prevented HFD-induced hepatic glycogen accumulation and also blocked hepatic steatosis in HFD-fed mice and reduced the expression of numerous lipogenic genes. These results indicated the crosstalk of glycogen synthesis and lipid synthesis. It has been shown that mTORC1 and SREBP activity were decreased in PTG knockout mice. It has been concluded that mTORC1/SREBP pathway can sense elevated 5 levels of glycogen and then initiate the transcriptional changes that increase lipid synthesis (18).

(c) Liver-specific overexpression of PPP1R3G increases hepatic glycogen accumulation and decreased hepatic triglyceride level.

Chen Y's group established transgenic mice with PPP1R3G specifically expressed in the liver (12-fold increase in PPP1R3G mRNA level in the transgenic mice compared with the wild-

type controls). They found that the liver glycogen level increased two-fold, whereas the glycogen level was not altered in the skeletal muscle and the brain in the transgenic mice. Further PPP1R3G overexpression reduces hepatic triglyceride in the fed state (15).

2. Function of GADD34 in metabolism.

PPP1R15A has been extensively studied as GADD34. Because GADD34 has multiple substrate-targeting domains (19), GADD34 has several functions in different stimulations. Among them recovery from shutoff of protein synthesis induced by ER stress has been widely studied in various disease models. We and Ron's group first reported GDD34 function in vivo by GADD34 knockout mice. It have shown that GADD34 dephosphorylates the translation initiation factor eIF2 α and inhibits the shut-off of protein translation (20,21). Sephin1, a selective inhibitor of PPP1R15A, has been used to treat mouse model of misfolded diseases (23).

Recently, we examined the effects of GADD34 on natural life span by using GADD34-deficient mice. We found that with age GADD34-deficient mice become obese, developing fatty liver followed by liver cirrhosis, hepatocellular carcinoma, and insulin resistance. Insulin signaling in young GADD34-deficient mice was higher than that in WT mice. This finding explains two pathways to become type II diabetes by aging. One is that insulin induces fat differentiation. Another is that the insulin act to store fat by facilitating fatty acid synthesis from Acetyl-CoA. Differentiation into fat is dependent on insulin signaling (24,25). We found that higher numbers of adipocytes were observed in GADD34-deficient MEFs compared with WT MEFs. We examined insulin 6 signaling in both WT and GADD34-deficient MEFs before differentiation. Because Akt is one of key insulin signaling molecule, we examined the phosphorylation of Akt after insulin stimulation of MEF. After insulin stimulation, GADD34-deficient MEFs showed higher levels of p-Akt compared with WT MEFs. It has been shown that constitutively active Akt can promote the differentiation of 3T3-L1 cells into adipocytes (26). It has been shown that primary target of Akt by insulin signaling in adipocyte differentiation is TSC2, which is a critical negative regulator of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1). Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway and increases PPAR γ (27). Because GADD34 has multiple substrate-targeting domains (19), several insulin signaling molecules and enzymes related to lipid metabolism may be engaged. Currently we are investigating effects of GADD34 on each steps of insulin signaling from insulin receptor and effects of GADD34 on metabolic enzymes. Through aging or a HFD, insulin signaling in GADD34-deficient liver converted to be down regulated. Neutrophils and macrophages are gathered to the accumulated fat, which produces TNF- α . TNF- α induces TNF- α receptor signaling, which inhibits insulin signaling (28).

Future remarks

Here we only describe glucose metabolism. Lipogenesis occurs from Acetyl-CoA from glucose by the stimulation of insulin. However, lipogenesis also occurs through direct meal. These aspects of obesity and insulin resistance are sure to be important. By using GADD34 deficient mice we are currently investigating both in vivo lipogenesis and lipogenesis from meal.

References:

