employing Bonferroni's correction for five haplotype-comparisons remained statistically significant at 0.0065 and 0.0035, respectively.

DISCUSSION

Kidney stone is found to be endemic, causing a major health problem and economic burden, in the NE Thai population. A recent study by our group has emphasized that the relative risk of having the disease is higher among members of affected families compared to that of the normal control population, indicating a contribution of genetic factor, although its actual etiology is still unidentified.[37] Interestingly, hypercalciuria, hyperoxaluria, and hyperuricosuria which are common in the Western and other ethnic groups were not demonstrated in most of these patients[36] but hypocitraturia and abnormality of some stone-forming inhibitor proteins, nephrocalcin and trefoil factor 1, had been reported.[32,34,19,38] These indicate a unique characteristic of the disease in this population and possibly cause from different etiology as ever reported elsewhere.

A number of proteins present either in urine or stone matrix have been implicated in the stone formation due to their ability to inhibit crystallization processes.[20,21] The important urinary stone-inhibitor proteins include Tamm-Horsfall protein (THP), osteopontin, urinary prothrombin fragment 1 (UPTF1), bikunin, calgranulin, and trefoil factor 1. Owing to potent inhibition of these proteins in stone formation, they have been proposed to be involved in pathogenesis of kidney stone. Since kidney stone is a complex disease and genetic predispositions have been suggested to be involved in its pathogenesis, these encourage us to investigate the association between kidney stone and SNPs in genes encoding for known stone-forming inhibitor proteins in the NE Thai population. The candidate genes in this study are *TFF1*, *S100A8*, *S100A9*, *S100A12*, *AMBP*, *SPP1*, *UMOD*, and *F2* which encode eight urinary stone-inhibitor proteins: trefoil factor 1, calgranulin (A, B, and C), bikunin, osteopontin, Tamm-Horsfall protein, and urinary prothrombin fragment 1, respectively.[19,39-40]

Our case-control candidate-gene association study, examined SNPs distributed within and flanking these eight candidate genes in 112 cases and 112 controls, discovered the association of polymorphisms of one candidate - *F2* gene and kidney stone (Table 2, Table 3, and Figure 1). The results revealed significant differences between control and case groups in allele frequencies of seven

SNPs (P = 0.006-0.033) and in genotype frequencies of eight SNPs (P = 0.0036-0.016) of the *F2* gene. When haplotypes were constructed from ten SNPs studied, one haplotype each associated with either increased or reduced risk of kidney stone, TGCCGCCGCG and CGTTCCGCTA, respectively, were detected (P = 0.0468 and 0.0045). This association was then confirmed when more sample of both case and control groups were added for the SNPs genotyping in the *F2* gene. The haplotypic association analysis obtained from the genotyping data of 164 cases and 216 controls revealed that the haplotype associated with increased risk (TGCCGCCGCG) was more represented in case group while the haplotype associated with reduced risk (CGTTCCGCTA) was more represented in control group (P = 0.0013 and 0.0007, respectively) (Table 3). The P-values remained significant at 0.0065 and 0.0035, respectively, after correction for multiple testing. These indicate that *F2* may influence genetic susceptibility to kidney stone in the patients studied and therefore it is worthwhile pursuing further studies on the functional roles of these genetic variations due to their involvement in the pathogenesis of kidney stone.

Urinary prothrombin fragment 1 (UPTF1) is a product of *F2* gene (Genbank NM_000506, NP_000497) which is located on 11p11-q12. *F2* gene encompasses approximately 20.3 kb containing 14 exons and its mRNA is 1,997 nt long encoding the protein with 622 amino acids. UPTF1 was found to be the major protein occluded within CaOx crystals generated from human urine *in vitro*.[28] This glycoprotein, with a molecular mass of approximately 31 kDa, was initially described as crystal matrix protein (CMP) and its N-terminal amino acid sequence was subsequently demonstrated identical with human prothrombin.[41] It was shown conclusively that the protein is F1 activation peptide of prothrombin by the later study.[42] UPTF1 was found in thick ascending limb of the loop of Henle and a distal convoluted tubule.[43] Amino acid analysis revealed that this protein contains ten γ-carboxyglutamic acid (Gla) residues located near its N-terminus, which are responsible for the calcium-binding activity of the protein. It has been shown to be a potent inhibitor of CaOx crystal growth and

aggregation in undiluted human urine[28] and under inorganic conditions.[30] Furthermore, a crude precursor to the purified protein has been shown to inhibit the crystallization process potently.[44]

Although UPTF1 processes several hallmarks expected of a regulatory protein in urolithiasis, its precise role remains unknown. Grover *et al*.[45] demonstrated that the renal prothrombin mRNA is significantly reduced in a hyperoxaluric rat model of nephrolithiasis and firstly reported a significant decreased in the renal expression of a urinary protein well documented to inhibit CaOx crystal growth and aggregation in undiluted human urine *in vitro*. Protein function of the UPTF1 has been studied in many features. Decreased inhibitory activity of prothrombin against CaOx crystallization by specific chemical modification of its Gla residues was reported, suggesting that the Gla composition might play an important role in inhibiting the formation of CaOx calculus.[46] In addition to the Gla domain, the UPTF1's carbohydrate moiety is considered to influence its functionality. The glycans on UPTF1 have been reported to play a pivotal role in the protein's ability to retard CaOx crystallization.[47] However, further evaluation of the role of this protein in kidney stone formation is still required. In the view of genetic, the finding of our association study demonstrated the contribution of *F2* gene polymorphisms to susceptibility to kidney stone in NE Thai population emphasized that UPTF1 plays role in kidney stone disease.

Although our result of single SNP analysis revealed significant differences between control and case groups of 3 SNPs (SNP7, SNP10, and SNP11) of the *S100A9-12-8* genes cluster (Supplementary Table S1), however the difference of these SNPs frequencies in both groups did not reach a statistical level when increased sample sizes were analyzed. This finding suggests that *S100A9-12-8* genes polymorphisms may not contribute to susceptibility to kidney stone in NE Thai population.

Only one of eight candidate genes proposed in this study, *SPP1*, has been previously studied for its association to kidney stone in other population which the result showed that a nonsynonymous SNP (rs4660), located in the exon 16, was highly associated with urinary calcium stone disease.[48] Another report also demonstrated that two novel SNPs (novel 2 and novel 3) located in the promoter region

were significantly associated with kidney stone risk. Two haplotypes, one associated with reduced risk and the other associated with increased risk of kidney stone, were detected, suggesting that the *SPP1* gene is dually associated with the risk of kidney stone and may have a different function in crystal formation during the development of the disease.[49] However, genotyping result of 14 SNPs of *SPP1* in our study, including those three SNPs (SNP4, SNP5, and SNP12) from those reports, did not show significant different between case and control groups. All three SNPs were monomorphic, showing only one genotype in all cases and controls studied (Supplementary Table S1). This finding suggests that *SPP1* gene polymorphisms may not contribute to susceptibility to kidney stone in NE Thai population. Though, a larger size of samples should be analyzed to increase the power of test.

In conclusion, to the best of our knowledge, this is the first association study of the genes encoding for stone-forming inhibitor proteins and kidney stone disease in Thailand. The result of our study in NE Thai population has demonstrated that *prothrombin* (*F2*) haplotype is significantly associated with kidney stone disease. Thus, *F2* may influence genetic susceptibility to the kidney stone disease in the patients studied. Further study on the functional role of the *F2* variation associated with the kidney stone disease is in progress.

CONCISE METHODS

Study groups

This study was performed after the approval of Siriraj Insititutional Review Board (SIRB) and the Ethical Committee of the Ministry of Public Health, Thailand, and informed consents were obtained from all subjects. The SIRB consideration was adhered to Declaration of Helsinki. A group of patients with kidney stone (age range of 20-80 years) recruited from Khon Kean Regional Hospital, in the northeastern part of Thailand, during 2004-2006 were enrolled in the study. The control group consisted of age-matched unrelated individuals with no history of kidney stone were recruited from the same area of patients.

Diagnosis of kidney stone was determined by roentgenography of kidney-ureter-bladder (KUB), the scar of stone removal surgery, and in some suspicious cases by additional ultrasonography. The exclusion criteria of subjects were the presence of kidney stone secondary to all known causes (including renal tubular acidosis, primary hyperparathyroidism, inflammatory bowel disease, Cushing disease, hyperthyroidism, and drug-induced kidney stone) diagnosed by clinical history and symptoms, physical and laboratory examinations, acute acid loading test, and serum electrolytes. Urine and blood samples were collected for electrolyte analyses. Stones after removal from patients were analyzed by using Nicolet™ 380 Fourier Transform Infrared (FTIR) Spectrometer. Genomic DNA from patients and controls was extracted from peripheral blood using standard phenol-chloroform method.

Selection of SNPs and primer design

SNPs distributed within and flanking eight candidate genes including *TFF1*, *S100A9*, *S100A12*, *S100A8*, *AMBP*, *SPP1*, *UMOD*, and *F2*, encoding the eight urinary stone-inhibitor proteins (trefoil factor 1, calgranulin (B, C, and A), bikunin, osteopontin, Tamm-Horsfall protein, and urinary prothrombin fragment 1) were selected for genotyping in 112 DNA samples each of the case and control groups. An average of 5-14 SNPs per gene were selected based on SNP data obtained from

SNP database of the National Center for Biotechnology Information and the International HapMap Project, focused on the data from Han Chineses in Beijing that genetic background is closely related to Thai population. SNPs were selected according the following criteria: spread throughout the gene, minor allele frequency more than 5%, and, if possible, located in coding region, (as a non-synonymous > synonymous > regulatory element > intron). Data of haplotype tagging SNPs was also considered to avoid redundant genotyping.

PCR primers were designed from nucleotide sequences of the corresponding genes (accession number AP001623, AL591704, AL137850, AC131944, AC106796, and AC115088 for *TFF1*, *S100A9*-12-8, *AMBP*, *SPP1*, *UMOD*, and *F2*, respectively) by Primer3 program. Extension primers, the oligonucleotide primer with 3'end complementary to the nucleotide sequence preceding the SNP site, for using in SNP genotyping were designed. The sequences of primers are shown in Supplementary Table S3. All primers were purchased from the BioService Unit-National Center for Genetic Engineering and Biotechnology, Thailand or from the Operon Biotechnologies, Germany.

Genotyping

To genotype SNPs in the eight candidate genes, primer extension (PE) reaction and denaturing high-pressure liquid chromatography (DHPLC) analysis was performed. Altogether 67 SNPs, on average 5-14 SNPs per gene, were analyzed in each group. Single or multiplex PCR (3- or 4- plex per reaction) were performed to obtain DNA fragments containing selected SNPs of each gene. These PCR products were treated with ExoSAP-IT (Amersham Biosciences, USA) to remove excess primers and unincorporated dNTPs. PE method, both single base extension (SBE) and a very short extension (VSET) reactions, was conducted in a 20-µl volume containing 5 µl of ExoSAP-IT treated PCR product, 5-15 pmole of extension primers, 50 µM of ddNTPs or combination of appropriate dNTP and ddNTP (Amersham Biosciences UK Ltd, Buckinghamshire, UK), 1 µl of Thermo Sequenase buffer, and 0.5 U of Thermo Sequenase™ DNA polymerase (Amersham Biosciences UK Ltd). Thermal

cycling for all PE reactions was carried out in GeneAmp® PCR System 2400 or 9700, consisting of an initial denaturation at 96 °C for 1 min, followed by 50-60 cycles consisting of denaturation at 96 °C for 15 sec, annealing at 43 °C or 50 °C for 30 or 15 sec, and extension at 60 °C for 1 min. The PE product was denatured at 96 °C for 1 min before loading and analysis by DHPLC machine (Wave, Transgenomic, USA) using fully denaturing condition with a column temperature of 70°C.

Initially, an individual SNP was typed by PE reaction and DHPLC and their results were used as guidance for multiplex primer extension reaction. Optimization of multiplex PE reactions was performed by testing with various combinations of extension primers until maximum multiplexing was obtained. The amount of each primer in the reaction was adjusted to obtain high PE product leading to high peak of elution profile. Finally, 2-6 sets of PE reaction for SNPs typing in 8 candidate genes were obtained (Supplementary Table S2).

SNPs that could not successfully be genotyped by PE method were analyzed by heteroduplex analysis. After amplification of DNA fragments containing SNPs, the PCR product was denatured by heating at 95°C for 1 min followed by slowly re-annealing and then applied for analysis by DHPLC for the first screening. The difference in melting temperature or elution profile between homoduplex and heteroduplex is the basis for identification of SNP variations. To differentiate between homozygous major allele and homozygous minor allele, the samples with homozygous genotype from the first screening were re-screened after premixed the samples with that of known homozygous genotype confirmed by DNA sequencing (in 1:1 ratio), prior to denaturation and subsequent heteroduplex analysis by DHPLC machine for second time.

Statistical analysis

Data of SNP genotyping from both case and control groups were collected and analyzed for association with the disease phenotype. Statistical tests for deviation from Hardy-Weinberg equilibrium and for association between SNP frequencies and disease phenotype were performed by using DeFinetti

(http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl), SNPStats (http://bioinfo.iconcologia.net/snpstats/start.htm), and Haploview (http://www.broad.mit.edu/mpg/haploview/) web-based programs. $P < 0.05$ was considered as significant.

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DISCLOSURE

The authors have no conflict of interests to declare.

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FIGURE LEGENDS

Figure 1

Linkage disequilibrium (LD) plots showing D' and LD block of 10 genotyped SNPs in *F2* gene from 164 cases and 216 controls determined by the Haploview program. Genomic structure of *F2* gene and location of SNPs are indicated above the LD plot. Exons are indicated by black boxes and untranslated regions are represented in blue. LD block is indicated by the black pentagon line. Squares represent LD and LOD score of between SNPs. Numbers in boxes represent D' (x 100). Bottom left panel displays the frequency of haplotype with htSNPs indicated by arrow head. The strength of LD is indicated with the bottom right-color scheme.

Supplementary Figure 1

DHPLC chromatograms of primer extension products for genotyping ten SNPs in *TFF1* gene in five sets, A-E, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 8-10-1, (B) SNP 2-9*-5, (C) SNP 6-7, (D) SNP 3, and (E) SNP 4. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 8-10-1- 2-9*-5- 6-7-3-4.
(* using of anti-sense extension primer)

Supplementary Figure 2

DHPLC chromatograms of primer extension products for genotyping fifteen SNPs in *S100A9-12-8* genes cluster in six sets, A-F, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 8-7, (B) SNP 9-5*, (C) SNP 4-10-15*, (D) SNP 2-11, (E) SNP 12-1-14*, and (F) SNP 3-13*-6. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 8-7-9-5*-4-10-15*-2-11-12-1-14*-3-13*-6. (* using of anti-sense extension primer)

Supplementary Figure 3

DHPLC chromatograms of primer extension products for genotyping nine SNPs in *AMBP* gene in three sets, A-C, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 3-4-2*, (B) SNP 6-7-8, and (C) SNP 9-10*-5*. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 3-4-2*-6-7-8-9-10*-5*.

(* using of anti-sense extension primer)

Supplementary Figure 4

DHPLC chromatograms of primer extension products for genotyping fourteen SNPs in *SPP1* gene in three sets, A-C, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 2-11-6-5*-12*-4, (B) SNP 10*-7-8-9*-13-14, and (C) SNP 1-3. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 2-11-6-5*-12*-4-10*-7-8-9*-13-14-1-3.

(* using of anti-sense extension primer)

Supplementary Figure 5

DHPLC chromatograms of primer extension products for genotyping nine SNPs in *UMOD* gene in two sets, A-B, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 9*-2*-3*-10* and (B) SNP 5*-8*-6*-4*-7*. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 9*-2*-3*-10*-5*-8*-6*-4*-7*.

(* using of anti-sense extension primer)

Supplementary Figure 6

DHPLC chromatograms of primer extension products for genotyping eight SNPs in *F2* gene in two sets, A-B, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 6*-7-2 and (B) SNP 10-1-4*-8-3. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 6*-7-2-10-1-4*-8-3.

(* using of anti-sense extension primer)

Supplementary Figure 7

DHPLC chromatograms of heteroduplex analyses of (A) SNP 5 (rs2070852) and (B) SNP 9 (rs2282687) of *F2* gene. DHPLC elution profile showed 2 different patterns in the first screening (left panel), sample 1 and 3 with the homozygous genotypes and sample 2 with the heterozygous genotype. The homozygous genotypes of sample 1 and 3 represented different patterns in the second screening (right panel) after mixed with the PCR product of known homozygous genotype sample to make a distinction between homozygous major allele and homozygous minor allele.

