

Effect of type 2 diabetes mellitus caveolin-3 K15N mutation on glycometabolism

YIYUAN HUANG¹, YUFENG DENG¹, LINA SHANG², LIHUI YANG², JUANJUAN HUANG², JING MA², XIANSHAN LIAO², HUI ZHOU², JING XIAN³, GUINING LIANG² and QIN HUANG²

¹School of Nursing, Youjiang Medical University for Nationalities, Baise, Guangxi 533000;

²Department of Physiology, Guangxi Medical University; ³Department of Endocrinology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi 530021, P.R. China

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Abstract. Caveolin-3 (CAV3) is a muscle-specific protein present within the muscle cell membrane that affects signaling pathways, including the insulin signaling pathway. A previous assessment of patients with newly developed type 2 diabetes (T2DM) demonstrated that CAV3 gene mutations may lead to changes in protein secondary structure. A severe CAV3 P104L mutation has previously been indicated to influence the phosphorylation of skeletal muscle cells and result in impaired glucose metabolism. In the present study, the effect of CAV3 K15N gene transfection in C2C12 cells was assessed. Transfection with K15N reduced the expression of total CAV3 and AKT2 proteins in the cells, and the translocation of glucose transporter type 4 to the muscle cell membrane, which resulted in decreased glucose uptake and glycogen synthesis in myocytes. In conclusion, these results indicate that the CAV3 K15N mutation may cause insulin-stimulated impaired glucose metabolism in myocytes, which may contribute to the development of T2DM.

Introduction

A member of the caveolin family, caveolin-3 (CAV3), is specifically expressed in muscle cells, including cardiomyocytes, skeletal muscle cells and smooth muscle cells (1,2). CAV3 interacts with and regulates various signaling molecules in the caveolae of the cell membrane, which include the insulin receptor (IR), IR substrate-1 (IRS-1), G-protein-coupled receptors and protein kinase C (3-8). CAV3 gene mutations lead to a variety of clinical phenotypes including limb-girdle muscular dystrophy, rippling muscle disease, distal myopathy,

hyperCKemia and cardiomyopathy (9,10). CAV3-null mice have been demonstrated to exhibit insulin resistance, characterized by reduced glucose uptake in skeletal muscles, impaired glucose tolerance and elevated serum lipids (4). Furthermore, the injection of CAV3 proteins restored insulin signaling in skeletal muscles (4). Studies have demonstrated that CAV3-knockout mice have increased adiposity, postprandial hyperinsulinemia, whole-body insulin resistance and whole-body glucose intolerance (4,11,12). More importantly, insulin-stimulated whole-body glucose uptake and whole-body glycogen synthesis decreased in CAV3-knockout mice compared with wild-type mice (12). These results indicate that CAV3 serves an important role in maintaining blood glucose balance.

Type 2 diabetes mellitus (T2DM) is the most common type of diabetes. Insulin is produced in T2DM, but production is either insufficient or the body does not respond with the appropriate sensitivity, which leads to a high blood glucose concentration. High blood glucose concentration is an important factor in the development of T2DM (13). A previous study demonstrated that among ~1,000 patients with T2DM, a variety of mutations were present in the CAV3 gene of 50 patients that exhibited blood glucose levels >20 mmol/l and no obvious genetic disease (14). The results of full-gene scans indicated that the total number of gene variations in the CAV3 gene in patients with T2DM was 48%, compared with 7% in healthy patients (14). A previous study also assessed the transfection of wild-type CAV3 (WT) in muscle cells and found that the PI3K/AKT signaling pathway were activated, increasing the plasma membrane localization of glucose transporter type 4 (GLUT-4) and increasing glucose uptake, cell growth and proliferation (15). One mutation of the CAV3 gene (P104L) in patients with myasthenia has been revealed to be associated with the inhibition of insulin-stimulated glucose uptake and glycogen synthesis in myocytes (16). Muscles constitute ~40% of human body weight and are critical tissues for glucose metabolism and important sites for insulin resistance in diabetic patients (17,18). The aim of the current study was to assess the extent to which the CAV3 gene mutations in patients with T2DM impaired glycometabolism in muscle cells and subsequently contributed to T2DM development. The CAV3 K15N (G/C) mutation was assessed in the present study. The mutant was transfected into C2C12 muscle cells and the effects were compared with cells transfected with WT.

Correspondence to: Dr Qin Huang, Department of Physiology, Guangxi Medical University, Building 102, 22 Shuangyong Road, Nanning, Guangxi 530021, P.R. China
E-mail: hqgxmu@163.com

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Materials and methods

Predicted secondary structure of the mutant CAV3 protein.

