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Polymerase Chain Reaction amplification of the Calpain-asd gene in *Plasmodium falciparum* to determine association with diabetes mellitus

ABSTRACT

Calpain-10 is important in the regulation of the transformational production of insulin, whose deficiency ultimately causes development of diabetes mellitus. The Non-Insulin Dependent Diabetes Mellitus (NIDDM) is associated with the production and conformational structure of calpain proteases, which shows a link between the prevalence of diabetes mellitus and calpain production. This study was carried out to determine the association between calpain-10 in the human body and the susceptibility of individuals to diabetes mellitus. The study was carried out by initial cloning of the calpain-active site domain from the *Plasmodium falciparum* g Deoxyribonucleic acid and amplifying it through the Polymerase Chain Reaction technique. The amplified gene was then used to determine whether or not the composition of calpain-10 genes and their active sites are necessary to enhance insulin production in the human body. The results showed that there is a high correlation between calpain-10 production in the body and the development of diabetes. This experiment shows the possibility of inhibiting diabetes by enhancing the body's ability to produce calpain proteases.

Keywords: *Calpain-10, insulin, Non-Insulin Dependent Diabetes Mellitus, diabetes mellitus, calpain-active site domain, polymerase chain reaction.*

INTRODUCTION

The first calpain was identified in 1964. Calpains have been proven to exist throughout the nature of most living organisms, showing evolutionary and physiological phenomena over the years. A calpain is an intracellular calcium ions (Ca^{2+}) dependent protease, which belongs to the cysteine family (Soh *et al,* p. 8). Calpains have been found to exist in all types of organisms, ranging from the higher and lower eukaryotes, and all the bacteria; with the only absence found in the archaebacteria.

The physiological role of calpain is yet to be fully understood. However, since calpains are regulated by calcium ions activity, it is believed that calpains are important in numerous cellular processes and systems including signal transduction, cell proliferation, progression of the cell cycle, cell differentiation, cell apoptosis, membrane fusion and activation processes of platelets (Suzuki, Hata, Kawabata & Sorimachi, p. 12).

The human genome has 14 known calpains (Pandurangan, Hwang, Orhirbat, Jieun & Cho, p. 162), which are isoformic. The primary calpains are two: milli (m)-calpain and the micro (μ)-calpain, and are also called conventional calpains; while the rest are unconventional calpains. Mammalian calpains are named in their order of CAPN1, CAPN2, CAPN3 and so on (Cui *et al*, p.450). Classical calpains have similarities based on their sequences (CAPN1, CAPN2, CAPN3, CAPN8, CAPN9, CAPN11, CAPN12 and CAPN14). Non-classical calpains like CAPN7 are divided into subfamilies like PalBH (humans), PalB (fungi) and Rim13 (yeast) (Pandurangan *et al.*, p. 162).

Diabetes mellitus is considered a metabolic syndrome, and the main characteristics are hyperglycemic activity as a result of insulin deficiency. Diabetes mellitus is mainly regarded

dangerous because it causes the body to shift from using carbohydrates for metabolic activities, to fats. Diabetes mellitus is among the five leading cause of death of the world (WHO, 2017), and is linked with other fatal conditions of the body including nephropathy, cardiovascular diseases, neurology, vascular diseases affecting body organs like the kidney, the brain, and retinopathy (Pandurangan *et al.*, p. 162). Diabetes mellitus is classified into two main types: Insulin Dependent Diabetes Mellitus (IDDM) and Non-Insulin Dependent Diabetes Mellitus (NIDDM).

NIDDM is due to the reduced activity of G-6-P which is affected due to the deficiency of the activity or the availability of the hexokinase enzyme, or due to the deficiency of the glucose transportation mechanism. NIDDM is often associated with old age, a manifestation of problems with glucose metabolism, and is sometimes linked to obese conditions, overeating and inadequate intense physical activity (Sorimachi & Ono, p. 19).

