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Variants of calpain-10 gene and it's association with type 2 diabetes mellitus in a Chinese population

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【 163 】

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【 学位論文題目 】

Variants of Calpain-10 Gene and It's Association with type 2 Diabetes Mellitus in a chinese population
(中国人における2型糖尿病とCalpain-10遺伝子変異に関する研究)

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論文審査の結果の要旨

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論文題目	Variants of Calpain-10 Gene and Its Association with type 2 Diabetes Mellitus in a chinese population （中国人における2型糖尿病とＣａｌｐａｉｎ－１０遺伝子変異に関する研究）		
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要　旨

２型糖尿病の発症機構は未だ明らかではないが、最近、テキサスのメキシコ系アメリカ人の２型糖尿病患者及び対照者について、ゲノムレベルの遺伝子解析が実施され、第２染色体上のカルパイン 10（カルパイン様カルシウム依存性システイン型タンパク質分解酵素の１種）遺伝子が２型糖尿病に関係し、さらに、カルパイン遺伝子の３つのイントロン（介在配列、３、６、１３）内部の３箇所の多型（異型）（個人による塩基または塩基配列の違い）のある組み合わせ（ハプロタイプの組合せ）をもった人に２型糖尿病が発症している頻度が高いとの報告がなされている。つまり、それら３箇所での多型には、それぞれ２種類の多型があり、それらを１型、２型と表現すると 112/121 というハプロタイプの組み合わせをもった人に糖尿病が多いという報告がなされている。この研究は、中国南部の 168 人の２型糖尿病患者と 104 人の対照者のカルパイン 10 遺伝子について、同様の遺伝学的解析を行なったものであり、その結果、糖尿病患者と対照者との間で、112/121 の組合せの対立遺伝子をもっている頻度には有意な差がなく、むしろ、高コレステロール血症の患者にその組合せが多いという結果を得ている。また一方で、糖尿病患者ではなく、対照者には、112/221 という別の組合せをもった人の頻度が有意に高いという結果を得ている。これらの結果は、カルパイン 10 遺伝子多型のハプロタイプの組合せと糖尿病の発症率との間には、人種による違いがあることを明らかにするとともに、異なった別の要因が存在することを示唆するものでもあり、２型糖尿病の発症機構を探求する上で有用な結果である。

この研究結果は、“Diabetes Research and Clinical Practice”（Elsevier）に印刷中である。

よって、学位申請者の呉斌は、博士（保健学）の学位を得る資格があると認める。

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（別紙様式 3）

論文内容の要旨

専攻領域　病態解析学

専攻分野　病態医化学

氏　名　呉 斌

論文題目（外国語の場合は、その和訳を併記すること。）

Variants of Calpain-10 Gene and It's Association with Type 2 Diabetes Mellitus in a Chinese population.
（中国人における2型糖尿病とCalpain-10遺伝子変異に関する研究）

論文内容の要旨

Type 2 Diabetes(T2DM) is a major public health problem which affect over 1.3 billion people worldwide. Familiar and twin studies provided convincing evidences that genetic factors involved in the development of T2DM. For example, clusters of T2DM in families, higher concordance rate in monozygotic twins than in dizygotic twins, and there are specific ethnic groups with a very high prevalence of T2DM. Both the impairment of insulin secretion and action constitute the major Pathophysiological defects of this disease. The identification of susceptibility genes responsible for T2DM could be of great significance to elucidate the underlying pathophysiological mechanisms leading to T2DM.Thus is essential to the development of more effective preventive and therapeutic strategies for this condition. Genome-wide scan studies on 330 affected sib pairs identified a susceptibity locus for T2DM on chromosome 2q37,which was designated as NIDDM1.Subsequent fine mapping study revealed that NIDDM1 was a single gene, calpain-10(*CAPN 10*). Three polymorphisms, UCSNP 43(G→A with intron 3;allele 1=G, allele 2=A), UCSNP 19(two repeats of 32-bp sequence, three repeats of 32- bp sequence within intron 6; allele 1 = 2 repeats and allele 2 = 3 repeats), UCSNP 63 (C→T within intron I3; allele 1 = C and allele 2 = T) were identified to define the at-risk combination haplotypes in Mexican-Americans. Heterozygosity for two haplotypes(112 and 121), defined by UCSNP-43, -19, and -63, was associated with the highest risk for T2DM in Mexican-Americans, German and finnish populations.