Table 1 Characteristics of patients with kidney stone

Characteristics	Cases (n)	%
Gender		
Male	32	28.57
Female	80	71.43
Age		
Mean (years)	48.26 ±11.69	-
Range (years)	22-80	-
Familial history		
Yes	69	61.61
No	43	38.39
Stone number		
Single	28	25.00
Multiple	72	64.29
Unknown	12	10.71
Position of stone		
Kidney	85	75.89
Ureter	11	9.82
Kidney and ureter	15	13.39
Kidney and bladder	1	0.89
Main stone type		
Whewellite	23	20.54
Dahllite	15	13.39
Weddellite	2	1.79
Uric acid	0	0
Struvite	0	0
Unknown	72	64.29

Table 2 Allele and genotype frequencies of 10 SNPs in *F2* gene observed in 112 controls and 112 cases

SNP No.	Ref. SNP ID	Allele	Allele frequency (%)		OR (95% CI)	*P*-value	Genotype	Genotype frequency (%)		OR (95% CI)	*P*-value
			Control	Case				Control	Case		
SNP1	rs2070850	T	54.5	65.2	1.00	**0.021***	T/T+C/T	77.7	90.2	1.00	**0.01***
		C	45.5	34.8	0.64 (0.44-0.94)		C/C	22.3	9.8	0.38 (0.18-0.81)	
SNP2	rs3136435	G	88.8	86.6	1.00	0.472	G/G+A/G	100	99.1	1.00	0.24
		A	11.2	13.4	1.23 (0.70-2.17)		A/A	0	0.9	NA (0.00-NA)	
SNP3	rs3136441	C	56.7	67.4	1.00	**0.019***	C/C+C/T	80.4	92.9	1.00	**0.0052****
		T	43.3	32.6	0.63 (0.43-0.93)		T/T	19.6	7.1	0.31 (0.13-0.74)	
SNP4	rs2070851	C	55.8	68.3	1.00	**0.006****	C/C+C/T	78.6	92	1.00	**0.0041****
		T	44.2	31.7	0.59 (0.40-0.86)		T/T	21.4	8	0.32 (0.14-0.73)	
SNP5	rs2070852	G	67.0	78.6	1.00	**0.006****	G/G+G/C	89.3	98.2	1.00	**0.0037****
		C	33.0	21.4	0.55 (0.36-0.84)		C/C	10.7	1.8	0.15 (0.03-0.69)	
SNP6	rs3136456	C	89.3	87.1	1.00	0.464	C/C+A/C	100	98.2	1.00	0.095
		A	10.7	12.9	1.24 (0.70-2.20)		A/A	0	1.8	NA (0.00-NA)	
SNP7	rs3136457	C	56.2	66.1	1.00	**0.033***	C/C+C/G	78.6	91.1	1.00	**0.0083****
		G	43.8	33.9	0.66 (0.45-0.97)		G/G	21.4	8.9	0.36 (0.16-0.79)	
SNP8	rs3136460	G	56.2	67.4	1.00	**0.015***	G/G+C/G	78.6	92	1.00	**0.0041****
		C	43.8	32.6	0.62 (0.42-0.91)		C/C	21.4	8	0.32 (0.14-0.73)	
SNP9	rs2282687	C	56.2	65.2	1.00	0.053	C/C+C/T	78.6	90.2	1.00	**0.016***
		T	43.8	34.8	0.69 (0.47-1.01)		T/T	21.4	9.8	0.40 (0.19-0.86)	
SNP10	rs3136516	G	80.8	89.3	1.00	**0.012***	G/G+G/A	94.6	100	1.00	**0.0036****
		A	19.2	10.7	0.50 (0.30-0.87)		A/A	5.4	0	0.00 (0.00-NA)	

* $P < 0.05$. ** $P < 0.01$.

Table 3 Association between haplotypes consisting of 10 SNPs of *F2* gene and kidney stone risk

Haplotype	Frequency of haplotype		OR (95% CI)	X^2	*P*-value
	Case (n=164)	Control (n=216)			
TGCCGCCGCG	0.625	0.509	1.6119 (1.2029-2.1601)	10.273	**0.0013**
CGTTCCGCTA	0.091	0.178	0.4637 (0.2958-0.7269)	11.577	**0.0007**
CATTGAGCTG	0.115	0.136	0.8271 (0.5346-1.2796)	0.724	0.3950
CGTTCCGCTG	0.096	0.121	0.7765 (0.4864-1.2396)	1.130	0.2879
CGCCGCCGCG	0.024	0.019	1.3112 (0.4883-3.5207)	0.305	0.5808

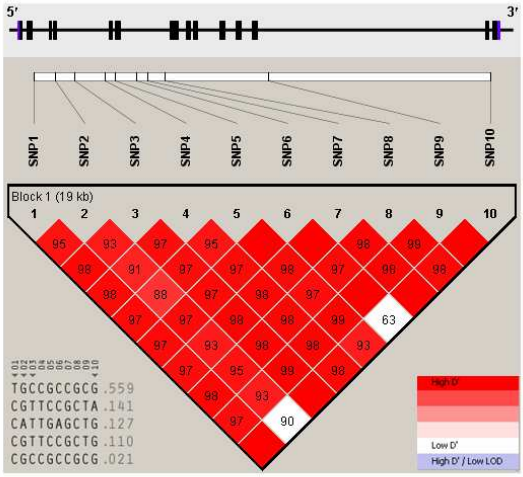


Figure 1

Linkage disequilibrium (LD) plots showing D' and LD block of 10 genotyped SNPs in F2 gene from 164 cases and 216 controls determined by the Haploview program. Genomic structure of F2 gene and location of SNPs are indicated above the LD plot. Exons are indicated by black boxes and untranslated regions are represented in blue. LD block is indicated by the black pentagon line. Squares represent LD and LOD score of between SNPs. Numbers in boxes represent D' (x 100). Bottom left panel displays the frequency of haplotype with htSNPs indicated by arrow head. The strength of LD is indicated with the bottom right-color scheme.
254x190mm (96 x 96 DPI)

Supplementary Table S1 Distribution of SNPs and association analysis of 67 SNPs in 8 candidate genes in 112 controls and 112 cases

Gene	SNP No.	Ref. SNP ID	Position[a]	Location	Major/Minor Alleles	Allele frequency		OR	95% CI	*P*-value	Chi-Square
						Controls	Cases				
TFF1	SNP1	rs184432	g.-958C>T	5'UTR	C/T	165/59	172/52	0.845	0.550-1.299	0.444	0.59
	SNP2	rs225359	g.-832C>T	5'UTR	C/T	172/52	171/53	1.025	0.662-1.587	0.911	0.01
	SNP3	rs2156310	g.-2C>T	Exon 1	C/T	119/105	114/110	1.094	0.755-1.584	0.636	0.22
	SNP4	rs225358	c.85+8G>A	Intron 1	G/A	169/55	172/52	0.929	0.602-1.434	0.739	0.11
	SNP5	rs13051704	c.85+149C>G	Intron 1	C/G	152/72	143/81	1.196	0.809-1.768	0.370	0.80
	SNP6	rs4920094	c.85+187C>T	Intron 1	T/C	158/66	160/64	0.958	0.637-1.440	0.835	0.04
	SNP7	rs2839488	c.85+334C>G	Intron 1	G/C	157/67	160/64	0.937	0.624-1.408	0.755	0.10
	SNP8	rs225355	c.86-966C>T	Intron 1	C/T	167/57	160/64	1.172	0.772-1.779	0.456	0.55
	SNP9	rs225354	c.86-685C>T	Intron 1	C/T	168/56	157/67	1.280	0.844-1.941	0.244	1.36
	SNP10	rs225353	c.238+309C>G	Intron 2	C/G	166/58	158/66	1.196	0.790-1.810	0.398	0.71
S100A9	SNP1	rs2916196	g.-2414T>C	LR	T/C	171/53	178/46	0.834	0.533-1.304	0.425	0.64
	SNP2	rs11205276	g.-1877G>C	LR	G/C	192/32	191/33	1.037	0.613-1.754	0.893	0.02
	SNP3	rs3014866	g.-1690C>T	LR	C/T	131/93	132/92	0.982	0.674-1.430	0.923	0.01
	SNP4	rs1063933	c.286G>A	Exon 3	G/A	224/0	224/0	-	-	-	-
	SNP5	rs2070864	g.*488A>G	LR	A/G	128/96	140/84	0.800	0.548-1.168	0.247	1.34
S100A12	SNP6	rs3006475	g.*1668A>C	LR	A/C	224/0	223/1	-	-	-	-
	SNP7	rs2916191	g.*393A>G	LR	A/G	205/15	218/6	0.376	0.143-0.988	**0.040**	4.22
	SNP8	rs2233864	g.139-82G>A	Intron 2	G/A	220/0	224/0	-	-	-	-
	SNP9	rs3014883	c.-20-372C>A	Intron 1	C/A	220/0	224/0	-	-	-	-
	SNP10	rs3006476	g.-2043G>A	LR	G/A	208/16	218/6	0.358	0.137-0.932	**0.029**	4.78
S100A8	SNP11	rs3006488	g.*73A>G	3'UTR	A/G	209/15	218/6	0.383	0.146-1.007	**0.044**	4.05
	SNP12	rs3795391	c. -22-72A>G	Intron 1	A/G	212/12	218/6	0.486	0.179-1.319	0.149	2.08
	SNP13	rs3806232	g.-1120T>C	LR	T/C	212/12	218/6	0.486	0.179-1.319	0.149	2.08
	SNP14	rs11205282	g.-2300T>C	LR	T/C	212/12	218/6	0.486	0.179-1.319	0.149	2.08
	SNP15	rs12040625	g.-2538A>G	LR	A/G	223/1	224/0	-	-	-	-
AMBP	SNP2	rs2251680	c.117+169T>C	Intron 1	C/T	153/71	156/68	0.939	0.629-1.402	0.759	0.09
	SNP3	rs2856923	c.260+150C>T	Intron 2	C/T	135/89	124/100	1.223	0.840-1.781	0.293	1.11
	SNP4	rs16912311	c.557-515G>A	Intron 5	G/A	125/99	119/105	1.114	0.768-1.616	0.569	0.32
	SNP5	rs12377342	c.603+1660G>A	Intron 6	G/A	184/40	192/32	0.767	0.462-1.273	0.303	1.06
	SNP6	rs10817564	c.604-2041G>A	Intron 6	G/A	130/94	140/84	0.830	0.568-1.212	0.334	0.93
	SNP7	th2399	c.853+214C>A	Intron 8	C/A	174/50	180/44	0.851	0.539-1.342	0.486	0.48
	SNP8	rs2269454	c.1028-100A>G	Intron 9	A/G	178/46	185/39	0.816	0.508-1.310	0.399	0.71
	SNP9	rs2269455	c.1028-70C>T	Intron 9	C/T	177/47	183/41	0.844	0.529-1.346	0.475	0.51
	SNP10	rs2269456	c.1028-60T>C	Intron 9	C/T	132/92	139/85	0.877	0.601-1.282	0.499	0.46
SPP1	SNP1	rs2853744	c.-1800T>G	LR	T/G	109/115	119/105	1.196	0.825-1.733	0.345	0.89
	SNP2	rs11730582	c.-1627T>C	LR	T/C	156/68	158/66	0.958	0.639-1.436	0.836	0.04
	SNP3	rs7687316	c.-1340G>GG	LR	GG/G	109/115	120/104	1.217	0.840-1.764	0.298	1.08

	SNP4	novel 2	c.-1330G>T	LR	G/T	224/0	224/0	-	-	-	-
	SNP5	novel 3	c.-1329T>G	LR	T/G	224/0	224/0	-	-	-	-
	SNP6	rs2853749	c.-14-220C>T	Intron 1	T/C	109/115	120/104	1.217	0.840-1.764	0.298	1.08
	SNP7	rs11728697	c.93+692C>T	Intron 3	C/T	148/76	152/72	0.922	0.622-1.368	0.688	0.16
	SNP8	rs6839524	c.93+1284C>G	Intron 3	G/C	107/117	114/110	1.133	0.782-1.642	0.508	0.44
	SNP9	rs7695531	c.174+606A>G	Intron 4	A/G	221/3	216/8	2.497	0.654-9.532	0.166	1.91
	SNP10	rs4754	c.240T>C	Exon 5	C/T	179/45	182/42	0.918	0.575-1.466	0.720	0.13
	SNP11	rs1126616	c.708C>T	Exon 6	T/C	178/46	182/42	0.893	0.560-1.424	0.634	0.23
	SNP12	rs4660	c.860G>A	Exon 6	G/A	224/0	224/0	-	-	-	-
	SNP13	rs1126772	c.*138A>G	LR	A/G	162/62	164/60	0.956	0.631-1.449	0.832	0.05
	SNP14	rs9138	c.*294A>C	LR	C/A	178/46	183/41	0.867	0.542-1.385	0.550	0.36
UMOD	SNP2	rs4506906	c.1182+50C>T	Intron 5	C/T	193/31	203/21	0.644	0.358-1.159	0.140	2.18
	SNP3	rs9940449	c.1183-671C>A	Intron 5	C/A	224/0	224/0	-	-	-	-
	SNP4	rs11647727	c.1182+1283T>C	Intron 5	T/C	145/79	155/69	0.817	0.551-1.212	0.315	1.01
	SNP5	rs4780884	c.1332-469C>T	Intron 6	C/T	193/31	204/20	0.610	0.336-1.107	0.102	2.68
	SNP6	rs9646256	c.1332-98T>C	Intron 6	T/C	193/31	204/20	0.610	0.336-1.107	0.102	2.68
	SNP7	rs9923532	c.1577+817T>C	Intron 7	T/C	193/31	205/19	0.577	0.315-1.056	0.072	3.24
	SNP8	rs1123670	c.1862-918C>T	Intron 10	C/T	195/29	205/19	0.623	0.338-1.148	0.127	2.33
	SNP9	rs8060932	c.*559C>T	LR	C/T	220/4	219/5	1.256	0.333-4.739	1.000	0.11
	SNP10	rs8062123	c.*683C>A	LR	C/A	224/0	224/0	-	-	-	-
F2	SNP1	rs2070850	c.240+83C>T	Intron 2	T/C	122/102	146/78	0.639	0.437-0.935	**0.021**	5.35
	SNP2	rs3136435	c.316+36G>A	Intron 4	G/A	199/25	194/30	1.231	0.699-2.169	0.472	0.52
	SNP3	rs3136441	c.316+857T>C	Intron 4	C/T	127/97	151/73	0.633	0.431-0.930	**0.019**	5.46
	SNP4	rs2070851	c.317-202T>C	Intron 4	C/T	125/99	153/71	0.586	0.398-0.862	**0.006**	7.43
	SNP5	rs2070852	c.423-7G>C	Intron 5	G/C	150/74	176/48	0.553	0.362-0.844	**0.006**	7.61
	SNP6	rs3136456	c.559+793C>A	Intron 6	C/A	200/24	195/29	1.239	0.697-2.204	0.464	0.53
	SNP7	rs3136457	c.560-1151G>C	Intron 6	C/G	126/98	148/76	0.660	0.450-0.968	**0.033**	4.55
	SNP8	rs3136460	c.560-344C>G	Intron 6	G/C	126/98	151/73	0.622	0.423-0.913	**0.015**	5.91
	SNP9	rs2282687	c.1654+290T>C	Intron 12	C/T	126/98	146/78	0.687	0.469-1.006	0.053	3.74
	SNP10	rs3136516	c.1726-59G>A	Intron 13	G/A	181/43	200/24	0.505	0.295-0.865	**0.012**	6.34

a, Human Genome Variation Society (HGVS) name; LR, Locus region; CI, confidence interval; OR, odds ratio.
The boldface represents $P < 0.05$.

