Acta Transitional Medicine *An International Scientific Journal*	ISSN: 2521-8662	Volume 1	Issue 1	pp.1-7	March 2018

GENDER-SPECIFIC EFFECT OF DEFICIENT ABCG4 ON LIPOGENESIS IN A MOUSE'S BRAIN

Shailesh B. Patel
Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA.

Abstract: The mRNA levels of genes for lipid lipogenesis as well as other genes are quantified in the brain of Abcg4 knockout and wild type littermates using quantitative RT-PCR. The results showed that different sets of genes for lipid metabolism were activated in the brains of Abcg4 -/- male and female mice, when compared to those of the wild type counterparts. It is shown that Abcg4 is a sex-regulated gene with the function of differentially regulating fatty acid and/or cholesterol synthesis in the brain.
Keywords: ATP-binding cassette transporter (ABC), abcg4, cholesterol, lipogenesis, gene knockout mouse, quantitative PCR, brain

1. INTRODUCTION

Abcg4 is a member of the G subfamily of ATP-binding cassette transporters (ABC) which utilizes ATP hydrolysis to transport a wide variety of substrates across various cellular membranes. Abcg transporters have a unique domain structure consisting of one single nucleotide-binding domain (NBD) located N-terminally of the six transmembrane domain (TMD), the half-transporters playing their role by forming a homo- or hetero dimer. Cloning and characterization of Abcg4 was first reported in 2001 [1] and 2002 [2,3]. Abcg4 is highly expressed in the brain and neural retina of the eyes assessed by northern blot analysis [2]. Abcg1 and Abcg4 have been shown to mediate cholesterol efflux to HDL in the brain [4-6]. Abcg4 may play an important role in the development of Alzheimer's disease, since Abcg4 was found highly expressed in the microglia near the senile plaque in Alzheimer's patients brain [7]. Bojanic DD et al (2009) examined Abcg1 (another member of ABC transporter G subfamily) and Abcg4 expression and function during development and aging. They found that loss of both Abcg1 and Abcg4 results in accumulation in the retina and/or brain of oxysterols, and behavioral tests showed that Abcg4-/- mice had a general deficit in associative fear memory[8]. The data indicate that the Abcg4 plays a critical function in the central nervous system (CNS).

Sex-specific effect on the gene expression has been reported in different organisms [9-17]. Another member of ABCG transporters, Abcg2 or BCRP (breast cancer resistance protein), has also been found to have sex-dependent expression and activity in the liver [18]. The relationship of Abcg4 expression and lipogenesis with regard to gender difference in a mouse's brain as well as in the body remains much to be explored. In this study, we report the gender-specific effect of deficient Abcg4 on gene expression of lipogenesis in the brain using quantitative RT-PCR, the first report of Abcg4 on sex-dependant gene expression regulation.

2. MATERIAL AND METHODS

Gene-knockout mouse: Abcg4 knock-out/GFP knock-in mice were made and genotypically verified with southern blot at Dr. Patel's lab in Medical College of Wisconsin, Milwaukee, Wisconsin, USA. Age- and sex- matched mice (littermates) were chosen for the study.

RNA isolation and analysis: Total RNA was isolated from brains of 1-2 month old or 3-4 month old mice using Trizol reagent (Invitrogen), and 1ug of total RNA was reverse transcribed. Quantitative PCRs were performed with SYBR-Green PCR Master Mix of Applied Biosystems using the 7300 Real-time PCR machine. Cyc2 was used as the internal control gene for the

relative quantification of gene expression in mouse brain (standard curve was first made and analyzed with the cyc2 in the brain, data not shown). The real-time PCR results were automatically analyzed and produced by ABI 7300 SDS software and were saved in the "print screen" format or in an exported raw-data form.