Calpain-10 is the first gene to be linked to diabetes. The haplotype combination of three single-nucleotide polymorphisms, that is, SNP-43, -19 and -63 are associated with a tripled susceptibility to diabetes, and are found in the non-coding regions of CAPN10. The aim of this experiment is to amplify the target DNA through Polymerase Chain Reaction (PCR) and extract the gene of interest, which is the CAPN10 part of the calpain protease. The extracted gene can then be examined to determine the direct effects of the gene in insulin regulation in the human body.

HYPOTHESIS

The null hypothesis postulated is that the CAPN10 gene directly increases the susceptibility of individuals to diabetes mellitus.

MATERIALS AND METHODS

MATERIALS

BUFFERS AND SOLUTIONS

1) 10x amplification buffer
2) Chloroform
3) dNTP solution (20Mm) containing four dNTPs, at a pH of 8.0
4) Tris-chloride
5) Sodium phosphate

ENZYMES AND BUFFERS

a) *Taq* Thermostable DNA polymerase enzyme
b) Nucleic acids and oligonucleotides
c) Forward primer (20µM) in H_2O
d) Reverse primer (20µM) in H_2O
e) *Plasmodium falciparum gDNA* as template DNA

METHODOLOGY

The calpain genes were amplified using full-length DNA samples obtained from *P. falciparum* mosquito species strain FCR-3. In the experiment, the PCR primers were:

i *rPfcal-IIa*
ii *rPfcal-IIb* and,
iii *rPfcal-IIab*

The forward primers used the *BamHI* site whilethe reverse primers used the *Xhol* site. The cloning vector used was the pGEM-T vector and the lytic sites were *BamHI* and *Xhol.* The ligation of the calpain genes was done with the pET21b+ vector.

After the ligation process, the highly proliferating bacteria *Escherichia coli* was used fro te transformation process. The ligase was then induced using 1mM isopropyl-β-D-thiogalactopyranoside (IPTG) for about 4 hours. Harvesting was then done by centrifuging and suspending in 6M Gu-HCl, 0.1M sodium phosphate as the buffer solution and 0.01M Tris-Cl at a pH 0f 8.0 for one hour. Centrifugation was performed to the cell lysate and incubation of the supernatant was done in slurry for one hour.

After further treatment in ELISA assay, the recombined vector was passed through gel electrophoresis to determine the gene portion of interest. After obtaining the portion of interest, the calpain gene passed through the gelatin zymography technique and later through the fluorogenic substrate assay using the Tris-HCl buffer at pH 6.5. 7.5, 8.5 and 9.5.

RESULTS

The PCR results obtained gave the following output.

