PCSK9 gene variants in West – African type 2 diabetes mellitus (T2DM) patients

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Introduction

- Type 2 diabetes mellitus (T2DM) is associated with lipid profile impairments and increased risk of atherosclerotic cardiovascular diseases (ACVD).
- Proprotein convertase subtilisin/kexin 9 (PCSK9) is an important regulator of low-density lipoprotein cholesterol (LDL-C) via LDL-receptor (LDLR) down regulation.
- PCSK9 inhibitors (PCSK9i) have emerged as the most effective LDL-C lowering drugs in diabetic and non diabetic hypercholesterolemic patients as well.
- In spite of PCSK9i high efficacy, their effect on glucose metabolism remains controversial.

- It has been demonstrated that the enhanced expression of LDLR promoted by PCSK9 knock-out in mice triggers the apoptotic death of pancreatic β-cells subsequent to cholesterol overcharge. This in turn reduces insulin secretion and impairs glucose metabolism (1). Moreover, a patient on anti-PCSK9 antibody treatment was described to have its Hemoglobin A1c doubled (2).
- But other studies revealed contradictory results. (3)

- Thence, the question of the PCSK9i long-term safety especially their link with T2DM onset or complications is still opened

The aim of this study is to identify the PCSK9 genetic variants and explore their association with the onset of T2DM or its complications

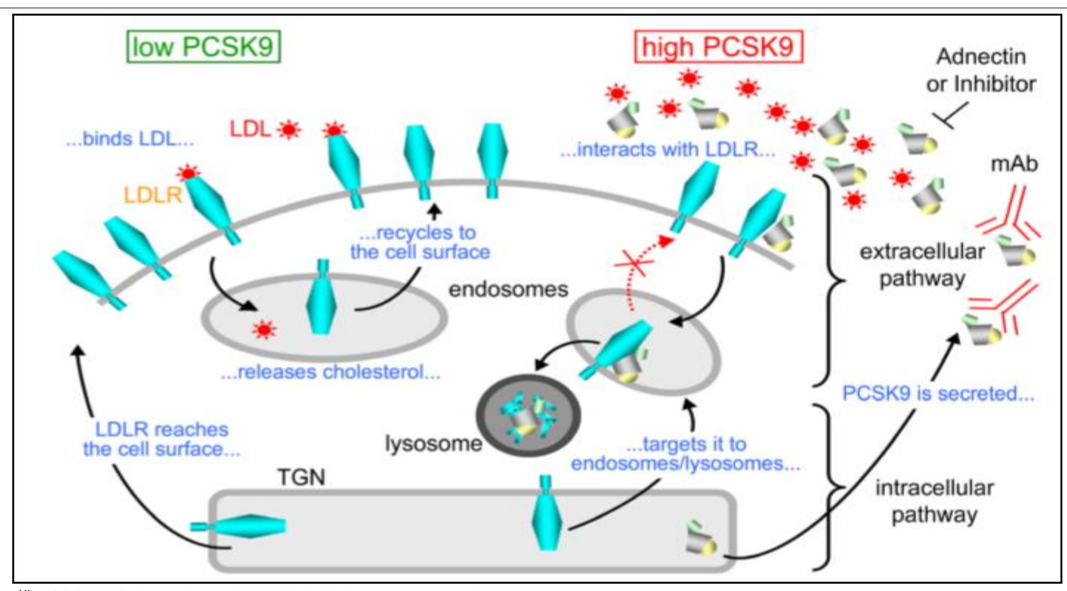

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Figure 1. Schematic representation of the intracellular and extracellular pathways of PCSK9 induced degradation of the LDLR. When PCSK9 levels are high or if it carries a gain-of-function mutation, it will enhance the degradation of the LDLR using both the intracellular and the extracellular pathways leading to the degradation of the PCSK9≡LDLR complex in lysosomes. This results in low levels of the LDLR at the cell surface and increased levels of circulating LDL-C. In absence or under low levels of PCSK9, cell surface LDLR levels are high and the LDLR can be recycled back to the surface after delivery of LDL particles to acidic endosomes. The extracellular pathway-specific treatments include the use of a monoclonal antibody (mAb), an inhibiting adnectin or other small-molecules. TGN: Trans Golgi Network

Methodology

❖**Patients:** T2DM subjects of both sexes aged of 40 years or more.