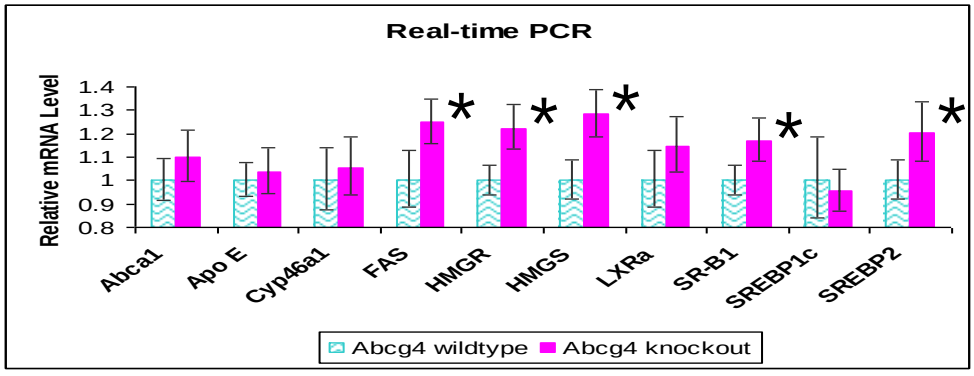
3. RESULTS

3.1. Gene Expression in the Brains of Female Mice

Expression of lipid-related genes in female mice of Abcg4 knockout, compared to that of wild type, is shown in Fig. (**1**). In the female mice, HMGS, FAS and HMGR, SREBP2 and SR-B1 were significantly upragulated in the brain of Abcg4 knockout mice, respectively 28%, 25%, 22%, 20% and 17% higher than that of wild type.

HMGS (3-hydroxy-3-methylglutaryl CoA synthase) is the rate limiting enzyme for ketone body formation. In the brain, the ketone bodies are a vital source of energy during fasting [19]. After the diet has been changed to lower blood glucose for 3 days, the brain gets 30% of its energy from ketone bodies. After about 40 days, this goes up to 70% (during the initial stages the brain does not burn ketones, since they are an important substrate for lipid synthesis in the brain). In time the brain reduces its glucose requirements from 120g to 40g per day.

While the lipogenesis was upregulated in the brain of G4 knockout mice, the enzyme related to glyconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK), was greatly suppressed in the brain of female G4 knockout mouse, with only 31% of that of wild type control (the same experiment, data not shown). It seems reasonable to deduce that the abcg4 knockout caused glucose deficiency due to extremely low PEPCK activity, the rate-controlling step of gluconeogenesis which is the process by which cells synthesize glucose from metabolic precursors. As the result of glucose starvation in the brain, the expression of fatty acid synthesase (FAS), the enzyme resposible for energy production and storage, was raised correspondingly. Our result showed that HMGR (HMG-CoA reductase) and SREBP2 (sterol regulatory element binding protein 2) were also significantly up regulated. HMGR is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids; SREBPs, the basic-helix-loop-helix leuzine zipper class of transcription factors, when activated, can bind to specific sterol regulatory element DNA sequences, thus up regulating the synthesis of enzymes involved in sterol biosynthesis. From the data above, we could conclude that the overall sterol synthesis was increased in the female brain of abcg4 knockout mouse.



