Polymorphisms of Calpain 10 (Capn10) In Type 2 Diabetes Mellitus – A Review

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I. INTRODUCTION

Type 2 Diabetes Mellitus is a complex disorder due to the actions and interactions of many genetic and environmental factors. Although exercise and changes in the diet can reduce the complications of Type 2 Diabetes Mellitus, identifying the genetic risk factors and non-genetic risk factors may help treat the disease better with its known physiology and effective therapies. Recent identification of different genetic risk locus of chromosomes, disease susceptibility genes and the genetic variations of each gene is not only a step towards knowing the disease but also help treate the disease, Type 2 Diabetes Mellitus better.

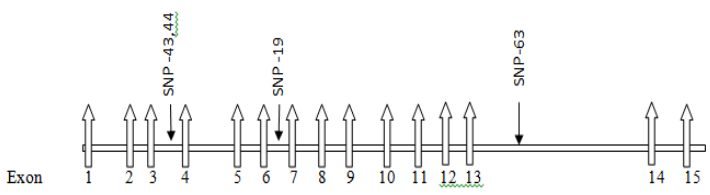
The first positionally cloned gene for Type 2 Diabetes Mellitus, Calpain 10, located on chromosone 2q37.3(NIDDM1) has undergone detailed study, four nucleotide polymorphisms (SNPs); UCSNP43, UCSNP44, UCSNP19, and UCSNP63 in the recent past with respect to other susceptibility genes; INS, INSR, PPARG etc. for Type 2 Diabetes Mellitus. Inheritance of specific haplotype combination defined by SNP-44, -43 -19 and -63 is found to be in association with increased risk to Type 2 Diabetes Mellitus. Different haplotypes and haplotype combinations such as four-locus (1112) and three locus (112) representing different alleles (G/G, G/A, T/C etc.) in different ethnic groups (South Indians, Africans-Americans, Samoans etc.) of different countries (Africa, Asia, India, UK etc.) has undergone as far as posible a through analysis to show the correlation between the disease and the other factors such as quantitative traits (hip size, waist to hip ratio etc), insulin resistence, glucose tollerence etc. in different ethnic groups.

Although Insulin secretion, insulin action, insulin stimulated glucose transport and insulin stimulated glycogen synthesis are the significant metabolic activities related to the disease of an individual, evidences from the studies show that there are susceptibility genes controlling these metabolic activities and calpain 10 and its variants contribute to the metabolic activites and Type 2 Diabetes Mellitus. Calpains are hetero-dimers, 80 KDa and 30 KDa, catalytic and regulatory sub-units respectively within the range of I to VI domains probably with calcium binding sites. Calpain 10, a cytoplasmic cystein protease probably requires calcium and phospholipids for its activity, is among one of 16 genes, 14 genes from 80K family and 2 genes from 30K family is expressed ubiquitously having probably its main function, protease activity and cellular signaling. Although Calpain 10 is highly expressed in heart, brain, liver, kidney and pancreas, the main role of it is in the tissues such as skeletal muscles.

II. SINGLE NUCLEOTIDE POLYMORPHISM

Calpain 10 has 15 exons, spanning around 31 kb with probably twelve SNP's located in different intron regions, of which only four have undergone detailed study with respect to the disease, Type 2 Diabetes Mellitus. All the SNP's are situated in the non-coding regions such as SNP-44: T/C and SNP 43; G/A in the third intron region, SNP-19; 2R/3R in the sixth intron region and SNP-63; C/T in the thirteenth intron region.

Calpain 10 increases susceptibility to Type 2 Diabetes Mellitus because of the strong involvement of its SNP-43, -44, -19 and -63. There is no uniformity on the study of SNP's in different populations with respect to the disease. In one study, SNP-19 genotype was reported risk genotype to Type 2 Diabetes mellitus by regression analysis in Tunisian Arab population(7) but in an another study, SNP-43 affects intra-abdominal obesity and insulin sensitivity in offspring of patients with Type 2 Diabetes Mellitus which differs between men and women after an adjustment for insulin sensitivity(25).