Supplementary Table S2 Sets of analyses by multiplex primer extension reaction for SNP genotyping in 8 candidate genes

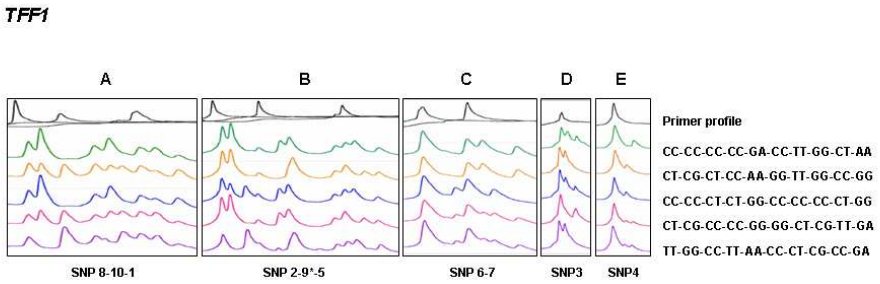
Gene	Set of primer extension reaction	Ref. SNP ID	SNP No.
TFF1	A	rs225355, rs225353, rs184432	8, 10, 1
	B	rs225359, rs225354, rs13051704	2, 9, 5
	C	rs4920094, rs2839488	6, 7
	D	rs2156310	3
	E	rs225358	4
S100A9-12-8	A	rs2233864, rs2916191	8, 7
	B	rs3014883, rs2070864	9, 5
	C	rs1063933, rs3006476, rs12040625	4, 10, 15
	D	rs11205276, rs3006488	2, 11
	E	rs3795391, rs2916196, rs11205282	12, 1, 14
	F	rs3014866, rs3806232, rs3006475	3, 13, 6
AMBP	A	rs2856923, rs16912311, rs2251680	3, 4, 2
	B	rs10817564, th2399, rs2269454	6, 7, 8
	C	rs2269455, rs2269456, rs12377342	9, 10, 5
SPP1	A	rs11730582, rs1126616, rs2853749, novel 3, rs4660, novel 2	2, 11, 6, 5, 12, 4
	B	rs4754, rs11728697, rs6839524, rs7695531, rs1126772, rs9138	10, 7, 8, 9, 13, 14
	C	rs2853744, rs7687316	1, 3
UMOD	A	rs8060932, rs4506906, rs9940449, rs8062123	9, 2, 3, 10
	B	rs4780884, rs1123670, rs9646256, rs11647727, rs9923532	5, 8, 6, 4, 7
F2	A	rs3136456, rs3136457, rs3136435	6, 7, 2
	B	rs3136516, rs2070850, rs2070851, rs3136460, rs3136441	10, 1, 4, 8, 3

Supplementary Table S3 PCR primers for amplifications of DNA fragments containing SNPs in eight candidate genes

Gene	Forward primer (5'→ 3')	Reverse primer (5'→ 3')	Product size (bp)	Ref. SNP ID	SNP No.	Extension primer (5'->3')
TFF1	AGCAGGGGAGGGGAGTGAG	GGAGGCCAAAGCAGGCGGA	453	rs184432	1	TCTAAAATACAGGAGTCCAGGGGAC
				rs225359	2	CTCCCTCCTGCCCCCAGC
	CCCGGGCCTCCTTAGGCAA	ATCCCAGGGGAGCCCAGGAA	722	rs2156310	3	GCCTTTGGAGCAGAGAGGAGG
				rs225358	4	GGCCGAGGCCCAGACAGGTAAGGC
				rs13051704	5	AAGTATATCCAATTTACAG GAT
				rs4920094	6	GTAACTCAGGGTGGCAGGG
				rs2839488	7	CAGAATAGTGCTTTTGA
	TGCCCTGCTTCAAGCTGTTTC	AAATTAGGCGGGCATTGTGG	491	rs225355	8	CCCCACCCGGTCCTCT
				rs225354	9	ACTCCAGCCTGAGTGACAGA*
	CAGTGGCCCCCCGTGAAAGA	TCCAGCTCAGGGGCAACGG	543	rs225353	10	TAGGATAGAGGGGGAAGGCATGA
S100A9	TGGCTGCTGGCTGGGTTTCC	CACCTCCCACCTTGCCACC	1137	rs2916196	1	CTTGAATACATGAGTCGCCTA
				rs11205276	2	GCACCGGCAATAAAGGAAG
				rs3014866	3	GCAAATCCGAGGGTGTC
	GCGTTTGGTATGTGCTCAGTG	GCGTTTGGTATGTGCTCAGTG	938	rs1063933	4	CACGAGAAGATGCAC
				rs2070864	5	TTTTCAAGGGGTAGAGATATGCC*
S100A12	TGTTTCTCACTATCCCATGCC	ATGTTTCAAGTGCTCAGTAGTC	497	rs3006475	6	CTTTCATCTCTGTATCCCTAGCATCCAG
	GACTCAAGCAATCCTCTCGC	AAGAATGGCTAAGCAGGGGTG	1694	rs2916191	7	GCTTGCATATTATTTGGGAGAG
				rs2233864	8	TGGACCTTCACAACCC
				rs3014883	9	CCACATATCGCCCACAAATG
	AAACCTGTGGCTTAGGCTGC	AGACCTACCTCTGGGCACGC	1113	rs3006476	10	TCTGGAGGCAAAAGTCTGTCTCTC
S100A8	ATACTACCACCCTGTTCCCAA	GTTTGGTTATTTGGAGAGTGC	934	rs3006488	11	ATTCCACATAAGCATAACAGAGAGGC
				rs3795391	12	AGGGCAGTACGCAGAGGA
	ACTATCGTTGTTGTTCTTTTGG	TACAGGCAGGAATAGGAAGTGG	1553	rs3806232	13	TAGAATGGATATAGCCCTTGGC*
				rs11205282	14	TTTTTATTTTGTCCCCAATGGCTCACATCC*
				rs12040625	15	CTTTTAGAACGCTGAATTACTATGTCTCTC*
AMBP	TACCTGGGTTCTGGTCTTGG	CTCCAATCGATCCCTGTAGC	335	rs2251700	1	CCCTGGAGCACCAGG*
	GGGCAAACACTGAGGTCAAC	GAAGGGCTGGGAGGTCTG	601	rs2251680	2	TTTATGCTCATAGAATGTCAGCCAAGAG*
	GAGAGACCCAGAGACCCACA	GGCAGACAACCAGGAAAGAA	398	rs2856923	3	TTACGCTCCTCCACCAGGA
	CTCCTCCCCATGACCTCTC	GCATCATGGAAAGAATAGCAA	450	rs16912311	4	TTTTGCAGTCACTGCTACCACCA
	TCTCCGTGACTTGAAAGACAGA	TGGCAAAGAAATGGCACTAA	260	rs12377342	5	TTTTTTATTTTGGATTATGGAGACAGAGAAGA*
	GCCCTGGAACTTTTACTACC	ACATCTGGATTAAAAGAGCA	330	rs10817564	6	ACGCTGAGCCATTCTATTC
	GGCAACGGTAACAACTTCGT	CTACCATCACCAGGGACACC	561	th2399	7	TTTTTAAACAGTTTCCCCATCTGTCAA
	TTGAAGATGTCTGTGCCTTGA	TTTATTTGGACCCAGGTTGC	431	rs2369454	8	TTTTTTATTTTTGGAATACCCTTTGGAGATAAATG
				rs2369455	9	GTTTCTGGCCCCAGG
				rs2369456	10	TTTTTTTCACTCAGGGAAGGGAA*
SPP1	ATAGGTAGGCTGGGCGATTT	ACATCCTCCACCAACACAGG	686	rs2853744	1	CTCAAGCAGTCATCCT
				rs11730582	2	GACAGAGGCAAGTT
				rs7687316	3	TTTAGGTATCAATTGTGTGTGTGCGTTTTTG
				novel 2	4	TTTTTTTTTTTTTTTTGATTGTGTGTGTGCGTTTTTGTTTTTTTTT
				novel 3	5	TTTTTTTATTAATCTGGTTTTGTGGTTAAA*
	AATGGTATAATTTCTGGAACTC	AATAGCTGACATCTTCATAGGT	262	rs2853749	6	TTTTAATACAGACTTGCAAAATTTC
	CCCAGAAGCTTGGACAAAAA	AGCTCCACGTGCCTTCTAAA	324	rs11728697	7	TCAGTTGAACAGAATAAAGG
	CATGTGTCATGAGAATAGATA	GGTGAAACCCCGTCTCTACTA	281	rs6839524	8	TTTATCATCTGGTCTCACTGTTTTT
	CGGATAAGTTTAGCATTACAG	CTCAAAACTGCCCATTGAATT	364	rs7695531	9	TTTTTTTTTAAAGGCAAAATCTTAGCTTTGATA*
	ATTAATTTTCCCGGCCATCT	GCTGAATGGATGAATGAATGG	462	rs4754	10	GCTGTCCACATGGTC*

	GGACATCACCTCACACATGG	TTTAATTGACCTCAGAAGATGCA	386	rs1126616	11	AGATTATATAAGCGGAAAGC
				rs4660	12	TTTTTTTTTTTTTTTGCACTATCTAATTCATGAGAAATA*
	TGAAAGAGAACATGAAATGCTTC	CCGTGGGAAAACAAATAAGC	410	rs1126772	13	TTTTTTTTTTTTAATTCTATTTGAGTCTGGAAATAACT
				rs9138	14	TTTTTTTTTTTTTTTTTTTATCTCATGAATAGAAATTTATGTAGA
UMOD	ATGGCACTGTGAGTGCTCC	TCACAGGGACAGACAGACAA	528	rs13335818	1	TTTTTTTTTATTTTTTTTATGCGTCCGTCCAGGT
	CGGGCTTCAATGACAGAGAC	GAGGTGGCAGGAGATAATGC	582	rs4506906	2	ATAGGAAGTGATAGAGCTGAA*
				rs9940449	3	TTTTATTTACCTCTGGGTTTCATTTGA*
	ATTCCATTGATGGTGACTTGG	ACCCCTCCTGAGTCATAGCA	218	rs11647727	4	TTTTTTTTTTTTTATTTTTCCCAGACAAGAAGACAG*
	TGTGGCTGTTTTCAGTGTGG	CAGATAGGCCACCTTCTCTG	513	rs4780884	5	AGGCCTACAACTTGC*
				rs9646256	6	TTTTTTTTTTAGGCAGTCCCTTGCAGTT*
	AGGCCGGAATACAGTGACTT	CCTACTCAGCTCTCTGGTGA	262	rs9923532	7	TTTTTTTTTTTTTTTATTTTTTTTTTTCAGCTACTCATGAGGCTA*
	CAGATGTACACACACTTATTGG	AGTAGCTGTTGCATAGATTTGC	340	rs1123670	8	TGGCATATAGTATTACTCAAC*
	GGATATAGCAGTGAACAAGCC	AGGCTCTGGTACCCCATTATTT	453	rs8060932	9	CTCTCCTACCAGACA*
				rs8062123	10	TTTTTTTTTTTTTTTATTTTTTTTTTTCAATCGATCCCCACCGCC*
F2	GAGCCAACACCTTCTTGGAG	CATGTCATGGAGCTGCACA	331	rs2070850	1	TTTTTTTTTAGAAGCAAGCGAGGAA
	GAGAATCTTCTGCTGCACCT	GAGGCAGAGAATTGCTTGAA	576	rs3136435	2	TTTTTTTTTTTTATGTTTGGGGAGCAGGACAT
	AGGACTGCTTCAAATGTCACC	TGAGCCCAGGAGTTCAAGAT	168	rs3136441	3	TTTTTTTTTTTTTTTTTTTAATTTTTTTTCTGTCTCTTATAAATAAACAAAG*
	GCCAAGAGAA GCCACAGAAT	CCCAGCTTACCACAGACAGG	644	rs2070851	4	TTTTTTTTTTTTATTCGCTAAGTGCCTACCAT*
				rs2070852	5	TTCCTGTTGGGCAATTTCCT
	GAAGCCCTCCCTAGCCATTT	GGAGGTTGCAGTGAGCTGAG	732	rs3136456	6	CAGTTGACCCTACAGAC*
				rs3136457	7	TTTGATTACCAGTCCACAAACATG
	CCAAAGTT CTGGGATTAC AGG	GCCACTTTTGCAGGTTTGTT	370	rs3136460	8	TTTTTTTTTTTTATTTTTTTTGAACAGCCTAGTGATCC
	GGTTATGGGAGGGTTAAATGAA	CTGGTCAGGGATTGCTGTTT	271	rs2282687	9	TTTTTTTTTTTTTTTTATTTTTACATTCATTCAGTCACTCAGCAAATA
	GGGTAAGTGG ACTCTCACCA G	TTCCAGGAGCCCAGAATATG	483	rs3136516	10	ACAGTTGCCTGGCAG

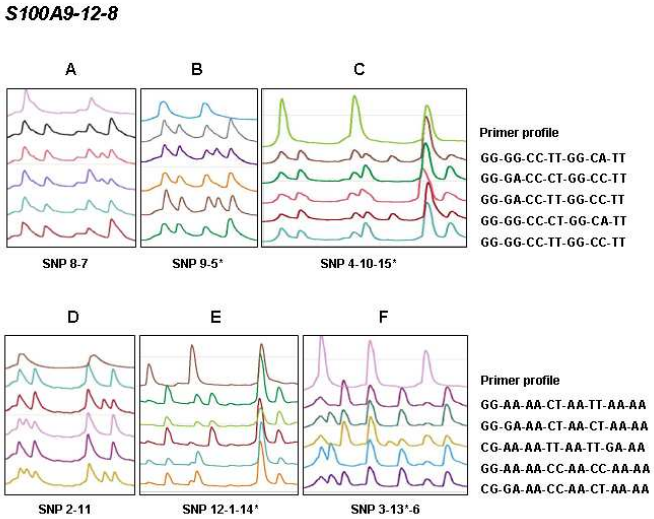
* anti-sense strand



Supplementary Figure 1

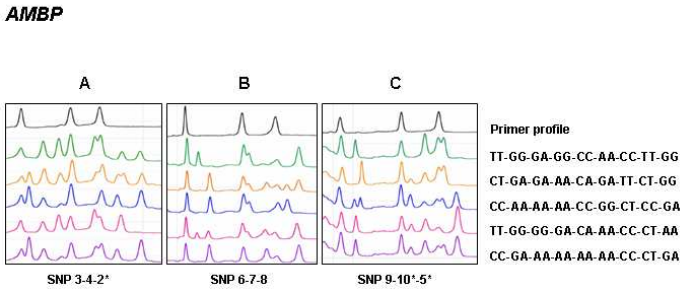
DHPLC chromatograms of primer extension products for genotyping ten SNPs in TFF1 gene in five sets, A-E, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 8-10-1, (B) SNP 2-9*-5, (C) SNP 6-7, (D) SNP 3, and (E) SNP 4. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 8-10-1- 2-9*-5- 6-7-3-4.
(* using of anti-sense extension primer)

254x190mm (96 x 96 DPI)



Supplementary Figure 2

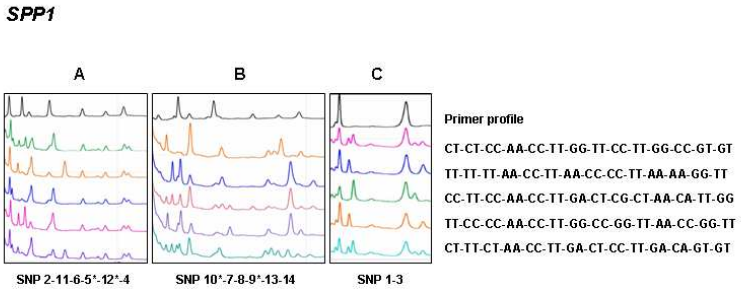
DHPLC chromatograms of primer extension products for genotyping fifteen SNPs in S100A9-12-8 genes cluster in six sets, A-F, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 8-7, (B) SNP 9-5*, (C) SNP 4-10-15*, (D) SNP 2-11, (E) SNP 12-1-14*, and (F) SNP 3-13*-6. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 8-7-9-5*-4-10-15*-2-11-12-1-14*-3-13*-6. (* using of anti-sense extension primer)
254x190mm (96 x 96 DPI)



Supplementary Figure 3

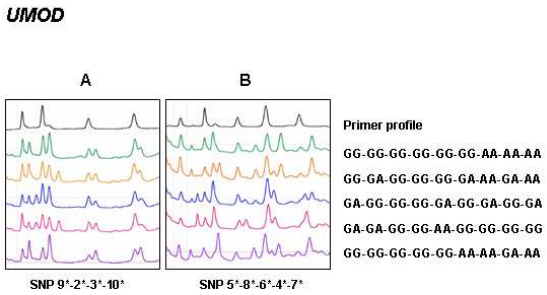
DHPLC chromatograms of primer extension products for genotyping nine SNPs in AMBP gene in three sets, A-C, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 3-4-2*, (B) SNP 6-7-8, and (C) SNP 9-10*-5*. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 3-4-2*-6-7-8-9-10*-5*.
(* using of anti-sense extension primer)

254x190mm (96 x 96 DPI)



Supplementary Figure 4

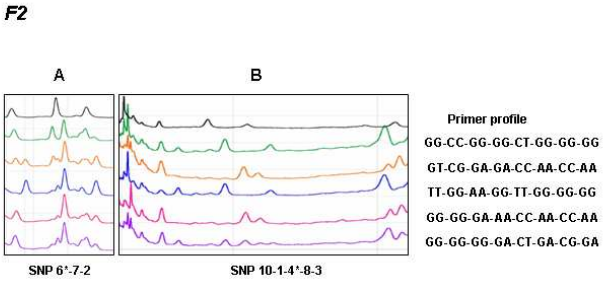
DHPLC chromatograms of primer extension products for genotyping fourteen SNPs in SPP1 gene in three sets, A-C, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 2-11-6-5*-12*-4, (B) SNP 10*-7-8-9*-13-14, and (C) SNP 1-3. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 2-11-6-5*-12*-4-10*-7-8-9*-13-14-1-3. (* using of anti-sense extension primer)
254x190mm (96 x 96 DPI)