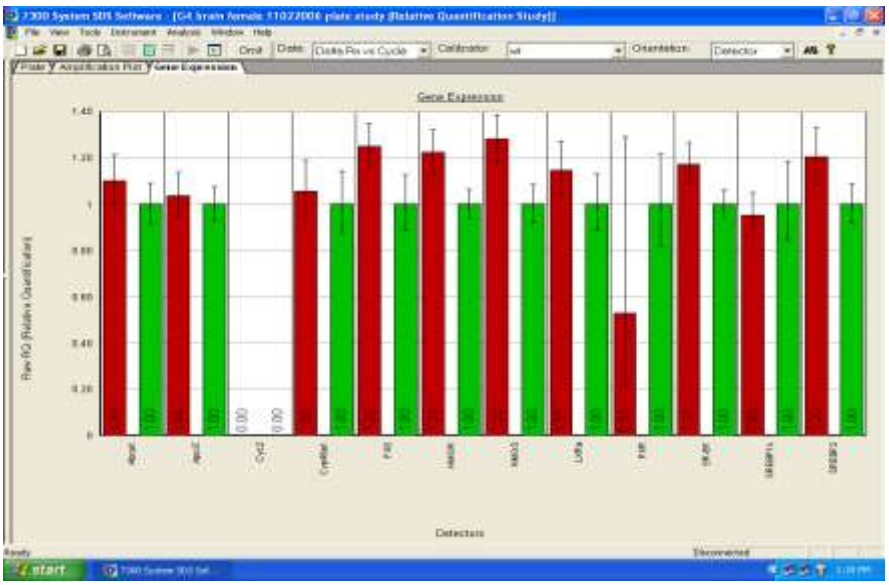


Fig. (1). Gene expression in the brains of female Abcg4 knockout mice, compared to those of wild type.
(The relative quantification, RQ, of the wild type is set as 1, and shown in green; RQ of the knockout is shown in red.)
The upper figure (manually drawn) and the lower one (ABI PRISM 7300 SDS software "print screen" result) are from the same experiment. HMGS, 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase; FAS, fatty acid synthase; HMGR, HMG-CoA reductase; SREBP2, sterol regulatory element binding protein 2; SR-B1, Scavenger receptor class B1.

3.2. Gene expression in the brains of male mice

The gene expression in the brains of male mice is shown in Fig. (**2**).
The result in Fig. (**2**) showed that mRNA expression of the genes for liver X receptor (LXRa), Apolipoprotein E (ApoE), ATP-binding cassette transporter A1 (Abca1) and low-density lipoprotein receptor (LDL-R) in male mice was significantly up regulated in the G4 knockout mice, and is 83%, 44%, 36% and 27% higher than their wild type mice respectively. SREBP1c were also remarkably (31% higher of that of wild type) up regulated in the brain.
Liver X receptor is a member of the nuclear receptor family of transcription factors. LXRs are important regulators of cholesterol, fatty acid, and glucose homeostasis. The target genes of LXRs are involved in cholesterol and lipid metabolism regulation[20], including: ATP binding cassette transporter isoforms A1, G1, G5 and G8; ApoE; FAS; LXR-α (a somewhat unusual example of receptor up-regulating its own expression), and SREBP1c. Consistent with this, the target genes measured in the experiment, such as LXR-α, ApoE, Abca1, SREBP1c were all significantly up regulated in the brain of male mouse, which were different from those of female mouse.
LXRs regulate fatty acid synthesis by modulating the expression of sterol regulatory element binding protein-1c (SREBP-1c) [21-22]. SREBP1c is responsible for regulating the genes required for *de novo* lipogenesis，SREBP2 mainly regulates the genes of cholesterol metabolism. In the brain of male knockout mouse, SREBP1c but not SREBP2 was upregulated; in the female mouse brain, SREBP2, but not SREBP1c was up regulated. This indicated that different mechanisms may exist in regulating lipogenesis both within the male and female mice brains.