Representation of different SNP's

III. GEOGRAPHY AND LINKAGE DISEQUILIBRIUM

The susceptibility variant at Calpain-10 showed an unusual pattern of geographic structure. The survey of Calpain 10 susceptibility variants indicate a complex and remarkable geographic structure at the locus which is in consistent with the effects of positive natural selection acting on the gene itself or on the closely linked sit or sites, a study on African, Asian and European populations (8). There is a strong Linkage Disequilibrium pattern reported to be increased susceptibility to disease. Linkage Disequilibrium pattern also differ substantially between the African and non-African Samples and showed that all three pairs of polymorphism were in strong Linkage Disequilibrium in polish population (13). Extensive Linkage Disequilibrium among sites SNP-56, -59, -19 and -30 are also found in appreciable frequencies of the main susceptibility genotype 111/221 in the samples of Asian and Native American origin and provide a strong evidence for the ancestral state of polymorphic site (8). The coding variants cause coding polymorphism, they are L34V, T504A, R555C and V6661 found in UK (6). Ala 504 allele of the polymorphism T504A of SNP 110 is in perfect Linkage Disequilibrium with C allele at SNP 44 (6).

IV. POPULATION AND HAPLOTYPE COMBINATIONS

Calpain 10 and its variants was studied in eleven different types of populations without much conclusion and the type of haplotype or allele involved in the increased risk to Type 2 Diabetes Mellitus. Different haplotypes increase the risk to type 2 Diabetes Mellitus in different ethnic groups. In UK population, only the 2111 haplotype containing SNP 44, a rare allele showed greater than expected transmission to diabetes offspring in contrast to other haplotypes and populations and 1112 haplotype had lower frequency and 2111 haplotype had higher frequency than expected. However, 112/121 haplotype combination of SNP-43, -19 and -63 is associated with Type 2 Diabetes Mellitus in Mexican-American, German, Gaza Strips populations (22) and it appears to increase type 2 Diabetes Mellitus in Samoans also(20). But 112/121 haplotype was less common in UK population and was less risk to Type 2 Diabetes Mellitus(6) and 112/121 haplotype was not associated with type 2 Diabetes Mellitus in Tunisian of Arab descent population(7) and Polish population (13). A well-established study shows that; haplotype combinations, 111/121 susceptible in Koreans and Northern Europeans, 112/221 susceptible in Chinese and 121/121 in Pan-European and Scandinavians. Moreover, these associations to Type 2 Diabetes Mellitus is in contrast to Tunisian (South East Tunisia) populations where 121/221 diplotype is with increased risk of Type 2 Diabetes Mellitus (7). In Samoans of the western pacific, the most common haplotypes are 121, 111, and 112 (20). In addition, 121 haplotype were more prevalent in Type 2 Diabetes Mellitus in Polish Population and 121/121 combination is associated with an increased risk of Type 2 Diabetes Mellitus in Polish Population, however the increased risk of Type 2 Diabetes Mellitus in Polish population is only in the presence of 111 haplotype (13). The analysis of the pooled data sets showed that SNP-43, SNP-19 and SNP-63 haplogenotypes were associated with type 2 Diabetes Mellitus in Europeans(21). The association of the 121/121 haplogenotype with type 2 Diabetes Mellitus was also observed by meta-analysis and the SNP-43 G allele and the 121 and 221 haplotypes showed significant association with type 2 Diabetes Mellitus in both the pooled and meta-analyses. The rare 112 haplotype reached significance in only the pooled analysis(21). The haplogenotypes 112/121 and 121/121 were associated with significantly increased risk and 111/221 with reduced risk in the pooled analysis (21). Finally, the knowledge of haplotypes is mostly limited to their presence like haplotype 121 were most common in non-African samples, haplotype 121 was not observed and haplotype 221 or 112 were much more common in two African samples (8).

V. INSULIN AND CHOLESTEROL

Calpain 10 was found in association with measure of insulin action in Pima Indians with normal glucose tolerance showing susceptibility through its effects on oxidation of glucose in skeletal muscles. Calpain 10 mRNA levels were elevated in pancreatic islets of patient with Type 2 Diabetes Mellitus and there was a positive correlation between Calpain 10 expression and insulin release in response to arginine in non-diabetic but not in diabetic donors (10). Tissues other than muscles also may play an important role in insulin-regulated glucose disposal (23). This idea is consistent with the hypothesis that mice store a larger percentage of glycogen in liver than in muscle, as compared to humans. Calpain 10 expression correlates with insulin release in non-diabetic islets and the correlation is lost if it is in the case of Type 2 Diabetes Mellitus. SNP-19 significantly alters the insulin sensitivity index by synthesis of a mutant protein and/or altered transcriptional regulation, which could contribute to the diabetes risk. One of the main tests to Diabetes Mellitus is cholesterol test. The total cholesterol level in heterozygous patients was higher than in homozygous patients for G allele of Type 2 Diabetes Mellitus. Patients with G/A type have higher total cholesterol levels in comparison with G/G allele and SNP-19 increases highest total cholesterol level (22); haplotype combinations, 111/111 has lowest total cholesterol, 121/221 has highest level of cholesterol and Control 111/121 has highest level of total cholesterol in the populations of Gaza Strip (22).