❖**Socio-demographic, anthropometric and clinical data:** Age, sex, ethnicity, socio-professional category, religion, waist circumference (WC), height, weight, blood pressure (systolic, SBP; diastolic, DBP), consumption of tobacco or alcohol, physical activities practice, personal and familial history of diabetes, hypertension or other cardiovascular diseases, hormonal contraception, use of antidiabetic or anti hypertension drugs.

❖**Serum glucose:** Serum glucose level was measured by Glucose Oxidase and Peroxidase method (ELITech Group, Puteaux, France),

❖**Insulin dosage:** Insulin level was measured by ELISA sandwich method (ALPCO, Salem, New Hampshire, USA)

❖**DNA Analysis:** DNA was extracted by the classical phenol-chloroform method. An amplicon of 734 bp comprising PCSK9 exon7 was generated using a high proofreading and hot start polymerase (Primestar® Max, Takara BIO INC.) and the following primers: forward TTTCCTATCTCCCCCACTG; reverse ATCCATCAAGCTCCCGATC. The obtained amplicon was sequenced by Sanger method.

❖**Statistical analysis:** BMI was calculated and subjects were categorized according to World Health Organization (WHO) BMI classification. Data were presented as mean±SD for quantitative variables and percentage (%) for qualitative variables. Correlation was tested with Pearson 's coefficient. The results were considered significant when $p<0.05$.

Results

Characteristics of the study subjects according to the variants portage

Table I: Clinical and anthropometric characteristics of the study subjects

	Male				Female			
	Non carriers	Rs533375 alone carriers	rs509504 alone carriers	Both variants carriers	Non carriers	Rs533375 alone carriers	Rs509504 alone carriers	Both variants carriers
Age (years)	61				58			
BMI (Kg/m²)	29.28	-	21.99	29.75	-	-	-	26.37
WC (cm)	95	-	80	98	-	-	-	100
Alcohol consumption (%)	-	-	-	-	-	-	-	-
Smokers (%)	-	-	-	-	-	-	-	-
Physical activities pratcice (%)	-	-	100	100	-	-	-	40
Hypertension (%)	100	-	-	100	-	-	-	20
SBP (mmHg)	150	-	110	150	-	-	-	132
DBP (mmHg)	70	-	60	90	-	-	-	82
Use of anti-hypertension drug (%)	-	-	-	-	-	-	-	20
Use of Metformin (%)	100	-	100	100	-	-	-	100
Use of Glybenclamide (%)	-	-	100	-	-	-	-	60
Glycemia (mg/dL)	187	-	184	115	-	-	-	214
Insulin (µIU/mL)	8.05	-	4.00	5.04	-	-	-	7.57**
HOMA2-IR	1.1	-	0.3	0.6	-	-	-	2.5

- The mean age of the study subjects was 61±9 years. The female sex was predominant with a sex ratio of 1.9
- No subject carries rs533375 alone.
- Frequency of hypertension was higher in men than women.
- All the subjects (100%) are on metformin therapy. 60% of women carrying both variants received additionally glibenclamide, an insulino-secretagogue.
- Women carrying both variants showed high SBP and DBP. 20% of them are on anti-hypertension drugs.
- The fasting insulin and glycemia was respectively 6.09 ±5.19 µIU/mL and 219 ±92 overall.
- Women carrying both PCSK9 variants exhibited insulin impairment, including a case of high insulinemia and a case of a very low insulinemia.