Calpain-10 is a member of a superfamily of calcium-activated cysteine protease, which are ubiquitously distributed in mammalian. The molecular mechanism of how the variations of *CAPN 10* contribute to the development of T2DM have yet to be elucidated. However, most likely, the DNA variation of this gene affect the both insulin secretion and insulin sensitivity. *In vitro* studies demonstrated different calpain inhibitors enhanced glucose-induced insulin secretion in pancreatic islets and reduced insulin-mediated glucose uptake into muscle and adipose tissue. Baier et al found UCSNP-43 among Pima Indians was not associated with T2DM. But among glucose tolerant individuals , G/G carriers had higher fasting plasma glucose and lower glucose turnover during a low-insulin euglycemia clamp than carriers of both G/A and A/A. G/G phenotype also reduced *CAPN 10* messenger RNA in muscle biopsy specimens in Pima Indians. The abovementioned observations suggested three *CAPN 10* SNPs are likely to alter insulin secretion and action through influence of *CAPN 10* gene expression.

Up to now, various studies in different ethnic populations have produced conflicting results about the role of *CAPN 10* in relationship with T2DM. Whereas little is known about *CAPN 10* variations in Chinese, since ethnic-related genetic backgrounnd may affect the phenotype expression of a given susceptibility gene variant.

We included 168 T2DM patients and 104 age- and sex- matched glucose tolerant controls. All subjects are living in the south area of China. The diagnosis of T2DM was based on the 1985 WHO criteria. A standard questionnaire was given to all the subjects regarding the age, family history of T2DM, the treatment method and other medical issues. The study individuals underwent a physical examination that include the measurement of height, weight, body mass index(BMI), hip and waist circumference, waist to hip ratio (WHR), blood pressure. Biochemical examinations included blood lipid profile(triglyceride TG, total cholesterol TC, high density lipoprotein HDL-cholesterol, and low density lipoprotein LDL-cholesterol), 0 and 2-h plasma glucose and serum insulin. Plasma glucose was analyzed by a glucose oxidase method, serum insulin was measured by enzyme-linked immunosorbent assay. Homeostasis model assessment (HOMA) of insulin resistance was calculated by the following formula: HOMA-IR=[fasting insulin(μU/ml)×fasting glucose(mmol/L)/22.5]. Genomic DNA was isolated from peripheral blood leucocytes. Genotyping of SNP-43 and SNP-19 were done by a mutagenically separated PCR (MS-PCR) method. PCR- restriction fragment length polymorphism (PCR-RFLP) method was applied for genotyping of SNP-63.

Distribution of alleles and genotypes at three loci were not significant different between the two groups. Eight possible haplotypes was inferred ,but the distribution had no significant differences between two groups. Analysis of haplotype combinations indicated heterozygotes of 112 and 221 haplotype were more frequent in control group than in T2DM patients.(16.4% vs 7.1%, *P*=0.025, OR=0.394). Haplotype combination(112/121) conferred the highest risk among Mexican-Americans, but we could not find the distribution of this haplotype combination were more prevalent in T2DM group than in nondiabetic group.(29.8% vs 25.0%, p=0.408, OR=1.271). Among glucose-tolerant control subjects, UCSNP-43 variation had no effect on fasting plasma glucose, serum insulin, TG, TC, HDL-cholesterol, LDL-cholesterol as well as 2-h plasma glucose and serum insulin, BMI and WHR.(G/G vs combined G/A and A/A).Also we found no effect of UCSNP-43 on insulin resistance estimated by HOMA. In regarding to haplotype combinations, carriers of at-risk haplotype combination (112/121) had higher TC(5.7±1.4 vs 5.2±0.7,*P*=0.011) and LDL-cholesterol(3.5±0.5 vs 3.2±0.5,*P*=0.017) than other carriers without 112/121 haplotype combination. However, haplotype combination 112/121 had no effect on fasting and 2-h plasma glucose, serum insulin, as well as BMI, WHR and HOMA-IR. Haplotype combination 112/221 had no effect on the clinical and biochemical variables we measured.

In conclusion, present study we found haplotype combination 112/221 of CAPN 10 was related with reduced risk among Chinese population, but we were not able to find significant association between previously reported at-risk variants of CAPN 10 (UCSNP-43, haplotype combination 112/121)with T2DM. UCSNP-43 and haplotype combination 112/121 had no effects no plasma glucose level and insulin sensitivity, but nondiabetic subjects with haplotype combination 112/121 had higher serum cholesterol level, indicating haplotype combination 112/121 might be a risk factor for increased serum cholesterol in Chinese population. This study we did not genotype UCSNP-44 because this polymorphism is also exclusively associated with haplotype 111 created by UCSNP-43,UCSNP-19, and UCSNP-63. And it should be noted that there was no significant difference between T2DM and control group in regard with the frequency of haplotype 111 in our sample. Further study should genotype more SNPs of *CAPN 10* to identify whether other alleles is associated with T2DM and should pay more attention to the effects of calpain-10 on the lipid metabolism.

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（注）1000~2000字でまとめること。