VI. NUTRI-GENETICS AND INSULIN RESISTANCE

Nutri-Genetics is a study of interaction of nutrients with gene. Calpain10 may be a fuel sensor, and a determinant of insulin exocytosis in the beta cells, with the actions in the mitochondria and plasma membrane. In diabetes, it is established that insulin resistance is the primary abnormality and that beta cell dysfunction is a late event that arises from the prolonged increased secretion of insulin. Lower level of Calpain 10 mRNA leads to insulin resistance (4). Gene-nutrient interaction modifies only insulin sensitivity but not insulin secretion. Although it is not possible to change the genetic constitution by interventions, environmental interventions such as dietary fat may play the same role (16). Saturated fatty acids may play a contributing role

in triggering insulin resistance by interacting with genetic variants at the Calpain 10 gene locus. It is found that the rs2953171 of Calpain 10 genetic variant influences insulin sensitivity by interacting with plasma saturated fatty acids in Metabolic Syndrome patients (16). Thus, the G/G genotype was associated with lower fasting insulin concentrations, lower HOMA-IR, and higher glucose effectiveness in subjects with low plasma saturated fatty acid concentrations than was the minor A allele (G/A and A/A). In contrast, higher fasting insulin and HOMA-IR and lower glucose effectiveness were observed in subjects with the G/G genotype with the highest concentration of plasma saturated fatty acids than in subjects with the A allele (16). One of the evidences as Calpain 10 a diabetes predisposition factor is gene–fatty acid interaction between the rs2953171 polymorphism of Calpain 10 and the proportion of saturated fatty acids in the plasma. This SNP interacted with plasma-saturated fatty acids to determine insulin sensitivity, which suggests the potential sensitivity of this SNP to dietary factors (16). The beneficial effect of decreasing the amount of saturated fatty acids in the diet of persons with the G/G genotype in comparison with persons with the A allele may help to improve the therapeutic efficacy of dietary recommendations with a personalized nutrition approach, where the genetic profile may determine the choice of dietary therapy.

VII. GLUCOSE TOLERANCE

There are haplotypes associated with even glucose tolerance test, 1112/1121 heterozygous haplotype combination of the 1112 haplotype with either 1121 or 1221 appears to influence susceptibility to glucose tolerance and confers both increase in risk of IFG/IGF and in Type 2 Diabetes Mellitus (1). The transmission of the 1121 haplotype to offspring was associated with a decrease in BMI and narrower hip size and was weakly associated with decreased fasting blood glucose, and transmission of 1121 haplotype was associated with larger hips (1). Analysis of traits in the urban survey found no association between either individual SNPs or haplotypes of Calpain 10 and quantitative traits: weight, height, BMI, waist circumference, hip, WHR, fasting blood glucose, and 2-h glucose and association analyses of haplotype combinations were all negative (1). This polymorphism data alone strongly suggest that the protein is functionally important and probably harbors adaptive variation (8). The results of study of Calpain 10 from muscles suggest that Calpain-10 mRNA and protein levels in human skeletal muscle are not affected by insulin resistance (15). One of the main findings from this study was that insulin infusion reduced Calpain-10 mRNA content, and not by lipid infusion (15). Calpain 10 has an influence on insulin sensitivity and fasting glucose as one of several loci influencing the risk of Type 2 Diabetes Mellitus. However, this increased risk in families, which was ascertained for a strong history of Type 2 Diabetes Mellitus, seems to be modest (5). No evidence was found for increased transmission of any allele for three variants of Calpain 10, and found only marginal evidence for an increased risk of the 111/ 221 haplotype combination (5)