Supplementary Figure 5

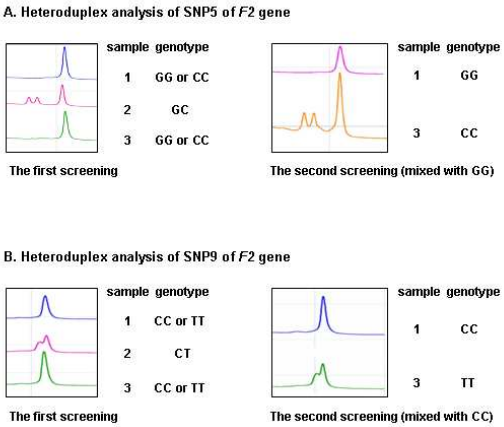
DHPLC chromatograms of primer extension products for genotyping nine SNPs in UMOD gene in two sets, A-B, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 9*-2*-3*-10* and (B) SNP 5*-8*-6*-4*-7*. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 9*-2*-3*-10*-5*-8*-6*-4*-7*.
(* using of anti-sense extension primer)

254x190mm (96 x 96 DPI)



Supplementary Figure 6

DHPLC chromatograms of primer extension products for genotyping eight SNPs in F2 gene in two sets, A-B, of multiplex primer extension reaction. Each set is designed to detect: (A) SNP 6*-7-2 and (B) SNP 10-1-4*-8-3. Genotypes of all SNPs calling from each sample are presented beside the chromatograms in order: SNP 6*-7-2-10-1-4*-8-3.
(* using of anti-sense extension primer)
254x190mm (96 x 96 DPI)



Supplementary Figure 7

DHPLC chromatograms of heteroduplex analyses of (A) SNP 5 (rs2070852) and (B) SNP 9 (rs2282687) of F2 gene. DHPLC elution profile showed 2 different patterns in the first screening (left panel), sample 1 and 3 with the homozygous genotypes and sample 2 with the heterozygous genotype. The homozygous genotypes of sample 1 and 3 represented different patterns in the second screening (right panel) after mixed with the PCR product of known homozygous genotype sample to make a distinction between homozygous major allele and homozygous minor allele.
254x190mm (96 x 96 DPI)

AP-1 mu1A is a binding protein of human kidney anion exchanger 1

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Keywords: Kidney anion exchanger 1, band 3, AP-1 mu1A, distal renal tubular acidosis, protein-protein interaction, protein trafficking

Summary

Kidney anion exchanger 1 (kAE1) mediates chloride (Cl^-) and bicarbonate (HCO_3^-) exchange at the basolateral membrane of kidney α-intercalated cells. Impaired trafficking of kAE1 leads to defect of the Cl^-/HCO_3^- exchange at the basolateral membrane and failure of proton (H^+) secretion at the apical membrane, causing a kidney disease – distal renal tubular acidosis (dRTA). To gain a better insight into kAE1 trafficking, we searched for proteins binding to the C-terminal region of kAE1 (Ct-kAE1) containing motifs crucial for intracellular trafficking by a yeast two-hybrid (Y2H) screen. An adaptor-related protein complex 1 μ1A subunit (AP-1 mu1A) was found to interact with Ct-kAE1 by the Y2H screen. The interaction between Ct-kAE1 or full-length kAE1 with AP-1 mu1A was confirmed by *in vitro* and *in situ* studies, including GST pull-down assay, co-immunoprecipitation, affinity co-purification, co-localization, and yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA). Suppression of endogenous AP-1 mu1A in human embryonic kidney (HEK) 293T by RNA interference decreased membrane localization of kAE1, suggesting a role of AP-1 mu1A in the kAE1 trafficking in kidney α-intercalated cells.

1. Introduction

Human anion exchanger 1 (AE1 or band 3), encoded by *solute carrier family 4*, *anion exchanger*, *member 1 (SLC4A1)* gene, is a plasma membrane transporter functioning in Cl^-/HCO_3^- exchange to regulate intracellular pH and acid-base homeostasis in the human [1, 2]. Two AE1 isoforms have been characterized. Erythroid AE1 (eAE1), a major protein on red cell membrane, functions in both electroneutral anion (Cl^-/HCO_3^-) exchange and cytoskeletal anchorage. It contains 911 amino acids which organizes into three structurally and functionally distinct domains: a cytoskeleton-associated amino- (N-) terminal domain, which interacts with ankyrin-1, proteins 4.1 and 4.2, glycolytic enzymes and hemoglobin, a central anion-transporting transmembrane segment, and a short cytoplasmic carboxyl- (C-) terminal domain known to interact with carbonic anhydrase II [3]. Kidney AE1 (kAE1), which lacks the first 65 amino acids, is expressed at the basolateral membrane of acid-secreting α-intercalated cells of kidney and mediates Cl^-/HCO_3^- transport across the basolateral membrane to balance H^+ secretion across the apical surface into urine [4].

Failure of either acid excretion or bicarbonate reabsorption due to mutations in the gene encoding H^+-ATPase or kAE1, respectively, leads to distal renal tubular acidosis (dRTA), a kidney disorder characterized by an inability to acidify urine resulting in systemic metabolic acidosis and several clinical manifestations such as muscle weakness, failure to thrive, hypokalemia, hypercalciuria, hypocitraturia, and nephrocalcinosis/nephrolithiasis [5]. Genetic studies revealed two modes of inheritance of dRTA attributable to *SLC4A1* mutations: autosomal dominant (AD) [6] and autosomal recessive (AR) dRTA [7]. The *SLC4A1* mutations causing both forms of dRTA generate mutant kAE1 that still maintains functional anion-exchange activity but exhibits basolateral trafficking defect and intracellular retention when they were expressed in non-polarized HEK293 cells [8-11] and either intracellular retention or apical mistargeting of kAE1 in polarized MDCK cells [12-14].

The involvement of the C-terminal portion of kAE1 in proper basolateral trafficking was reported. A 20-bp deletion in exon 20 of *AE1* leading to mutation in codon 888 followed by a premature termination codon at position 889 (A888L+889X) was identified in two affected brothers with dRTA [15]. Furthermore, Y_{904} is crucial for polarized transport of kAE1 as Y904A or Y904A+V907A mutation caused non-polarized distribution of kAE1 in polarized MDCK cells [12, 13]. Removal of the last 5 amino acids was sufficient to retard kAE1 trafficking in HEK 293 and LLC-PK1 cells [19]. Despite many pieces of evidence suggest that C-terminal portion of kAE1 is involved in basolateral membrane trafficking, very little information is known for proteins that physically interact with the C-terminal tail of kAE1 [3]. We reported here that kAE1 interacts with AP-1 mu1A, a subunit of AP-1A adaptor complex, in a yeast two-hybrid screen. The interaction was further confirmed by GST pull-down assay, co-immunoprecipitation, affinity co-purification, immunofluorescence staining, and protein fragmentation complementation assay (PCA) [20]. Trafficking defect of kAE1 in AP-1 mu1A suppressed HEK 293T cells using RNA interference was also investigated.

2. Materials and methods

2.1 Plasmid constructions

pcDNA3-kAE1 (a kind gift from Professor Reinhart Reithmeier, University of Toronto, Canada) containing full-length *kAE1* cDNA was used as a template for amplification by polymerase chain reaction (PCR) of a sequence consisting of 108 base pairs (bp) encoding the C-terminal 36 amino acids of AE1 (Ct-kAE1). The *Eco*RI/*Sal*I-digested Ct-kAE1 was subsequently inserted in-frame into pGBKT7 plasmid (Clontech, Mountain View, CA, USA) to generate a bait construct (pGBKT7-Ct-kAE1) expressing a fusion of GAL4-DNA binding domain (DBD) and Ct-kAE1 in a yeast two-hybrid system. The bait construct was tested for correct protein expression prior to library screening. No intrinsic transcriptional activity of the bait construct was observed as measured in an autoactivation test by growing on synthetic dropout (SD)/-His-Ade medium [21] supplemented with X-α-gal.

To generate pGEX4T-2-Ct-kAE1, a plasmid construct expressing GST-Ct-kAE1 fusion protein, cDNA fragment encoding Arg^{870}-Val^{911} for Ct-kAE1 were amplified and subcloned into *Sma*I/*Xho*I sites of pGEX-4T-2. The full-length cDNA encoding AP-1 mu1A, amplified from a cDNA pool derived from human kidney tissue (Invitrogen, Carlsbad, CA, USA), was subcloned into *Bam*HI/*Hind*III sites of pTrcHisA to generate pTrcHisA-AP-1 mu1A construct for the expression of AP-1 mu1A tagged with 6x-His at the N terminal. pcDNA3.1/His-AP-1 mu1A, a mammalian plasmid construct expressing human AP-1 mu1A N-terminally tagged with 6x-His, was generated by PCR-amplifying a full-length AP-1 mu1A sequence from the cDNA pool and cloned into pcDNA3.1/HisC.

The sequences encoding two separate fragments of yellow fluorescent protein (YFP) fused with leucine zipper protein, YFP [1]-GCN leucine zipper and YFP [2]-GCN leucine zipper, inserted between the *Not*I/*Xba*I sites of the pcDNA3.1/Zeo vectors (Invitrogen, San Diego, CA, USA) which were named as pcDNA3.1/Zeo-YFP [1]-Zip and pcDNA3.1/Zeo-YFP [2]-Zip,

were the gifts from Professor Stephen W. Michnick, University of Montreal, Canada. These constructs consisted of the sequences encoding YFP fragments (either YFP [1] – amino acids 1 to 158, or YFP [2] – amino acids 159 to 240) and *Zip* cDNA encoding leucine zipper. A sequence encoding flexible 10 amino-acid linker $(GGGGS)_2$ was inserted between the sequences encoding YFP fragment and leucine zipper. The full-length cDNAs encoding kAE1 and AP-1 mu1A were amplified by PCR and subcloned into the *Bsp*EI/*Xba*I sites of the pcDNA3.1/Zeo-YFP [1]-Zip and pcDNA3.1/Zeo-YFP [2]-Zip constructs, generating pcDNA3.1/Zeo-YFP [1]-kAE1, pcDNA3.1/Zeo-YFP [2]-kAE1, pcDNA3.1/Zeo-YFP [1]-AP-1 mu1A, and pcDNA3.1/Zeo-YFP [2]-AP-1 mu1A, which expressed YFP[1]-kAE1, YFP [2]-kAE1, YFP [1]-AP-1 mu1A, and AP1 mu1A, YFP [2]-AP-1 mu1A fusion proteins, respectively.

2.2 Yeast two-hybrid screening

To generate the bait strain, pGBKT7-Ct-kAE1 was transformed into the yeast AH109 strain. The prey strain, Y187, pre-transformed with the prey plasmids, pACT2, which carried the GAL4-activation domain [6] fused to fragments from a human kidney cDNA library, was purchased from Clontech (Mountain View, CA, USA). The yeast two-hybrid screening was performed according to the manufacture protocol (Clontech, Mountain View, CA, USA). Mated diploids whose cDNA-encoded products interacted with the bait protein were selected by growth and galactosidase activity on SD/-Trp-Leu-His-Ade plates and SD/-Trp-Leu-His-Ade plates supplemented with X-α-gal (Clontech, Mountain View, CA, USA) to assay for activation of reporter genes [*HIS3, ADE2, MEL1* (α-galactosidase)]. The positive colonies with strong reporter activities were selected. The prey plasmids rescued from positive colonies were transformed into *E. coli* for PCR amplification. The *Alu*I restriction patterns of PCR products were generated and representatives from different restriction patterns were chosen for

the specificity tests. Specific interactions between the bait protein and the encoded products of isolated preys were tested by re-transforming both bait and prey constructs into opposite yeast mating types for re-mating. The cDNA fragments of the positive clones from the specificity tests were sequenced and aligned with sequences in the database using BLAST homology search (http://www.ncbi.nlm.nih.gov) in order to obtain full-length sequences of corresponding genes.

2.3 GST pull-down binding assay

E. coli BL21 (DE3) containing plasmid constructs encoding either GST, GST-Ct-kAE1-WT, or 6x-His-tagged AP-1 mu1A were expressed by induction with IPTG. The bacterial cells were lysed by sonication in lysis buffer (1xPBS, 1% Triton X-100, Protease Inhibitors Cocktail (Roche Molecular Biochemicals, Mannheim, Germany) and 1 mM phenylmethylsulfonyl fluoride). GST or GST-fusion proteins in the lysates were purified by binding with Glutathione-Sepharose 4B beads (Amersham Biosciences, Piscataway, NJ, USA). Unbound proteins were eliminated by the serial washing with 1%Triton X-100 in PBS for 2 times, PBS for 1 time, and 0.1%Triton X-100 in 1xPBS for 1 time. GST pull-down assay was conducted as previously described [21]. GST or GST-Ct-kAE1 fusion protein fixed on Glutathione-Sepharose 4B beads was incubated with His-tagged AP-1 mu1A protein from the bacterial lysate in binding buffer (1xPBS, 0.1%Triton X-100, Protease Inhibitors Cocktail and 1 mM phenylmethylsulfonyl fluoride) by gently rocking at 4°C overnight. The beads were recovered by centrifugation and washed for three times with the binding buffer to eliminate the unbound proteins. The binding protein complexes were eluted, subjected to SDS-PAGE and analyzed by immunoblotting.

2.4 Cell culture and transfection

HEK 293T cells were maintained in complete Dulbecco's Modified Eagle Medium (DMEM, Gibco Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (Perbio, Chester UK), 100 units/ml penicillin and 100 μg/ml streptomycin. The cells were cultured in 25-cm^2 flask at 37ºC with 5% CO_2 and sub-cultured twice per week following a standard trypsinization protocol. Two days before transfection, the HEK 293T cells were collected by trypsinization and seeded in 6-well plates. The cultured cells were transiently transfected with pcDNA3.1 vector or different constructs according to experiments by DEAE-dextran as previously described [21] or lipofection transfection method following the manufacture protocol (Invitrogen). After transfection for 2 days, the cells were collected for further experiments.

2.5 Co-immunoprecipitation and affinity co-purification

Two days post-transfection, the cells were lysed in 500 μl of IPB^+ lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 0.2% BSA and protease inhibitor). Co-immunoprecipitation procedure was performed as described in our previous study [21]. Affinity co-purification using Co^{2+}-chelated resins (BD Biosciences, Franklin Lakes, NJ, USA) was carried out as described [21]. Immunobloting was performed using rabbit anti-Ct-kAE1 (a gift from Dr. Joseph Casey, University of Alberta, Canada), mouse anti-His (Amersham Biosciences Corp., Piscataway, NJ, USA), and mouse anti-AP1 mu1A (Abnova, Taipei, Taiwan) as primary antibodies and swine anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit anti-mouse IgG-HRP (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as secondary antibodies and detected by SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL USA).

2.6 Yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA)

Two days before transfection, the HEK 293T cells were collected by trypsinization and seeded in 6-well plates. The cultured cells were individually transfected with 1 μg of each recombinant plasmids, pcDNA3.1/Zeo-YFP [1]-kAE1, pcDNA3.1/Zeo-YFP [2]-kAE1, pcDNA3.1/Zeo-YFP [1]-AP-1 mu1A, and pcDNA3.1/Zeo-YFP [2]-AP-1 mu1A, and were also co-transfected with pcDNA3.1/Zeo-YFP [1]-kAE1 and pcDNA3.1/Zeo-YFP [1]-AP-1 mu1A, pcDNA3.1/Zeo-YFP [2]-kAE1 and pcDNA3.1/Zeo-YFP [2]-AP-1 mu1A, pcDNA3.1/Zeo-YFP [1]-kAE1 and pcDNA3.1/Zeo-YFP [2]-kAE1, pcDNA3.1/Zeo-YFP [1]-AP-1 mu1A and pcDNA3.1/Zeo-YFP [2]-AP-1 mu1A, pcDNA3.1/Zeo-YFP [1]-kAE1 and pcDNA3.1/Zeo-YFP [2]-AP-1 mu1A, and pcDNA3.1/Zeo-YFP [2]-kAE1 and pcDNA3.1/Zeo-YFP [2]-AP-1 mu1A by using lipofectamine 2000 (Invitrogen, USA) transfection method following the manufacture protocol as previously described [21]. After transfection for 2 days, the cells were fixed *in situ* in 3% formaldehyde for 20 min, followed by thrice washing in PBS for 10 min and mounted with fluorescence mounting medium (Dako, USA). Cell fluorescence and phase contrast images were captured using a confocal Zeiss LSM 510 microscope (Carl Zeiss, Göttingen, Germany).

2.7 Co-localization

HEK-293T cells were grown on coverslips in a 6-well plate for one day prior to transfection. Cells were transfected using lipofectamine™ 2000 (Invitrogen) and cultured for two days. As a negative control, the transfected cells were fixed with 3.7% formaldehyde for 10 min, rinsed twice in PBS, mounted with Fluorosave for image capture by a confocal microscope. For immunofluorescence staining, the transfected cells were fixed, rinsed, permeabilized with 0.1% Triton X-100 and blocked with 1% BSA. Rabbit anti-Ct-kAE1 antibody (1:1,000 dilution), mouse anti-His antibody (1:1,000 dilution), and mouse anti-AP1 mu1A (1:1,000 dilution) were used as primary antibodies. Donkey anti-rabbit IgG antibody conjugated with Cy3 fluoresceine (Jackson Immunoresearch Laboratories, West Grove, PA, USA, 1:8,000 dilution) and goat anti-mouse IgG antibody conjugated with Alexa 488

fluoresceine (Molecular Probes, Eugene, OR, USA, 1:1,000 dilution) were used as secondary antibodies. The coverslips were washed with PBS and mounted with Fluorosave. Cellular localization of kAE1 and His-AP-1 mu1A was observed by using a laser scanning confocal Zeiss LSM 510 microscope (Carl Zeiss, Göttingen, Germany).