The CAV3 gene sequence was retrieved from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov; Human CAC3: Chromosome 3, location NC_000003.12; Mouse CAV3: Chromosome 6, location NC_000072.6; Rat CAV3: Chromosome 4, location NC_005103.4), and then the open reading frame sequence was searched by DNASTAR 7.1 software (<https://www.dnastar.com/>), and finally translated into amino acid sequence. Sequence alignment was conducted using CLUSTAL 2.1 (www.clustal.org) and T-COFFEE 12.00 software (<http://tcoffee.crg.cat/>). The secondary structure of the CAV3 protein was predicted using PSIPRED 4.0 software (bioinf.cs.ucl.ac.uk/psipred).

Cell culture. The mouse skeletal muscle cell line C2C12 (Type Culture Collection of the Chinese Academy of Sciences) was cultured in an incubator at 37°C, in DMEM (cat. no. 12800-017; Gibco; Thermo Fisher Scientific, Inc.) supplemented with high glucose (25 mM D-Glucose), 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 5% CO₂. Cells in the logarithmic phase (the 4 and 5th generations) were collected for transfection.

Stable transfection. A total of 0.5x10⁵ cells/well were seeded into 24-well plates in antibiotic-free DMEM containing 10% FBS. When cells reached 80% confluence, 5,368 ng/µl of an expression plasmid (EX-T1783-M98; GeneCopoeia, Inc.) containing wild-type CAV3 + enhanced green fluorescent protein (eGFP; WT) or 1,289 ng/µl CAV3 K15N + eGFP (K15N) were transfected into C2C12 cells with Lipofectamine[®] 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) following manufacturer's protocol. The transfection was as follows: 50 µl DMEM + 1.5 µl Lipofectamine 3000 + 0.5 µg plasmid DNA + 1 µl P3000TM. The negative control (NC) group was transfected with a vector carrying eGFP alone. The CAV3 genes in the expression vectors were homozygotes. At 24 h post-transfection, cells were cultured in high-glucose DMEM containing 400 µg/ml G418 for 7 days at 37°C prior to selection of positive clones. To obtain a stable transfection cell line, cells were subsequently transferred to high-glucose DMEM containing 200 µg/ml G418 at 37°C in a 5% CO₂ incubator for 45 days. After the cell line was constructed, the stability of the cell line was assessed using western blot analysis 55 days after transfection by analyzing whether the eGFP-CAV3 fluorescent protein was successfully integrated into the cell genome.

Western blot analysis. Cells were stimulated with 100 nmol/l insulin (cat. no. 16634; Sigma-Aldrich; Merck KGaA) for 30 min at 37°C. Cells were subsequently rinsed with cold PBS and total protein was extracted using 1X RIPA lysis buffer (cat. no. P0013; Beyotime Institute of Biotechnology) and protease inhibitors (Beijing Leagene Biotech Co., Ltd.). After 30 min of incubation on ice, cells were centrifuged at 10,142 x g for 10 min at 4°C and supernatant was collected for subsequent quantification. The protein concentration was obtained by the BCA kit (cat. no. P0010S; Beyotime Institute of Biotechnology). A total of 25 or 50 µg of protein was separated by SDS-PAGE on a 12% gel and transferred to PVDF membranes. Membranes were

blocked with 5% dried skim milk and TBS plus 0.01% Tween 20 for 1 h at room temperature and subsequently incubated overnight at 4°C with mouse anti-GFP monoclonal antibody (1:500; cat. no. J20625; Beijing Transgen Biotech Co., Ltd.), mouse anti-CAV3 monoclonal antibody (1:100; cat. no. sc-5310; Santa Cruz Biotechnology, Inc.), rabbit anti-caveolin-1 (CAV1) polyclonal antibody (1:1,000; cat. no. sc-894; Santa Cruz Biotechnology, Inc.), mouse anti-phosphorylated (p)-AKT ser473 monoclonal antibody (1:1,000; cat. no. 12694; Cell Signaling Technology, Inc.), mouse anti-AKT monoclonal antibody (1:1,000; cat. no. 2920; Cell Signaling Technology, Inc.), goat anti-AKT1 polyclonal antibody (1:1,000; cat. no. sc-1618; Santa Cruz Biotechnology, Inc.), mouse anti-AKT2 monoclonal antibody (1:1,000; cat. no. 5239; Cell Signaling Technology, Inc.), goat anti-GLUT-4 polyclonal antibody (1:100; cat. no. sc-01608; Santa Cruz Biotechnology, Inc.), rabbit anti-p glycogen synthase kinase 3 beta (GSK3β) monoclonal antibody (1:1,000; cat. no. 9323; Cell Signaling Technology, Inc.) or rabbit anti-GSK3β monoclonal antibody (1:1,000; cat. no. 9315; Cell Signaling Technology, Inc.). After washing with TBST (50 mg Tris-HCl, pH 7.6, 150 mM NaCl, 0.2% Tween 20), the membranes were incubated with Dylight[™] 800-conjugated secondary antibodies: Anti-mouse (1:1,000; cat. no. 5257P; Cell Signaling Technology, Inc.), anti-rabbit (1:1,000; cat. no. 5151P; Cell Signaling Technology, Inc.) and anti-goat (1:500; cat. no. E032830-01; EarthOx, Inc.) at room temperature for 2 h. Protein bands were subsequently visualized and quantified using a Li-Cor Odyssey infrared imager (LI-COR Biosciences). Integrated intensities of the 800 nm infrared signal for each band were calculated using the Odyssey system.