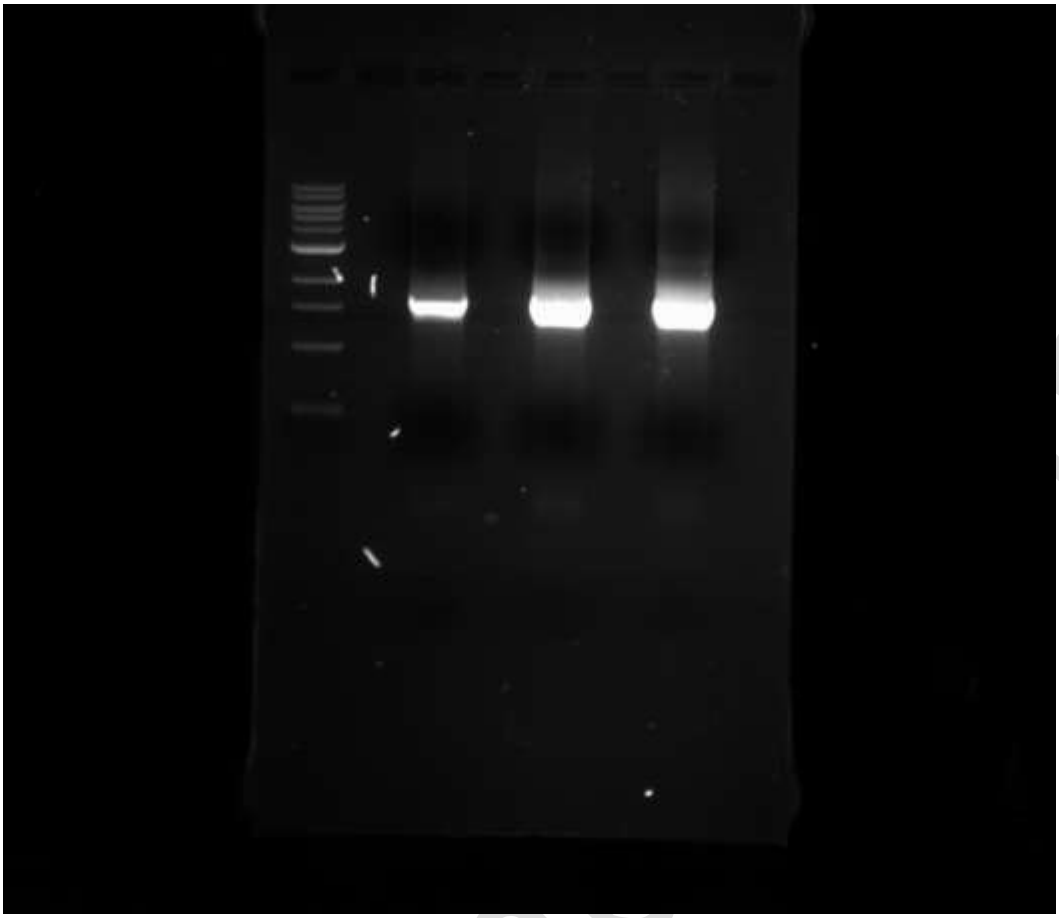

Figure 1: The image shows the image output of the DNA samples subjected to gel electrophoresis.