2.8 RNA interference

The siRNA directed against mu1 subunit of AP-1 was described previously [22] (Invitrogen) and its target sequence was 5'-TCCGAAGGCATCAAGTATCGGAAGA-3'. HEK-293T cells were transfected using lipofectamine™ 2000 (Invitrogen) as specified by the manufacturer. The transfection mixture was left on the cells for 6 h, after which complete medium was replaced to each well. Second transfection was performed for efficient knockdown with pcDNA3.1/His-kAE1 co-transfection, and experiments were carried out 48 h after the second knockdown.

Total RNA was prepared by Trizol reagent (Invitrogen) and chloroform extraction. Assays were performed using LightCycler® 480 SYBR Green I Master (Roche, Mannheim, Germany), and the reactions were recorded and analyzed using a LightCycler® 480 Instrument equipped with a 96-well thermal cycler (Roche, Mannheim, Germany). RNA samples (1 μg) were reverse transcribed using the SuperScript ^{III} First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) as specified by the manufacturer. Complementary DNA (cDNA) templates were then subjected to a 10-minute initial denaturation at 95°C prior to 50 cycles of PCR (95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 20 seconds, per cycle) in the presence of *Taq* DNA polymerase and primers spanning *AP-1 mu1A* gene (forward primer, 5'-CTAGTGTGGAGGCCGAAGAC-3'; reverse primer, 5'-CGGAGCTGGTAATCTCCATT-3'). Experiments were performed in triplicate for each data point and beta-actin (ACTB) mRNA was used as a reference.

3. Results

3.1 Identification of AP-1 mu1A as a Ct-kAE1-binding protein in a yeast two-hybrid screen

To explore the molecular trafficking machineries involved in the basolateral sorting and transport of kAE1, a yeast two-hybrid screen was carried out to search for proteins that bind to the C-terminus of kAE1 (Ct-kAE1, amino-acids 876-911). A bait construct, pGBKT7-Ct-kAE1, was used to screen a human kidney cDNA library. One plasmid isolated from the library represented a fragment of AP-1 mu1A, a medium subunit of clathrin-associated AP-1A adaptor complex (Fig. 1A). Interestingly, this fragment is a part of the C-terminal domain of AP-1 mu1A that directly recognizes and binds to tyrosine-based sorting signals present in the cytoplasmic domains of cargo proteins [23]. We next confirmed the specific interaction between Ct-kAE1 and the identified AP-1 mu1A fragment by plasmid-swapping transformation and re-mating. The prey plasmid containing partial AP-1 mu1A cDNA sequence isolated from the initial screen was re-transformed into the yeast strain AH109 and then re-mated with the opposite mating strain Y187 harboring either the bait plasmid (Ct-kAE1), empty vector, or two other plasmids containing unrelated genes whose protein products do not interact with AP-1 mu1A. Mated diploid cells were plated on SD/-Trp-Leu-His-Ade and SD/-Trp-Leu-His-Ade/X-α-Gal plates as shown in Fig. 1B. Only the diploid cells with both Ct-kAE1 and AP-1 mu1A-containing plasmids activated the expression of reporter genes, hence, grew and turned blue on SD/-Trp-Leu-His-Ade and SD/-Trp-Leu-His-Ade/X-α-Gal plates, respectively.

3.2 AP-1 mu1A interacts with Ct-kAE1 in GST pull-down assay

The interaction between Ct-kAE1 and AP1 mu1A was confirmed by GST pull-down assay. His-AP-1 mu1A was detected when GST-Ct-kAE1 was used as a bait (Fig. 2A), indicating that AP-1 mu1A directly binds to Ct-kAE1.

3.3 AP-1 mu1A interacts with kAE1 in HEK 293T cells as detected by co-immunoprecipitation and affinity co-purification

To establish whether AP-1 mu1A associates with kAE1 in mammalian cells, full-length cDNA sequences of kAE1 and AP-1 mu1A were subcloned into mammalian expression vectors. HEK 293T cells were transfected with either pcDNA-kAE1 or pcDNA3.1/His-AP1mu1A or co-transfected with both constructs and then subjected to co-immunoprecipitation and affinity co-purification. Fig. 2B showed that immunoprecipitation with anti-His antibody, kAE1 co-isolated with the immunoprecipitated complex when co-transfected with His-AP-1 mu1A. Similarly, in the affinity co-purification assay using Co^{2+} column, kAE1 co-purified with His-AP-1 mu1A when co-transfected together (Fig. 2C).

3.4 AP-1 mu1A interacts with kAE1 in HEK 293T cells as examined by yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA)

This interaction was also examined by yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA). Two separate fragments of YFP were fused to the N-terminus of kAE1 and to the N-terminus of AP-1 mu1A. pcDNA3.1/Zeo-YFP [1]-kAE1, pcDNA3.1/Zeo-YFP [2]-kAE1, pcDNA3.1/Zeo-YFP [1]-AP-1 mu1A and pcDNA3.1/Zeo-YFP [2]-AP-1 mu1A were transfected or co-transfected into HEK 293T cells and captured interaction by confocal microscopy. The interaction between kAE1 and AP-1 mu1A in HEK 293T cells was demonstrated by intracellular yellow fluorescent signals (Fig. 3).

3.5 Subcellular localization of kAE1 and AP-1 mu1A in HEK 293T cells

Subcellular localization of kAE1 and His-AP-1 mu1A was investigated in HEK 293T cells individually transfected with pcDNA-kAE1 or pcDNA3.1/His-AP1mu1A, or co-transfected with both plasmid constructs by immunofluorescence staining and confocal microscopy. In single-transfected HEK 293T cells, kAE1 and AP-1 mu1A showed differential distributions;

kAE1 localized predominantly on the cell surface (Fig. 4A) whereas His-AP-1 mu1A dispersed throughout the cytoplasm (Fig. 4B). In cells co-transfected with both plasmids, however, overlapped localization of His-AP-1 mu1A and kAE1 was observed at the plasma membrane (Fig. 4C-H).

3.6 Supression of AP-1 mu1A accumulates kAE1 in cytoplasm of HEK 293T cells

To address the functional importance of AP-1 mu1A, we employed RNAi to transiently deplete the expression of endogenous AP-1 mu1A in HEK 293T cells with kAE1 co-transfection. siRNAs of 25-mer oligoribonucleotides targeting AP-1 mu1A, was designed to suppress AP-1 mu1A expression. The efficiency of the siAP-1 mu1A as shown by real-time PCR (Fig. 5A) and immunoblotting (Fig. 5B). Transfection of HEK 293T cells with siAP-1 mu1A reduced AP-1 mu1A mRNA and protein >80% within 48 h. Moreover, siAP-1 mu1A treatment affected kAE1 localization. In condition with low expression of endogenous AP-1 mu1A, kAE1 was predominantly in cytoplasm where as in kAE1-trasfected HEK293T alone or co-trasfected with siControl showed that kAE1 localizes mostly at cell membrane (Fig. 6). AP-1 mu1A might be a key factor of membrane transport of kAE1.

4. Discussion

To date, it has not been clearly described the trafficking itinerary of kAE1 to the polarized basolateral cell surface or the molecular pathogenesis of dRTA-associated AE1 mutations. Trafficking defect caused by AE1 mutations has been explored as a potential molecular basis as all mutants showed near normal anion-transport activities [6, 10, 11, 21, 24]. To unravel defective steps in kAE1 transport process, identification of proteins involved in kAE1 intracellular transport was essential. In this study, we reported a novel interaction between kAE1 and AP-1 mu1A, a medium subunit of an adaptor protein complex AP-1A.

Clathrin-associated adaptor protein complexes are heterotetrameric complexes comprising two large, one medium and one small subunit [16, 25]. They play crucial roles in cargo selection and vesicle formation in post-Golgi trafficking pathways. So far, four types of adaptor complexes have been described: AP-1, AP-2, AP-3 and AP-4. AP-1, AP-3 and AP-4 function in protein sorting at the TGN or endosomes, while AP-2 mediates endocytosis at the plasma membrane. In mammalian, two forms of AP-1 complexes exist: AP-1A and AP-1B. Both share the same subunits except the medium chain, which is a central subunit that mediates cargo selection via direct binding with tyrosine-based sorting motifs on the cytoplasmic domain of cargo proteins [26-28]. AP-1A contains an ubiquitiously expressed mu1A whereas AP-1B contains an epithelial-specific mu1B subunit [23]. Functionally, the ubiquitous AP-1A facilitates vesicle transport between the TGN and early endosomes, and the epithelial-specific AP-1B is required for polarized trafficking of many basolateral membrane proteins [17].

The role of the AP-1B complex in kAE1 basolateral sorting was previously examined by expression of chimeric constructs harbouring wild-type C-terminal kAE1 or kAE1 R901X (with 11-amino acid deletion) in LLC-PK1 cells, which lack the mu1B subunit [13]. Although the AP-1B complex cannot form in these cells, wild-type kAE1 was correctly targeted to the basolateral membrane [13]. Another study in LLC-PK1 cells demonstrated that the C-terminus

of kAE1, which contain a tyrosine-based basolateral-sorting signal, did not recognize the AP-1B complex [12]. These studies reveal that another adaptor complex other than AP-1B is responsible for the basolateral sorting of kAE1. Using the C-terminal tail of kAE1, which contains a putative tyrosine-based sorting signal $Y_{904}DEV_{907}$, as bait in the yeast two-hybrid screen, we identified the C-terminal fragment of AP-1 mu1A (amino acids 306-408). Importantly, this region is part of the AP-1 mu1A segment that directly interacts with tyrosine-based sorting signals present in cargo proteins [23]. Using GST pull-down assay, we found that AP-1 mu1A could interact with Ct-kAE1 (Fig. 2A). Moreover, the interaction of AP-1 mu1A and Ct-kAE1 was confirmed in HEK 293T cell using co-immunoprecipitation (Fig. 2B), affinity co-purification (Fig. 2C), and yellow fluorescent protein (YFP)-based protein fragment complementation assay (Fig 3H and I). The minimal motif of interaction between Ct-kAE1 and AP-1 mu1A should be further investigated. $Y_{904}DEV_{907}$ motif conforms to a subset of YXXØ motif presenting on the cytoplasmic C terminal tail of human AE1 and plays an important role in kAE1 distribution in polarized cells. Tyr904 in the YXXØ motif of kAE1 has been reported for the basolateral signal in MDCK cells and rat inner medullary collecting duct (IMCD) cells [12, 13]. kAE1 with substitution of Tyr904 by alanine (Y904A) or with an 11 amino acid deletion (R901X) is mis-targeted to the apical membrane of MDCK cells [12, 13]. The abnormal of kAE1 trafficking might due to loss of AP-1 mu1A binding. The previous study showed the medium chains (mu1 and mu2) of two clathrin-associated protein complexes (AP-1 and AP-2, respectively) specifically interacted with tyrosine-based signals of several integral membrane proteins [27]. The “dileucine” motif DxxxLL was reported to interact with the beta1 subunit of AP1, not mu1 [30]. However, the dileucine motif may also bind indirectly to the mu subunit via an “adaptor” protein [31, 32]. Thus, there are at least two physically separate binding sites for sorting signals on kAE1 in associated to AP-1 mu1A, the first, and

most common, contains a critical tyrosine residue, and members of this group mostly conform to the consensus sequence YXXØ, the second is the “dileucine” motif.

The mu1A protein is the medium subunit of the ubiquitous adaptor complex protein AP-1A, which interacts with membrane proteins to be directed to their final compartment. AP-1A has been involved in the trafficking of the cation-independent mannose-6-phosphate receptor (CI-M6PR) between the TGN and endosomes [33]. AP-1 is required for the transport of sortilin to the endosomes, while RNAi depletion of AP-1 causes accumulation of sortilin in the Golgi apparatus [34]. As AP-1 mu1A interacts with kAE1, we next examine whether AP-1 mu1A is necessary for kAE1 targeting in HEK293T cells. Knocking down of endogenous AP-1 mu1A by siRNA showed the accumulation of kAE1 in cytoplasm of HEK 293T cells.

In conclusion, we identify a novel binding protein of kAE1 which is AP-1 mu1A and identify the amino acid motif as an essential sequence of kAE1 for their interaction. We also demonstrate a critical role for AP-1 mu1A in the targeting of kAE1. Since dRTA mutations are carboxyl-terminal truncations of kAE1 protein and at least one of these two mutants is mis-targeted to the apical instead of basolateral membrane, we showed that the AP-1 mu1A interacts with kAE1 and plays an important role for the targeting of kAE1 to the cell membrane. Understanding of the molecular mechanisms overriding the trafficking of kAE1 to the basolateral membrane will explore new strategies for the treatment of this renal disease.

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Figure legends

Fig. 1. (A) Schematic diagram of the identified AP-1 mu1A fragment. Top bar represents full-length mu1A peptide sequence. Middle bar is the C-terminal two-thirds of mu1A reported to bind to a tyrosine-based sorting signal (YQRL) [19]. Bottom bar is the mu1A fragment identified by yeast two-hybrid screen in this study. (B) Specificity test of the interaction between AP-1 mu1A and Ct-kAE1. Positive interaction between AP-1 mu1A and Ct-kAE1 was confirmed by growth of yeast diploids on synthetic dropout [21]/-Trp-Leu-His-Ade medium and SD/-Trp–Leu-His-Ade/X-α-Gal agar plates (column B). +ve represents mated diploids between Y187 harboring pGBKT7-p53 and AH109 containing pTD1-SV40-T Ag. -ve represents mated diploids between Y187 harboring pGBKT7-laminC and AH109 containing pTD1-SV40-T Ag. B, V, p53, and lamC are mated diploids between AH109 harboring prey plasmid with AP-1 mu1A sequence and Y187 containing pGBKT7-Ct-kAE1 (bait plasmid), pGBKT (empty vector), pGBKT7-p53 (unrelated gene), or pGBKT7-laminC (unrelated gene), respectively.

Fig. 2. GST pull-down assay of kAE1 and AP-1 mu1A. (A) GST pull-down of Ct-kAE1 and His-AP-1 mu1A which was detected using anti-His by immunobloting. (B) Co-immunoprecipitation of kAE1 and AP-1 mu1A in HEK-293T cells. Lysate from single-transfected or co-transfected HEK-293T cells were incubated with anti-His antibody to immunoprecipitate His-AP-1 mu1A and associated complexes. Input and immunoprecipitated samples were resolved on SDS-PAGE and analyzed by Western blot. Lane 3 illustrated the co-isolation of kAE1 with His-AP-1 mu1A. The presence of co-immunoprecipitated kAE1 was detected with anti-Ct-kAE1 antibody. Lanes 1, 2 and 5 are cell lysate inputs. Lanes 3, 4 and 6 are immunoprecipitated samples. (C) Affinity co-purification of kAE1 and AP-1 mu1A in HEK-293T cells. Lysate from single-transfected or co-transfected HEK-293T cells were incubated with Co^{2+} beads. Input and affinity-purified samples were resolved on SDS-PAGE and analyzed

by Western blotting. The presence of co-purified kAE1 with His-AP-1 mu1A was shown in lane 3. Anti-Ct-kAE1 antibody was used to detect kAE1. Lanes 1, 2 and 5 are cell lysate inputs. Lanes 3, 4 and 6 are purified samples.

Fig. 3. The interaction between kAE1 and AP-1 mu1A in HEK 293T cells examined by yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA). Transfections of the cells with YFP[1]-AE1+YFP[2]-AP-1 mu1A or YFP[2]-AE1+YFP[1]-AP-1 mu1A (H and I) show intracellular yellow fluorescent signals indicating their interactions. Single transfection (A, B, D, and E) cannot detect signal while transfection of YFP[1]-AE1+YFP[2]-AE1 (C) which is positive control show yellow fluorescent signal.

Fig. 4. Subcellular co-localization of kAE1 and AP-1 mu1A in HEK-293T cells. HEK-293T cells were single-transfected with pcDNA-kAE1 (A) or pcDNA3.1/His-AP-1 mu1A (B) or co-transfected with pcDNA-kAE1 and pcDNA3.1/His-AP1 mu1A (C-H). Representative images are shown. Co-localization of kAE1 and His-AP-1 mu1A at the plasma membrane marked by yellow color was demonstrated in the merged pictures (E, H). F-H are enlarged images from insets in C-E. pcDNA3.1/His empty vector show no background staining (data not shown).