Immunofluorescence. Cells were induced with 100 nmol/l insulin for 30 min at 37°C, washed with PBS three times, fixed with 4% paraformaldehyde for 30 min at room temperature and blocked in 10% donkey serum (cat. no. CJ-326; Beijing Biotopped Biotech Co., Ltd.) in PBS at room temperature for 30 min. Mouse monoclonal anti-CAV3 (1:500; cat. no. sc-5310; Santa Cruz Biotechnology, Inc.) and goat polyclonal anti-GLUT4 (1:500; cat. no. sc-01608; Santa Cruz Biotechnology, Inc.) were used as primary antibodies. Alexa Fluor 594-conjugated donkey anti-goat (cat. no. A11058) and Alexa Fluor 647-conjugated donkey anti-mouse (cat. no. A31571; both 1:1,000; Invitrogen; Thermo Fisher Scientific, Inc.) were used as secondary antibodies. The cells were then washed with PBS three times and incubated with 0.5 µg/ml DAPI (cat. no. c0065-10; Solarbio Inc.) at 37°C for 5 min in the dark. Cells were subsequently washed three times in PBS and visualized under confocal laser scanning microscopy at a magnification of x40.

Glucose uptake and glycogen synthesis assays. Stably transfected cells were seeded at a density of 1.25x10⁵ in 60-mm culture dishes with high-glucose DMEM containing 10% FBS under conventional culture conditions. After 2 days, cells were moved to low-glucose DMEM (5.55 mM D-Glucose; cat. no. 31600-034; Gibco; Thermo Fisher Scientific, Inc.) for 12 h at 37°C and subsequently cultured in high-glucose DMEM containing 100 nmol/l insulin at 37°C. A total of 10 µl supernatant was removed from the medium to measure glucose uptake at 12 and 24 h using the glucose oxidase/hydrogen peroxide method (19) and a glucose assay kit (Nanjing Jiancheng Bioengineering Institute) according