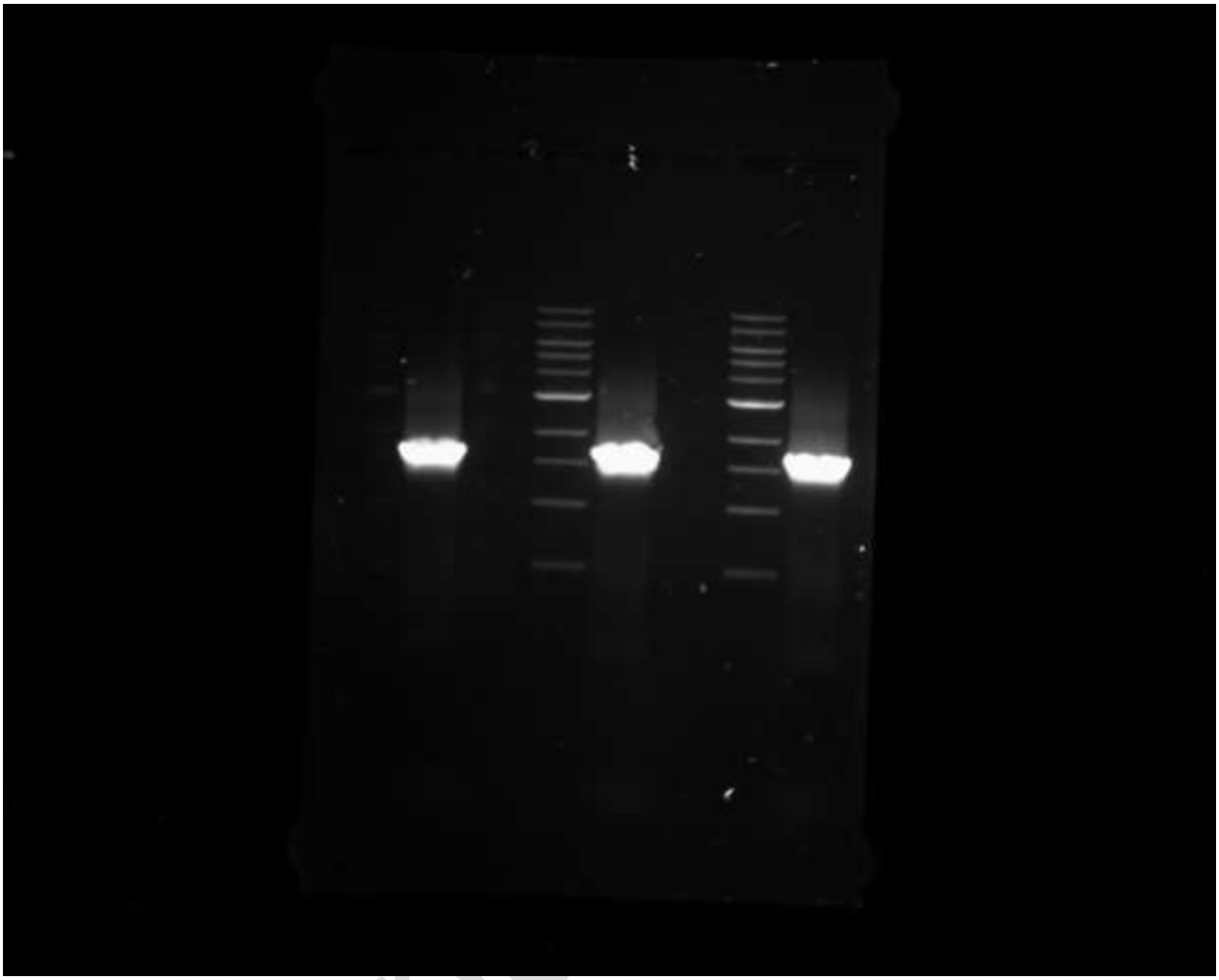

Figure 2: The image shows the output after observation of DNA samples in a gel electrophoresis machine

The level of activity of calpains within the DNA fragment was determined using the fluorogenic substrate assay combined with gelatin zymgraphy.it was shown that there was a high level of activity in calpains when insulin production is ongoing. The images show that the Calpain-10 is the most fluorescent and travels the farthest

DISCUSSION

Several studies have linked the prevalence of diabetes mellitus to the function of calpain-10 in the human body. Calpain-10 is expressed ubiquitously in all human beings in spite of the age. This is even been observed in rodents such as mouse and rats. The activity level of calpain-10 is highest around the heart, and lowers from the heart to the brain, liver, kidney and then the pancreas (Suzuki *et al,* p. 17). The variations of calpain-10 in terms of genetic complexity is linked to increased free fatty acids in the body. This phenomenon has also been noted to have a direct relationship with insulin resistance. The presence of free fatty acids within the bloodstream lead to the activation of the enzyme kinase C, which induces hyperphosphorylation of insulin receptors (Castro-Martinez *et al,* p. 84). This in turn lowers the activity of the enzyme kinase and thus increases insulin resistance (Pandurangan *et al,* p. 163). It therefore appears that the downregulation of kinase C allows effective levels of phosphorylation in insulin receptors.

Although the actual role of calpain-10 in inhibiting insulin production and enhancing insulin resistance is not yet understood, it is evident that calpain-10 is involved in anomalies in insulin production by the body. This is supported by the fact that the roles of calpains appear to have a functional connection, even when individual calpains are structurally and functionally diverse. For instance, calpains are essential in the formation of the membrane during membrane fusion, and they hydrolyze various proteins (Khodaeian *et al*, p. 16). Additionally, calpains significantly trigger cellular signaling mechanisms such as enzyme kinases, receptors and transcriptional factors. The roles of calpains therefore provide evidence that calpain-10 could have an integral role in the secretion, transport and activity of insulin in the body (Tkac, p. 60).

CONCLUSION

There is significant evidence of a relationship between calpains in general and calpain-10 in particular to hypothesize that calpains are partly responsible for diabetes mellitus. The overwhelming evidence that diabetes is caused, in part, by a sedentary lifestyle and poor dietary habits, is not refuted by this paper. However, this proves that diabetes could be a potentially genetic condition that may or may not be inherited in the Mendelian way. Thus, more research is needed to determine the exact role of calpain-10 in the body, especially concerning its activity and effects on the mechanisms and/or activity of insulin receptors and insulin in the body.

ACKNOWLEDGEMENTS

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