- 1) International Diabetes Federation. IDF diabetes atlas. 6th ed. Brussels: International Diabetes Federation, 2014.
- 2) Goldberg J, Huang, HB, Kwon, YG, Greengard, P, Nairn, AC, Kuriyan, J. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. *Nature* 376 (6543): 745–53. 1995.
- 3) <http://www.genenames.org/cgi-bin/genefamilies/set/694> HGNC; Human Genome Nomenclature Committee
- 4) Heroes E et al. The PP1 binding code: a molecular-lego strategy that governs specificity. *FEBS J.* 2013 Jan; 280(2):584-595.
- 5) Mathieu Bollen, Wolfgang Peti, Michael J. Ragusa, Monique Beullens. The extended PP1 toolkit: designed to create specificity. *Trends Biochem Sci.* 2010 August; 35(8): 450–458.
- 6) MGI: Mouse Genome Informatics; <http://www.informatics.jax.org/>
- 7) Roach PJ, Depaoli-Roach AA, Hurley TD, Tagliabracci VS. Glycogen and its metabolism: some new developments and old themes. *Biochem J* 2012; 441: 763–787.
- 8) Newgard CB, Brady MJ, O'Doherty RM, Saltiel AR. Organizing glucose disposal: emerging roles of the glycogen targeting subunits of protein phosphatase-1. *Diabetes* 2000; 49:1967–1977.
- 9) Delibegovic M, Armstrong CG, Dobbie L, Watt PW, Smith AJ, Cohen PT. Disruption of the striated muscle glycogen targeting subunit PPP1R3A of protein phosphatase 1 leads to increased weight gain, fat deposition, and development of insulin resistance. *Diabetes.* 2003;52(3):596–604.
- 10) Kelsall IR, Rosenzweig D, Cohen PT. Disruption of the allosteric phosphorylase a regulation of the hepatic glycogen-targeted protein phosphatase 1 improves glucose tolerance in vivo. *Cell Signalling.* 2009;21(7):1123–1134.
- 11) Crosson SM, Khan A, Printen J, Pessin JE, Saltiel AR. PTG gene deletion causes impaired glycogen synthesis and developmental insulin resistance. *J Clin Invest.* 2003; 111(9):1423–1432.
- 12) Rubio-Villena C, Garcia-Gimeno MA, Sanz P. Glycogenic activity of R6, a protein phosphatase 1 regulatory subunit, is modulated by the laforin-malin complex. *Int J Biochem Cell Biol.* 2013;45(7):1479–1488.
- 13) Munro S, Ceulemans H, Bollen M, Diplexcito J, Cohen PT. A novel glycogen-targeting subunit of protein phosphatase 1 that is regulated by insulin and shows differential tissue distribution in humans and rodents. *FEBS J.* 2005; 272(6):1478–1489.
- 14) Kelsall IR, Voss M, Munro S, Cuthbertson DJ, Cohen PT. R3F, a novel membrane-associated glycogen targeting subunit of protein phosphatase 1 regulates glycogen synthase in astrocytoma cells in response to glucose and extracellular signals. *J Neurochem.* 2011; 118(4):596–610.
- 15) Zhang Y, Xu D, Huang H, Chen S, Wang L, Zhu L, Jiang X, Ruan X, Luo X, Cao P, Liu W, Pan Y, Wang Z, Chen Y. Regulation of glucose homeostasis and lipid metabolism by PPP1R3G-mediated hepatic glycogenesis. *Mol Endocrinol.* 2014 Jan; 28(1):116-26.
- 16) Printen JA, Brady MJ, Saltiel AR. PTG, a protein phosphatase 1-binding protein with a role in glycogen metabolism. *Science* 1997; 275:1475–1478.
- 17) Brady MJ, Printen JA, Mastick CC, Saltiel AR. Role of protein targeting to glycogen (PTG) in the regulation of protein phosphatase-1 activity. *J Biol Chem* 1997; 272:20198–20204.
- 18) Binbin Lu, Dave Bridges, Yemen Yang, Kaleigh Fisher, Alan Cheng, Louise Chang, Zhuo-Xian Meng, Jiandie D. Lin, Michael Downes, Ruth T. Yu, Christopher Liddle, Ronald M. Evans, Alan R. Saltiel. Metabolic Crosstalk: Molecular Links between Glycogen and Lipid metabolism in Obesity. *Diabetes.* 2014 September; 63(9): 2935–2948.
- 19) Mathieu Bollen, Wolfgang Peti, Michael J. Ragusa, Monique Beullens. The extended PP1 toolkit: designed to create specificity. *Trends Biochem Sci.* 2010 August; 35(8): 450–458.
- 20) Kojima E, Takeuchi A, Haneda M, Yagi A, Hasegawa T, Yamaki K, Takeda K, Akira S, Shimokata K, Isobe K. The function of GADD34 is a recovery from a shutoff of protein synthesis induced by ER stress: elucidation by GADD34-deficient mice. *FASEB J.* 2003 Aug; 17(11): 1573-5.
- 21) Novoa, I. et al. Stress-induced gene expression requires programmed recovery from translational repression. *EMBO J* 22, 1180–1187 (2003).
- 22) Das I, Krzyzosiak A, Schneider K, Wrabetz L, D'Antonio M, Barry N, Sigurdardottir A, Bertolotti A. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science.* 2015 Apr 10; 348(6231):239-42.
- 23) Nishio N, Isobe K. GADD34-deficient mice develop obesity, nonalcoholic fatty liver disease, hepatic carcinoma and insulin resistance. *Sci Rep.* 2015 Aug 28; 5:13519.
- 24) Girard J., Perdereau D., Foufelle F., Prip-Buus C. & Ferré P. Regulation of lipogenic enzyme gene expression by nutrients and hormones. *FASEB J* 8, 36–42 (1994).
- 25) Smith P. J., Wise L. S., Berkowitz R., Wan C. & Rubin C. S. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. *J Biol Chem* 263, 9402–9408 (1988).
- 26) Kohn AD, Summers SA, Birnbaum MJ, Roth RA (1996) Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J Biol Chem* 271: 31372–31378.
- 27) Zhang HH, Huang J, Düvel K, Boback B, Wu S, Squillace RM, Wu CL, Manning BD. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. *PLoS One.* 2009 Jul 10;4(7):e6189. 28) Shi H, Kokoeva MV, Inouye K, Tzamelis I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest.* 2006 Nov; 116(11):3015-25.