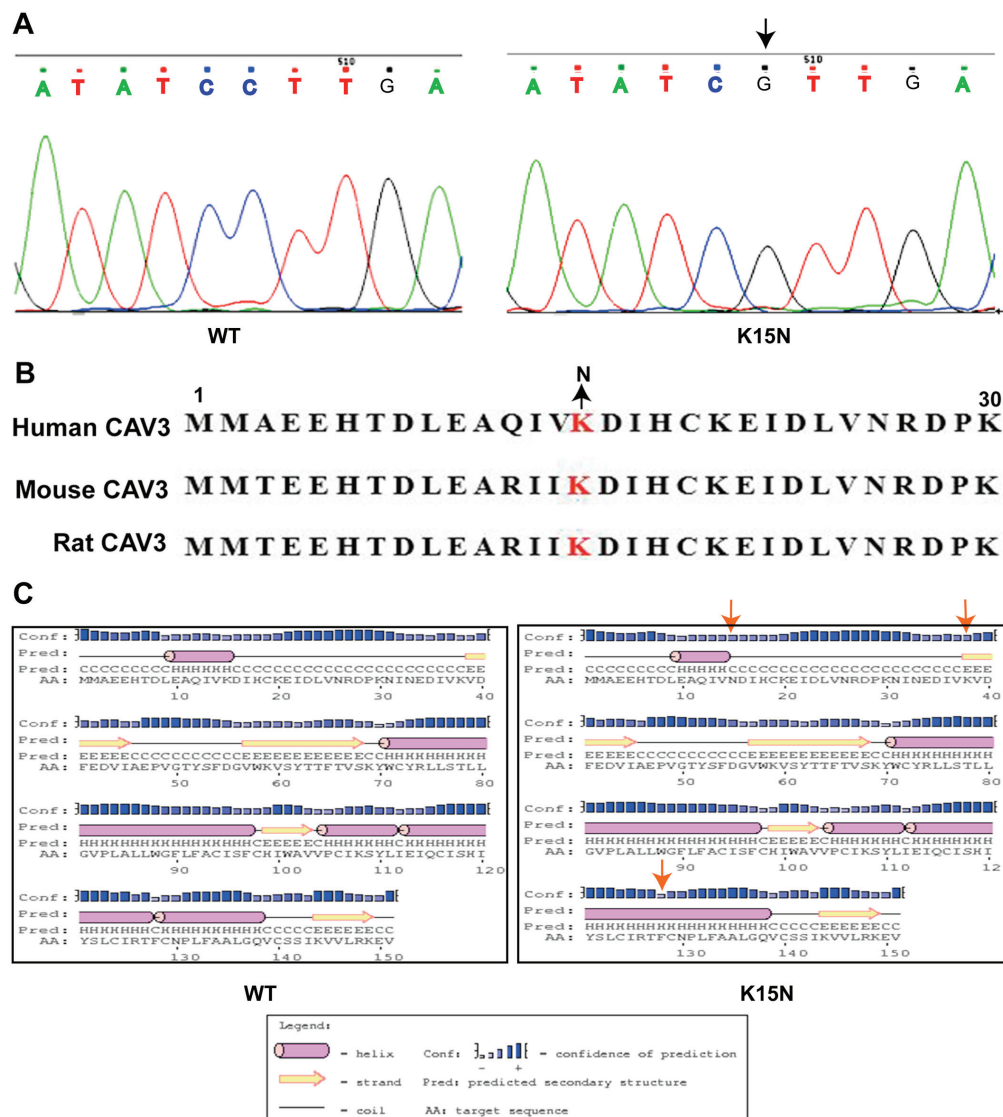


Figure 1. Mutation analysis of the CAV3 gene. (A) Sequencing diagram of WT and K15N mutant of CAV3. The arrow indicates a C to G nucleotide change on the reverse sequence diagram. (B) Alignment of CAV3 amino acid sequences at a topological domain (codons 1-30) from human and other species. The arrow indicates the 15th nucleotide of CAV3, which is conserved. (C) The predicted secondary structure of WT CAV3 and CAV3 K15N protein. Red arrows indicate changed areas. CAV3, caveolin-3; K15N, CAV3 K15N mutation; WT, wild-type.

to manufacturer's protocol. Glycogen synthesis was detected at 24 h after the insulin incubation with the glycogen assay kit (Nanjing Jiancheng Bioengineering Institute) following manufacturer's protocol (20,21).

Statistical analysis. All data are presented as the mean \pm standard deviation. An unpaired two-tailed Student's t-test was performed using SPSS 20.0 software (IBM Corp.) for comparisons between two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

K15N mutation alters the secondary structure of the CAV3 protein. Two exons exist within the CAV3 gene. The K15N mutation is located in the first exon and changes the 15th nucleotide from G to C, replacing the protein's amino acid lysine (AAG) with asparagine (AAC) (Fig. 1A). The 15th lysine is highly conserved in this protein region among humans and other species (Fig. 1B).

PSIPRED software predicted that the following three changes would occur in the proteins' secondary structure caused by the K15N mutation: One helix would change into one coil, a coil would change into a strand at the N-terminus and a further coil would change into a helix at the C-terminus (Fig. 1C).

Identification of stably transfected cells. The molecular weight of the human CAV3 protein is 25 kDa and the molecular weight of the eGFP protein is 27 kDa. The molecular weight of the recombinant eGFP-CAV3 protein was therefore ~ 52 kDa. As presented in Fig. 2, recombinant proteins with a molecular weight of 52 kDa were detected in the WT and K15N groups, respectively. However, no recombinant protein was detected in the empty vector that contained only eGFP. The results indicate that stable transfection was successful and the cells could therefore be confidently used in the subsequent experiments.

K15N mutation reduced recombinant CAV3 expression but did not affect the CAV1 protein levels. The recombinant protein

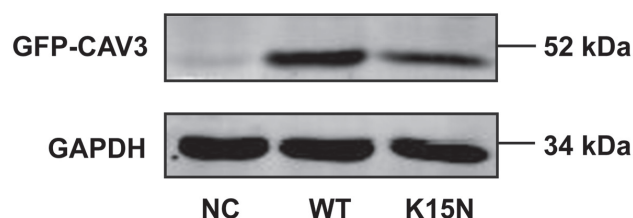


Figure 2. Identification of stably transfected cells. Western blot analysis of recombinant protein WT and K15N cells, detected using the eGFP antibody. WT, wild-type; eGFP, enhanced green fluorescent protein; NC, negative control; CAV3, caveolin-3.