VIII. ALLELE AND ITS EFFECT

More specific to find out the susceptibility is to analyze the allele of Calpain 10. The C allele at SNP-44 was transmitted more often to affected offspring and was associated with increased risk of Type 2 Diabetes Mellitus, UK (6) and Gaza Strip (22). A allele of SNP-43 was associated with intra abdominal fat area even after the adjustment for insulin sensitivity (25). The high frequency of G allele in all populations surveyed suggest that the G is the true ancestral State at a specific site (8) and the uncommon T allele of SNP-63 was significantly increased risk in both IFG/IGT subjects to Type 2 Diabetes Mellitus (1). In African-American, presence of G allele was slightly higher (24) but participants with G/G genotypes were more likely to have diabetes than those with at least one copy of A allele. Homozygocity G/G of SNP-43 of Calpain 10 gene is associated with modest increase in risk of Type 2 Diabetes Mellitus. The association is independent of age, sex and BMI, and in continuation for nine years of follow-up, there are changes noticed from participants having G/G allele with increase in triglycerides and fasting glucose leading to insulin resistance when compared with other A alleles without affecting the other diabetes or obesity affected traits (24). There is a strong argument that the change in genotype is not possible due to extrinsic factors such as physical exercise or nutrition for a gene-environment interaction (24). SNP-43 is associated with abdominal obesity in the waist circumference tent to be larger in carriers of the A allele than G/G (25). The G/G genotype has been associated with improved insulin action in isolated subcutaneous adipoctyes, and is associated with low calpain 10 expression in fat which can have a role in adipocyte metabolism associated with abdominal obesity and adipocyte differentiation (25).

IX. EXERCISE IMPROVES GLUCOSE UPTAKE

The effect of exercise is similar to the action of insulin on glucose uptake. Exercise training improves the insulin resistance, a study using rats and inhibition of calpain 10 using Calpain 10 inhibitors lead to sever insulin resistance. Since Calpain 10 is a susceptibility gene to Type 2 Diabetes Mellitus, exercise decreases its expression and influence to regulate the related pathways of glucose transport in skeletal muscles. Decreased expression of Calpains is associated with glucose transport to the muscles of type 2 diabetes rats. Probably, increased body weight might up-regulate the Calpain 10 mRNA level and exercise might down-regulate and higher expression of Calpains mRNA is a risk factor for Type 2 Diabetes Mellitus (9).

In conclusion, the expression level of Calpain 10 is different in different tissues. The study in each population was not coordinated to a particular haplotype combination, even if searched for the same combination of haplotype in different population, no doubt, but will not yield a good result in considering the above studies and results. Since each SNP is involved separately and in-combination with one another to increase susceptibility to Type 2 Diabetes Mellitus in different haplotypes and populations, the effort to predict and treat the polymorphic effect of Calpain 10 on the disease is probably difficult. However, the treatment to a particular ethnic group is

probably possible with personalized therapy rather than universal therapy due to the available genetic knowledge on the disease with respect to the ethnic group.

REFERENCES

[1] Cassell, P. G., A. E. Jackson, et al. (2002). "Haplotype combinations of calpain 10 gene polymorphisms associate with increased risk of impaired glucose tolerance and type 2 diabetes in South Indians." Diabetes**51**(5): 1622-1628.

[2] Cox, N. J. (2001). "Challenges in identifying genetic variation affecting susceptibility to type 2 diabetes: examples from studies of the calpain-10 gene." Hum Mol Genet**10**(20): 2301-2305.

[3] Cox, N. J., M. G. Hayes, et al. (2004). "Linkage of calpain 10 to type 2 diabetes: the biological rationale." Diabetes**53 Suppl 1**: S19-25.

[4] Ek, J., G. Andersen, et al. (2001). "Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant caucasians." Diabetologia**44**(9): 1170-1176.

[5] Elbein, S. C., W. Chu, et al. (2002). "Role of calpain-10 gene variants in familial type 2 diabetes in Caucasians." J Clin Endocrinol Metab**87**(2): 650-654.

[6] Evans, J. C., T. M. Frayling, et al. (2001). "Studies of association between the gene for calpain-10 and type 2 diabetes mellitus in the United Kingdom." Am J Hum Genet**69**(3): 544-552.

[7] Ezzidi, I., A. Turki, et al. (2010). "Common polymorphisms of calpain-10 and the risk of Type 2 Diabetes in a Tunisian Arab population: a case-control study." BMC Med Genet**11**: 75.

[8] Fullerton, S. M., A. Bartoszewicz, et al. (2002). "Geographic and haplotype structure of candidate type 2 diabetes susceptibility variants at the calpain-10 locus." Am J Hum Genet**70**(5): 1096-1106.