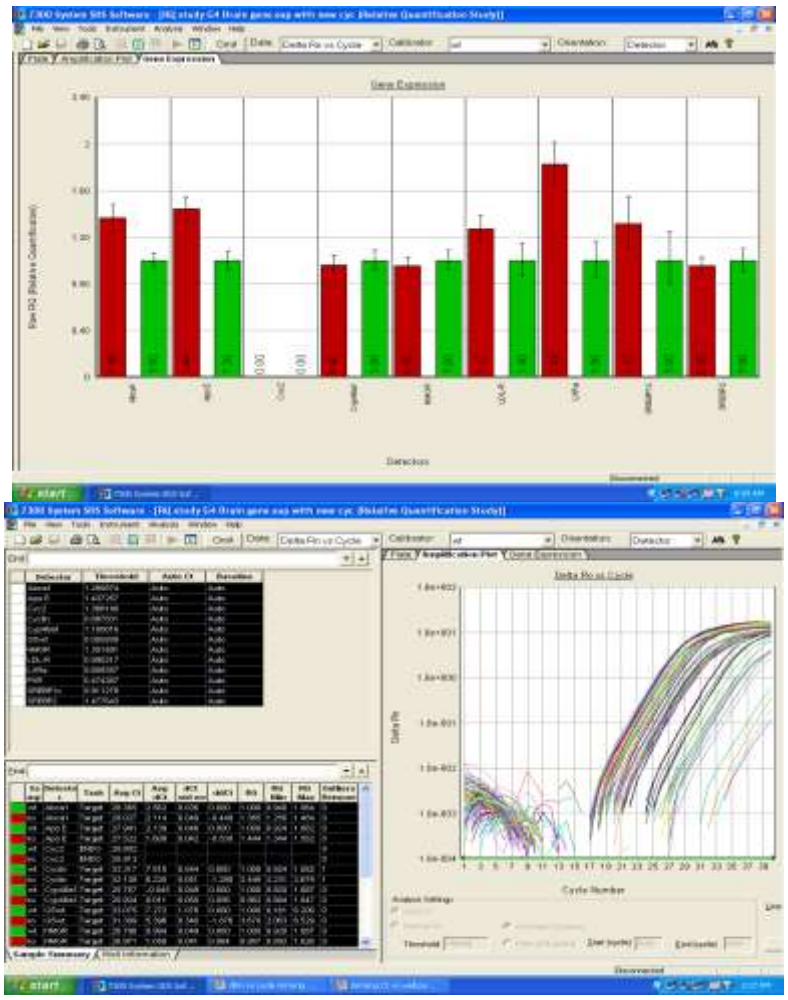


Fig. (2). Gene expression in the brains of male mice of Abcg4 knockout, compared to those of wild type.

The figure from the upper panel was from the same experiment with the one from the lower panel, but with different displaying parameters. LXRα, liver X receptor.; ApoE, Apolipoprotein E; Abca1, ATP-binding cassette transporter ABCA1; LDL-R, The Low-Density Lipoprotein (LDL) Receptor; SREBP1c, sterol regulatory element binding protein 1c.

ATP-binding cassette transporter Abca1, also known as the cholesterol efflux regulatory protein (CERP), is highly induced in G4 knockout (KO) brains, which is consistent with the observation by Bojanic DD et al[8].

Clearance of Alzheimer's amyloid-$\beta_{1\text{-}40}$ peptide from brain by LDL receptor–related protein-1 at the blood-brain barrier has been reported [23]. LDL is directly involved in the development of atherosclerosis, due to accumulation of LDL-cholesterol in the blood. And atherosclerosis is the process responsible for the majority of cardiovascular diseases. In the G4 KO male brain, LDL-R was up regulated, which may indicate a beneficial effect of raised LDL-R associated with the decreased risk of cerebral vascular atherosclerosis.

In the paper "increased LDL receptor lowers amyloid plaques via apolipoprotein E: study offers new insight on how APOE works in Alzheimer disease" by Robinson R (2010) [24], it was stated: "together, these results suggest that one of the normal functions of APOE is to bind to Abeta and bring it into the cell to clear it". David M. Holtzman, MD, professor and chair of neurology at the Washington University School of Medicine in Saint Louis, MO, who led the study, also said: "The big take-home message is that the LDL receptor plays an important role in regulating APOE levels in the brain, and when you increase its levels, it helps clear APOE and Abeta, and that results in less Alzheimer

pathology." In our study, if the up regulated LDL-R in the G4 KO male mouse brain could lower amyloid plaques, would be very interesting to see.

In the same paper, it was also pointed out that "one finding needing fuller explanation was a strong sex difference in the effect. Over expression of the LDL receptor reduced pathology by about 50 percent in females, and about 70 percent in males. In females, though not in males, even a low level of receptor over expression prevented Abeta accumulation. In both mice and humans, females usually show more extensive amyloid pathology than males, for reasons that are not yet clear." In our study, the Abcg4 might provide a clue regarding to the gender difference of LDL receptor expression on Abeta accumulation and amyloid pathology. From the data above, it can be seen that the genes up regulated significantly in the brains of G4 knockout female mice are different to those in the male mice.