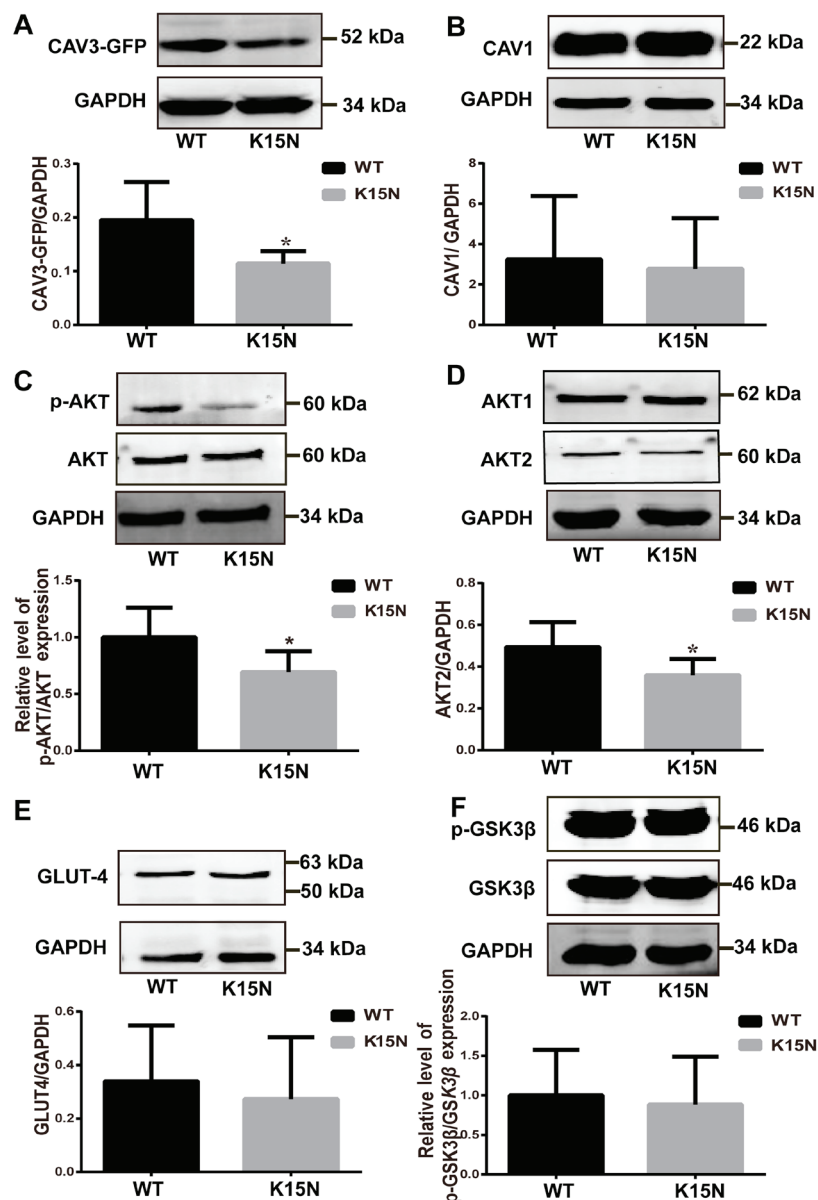


Figure 3. K15N mutation effect on protein expression in the AKT signaling pathway. Western blot and densitometry analysis of (A) CAV3-eGFP, (B) CAV1, (C) p-AKT, (D) AKT1 and AKT2, (E) GLUT-4 and (F) p-GSK3 β expression in WT and K15N cells. All data are presented as the mean \pm standard deviation. * P <0.05 vs. WT. CAV3, caveolin-3; eGFP, enhanced green fluorescent protein; CAV-1, caveolin-1; p, phosphorylated; GLUT-4, glucose transporter type 4; GSK3 β , glycogen synthase kinase 3 β ; WT, wild-type; K15N, CAV3 K15N mutation.

consists of eGFP and CAV3, therefore it can be detected by a eGFP antibody and a CAV3 antibody. After treatment with the CAV3 antibody, recombinant CAV3-eGFP protein expression in the K15N group decreased and was ~41.54% lower compared with the expression level in the WT-transfected group

(P =0.037; Fig. 3A). However, no significant difference in CAV1 expression was determined between the two groups (Fig. 3B).

K15N mutation reduces p-AKT and AKT2 levels but not total GLUT-4 or p-GSK3 β expression. AKT activation requires