Fig. 5. Endogenous AP-1 mu1A suppression by siAP-1 mu1A. HEK 293T cells were transfected with siAP-1 mu1A or siControl using Lipofectamine 2000. (A) Expression of AP-1 mu1A mRNA by real-time PCR. Relative AP-1 mu1A expression levels (% of parental) after siRNAtransfection for 24 h, 48 h, 72 and 96 h. Results are the mean $\pm$ SE (bars) of three independent experiments. *$P < 0.05$ compared with parental cells. (B) Expression of AP-1 mu1A protein using Western blotting after siRNAtransfection for 24 h, 48 h, 72 and 96 h.

Fig. 6. Subcellular co-localization of kAE1 and AP-1 mu1A in HEK-293T cells by confocal microscopy. HEK-293T cells were single-transfected with HA-kAE1 (A-C) or co-transfected with siControl (D- F) and si AP-1 mu1A (G- I). The presence of HA-kAE1 and AP-1 mu1A were detected with anti-HA antibody (red) and anti AP-1 mu1A (green), respectively.

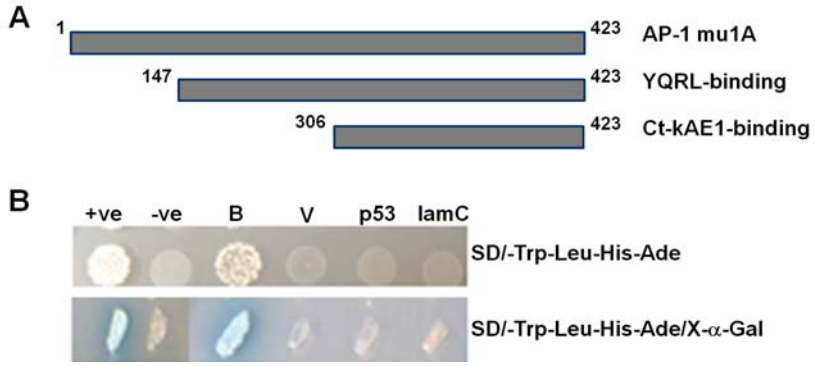
A
1
423
AP-1 mu1A
147
423
YQRL-binding
306
423
Ct-kAE1-binding
B
+ve
-ve
B
V
p53
lamC
SD/-Trp-Leu-His-Ade
SD/-Trp-Leu-His-Ade/X-α-Gal

Fig. 1

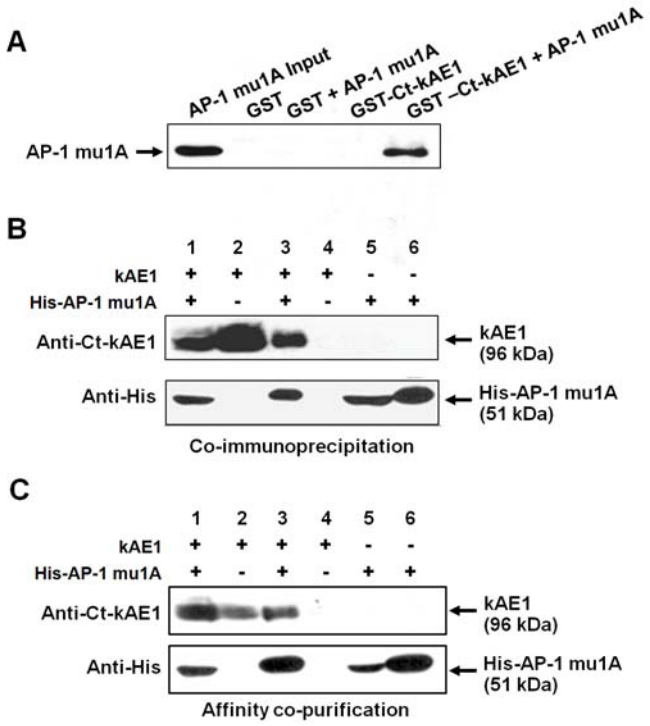
A
AP-1 mu1A Input
GST
GST + AP-1 mu1A
GST-Ct-kAE1
GST –Ct-kAE1 + AP-1 mu1A
AP-1 mu1A
B
1 2 3 4 5 6
kAE1 + + + + - -
His-AP-1 mu1A + - + - + +
Anti-Ct-kAE1
kAE1 (96 kDa)
Anti-His
His-AP-1 mu1A (51 kDa)
Co-immunoprecipitation
C
1 2 3 4 5 6
kAE1 + + + + - -
His-AP-1 mu1A + - + - + +
Anti-Ct-kAE1
kAE1 (96 kDa)
Anti-His
His-AP-1 mu1A (51 kDa)
Affinity co-purification

Fig.2

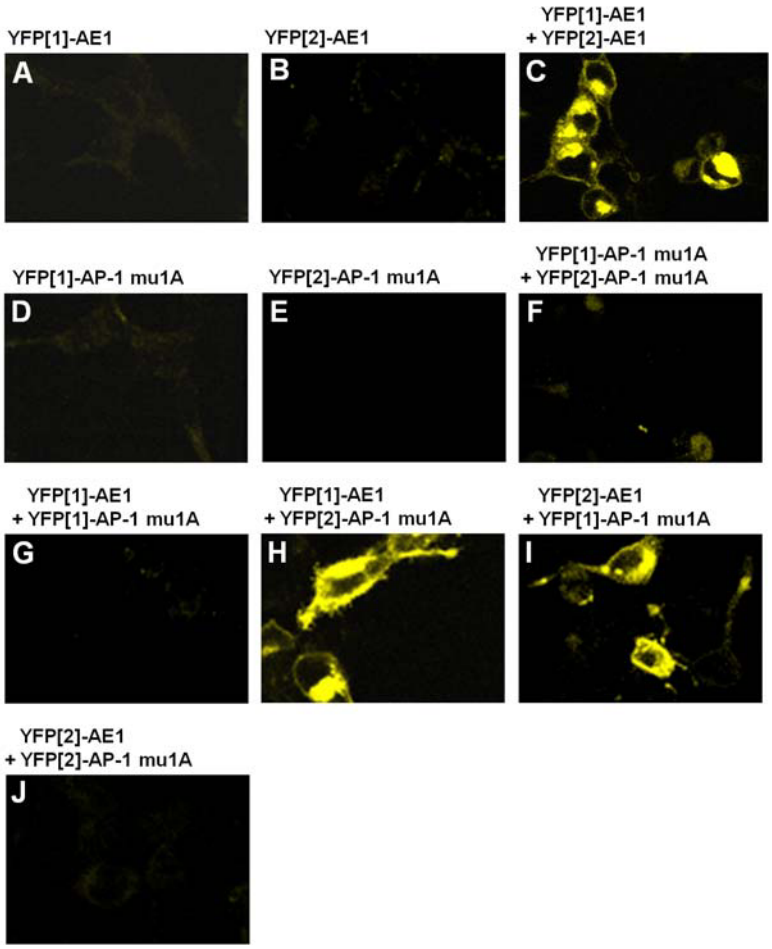
YFP[1]-AE1
A
YFP[2]-AE1
B
YFP[1]-AE1
+ YFP[2]-AE1
C
YFP[1]-AP-1 mu1A
D
YFP[2]-AP-1 mu1A
E
YFP[1]-AP-1 mu1A
+ YFP[2]-AP-1 mu1A
F
YFP[1]-AE1
+ YFP[1]-AP-1 mu1A
G
YFP[1]-AE1
+ YFP[2]-AP-1 mu1A
H
YFP[2]-AE1
+ YFP[1]-AP-1 mu1A
I
YFP[2]-AE1
+ YFP[2]-AP-1 mu1A
J

Fig. 3

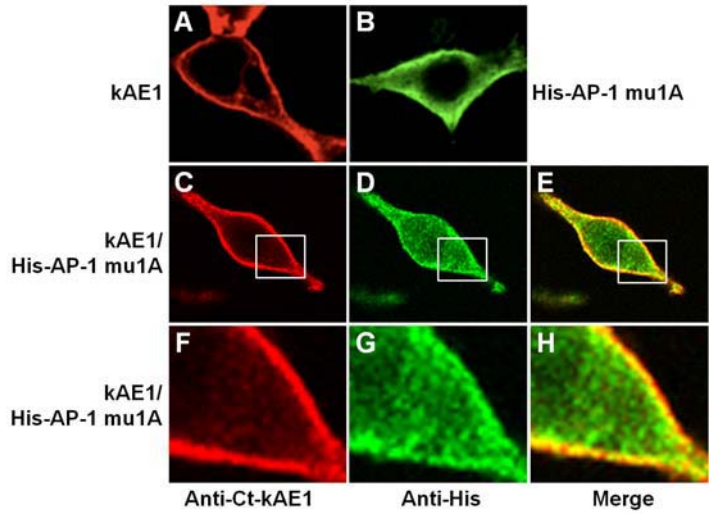
A
B
kAE1
His-AP-1 mu1A
C
D
E
kAE1/
His-AP-1 mu1A
F
G
H
kAE1/
His-AP-1 mu1A
Anti-Ct-kAE1
Anti-His
Merge

Fig. 4

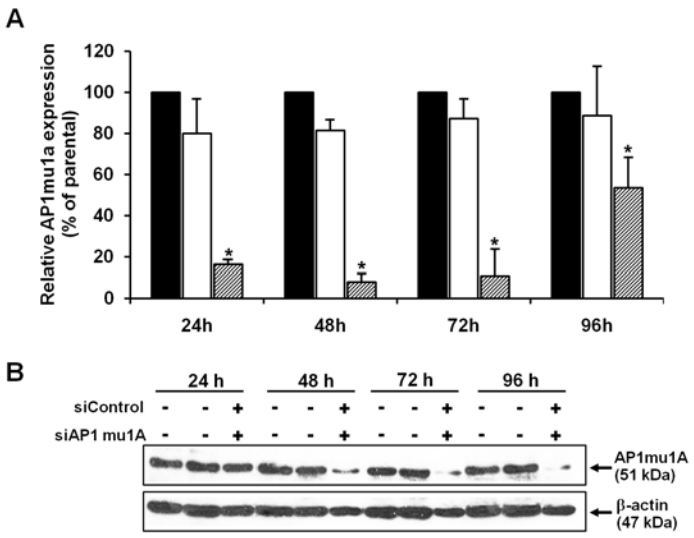
A
Relative AP1mu1a expression (% of parental)
120
100
80
60
40
20
0
24h
48h
72h
96h
B
24 h
48 h
72 h
96 h
siControl
siAP1 mu1A
AP1mu1A (51 kDa)
β-actin (47 kDa)

Fig. 5

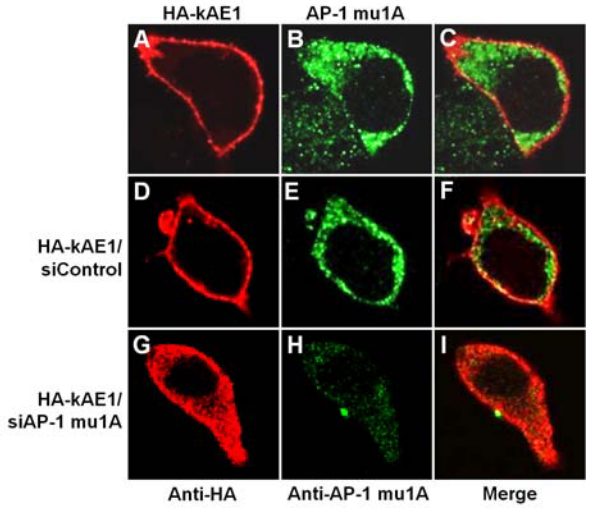
HA-kAE1
AP-1 mu1A
A
B
C
D
E
F
HA-kAE1/
siControl
G
H
I
HA-kAE1/
siAP-1 mu1A
Anti-HA
Anti-AP-1 mu1A
Merge

Fig.6

Appendix II

Publication in peer-reviewed national journals

Molecular Genetics and Genomics in Medicine

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E-journal: http://www.sirirajmedj.com

In the past few decades, a remarkable progression occurred in the field of Human Genetics – the discipline that provides essential knowledge to understand human biology in normal and abnormal conditions. This was importantly attributable to the strong impetus of the Human Genome Project (HGP), the internationally collaborative effort to sequence the whole human genome comprising about 3.2 gigabases (Gb) and to identify about 25,000 human genes. This project announced its success on April 14th, 2003[1] – two years ahead its original schedule which was coincidentally the 50th anniversary of Watson and Crick's discovery of the double helical structure of DNA[2].

Since the HGP produced enormous data of human and model-organism genomes, during the project **'Bioinformatics'** was originated from the necessity to manage, analyze, and understand the myriad amount of data using informatics tools. These data were deposited in public databases freely accessible via the World Wide Web. Before the end of HGP, the International Haplotype Mapping (HapMap) Project[3] – an effort to produce a genome-wide map of common human genetic variations with the aim to speed the search for genes that contribute to common diseases – was launched in November 2002. This project has successfully generated the data of over 3 million human single nucleotide polymorphisms (SNPs) from geographically diverse populations.[4,5]

The HGP has a great impact to the research and application in human molecular genetics, which finally leads to the origination of **'Genomics'** – a new discipline that studies functions and interactions of all the genes in the genome.[6] **Functional Genomics** is the division that involves the examination of global gene expression (transcriptome) and overall proteins (proteome) in the cells or their extracellular milieu. It is an extension to the understanding of genetic contributions to human health which gives rise to **'Genomic Medicine'**.[7,8] This new discipline not only provides an important insight into the biology of health and disease but also plays an increasingly important role in the development of new methods for prevention, diagnosis, monitoring, and treatment of diseases. It has started to fundamentally change the practice of medicine in the way that was not possible before and will revolutionize medicine in the 21st century.[6-9] The importance of the **'Genomic Revolution'** has been emphasized in two series of review articles recently published in The New England Journal of Medicine (November 2002 – September 2003) and the Mayo Clinic Proceeding (August 2002 – May 2004).

From Genetic to Genomic Medicine

The advances in molecular genetic techniques in the past few decades have assisted us to unravel more than one thousand monogenic (Mendelian) genetic disorders, which are caused by mutations of single genes. These diseases have a high-risk but segregate in rare families. Unlike monogenic disorders, common and complex genetic (previously classified as multifactorial) diseases result from susceptibility genes with only a minor individual effect on the disease *per se*. The diseases in this group are caused by interplay between multiple genes and environmental factors. As many of them run in families, they are thought to be inherited, but different from Mendelian disorders, since they do not show the definite inheritance patterns. The genetic variations contribute to susceptibility to these diseases causing increased individual's risk to diseases, but may not result in the diseases without environmental triggers. However, they are much more prevalent than monogenic diseases and are the focus of intense attention in current genetics and genomics research. The examples of diseases in this category include diabetes mellitus, hypertension, heart disease, cancer, autoimmunity, asthma, mental illness, neurodegenerative disorders, and many more.

Two main genetic approaches, linkage analysis and association study (Fig 1), have been complementarily used to detect the specific genetic regions or loci associated with the diseases, which can be used with candidate-gene and genome-wide studies. The known (parametric) or unknown (non-parametric) mode of inheritance may be applied in the linkage calculation. Linkage analysis is powerful for localization and identification of causative genes in monogenic disorders but it is less efficient for identification of susceptible genes in common and complex genetic diseases which occur

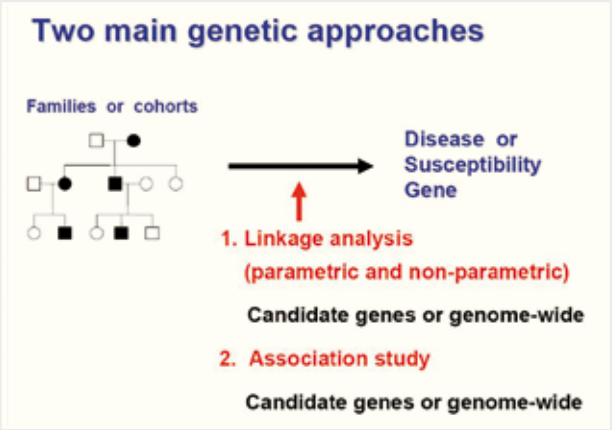

Fig 1. Two main genetic approaches, linkage analysis and association study, can be used for chromosomal localization and identification of disease or susceptibility gene.

from variations of several or many loci.

The availability of the human genome resources and the recent development of high-throughput genomic technologies (such as microarray) for simultaneous analyses of genomic variations, in combination with new analytical methods, have now made it possible to use a genome-wide association study (GWAS), which is the most powerful and efficient approach thus far, for identifying genetic variants associated with human common and complex genetic diseases (Fig 2).