4. DISCUSSION

Mammalian brain contains 25% of total body cholesterol, making it the most cholesterol-rich organ in the body; earlier experiments have shown abcg4 is most abundant in the brain and eyes; and that Abcg4 was capable of transporting cholesterol to HDL and other receptors. Thus, Abcg4 may play an important role in lipid metabolism within the brain.

Gender-specific differentiation on gene expression has been reported in many organisms [9-17]. This phenomenon is important for us to take into consideration when we design our experiments using both male and female knockout animals, as the varying number of male and female animals between different experiments may affect our results, conclusions and explanations.

The effect of deficient Abcg4 on differential gene expression between male and female mouse brain is first reported in the study. Using the gender- and age-matched experiment design, we observed some interesting results which were produced by the ABI 7300 SDS software. Our results have shown that Abca1 and ApoE is up regulated in G4 -/- mouse compared to the wild type, which is consistent with the result by Bojanic DD et al (2010), however in our result, the up regulated levels of Abca1 and ApoE in the male knockout mice compared to wild type is much more significant, while those in the G4 knockout female mice, Abca1 and ApoE did not show significant difference when compared to their female wild type. The abnormal cholesterol/lipid metabolism in Abcg4 knockout mice has been reported by several labs, but the details about how Abcg4 affects the process and the effect of such abnormal regulation in the brain is unclear. Our results showed for the first time that Abcg4 knockout had a differential effect on lipid-related gene expression in the female and male mouse brains, in conclusion we believe that this is an interesting and important phenomenon and consider it worthy of further exploration especially in regards to gender-specific lipid regulation and its related disease control in the brain as well as other organs within a mammal's body.

REFERENCES

[1] Annilo T., Tammur J., Hutchinson A., Rzhetsky A., Dean M., and Allikmets R.. Human and mouse orthologs of a new ATP-binding cassette gene, ABCG4. Cytogenetics and Cell Genetics. 94(3-4):196-201, 2001.

[2] Oldfield S., Lowry C.A., Ruddick J., and Lightman S.L.. ABCG4: a novel human white family ABC-transporter expressed in the brain and eye. Biochimica Et Biophysica Acta-Molecular Cell Research, 1591(1-3):175-179, 2002.

[3] Yoshikawa M., Yabuuchi H., Kuroiwa A, Ikegami Y., Sai Y., Tamai I., Tsuji A., Matsuda Y., Yoshida H., and Ishikawa T.. Molecular and cytogenetic characterization of the mouse ATP-binding cassette transporter Abcg4. Gene, 293(1-2): 67-75, 2002.

[4] Wang N., Lan D., Chen W.G., Matsuura F., Tall A.R.. ABCG1 and ABCG4 promote cellular cholesterol efflux to HDL. Circulation, 110(17): 1306, 2004.

[5] Wang N., Lan D.B., Chen W.G., Matsuura F., and Tall A.R.. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proceedings of the National Academy of Sciences of the United States of America, 101(26): 9774-9779, 2004.

[6] Wang N., Yvan-Charvet L., Lutjohann D., Mulder M., Vanmierlo T., Kim T.W., Tall A.R.. ATP-binding cassette transporters G1 and G4 mediate cholesterol and desmosterol efflux to HDL and regulate sterol accumulation in the brain. Faseb Journal, 22(4): 1073-1082, 2008.

[7] Uehara Y., Yamada T., Baba Y., Miura S.I., Abe S., Kitajima K., Higuchi M.A., Iwamoto T., and Saku K.. ATP-binding cassette transporter G4 is highly expressed in microglia in Alzheimer's brain. Brain Research, 1217:239-246, 2008.