[9] Hsieh, Y. Y., C. C. Chang, et al. (2008). "Effect of exercise training on calpain systems in lean and obese Zucker rats." Int J Biol Sci**4**(5): 300-308.

[10] Ling, C., L. Groop, et al. (2009). "Calpain-10 expression is elevated in pancreatic islets from patients with type 2 diabetes." PLoS ONE**4**(8): e6558.

[11] Lynn, S., J. C. Evans, et al. (2002). "Variation in the calpain-10 gene affects blood glucose levels in the British population." Diabetes**51**(1): 247-250.

[12] Ma, H., C. Fukiage, et al. (2001). "Characterization and expression of calpain 10. A novel ubiquitous calpain with nuclear localization." J Biol Chem**276**(30): 28525-28531.

[13] Malecki, M. T., D. K. Moczulski, et al. (2002). "Homozygous combination of calpain 10 gene haplotypes is associated with type 2 diabetes mellitus in a Polish population." Eur J Endocrinol**146**(5): 695-699.

[14] Nicklas, B. J., E. F. van Rossum, et al. (2001). "Genetic variation in the peroxisome proliferator-activated receptor-gamma2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain." Diabetes**50**(9): 2172-2176.

[15] Norton, L., T. Parr, et al. (2008). "Calpain-10 gene and protein expression in human skeletal muscle: effect of acute lipid-induced insulin resistance and type 2 diabetes." J Clin Endocrinol Metab**93**(3): 992-998.

[16] Perez-Martinez, P., J. Delgado-Lista, et al. (2011). "Calpain-10 interacts with plasma saturated fatty acid concentrations to influence insulin resistance in individuals with the metabolic syndrome." Am J Clin Nutr**93**(5): 1136-1141.

[17] Permutt, M. A., E. Bernal-Mizrachi, et al. (2000). "Calpain 10: the first positional cloning of a gene for type 2 diabetes?" J Clin Invest**106**(7): 819-821.

[18] Radha, V. and V. Mohan (2007). "Genetic predisposition to type 2 diabetes among Asian Indians." Indian J Med Res**125**(3): 259-274.

[19] Suzuki, K., S. Hata, et al. (2004). "Structure, activation, and biology of calpain." Diabetes**53 Suppl 1**: S12-18.

[20] Tsai, H. J., G. Sun, et al. (2001). "Type 2 diabetes and three calpain-10 gene polymorphisms in Samoans: no evidence of association." Am J Hum Genet**69**(6): 1236-1244.

[21] Tsuchiya, T., P. E. Schwarz, et al. (2006). "Association of the calpain-10 gene with type 2 diabetes in Europeans: results of pooled and meta-analyses." Mol Genet Metab**89**(1-2): 174-184.

[22] Zaharna, M. M., A. A. Abed, et al. (2010). "Calpain-10 gene polymorphism in type 2 diabetes mellitus patients in the Gaza Strip." Med Princ Pract**19**(6): 457-462.

[23] Fernández, A. M., J. K. Kim, S. Yakar, J. Dupont, C. Hernandez-Sanchez, A. L. Castle, J. Filmore, G. I. Shulman and D. Le Roith (2001). "Functional inactivation of the IGF-I and insulin receptors in skeletal muscle causes type 2 diabetes." Genes Dev **15**(15): 1926-1934.

[24] Garant, M. J., W. H. Kao, F. Brancati, J. Coresh, T. M. Rami, C. L. Hanis, E. Boerwinkle, A. R. Shuldiner and A. R. i. C. Study (2002). "SNP43 of CAPN10 and the risk of type 2 Diabetes in African-Americans: the Atherosclerosis Risk in Communities Study." Diabetes **51**(1): 231-237.

[25] Pihlajamäki, J., U. Salmenniemi, M. Vänttinen, E. Ruotsalainen, J. Kuusisto, I. Vauhkonen, S. Kainulainen, M. C. Ng, N. J. Cox, G. I. Bell and M. Laakso (2006). "Common polymorphisms of calpain-10 are associated with abdominal obesity in subjects at high risk of type 2 diabetes." Diabetologia **49**(7): 1560-1566.

[26] Saltiel, A. R. and C. R. Kahn (2001). "Insulin signalling and the regulation of glucose and lipid metabolism." Nature **414**(6865): 799-806.

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