GWAS became feasible because of the availability of large collections of cases and controls, in addition to the advancement in genetic technologies and statistical analysis methods. Recently, many common and complex genetic diseases and quantitative traits were successfully studied by GWAS and many more studies are in progress. At the initial stage of this technology, nearly 100 loci for nearly 40 common diseases and traits have been identified.[10] Publications on the use of GWAS for this group of diseases includes macular degeneration and exfoliative glaucoma, type 1 and type 2 diabetes, inflammatory bowel disease, prostate cancer, breast cancer, colorectal cancer, cardiovascular disease, neuropsychiatric conditions, autoimmune and infectious diseases, and others. These have provided new insights into disease etiologies and have suggested previously

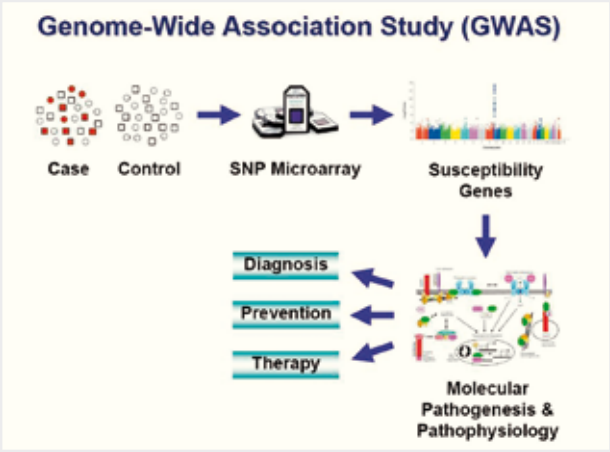

Fig 2. Genome-wide association study (GWAS) for identification of susceptibility genes in human common and complex genetic diseases.

unsuspected molecular pathophysiology pathways for common diseases useful for identifying new therapeutic targets and developing targeted interventions based on genetically defined risk.[10]

Genomic Revolution

Genomics makes its greatest contribution to medicine by revealing pathogenic and pathophysiologic mechanisms underlying the human common and complex diseases, which promisingly leads to the development of new approaches for prevention, diagnosis, and therapy.[7,8] The capability to rapidly analyze an individual's genomic sequence variation is useful for acquiring genetic information related to human health including susceptibility to diseases. Microarray-based technology (DNA chips) has currently been used for this rapid analysis. This information is important for determination of preventive measures for the disease that has genetic susceptibility, but has not yet occurred.

A better understanding of the multiple and interacting genetic components of disease pathogenesis and pathophysiology will make it possible to specifically design targeted therapies.[8,9] Moreover, it will be possible to customize drug and other treatments to accommodate both individual patient's inherited differences in the disease process and individual patient's genetic variations in their ability to metabolize drugs, which are the issues addressed by '**Pharmacogenomics**'. Genetic variations in the genes involved in drug metabolism, particularly the cytochrome P450 (CYP450) multigene family, encoding enzymes responsible for metabolizing most drugs used today, will affect their functions and patients' responses to the drug and the dose administered.[9] A rapid testing to determine individual patient's genotypes will guide treatment with the most effective drugs and reduce adverse reactions. This will lead to a paradigm shift from '**one-size-fits-all**' treatment to '**customized therapy**' and '**individualized medicine**'.

Genomic data and technologies will also accelerate the rate, reduce the cost, and increase the efficiency of new drug development. The targets of most drugs today are about 500 molecules.[9] The knowledge of genes involved in the disease process, pathophysiologic pathways, and response molecules will lead to the discovery of new drug targets. Ideally, the new drugs should aim at specific sites of cells and at a specific biochemical pathway or molecule implicated in the disease process and they should also cause fewer side effects than many current medicines.

Ethical and Societal Concerns

The promise of genomic revolution in medicine is unprecedented but this excitement is modest by public concerns regarding the potential misuse of genomic information and technologies. Several ethical, legal, and societal issues that must be addressed include the possibility of loss of personal confidentiality, social and employment discrimination, group stigmatization, and the more complicated issue of genomic determinism. From its first initiation, the HGP dedicated a proportion of about 5% of its funds toward identifying and addressing these issues arising from the availability of new information and capabilities.[9]

The incomplete understanding of the pathogenesis of common and complex genetic diseases leads to problems in the translation of genomic information into clinical

practice. The difficulties in identifying genetic susceptible loci and alleles of the diseases result in uncertainty in interpretation. Further research and technological development are still needed for many common and complex genetic diseases before the clinical applications will be possible. For the diseases that the clinical applications of genomic technologies are available, the provision of adequate counseling and support for individuals at risk is required for the presymptomatic screening.

To gain a full benefit and efficiently apply the genomic technologies, it is essential that the genomic knowledge and technological capabilities appropriate to each ethnic population and country or geographical area should be created and developed, because it has now been realized that different genomic variations causing the same diseases exist in each population. These appropriate genomic knowledge and technological capabilities should also be effectively transferred to the local health-care providers in each country by incorporating them into normal educational curriculums and training systems.

Future Trend

Routine generation of whole-genome sequences will greatly affect biomedical research and clinical care. The scientists who were involved in the HPG and who are studying genomics wish to have new technologies that can sequence the entire genome of any person for less than $1,000.[1] It has been predicted that within 5 years DNA sequencing technologies will be affordable enough that personal genomics will be integrated into routine clinical care.[11] On January 22, 2008, an international research consortium announced the '**1000 Genomes Project**', which is an effort to sequence the genomes of at least a thousand people from around the world to create the most detailed and medically useful picture of human genetic variation.[12,13] Personal genomes from two well-known scientists have recently been sequenced and reported.[14,15] When the technologies for whole-genome sequencing of any person become feasible, many benefits such as presymptomatic screening, genetic susceptibility and risk evaluation, disease prediction and prevention, and pharmacogenomic applications are anticipated. Other applications include ancestral tracing, personal identification and forensics, nutritional choice and advice, and reproductive assistance. The medical community may need to consider and provide guidelines for efficient routine use of personal genomic information, its effect to the health system, and ethical, legal, and social implications – in addition to those that have been addressed in the preceding projects. Two important questions concerning the genomic knowledge and technologies are: (*i*) how to efficiently and ethically use them, and (*ii*) how to make them economical and accessible, so they will be widely beneficial to most people.

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Molecular Genetics of Diabetes Mellitus

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Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1] DM is a threat to the world population and a global burden in the 21st century. Its prevalence in adults worldwide was estimated to be 4.0% in the year 1995 and will have risen to 5.4% by the year 2025. There will be a 42% increase, from 51 to 72 million people affected, in the developed countries and a 170% increase, from 84 to 228 million people affected, in the developing countries.[2] It was estimated in 2000 that 9.6% (2.4 million) of Thai adults were affected with DM and 5.4% (1.4 million) had impaired fasting glucose[3]. These have indicated that DM is an enormous global public health problem and also an increasingly significant public health problem in Thailand. Since the disease is very heterogeneous, abnormality at different biological pathways can lead to hyperglycemia and subtypes of the disease requiring precise diagnostic criteria. Several research studies have concentrated on identifications of diabetic susceptibility genes. These efforts facilitate a better understanding in molecular pathogeneses and pathophysiologies underlying DM subtypes, thereby leading to the development of appropriate and effective therapeutic approaches.

Classification and Pathogenesis of Diabetes Mellitus – Vital for Genetic studies

A major requirement for epidemiological and clinical research and for clinical management of diabetes is an appropriate system of classification that provides a framework within which to identify and differentiate its various forms and stages. DM generally presents in two major forms, type 1 diabetes (T1D) and type 2 diabetes (T2D). T2D is more common, accounting for approximately 90% of diabetic cases. The onset of T1D is in childhood while that of T2D is predominantly after 40 years of age and generally occurs in obese people. T1D (also autoimmune diabetes mellitus) is usually caused by autoimmune destruction of islet beta cells in the pancreas. The destruction of islet beta cells causes insulin deficiency and thereby dysregulation of anabolism and catabolism. T1D has a characteristic feature for most of the autoimmune diseases.[4] (i) It is associated with specific human leukocyte antigens (HLA) class II haplotypes (particularly HLA DRB1*04–DQB1*0302, but also HLA DRB1*03–DQB1*0201), (ii) autoantibodies against autoantigens of β-cells are found (GAD65, IA-2, ICA, IAA), and (*iii*) mononuclear cell infiltration of the pancreatic islets can be detected histologically. However, the exact mechanism involved in the initiation and progression of β cell destruction in T1D is still un clear.

While most T1D is characterized by the presence of autoantibodies identifying the autoimmune process that leads to β-cell destruction and insulin deficiency (Fig 1), T2D includes the most prevalent form of diabetes which results from insulin resistance or insulin secretory defect or both (Fig 2).[5] Generally, exogenous insulin is not required for the survival of T2D patients. This form of diabetes frequently goes undiagnosed for many years because hyperglycemia developed gradually. The earlier stage is often not severe enough to cause noticeable symptoms. Obesity is one of the principal risk factors for T2D. Weight gain leads to insulin resistance through several mechanisms. Insulin resistance places a greater

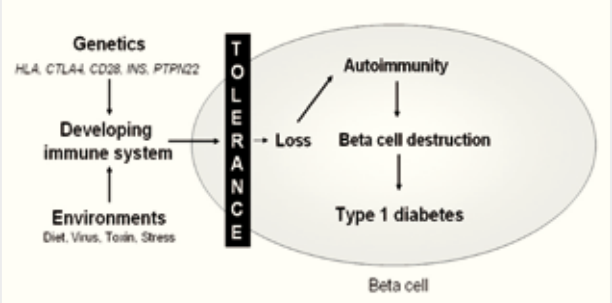

Fig 1. Pathogenesis of type 1 diabetes (T1D). Genetic predisposition and environmental factors are involved in failure of tolerance to β-cell (self) antigens and autoimmune de struction of pancreatic β-cells leading to T1D.

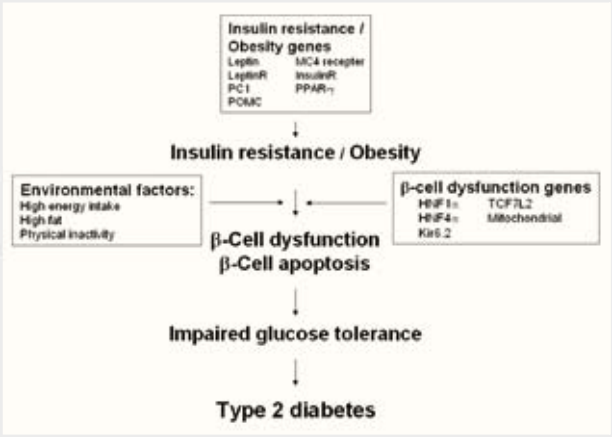

Fig 2. Pathogenesis of type 2 diabetes (T2D). The interaction of diabetic susceptible genes (causing insulin resistance, obesity, and β-cell defects) and environmental factors leads to relative insulin insufficiency. Impaired glucose tolerance and T2D are developed when pancreatic β-cells are unable to adequately compensate for the requirement of insulin in obesity and/or insulin resistance.

demand on the pancreatic capacity to produce insulin, which also declines with age, leading to the development of diabetes. Physical inactivity, both a cause and consequence of weight gain, also contributes to insulin resistance.[6,7]

Other specific types of DM, including several monogenic defects in β-cell function and a non-genetic form, account for approximately 1%-2% of cases. Maturity onset diabetes of the young (MODY) is one of monogenic form that is characterized by onset of hyperglycemia at an early age (generally before age 25). The term maturity-onset diabetes of the young was based on the old classification of diabetes into juvenile-onset and maturity-onset diabetes. The American Diabetes Association and the World Health Organization have introduced a revised, cause-based classification for diabetes. MODY is now defined as a genetic defect in β-cell function with subclassification according to the gene involved.[1] The characteristics used to differentiate T2D from T1D and MODY are shown in Table 1.

Type 1 Diabetes (T1D)

T1D is a complex and polygenic disease due to gene-environment interactions. The role of the genetic background of diabetes has been extensively investigated. However, final conclusions have not yet been reached. Family and twin studies have shown that genetic factors are important contributors to the disease risk. There is significant familial clustering of T1D with an average prevalence risk of 7% in siblings and 6% in a child of affected parents, compared to 0.4% in the general population. The degree of clustering of disease in families can be estimated from the relative risk (λs) which is the ratio between the prevalence of disease among the patients' siblings and the prevalence of the disease in the population. If this ratio (λs) is close to 1, there is no evidence for familial clustering. For T1D, the degree of familial clustering λs is about 15 (6%/0.4%), which means that if a family has a child with T1D, then the other siblings have a 15 times greater risk of developing diabetes than the general population.[8]

Although more than 18 T1D-predisposing genes have been reported to date, only the major histocompatibility complex (HLA) region on chromosome 6p21 (IDDM1) and the insulin (INS) gene on chromosome 11p15 (IDDM 2) have been conclusively associated with susceptibility to T1D. Alleles at the human leukocyte antigen (HLA) locus explain up to 50% of the familial clustering of T1D through a large variety of protective and predisposing haplotypes.[9]

However, it has recently been shown that there are other important loci associated with T1D but with much smaller effects than *HLA* and *insulin* (INS) genes. These genes encode protein tyrosine phosphatase (PTPN22), cytotoxic T-lymphocyte antigen 4 (CTLA4), interleukin-2 receptor A (IL2RA), interferon-induced helicase (IFIH1), C-type lectin domain family (CLEC-16A) and cytochrome P450, Subfamily XXVIIb, polypeptide 1 (CYP27B1)[10]. In addition, recent genome-wide association studies (GWAS) have uncovered two additional solidly replicated loci that, have not yet been mapped to individual genes (Table 2).

By identifying new loci and reanalysis of previous studies with the help of more powerful genetic analysis tools, it is highly likely to shed more light on the complex field of the genetics of T1D and to further understand the molecular pathophysiology of the disease, which will allow us eventually to treat or prevent it.

Type 2 Diabetes (T2D)

Clearly, genetic variants predispose an individual toward reduced insulin secretion or increased insulin resistance. Sequence variations of several candidate genes involved in insulin sensitivity, β-cell function and obesity were investigated by a genetic case-controlled association approach. More than 40 different genes have been reported to be associated with T2D but few have been replicated in additional populations.[9] Study of *peroxisome proliferators-activated receptor-gamma*

TABLE 1. Differentiation of MODY from T2D and T1D (adapted from Hattersley et al[30]).

Characteristics	T1D	T2D	MODY
Relative frequencies	90%	<10%	1-3%
Genetics	Polygenic	Polygenic	Monogenic
Clinical presentation onset	Acute	Variable	Variable
Age	Throughout childhood	Usually pubertal or later	Often postpubertal
Obesity	Same risk as general population	Common	Same risk as general population
Ketosis	Common	Rare	Rare
Antibodies to islets antigens	Positive ICA, GAD, IA2	Rare	No
Associated autoimmune disease	Yes	No	No
Parent with diabetes	2-4%	40-80%	90%

TABLE 2. T1D-susceptible loci (modified from Ounissi-Benkalha and Polychronakos[10]).

Locus	Gene	Odd ratio Het	Hom	Postulated mechanism
6p21 *(IDDM1)*	*HLA DR-DQ*	0.02-11.4	0.02-49.2	Present exogenous antigen processed by APCs, with some antigen specificity. Predisposing alleles might bind autoantigens poorly, compromising adaptive self tolerance.
	HLA-A	0.29-1.23		Present endogenously synthesize antigen (e.g. viral) by all cells.
	HLA-B	0.73-3.6		
11p15 *(IDDM2)*	*INS*	2.68	3.27	Modulation of thymic expression and central tolerance to insulin.
1p13	*PTPN22*	1.95	4.16	Moderates TCR signaling by dephosphorylation. Gain of function might inhibit proper development of tolerance.
2q31 *(IDDM12)*	*CTLA4*	1.14	1.5	Moderates T-cell activation. Functional effect of locus to be determined.
10p15	*IL2RA*	1.87	3.89	Modulation of the effect of IL2 on regulatory and/or effector T lymphocytes.
2q24	*IFIH1*	1.18	1.37	Triggers interferon response upon recognition of viral RNA. Might be involved in infectious etiology of T1D.
16p13	*CLEC16A*	1.29	1.42	Function unknown. Contains C-lectin and ITAM domains.
18q11	*PTPN22*	1.33	1.61	Phosphotyrosine phosphatase. Role likely similar to *PTPN2.*
12q24		1.24	1.74	Not mapped to specific gene.
12q13		1.31	1.58	Not mapped to specific gene.