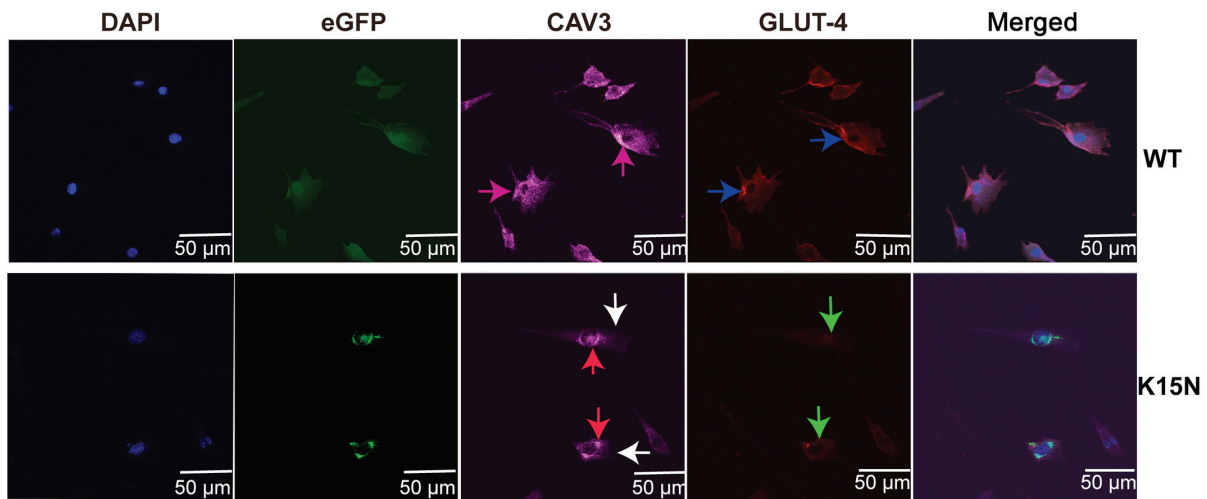


Figure 4. K15N mutation effect on the distribution of CAV3 and GLUT-4 in C2C12 cells. Confocal microscopy analysis of the distribution of total CAV3 and GLUT-4 in C2C12 cells (magnification, $\times 40$; $n=3$). Blue staining indicates DAPI (nucleus), green staining indicates fluorescent protein in successfully transfected cells, purple indicates CAV3 protein and red indicates GLUT-4 protein. The purple arrows indicate the even localization of CAV3 protein at the plasma membranes of the cells. White arrows indicate decreased protein levels on the cell membrane in the K15N mutation group. Red arrows indicate protein accumulation in vesicles surrounding the nucleus. Blue arrows indicate GLUT-4 at the surface of the wild-type CAV3. Green arrows indicate GLUT-4 proteins, which were reduced on the cell membrane in K15N cells. CAV3, caveolin-3; GLUT-4, glucose transporter type 4; eGFP, enhanced green fluorescent protein; WT, wild-type; K15N, CAV3 K15N mutation.

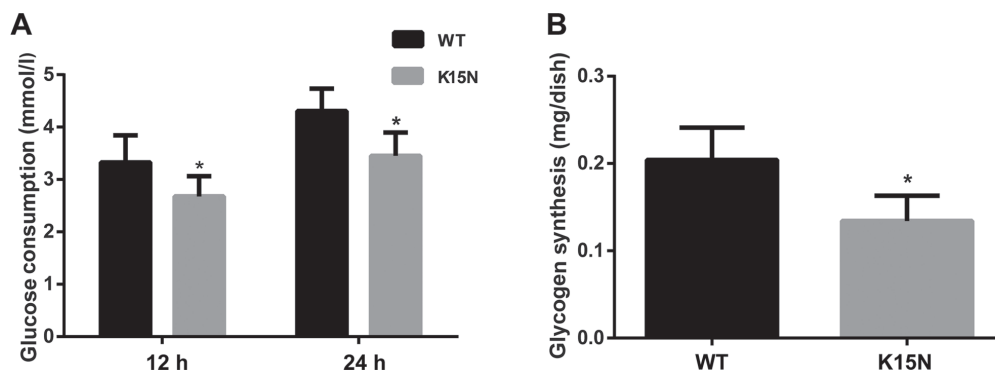


Figure 5. K15N mutation effect on glycometabolism in skeletal muscle cells. (A) Glucose consumption at 12 and 24 h after insulin stimulation. (B) Glycogen synthesis at 24 h after insulin stimulation. * $P<0.05$ vs. WT. WT, wild-type; K15N, caveolin-3 K15N mutation.

phosphorylation and is associated with the activation of the PI3K/AKT insulin signaling pathway. Two AKT phosphorylation sites exist, including T308 and S473 (22). However, optimal AKT activity requires the regulation of the Ser473 phosphorylation site (23-25). Therefore, S473 was used to detect AKT phosphorylation levels. AKT S473 phosphorylation in the K15N group was significantly decreased compared with the CAV3 WT group ($P=0.040$; Fig. 3C), however the total AKT protein content in the cell exhibited no marked difference between the two groups.

In the present study, AKT1 and AKT2 protein expression levels were assessed using western blot analysis and the results demonstrate that AKT2 expression was significantly lower in the K15N group when compared with the WT group ($P=0.042$; Fig. 3D), whereas AKT1 protein expression was not significantly different between the two groups. The results also demonstrated that the expression of downstream molecule GLUT-4 (Fig. 3E) and the relative p-GSK3 β /GSK3 β ratio (Fig. 3F) did not differ significantly between the WT and K15N group.