[8] Bojanic D.D., Tarr P.T., Gale G.D., Smith D.J., Bok D., Chen B., Nusinowitz S., Lovgren-Sandblom A., Bjorkhem I., and Edwards P.A.. Differential expression and function of ABCG1 and ABCG4 during development and aging. Journal of Lipid Research, 51(1): 169-181, 2010.

[9] Amador-Noguez D., Zimmerman J., Venable S., Darlington G.. Gender-specific alterations in gene expression and loss of liver sexual dimorphism in the long-lived Ames dwarf mice. Biochemical and Biophysical Research Communications 332(4): 1086-1100, 2005.

[10] Ahluwalia A., Clodfelter K.H., Waxman D.J.. Sexual dimorphism of rat liver gene expression: Regulatory role of growth hormone revealed by deoxyribonucleic acid microarray analysis. Molecular Endocrinology, 18(3):747-760, 2004.

[11] Bermejo-Alvarez P., Rizos D., Rath D., Lonergan P., Gutierrez-Adan A.. Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. Proceedings of the National Academy of Sciences of the United States of America, 107(8): 3394-3399, 2010.

[12] Chiba-Falek O., Nichols M., Suchindran S., Guyton J., Ginsburg G.S., Barrett-Connor E., and McCarthy J.J.. Impact of gene variants on sex-specific regulation of human Scavenger receptor class B type 1 (SR-BI) expression in liver and association with lipid levels in a population-based study. Bmc Medical Genetics, 11, 2010.

[13] Fujii S. and Amrein H.. Genes expressed in the Drosophila head reveal a role for fat cells in sex-specific physiology. Embo Journal 21(20): 5353-5363, 2002.

[14] McCarthy M.M., Auger A.P., Bale T.L., De Vries G.J., Dunn G.A., Forger N.G., Murray E.K., Nugent B.M., Schwarz J.M., Wilson M.E.. The Epigenetics of Sex Differences in the Brain. Journal of Neuroscience, 29(41): 12815-12823, 2009.

[15] Tower J. Sex-specific regulation of aging and apoptosis. Mechanisms of Ageing and Development, 127(9): 705-718, 2006.

[16] Wang J.J., Bingaman S., Huxley V.H.. Intrinsic sex-specific differences in microvascular endothelial cell phosphodiesterases. American Journal of Physiology-Heart and Circulatory Physiology, 298(4): H1146-H1154, 2010.

[17] Waxman D.J., Holloway M.G.. Sex Differences in the Expression of Hepatic Drug Metabolizing Enzymes. Molecular Pharmacology, 76(2): 215-228, 2009.

[18] Merino G., van Herwaarden A.E., Wagenaar E., Jonker J.W., Schinkel A.H.. Sex-dependent expression and activity of the ATP-binding cassette transporter breast cancer resistance protein (BCRP/ABCG2) in liver. Molecular Pharmacology, 67(5): 1765-1771, 2005.

[19] Hasselbalch S.G.K.G., Jakobsen J., Hageman L.P., Holm S., Paulson O.B.. Brain metabolism during short-term starvation in humans. In: GM K, editor. 14:125, 1994.

[20] Edwards P.A., Kennedy M.A., Mak P.A.. LXRs; Oxysterol-activated nuclear receptors that regulate genes controlling lipid homeostasis. Vascular Pharmacology, 38(4): 249-256, 2002.

[21] Yoshikawa T., Shimano H., Amemiya-Kudo M., Yahagi N., Hasty A.H., Matsuzaka T., Okazaki K., Tamura Y., Iizuka Y., and Ohashi K.. Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Molecular and Cellular Biology, 21(9): 2991-3000, 2001.

[22] Repa J.J., Liang G.S., Ou J.F., Bashmakov Y., Lobaccaro J.M.A., Shimomura I., Shan B., Brown M.S., Goldstein J.L., and Mangelsdorf D.J.. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXR alpha and LXR beta. Genes & Development, 14(22): 2819-2830, 2000.