Correlation of glucose with serum insulin level

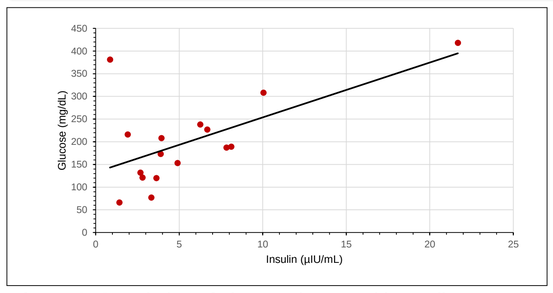

Figure 2: Correlation of plasma glucose with Insulin

A positive correlation was observed between fasting insulin and glycemia ($r=0.61$ $p<0.05$), which suggests that glucose homeostasis is generally not deregulated

Correlation of SBP with BMI

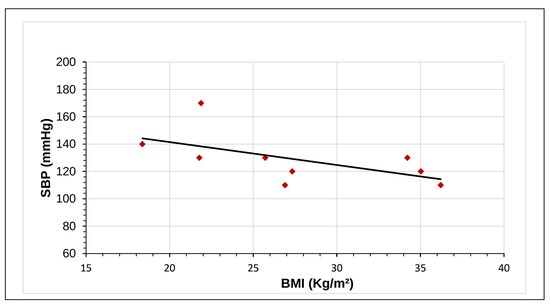

Figure 3: Correlation of SBP with BMI in female

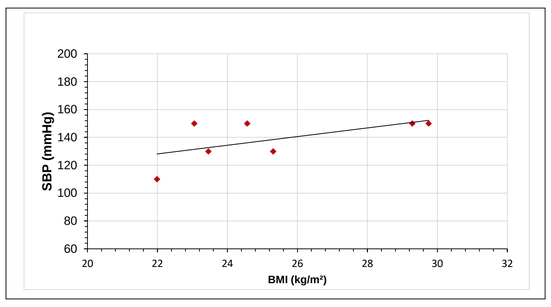

Figure 4: Correlation of SBP with BMI in male

The BMI correlated positively in men ($r=0.60$, $p<0.05$) but correlation did not attain significance in women ($r=-0.587$, $p>0.05$) (Figures 3&4)

Genetic features

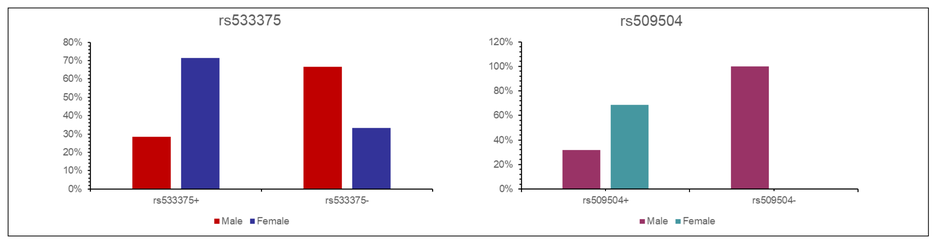

Figure 5: Sex distribution of the two variants in the cohort of study

Two genetic variants associated with conflicting conditions related to cholesterolemia (5,6) and already reported in African population were detected:

i) The rs533375 intronic variant c.1180+174 A>G present in 70% of the subjects (71%female; 29% male)

ii) The rs509504 synonymous single nucleotide polymorphism (SNP) c.1026A>G (p.Gln342=) observed in 95% of the subjects (68% female; 32% male)

Figure 6: Association of variants 1 and 2 within the male and female populations in the cohort

- 33% of the men carry both variants against 100% in women.
- All the women carrying variant1 also carry variant 2 and no subject carries variant 1 only.

Variant 1: rs533375; Variant 2: rs509504

Figure 6: Distribution of the genetic variants according to BMI categories

- Men carrying the rs509504 variant were normally weight.
- Women carrying both variants were obese

Conclusions & Perspectives

In the present study, the preliminary results showed two genetic variants of PCSK9 among the study subjects with high frequencies, namely the rs533375 intronic variant c.1180+174 A>G and the rs50904 synonymous single SNP c.1026A>G (p.Gln342=). Many of the carriers of the genetic variants were obese or overweight despite a diet dominated by plant products and a relatively low frequency of tobacco and alcohol use, suggesting the possibility of a genetic factor implication. Women carrying both variants exhibited insulin impairment. Among the latter, women showed higher SBP and DBP.

At this stage, more investigations at a larger scale are needed to confirm whether the variants are associated with T2DM.

References :

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