K15N mutation decreases the translocation of cell membrane GLUT-4. CAV3 protein was evenly localized in the cytoplasm and on the cell membrane (purple arrows) in the WT group; however, its expression on the cell membrane of the K15N group was decreased (white arrow) and accumulated in the vesicles around the nucleus (red arrow; Fig. 4). GLUT-4 immunolabeling strongly outlined the cell surface membrane in WT cells (blue arrows), while the translocation of GLUT-4 to the cell membrane of K15N group cells was markedly decreased as GLUT-4 was not localized to the cell membrane (green arrow).

K15N mutation impairs glycometabolism in muscle cells. In the present study, differences in glucose uptake between the WT group and the K15N group were observed at 12 and 24 h. The results indicate that the K15N mutation significantly reduces glucose uptake after insulin stimulation at 12 and 24 h ($P=0.021$ and $P=0.003$, respectively; Fig. 5A). The quantity of synthesized glycogen in the K15N group was also significantly lower than that in the WT group ($P=0.004$; Fig. 5B).

Discussion

The CAV3 protein is 151 amino acids long and is divided into five separate domains (9). The K15N mutation is located in the N-terminus, which is highly conserved in the CAV3 protein and may impact protein configuration (9,26). Mutations in this region may impair the location and maturity of the CAV3 protein in the cell membrane (27,28). This may induce effects on the signaling pathways, which are associated with this protein (29).

CAV3 proteins have been demonstrated to act as molecular scaffolds, regulating the activity of a variety of signaling molecules and modulating their function (3,30,31). CAV3 defects cause downstream signaling molecules (that are regulated by CAV3) to degrade at a faster rate (12). The K15N mutation reduces the CAV3 protein on the muscle cell membranes by ~95% (32). This reduction causes the abnormal localization of proto-oncogene tyrosine-protein kinase Src on the Golgi (33) and leads to CAV3 protein retention (15). Consistent with these data, western blot analysis performed in the current study indicated that the CAV3 K15N mutation led to decreased recombinant CAV3 protein expression in muscle cells. CAV1, another subtype of CAV, plays an important role in maintaining insulin signaling (4), especially in regulating glucose uptake in skeletal muscle cells (34). Therefore the authors also detected the expression of CAV1 protein. However, CAV1 expression was not significantly different between the two groups. Thus, in that experiment, the authors were able to rule out its effect on glucose metabolism. Furthermore, confocal scanning microscopy revealed that the K15N mutation caused CAV3 to accumulate around the nucleus and not the cell membrane. The Golgi body is the site of protein packaging and maturation (35). CAV3 protein retention indicates an increased likelihood that the K15N mutation may lead to an amino acid sequence change; retention may be due to the Golgi's inability to recognize the mutated protein, thus allowing the CAV3 to mature into the membrane protein (16). Subsequently, the stability and effects of related molecules on the skeletal muscle cell membrane may also be affected (12).

The IR/PI3K/AKT/GLUT signaling pathway is primarily associated with insulin signaling in skeletal muscle and liver cells (36). Additionally, this pathway has been demonstrated to be a major mechanism in the development of insulin resistance (37). However, IR and GLUT-4 are associated with glucose metabolism and are localized to membrane caveolae, with their expression being regulated by CAV3 on the cell membrane. CAV3 can enhance the expression of IR (38-40) by stimulating IR kinase activity, increasing the stability of IR at the sarcolemmal membrane and reducing its degradation (12), stimulating the phosphorylation of IRS-1 and activating the PI3K/AKT signaling pathway (15,18). Activated AKT not only promoted the translocation of GLUT-4 to the plasma membrane and enhanced glucose uptake, but also induced the phosphorylation of GSK3 β , which leads to glycogen synthesis via the activation of glycogen synthase (41,42). Therefore, any alteration of protein expression in the PI3K/AKT signaling pathway may influence insulin sensitivity.

Insulin resistance is a major factor in T2DM development and can result in the dysfunction of the insulin signaling

pathway (43-46). Therefore, to investigate the effect of the CAV3 K15N mutation on insulin-stimulated glucose metabolism, the expression and activation state of insulin signaling pathway-related molecules was assessed in the current study. There are three subtypes of AKT: AKT1, AKT2 and AKT3. AKT1 is associated with cell physiological growth and function, AKT2 is associated with the insulin-mediated regulation of glucose homeostasis and AKT3 is associated with various neurological conditions (47,48). The CAV3 K15N mutation decreased AKT phosphorylation and AKT2 levels, but total AKT, AKT1, GSK3 β and p-GSK3 β protein levels did not differ significantly between groups. A limitation of the current study is shown in the expression of these proteins, as some of the samples were large and caused double banding. The results indicate that the activation of AKT signaling may be inhibited when the CAV3 protein is decreased on the cell membrane. The overexpression of CAV3 enhanced the tyrosine phosphorylation of IRS-1 and activated the AKT signaling pathway (49). The K15N mutation resulted in decreased CAV3 protein expression on the cell membrane, which inhibited the phosphorylation of AKT and may subsequently affect the activation of various downstream signaling molecules. This result may be due to downregulated CAV3 expression, causing CAV3 to attach to the cell membrane abnormally, affecting the expression and stability of weakening the physiological effect of IR on the PI3K/AKT signaling pathway. Various downstream signaling pathways, including AKT phosphorylation and GLUT-4 protein translocation may also be selectively suppressed.

AKT serves a role in cell metabolism via its association with glucose transporters and glucose uptake, and the conversion of stored glycogen to glucose (50). Studies have demonstrated that mice with an AKT1 gene deletion show normal glucose metabolism (51), whereas mice with an AKT2 gene deletion or humans with an AKT2 gene mutation develop insulin resistance and a type 2 diabetes-like phenotype (51-53). Furthermore, *in vitro* studies have demonstrated that AKT2 silencing causes inhibition of insulin-induced GLUT-4 translocation to the plasma membrane (52,54). Due to the role of AKT2 in insulin-mediated glucose metabolism, the results of the present study indicate that AKT2 expression decreased in cells transfected with K12N compared with the WT. Decreased AKT2 expression may decrease the insulin sensitivity of skeletal muscle cells and may be an important molecular mechanism associated with insulin resistance in patients with T2DM.

Insulin can promote glucose transport in skeletal muscles by stimulating the translocation of GLUT-4 from intracellular storage vesicles to the plasma membrane (55). CAV3 promotes glucose uptake in skeletal muscle cells by enhancing GLUT-4 translocation to the plasma membrane (56). In individuals that exhibit insulin resistance, GLUT-4 expression is normal (57), but translocation to the cell membrane is decreased (58). In the present study, the results revealed that in CAV3 K15N cells, GLUT-4 was less concentrated on the cell membrane due to reduced translocation, but the total GLUT-4 expression of GLUT-4 protein did not change. These results indicated that K15N mutations affect glucose metabolism by reducing GLUT-4 translocation to the cell membrane and not through the overall expression of GLUT-4 protein.

CAV3-null myotubes exhibit low levels of insulin-stimulated glucose uptake as PI3K/AKT activation and plasma membrane GLUT-4 translocation are reduced (56). Consistent with this effect, the current study demonstrated that subsequent to insulin stimulation, the CAV3 K15N mutation reduced p-AKT and AKT2 expression, GLUT-4 translocation to the membrane, glucose uptake and glycogen synthesis. Therefore, the K15N mutation in patients with T2DM may impair glycometabolism in skeletal muscle cells and increase blood glucose.

In total, the authors hypothesized that the CAV3 gene K15N mutation, which was located in patients with T2DM, decreased total CAV3 expression, caused CAV3 proteins to aggregate in the Golgi and caused the proteins to lose their normal functional role. This may affect the stability of IR on the cell membrane and inhibit the AKT pathway, which is associated with AKT phosphorylation and GLUT-4 protein translocation (12,16). The IR/PI3K/AKT/GLUT signaling pathway may be restricted due the K15N mutation and may result in glucose accumulation, which is exhibited in hypoglycemia and during the development of T2DM (59).

The current study demonstrated an association between the CAV3 K15N mutation and the pathogenesis of T2DM. However, subsequent observations are required in other cell lines and animal models to further validate these results. The current study assessed the effect of CAV3 K15N mutation on impaired glucose metabolism induced by insulin. The K15N mutation exhibited the same effect as the P104L mutation in CAV3 (16), which demonstrated impaired glucose metabolism in the C2C12 cell line. These kinds of patients showed different symptoms, which may be attributed to one or more mutations in their genes, including the CAV3 gene, and the interactions of the genes (60,61). To the best of our knowledge, the current study is the first to assess the contribution of K15N mutation in T2DM.

The present study identified AKT is a key factor in the regulation of glucose metabolism in cells. The present study also assessed the downstream signaling factors of AKT, including GSK3 β , p-GSK3 β and GLUT-4. Other downstream effectors of AKT signaling including glucose transporter 2, glycogen synthase and phosphoinositide 5-phosphate 4-kinase type II remain to be investigated.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YH and QH designed and performed the experiments, analyzed the data, and wrote the manuscript. YD, LS, LY, JH and QH performed the research and analyzed the data. JM,

XL, HZ and JX designed and executed the search strategies and contributed to the critical revision of the manuscript and approved the final version. GL designed and supervised the research. All authors have read and approved the final draft of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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