(*PPARγ*), which encodes a transcription factor and plays a central role in adipocyte development, is the most widely reproduced association.[11] The Pro12Ala polymorphism in *PPARγ* had been conclusively associated with common T2D.[12] Strong candidate genes for T2D are *ATP-binding cassette, sub-family C, member 8 (ABCC8)* encoding sulfonylurea receptor (SUR1), a drug target for an oral hypoglycemic agent − sulfonylurea, and *potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11)* encoding Kir6.2, the gene in the same region of chromosome 11. Both proteins interact physically and functionally to regulate the potassium inward rectifier current and β-cell depolarization, the trigger for insulin release.[13]

Several T2D genome-wide association studies (GWAS) were reported in early 2007, initially in Europeans and later in more diverse populations. The combined data of the traditional candidate-gene approach together with recent information from five GWAS, as well as a partial meta-analysis that encompasses three of them (Table 3) have confirmed known T2D loci and introduced at least six novel diabetes genes. Of note, *TCF7L2* has been replicated as the genetic locus with the strongest signal. The first GWAS was performed in a French population and reported in early 2007.[14] It confirmed the impact of *TCF7L2* as a T2D susceptibility locus and also identified hematopoietically expressed homeobox *(HHEX)* and solute carrier family 30 member 8 *(SLC30A8)* as potential novel T2D loci. *HHEX* is thought to be involved in β cell development or function. SLC30A8, a zinc transporter gene initially cloned and sequenced in 2004, is expressed exclusively in pancreatic β cells, where it transports zinc from the cytoplasm into insulin secretory vesicles. This is a critical step in the final biosynthetic pathway of insulin production and secretion. Following this initial report, four GWAS were published at the same time. The deCODE group and collaborators[15] confirmed the strong signal of TCF7L2 and replicated the HHEX and SCL30A8 findings. The novel T2D susceptibility loci, CDK5 regulatory subunit associated protein 1−like 1 *(CDKAL1)* was identified in this study. This gene affects CDK5/CDK5R1 activity, and in so doing, may lead to β cell degeneration. The Diabetes Genetics Initiative (DGI)[16], the Wellcome Trust Case Control Consortium (WTCCC)[17], and the Finland−United States Investigation of NIDDM Genetics (FUSION)[18], studies represent, in aggregation, more than 32,000 individuals which confirmed the impact of *TCF7L2, KCNJ11, PPARG* loci as well as *HHEX, SCL30A8* and *CDKAL1* on the risk of development of T2D. These studies also discovered the novel diabetes loci; insulin-like growth factor 2 mRNA binding protein 2 *(IGF2BP2)* and cyclin-dependent kinase inhibitor 2B *(CDKN2B).*

Monogenic Diabetes

Some of the most compelling evidence that genetic variation can cause glycemic dysregulation comes from the clinical and genetic description of monogenic diabetes, including maturity-onset diabetes of the young (MODY), insulin resistance syndromes, mitochondrial diabetes, and neonatal diabetes. Although uncommon, these diabetic subtypes provide a framework for understanding and investigating the complex genetics underlying T2D.

Maturity-Onset Diabetes of the Young

The well-defined mode of inheritance with high penetrance and the early age of onset of diabetes allow the collection of multigenerational pedigrees, making MODY an attractive model for genetic studies. MODY is not a single entity but is a heterogeneous disease with regard to genetic, metabolic, and clinical features. The underlying pathophysiology of MODY is an insulin secretion defect usually without insulin resistance.[19] The concerted application of modern genetic linkage and positional cloning techniques led to the successive identification of six MODY genes including hepatocyte nuclear *factor-4α (HNF-4α), glucokinase (GCK), hepatocyte nuclear factor-1α (HNF-1α), insulin promotor factor-1 (IPF-1), hepatcyte nuclear factor-1β, (HNF-1β), and neurogenic dfferentiation 1/β-cell E-box transactivat or 2 (NeuroD 1/β 2).* Moreover, additional MODY genes remain to be discovered, since there are MODY fami-

lies which diabetes does not cosegregate with the known MODY loci, referred to as MODY-X.

The prevalence of MODY is estimated at less than 5% of patients who have T2D in most white populations. The prevalence of different subtypes of MODY varies greatly as demonstrated from studies in the British, French, German, and Spanish family cohorts. The estimated prevalence of MODY-X is 15-20% in European families[20] and 60-80% in Chinese[21] and Japanese families[22]. Analysis of genetic variability of MODY genes in Thai diabetic patients performed by Siriraj Diabetes Research Group (SiDRG) showed that sequence variations of the six known MODY genes account for a small proportion of both classic MODY (19%) and early-onset type 2 diabetes patients (10%) suggesting that MODY-X is also frequent in the Thai population.

Insulin Resistance Syndromes and Lipodystrophies

These syndromes are characterized biochemically by hyperinsulinemia and insulin resistance in fat, muscle, and liver. The insulin receptor was a logical initial candidate gene for the severe hyperinsulinemia described in index patients. The most severe form of insulin resistance is Donahue syndrome or leprechaunism, named for the dysmorphic appearance of the affected infants. The Rabson-Mendenall syndrome is characterized by pineal hyperplasia, acanthosis nigricans, accelerated growth, and dental dysplasia. Mutations of the insulin receptor gene allow incomplete receptor activation. The lipodystrophies are characterized by dysregulation of fat storage. Adipose tissue is deposited inappropriately in muscle and liver rather than the usual subcutaneous compartment.[23]

Mitochondrial Diabetes

Mitochondrial diabetes is another rare variant of monogenic diabetes. Maternally inherited diabetes and deafness (MIDD) is caused by an alanine-to-guanine mutation in the gene encoding the tRNA for leucine. This defect leads to ineffective oxidative phosphorylation.[23]

Neonatal Diabetes

Neonatal diabetes, which usually presents in the initial days or months of life, can be transient (resolving at a median of 12 weeks) or permanent.

TABLE 3. Genetic loci associated with T2D that have achieved genome wide significance (modified from Moore and Florez23).

Marker (location)	Nearest gene	Function	Risk allele	Odd Ratio (p-value)					
				Sladek *et al.* (n=6,794)	deCODE (n=10,056)	DGI (n=13,781)	WTCCC (n=13,965)	FUSION (n=4,808)	Meta-analysis* (n=32,554)
Previously known									
rs7903146 (Intron)	*TCF7L2*	Transcription factor; risk allele impairs insulin secretion	T	1.65 (3.3×10^{-10})	1.38 (1.9×10^{-10})	13.8 (2.3×10^{-31})	1.37 (6.7×10^{-13})	1.34 (1.4×10^{-8})	1.37 (1.0×10^{-48})
rs5219 (Exon)	*KCNJ11*	Kir6.2 potassium channel; risk allele impairs insulin secretion	T	1.34 (0.074)		1.15 (1.0×10^{-7})	1.15 (1.3×10^{-3})	1.11 (0.014)	1.14 (6.7×10^{-11})
rs1801282 (Exon)	*PPARG*	Nuclear hormone receptor; target for thiazolidinediones	C	1.22 (0.11)		1.09 (0.019)	1.23 (1.3×10^{-4})	1.20 (1.4×10^{-3})	1.14 (1.7×10^{-6})
Identified by GWAS									
rs4402960 (Intron)	*IGF2BP2*	Growth factor binding protein; pancreatic development	T			1.17 (1.7×10^{-9})	1.11 (1.6×10^{-4})	1.18 (2.4×10^{-4})	1.14 (8.9×10^{-16})
rs10811661 (Intergenic)	*CDKN2B*	Cyclin dependent kinase inhibitor and p15 tumor suppressor; islet development	T			1.20 (5.4×10^{-8})	1.19 (4.9×10^{-7})	1.20 (2.2×10^{-3})	1.2 (7.8×10^{-15})
rs7754840 (Intron)	*CDKAL1*	Homologous to CDD5RAP1, CDK5 inhibitor; islet glucotoxicity sensor	C	1.2 (7.7×10^{-9})		1.08 (2.4×10^{-3})	1.16 (1.3×10^{-8})	1.12 (9.5×10^{-3})	1.12 (4.1×10^{-11})
rs1111875 (Intergenic)	*HHEX*	Pancreatic transcription factor; pancreatic development	C	1.21 (8.6×10^{-6})	1.17 (0.001)	1.14 (1.7×10^{-4})	1.13 (4.6×10^{-6})	1.10 (0.025)	1.13 (5.7×10^{-10})
rs13266634 (Exon)	*SLC30A8*	Beta cell zinc transporter ZnT8; insulin biosynthesis	C	1.18 ($5.0 \times 10\text{-}7$)	1.19 (0.001)	1.07 (0.047)	1.12 (7.0×10^{-5})	1.18 (7.0×10^{-5})	1.12 (5.3×10^{-8})

*Meta-analysis of DGI, WTCCC and FUSION

Most cases of transient neonatal diabetes mellitus (TNDM) result from imprinting mutations of the *ZAC* and *HYMAI* genes on chromosome 6q24. This type of neonatal diabetes usually remits in childhood but is associated with an increased risk of T2D in adulthood. Permanent neonatal diabetes mellitus (PNDM), traditionally treated with insulin, has recently been shown to be associated with activating mutations in the *KCNJ11* gene, which encodes the islet ATP-sensitive potassium channel Kir6.2. The severity of the mutation correlates with the clinical phenotype—some KCNJ11 defects result in PNDM, and even more severe genetic mutations result in the DEND syndrome (developmental delay, epilepsy, neonatal diabetes). Mutations in this gene appear to be the most common cause of neonatal diabetes and account for about one third of PNDM cases.

Genetics of Diabetic Complications

A principal objective of the clinical management of diabetes is the prevention of long-term vascular complications. The complications of diabetes can be broadly categorized as microvascular (including retinopathy and nephropathy) and macrovascular (affecting the coronary, cerebral, and peripheral arterial vasculature). These complications can result in potential loss of vision, renal failure, coronary heart disease, stroke, foot ulcers, amputation, Charcot joints, and autonomic neuropathy. These complications are common to both T1D and T2D.

Not only diabetes but also their complications are influenced by genetic factors. There is mounting evidence for the role of genetic factors in several diabetic complications, particularly diabetic nephropathy (DN). DN is the most common cause of end-stage renal disease (ESRD) and accounts for a significant increase in morbidity and mortality in patients with diabetes. The familial clustering of overt DN and diabetic ESRD has been observed widely in multiple racial and ethnic groups, with the earliest reports of familial aggregation of diabetic kidney disease in patients with T1D. Family members with diabetes, even in the absence of clinical nephropathy, demonstrate similar patterns of glomerular involvement.

Accelerated atherosclerosis is a hallmark of macrovascular disease in both T1D and T2D. Although the pathophysiology of atherosclerosis in T1D has not been fully elucidated, the current concept supports a model in which circulating factors associated with the perturbed metabolic milieu of T1D (e.g. hyperglycaemia, glycation, and oxidation products) cause endothelial dysfunction, which in turn leads to vasoconstrictive, proinflammatory, and pro-thrombotic changes that contribute to atherosclerotic plaque development and an enhanced potential for thrombosis after plaque rupture.[24]

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes. The evaluation of retinopathy enables clinicians a unique opportunity to directly visualize and assess the actual morphology of diabetic microvascular damage. Extensive studies have shown that people with diabetic retinopathy have excess risks of systemic vascular complications, including subclinical and clinical stroke, coronary heart disease, heart failure, and nephropathy. There is also emerging evidence which suggests that diabetic retinopathy may share common genetic linkages with systemic vascular complications.[25]

Hundreds of loci have been studied so far in order to explain genetic susceptibility to diabetic complications. Most loci identified to date have not been replicated probably due to the complex etiologies of all diabetic complications. Recent information indicated that the most intriguing genes are those encoding aldose receptor, advanced glycation end products receptor, vascular endothelial growth factor, intercellular adhesion-molecule 1, β3-adrenergic receptor gene, hemochromatosis, reductase and α2β1 integrin, which may represent a fruitful area for further genetics studies of DN and DR.[26,27]

Recently, advanced glycation end-products (AGEs) have been shown to induce oxidative stress, increase inflammation by promoting NFkB activation, and enhance extracellular matrix accumulation. These biological effects translate to accelerated plaque formation in diabetes as well as increased cardiac fibrosis with consequent effects on cardiac function.[28] Several studies suggested that inflammation would be an essential component of T2D and its complications. An increased systemic inflammation in high glucose milieu is important in the pathogenesis of coronary artery disease (CAD) in patients with T2D. Recently, the role of genetic variability of *A20/TNFAIP3* has been show to modulate the CAD risk in T2D, which was mediated by allelic differences in *A20* expression.[29] The understanding of genetic factors predisposing to diabetic complications would help to unveil their pathogenesis and may lead to the development of novel preventive measures.

CONCLUSION

Diabetes mellitus affects more than 150 million people worldwide, and this number is expected to double within the next two decades. The genetic susceptibility to the disease and an individual genetic signature remain incompletely understood. However, during the past decade, genetic analysis of DM and its complications have been extremely productive. Multiple genes have been identified especially through a series of genome-wide association studies (GWAS). It is of important that results from various genetic studies must be replicated in different populations in the validation of individual findings. Demonstration of functional modifications of the encoded variant proteins will also be an important evidence to support identified susceptible genes – the step following GWAS. Understanding the complex interactions among genetic profiles, individual lifestyles, and environmental factors lies at the core of effective diabetes treatment. The successful means to identify the disease at an early stage, changes of life-style, and dietary behavior are important for prevention and control of the disease. It is expected that characterization of the genetic factors involved in the development of DM and its complications will lead to the understanding of their molecular pathogenesis and the development of novel therapeutic approaches.

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Molecular genetics of monogenetic beta-cell diabetes

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ABSTRACT

Monogenic β-cell diabetes – a rare form of diabetes mellitus (DM) is caused by defects in a group of genes controlling pancreatic β-cell development and function. The diabetic symptoms are manifested within a short period after birth as neonatal diabetes mellitus (NDM), in childhood or early adulthood as maturity-onset diabetes of the young (MODY) and mitochondrial diabetes. Several etiologic genes for this form of DM have been identified in many patients. The common etiologic genes encode β-cell transcription factors and proteins involving in glucose-stimulated insulin secretion. Owning to their nature of genetic heterogeneity, monogenic β-cell diabetes presents the characteristics of variable age at onset, degree of severity, and occurrence of diabetic complications. The study of this form of diabetes has provided new knowledge and a better insight into the molecular mechanism controlling normal and pathological states of β-cells as reviewed in this article.

Keywords: Monogenic diabetes, β-cell dysfunction, neonatal diabetes mellitus, maturity-onset diabetes of the young, early-onset type 2 diabetes, mitochondrial diabetes, transcription factor, hepatocyte nuclear factor

INTRODUCTION

Diabetes mellitus (DM) is a group of common metabolic disorder that at present affects over 171 millions people worldwide. The disease is characterized by chronic hyperglycemia resulted from b-cell defects in insulin secretion, defects in insulin action, or combination of both. DM is generally recognized as a complex or multifactorial disease in which several genetic abnormalities together with environmental triggering are required for its development. However, 1%-5% of cases with DM are caused by single-gene (monogenic) defects, of which the genes controlling β-cell function is predominant. The monogenic β-cell diabetes is also found to be heterogeneous, comprising neonatal diabetes mellitus (NDM), maturity-onset diabetes of the young (MODY), and mitochondrial diabetes. Among these, MODY is the most intensively investigated. Currently, six different genes have been

identified to be responsible for MODY. While most of them encode transcription factors required for β-cell development and function, one encodes glucokinase – an enzyme in a rate-limiting step of glycolysis. Extensively heterogeneous clinical manifestrations of MODY are attributable to defects of distinct genes. While MODY is usually developed in childhood or young adult, the onset of NDM is at an early infancy. The most common causes of NDM are defects in genes encoding molecule involved in insulin secretion but a few cases are caused by mutations in the genes encoding transcription factors that are required for the β-cell development. Defects in some particular genes can cause either NDM or MODY. Mutation in mitochondrial DNA associated with DM is rare and can be differentiated from MODY by the presence of maternal transmission in conjunction with deafness. Due to their extensive heterogeneity in clinical presentations, it has recently been suggested that the terms of MODY and NDM are obsolete and new terminologies based on molecular genetic classification are proposed (Murphy *et al.*, 2008). The study of 'monogenic β-cell diabetes' has provided a better understanding in the etiology of β-cell dysfunction, which is also involved in other subtypes of DM. Knowledge of the molecular pathology of diabetes is required for appropriate treatment, prediction of disease progression, family-member screening and genetic counseling. Thus, the study into molecular genetics and pathophysiology of monogenic β-cell diabetes as well as other subtypes of DM will not only fulfill an image of complex biological networks maintaining glucose homeostasis but also lead to development of novel methods for therapeutic management.

Monogenic β-cell diabetes

Monogenic β-cell diabetes is caused by defects of single genes critically responsible for pancreatic β-cell development or function. The patients with monogenic β-cell diabetes may develop the disease since childhood, similar to type 1 diabetes (T1D), or they may develop it later in early adulthood. It can be differentiated from T1D by the absence of islet autoantibodies – a marker for autoimmunity. A markedly obesity, insulin resistance and acanthosis nigrican (a skin condition characterized by dark thickened velvety patches), generally presented in type 2 diabetes (T2D), are not observed in the patients with monogenic β-cell diabetes. NDM and MODY are two main forms of monogenic β-cell diabetes. NDM is a rare disease occurred and diagnosed within six months after birth. The clinical manifestations may be transiently observed (transient neonatal diabetes, TND) or permanently appear throughout the life (permanent neonatal diabetes, PND). MODY is usually developed in childhood or young adulthood and occurred from defects of different genes. Because of distinct genetic etiologies, monogenic β-cell diabetes presents with heterogeneous clinical manifestations. Four broad categories are proposed (Murphy *et al.*, 2008) including (*i*) neonatal diabetes with the disease diagnosed within the first 6 months of life, (*ii*) familial diabetes with mild fasting hyperglycemia, (*iii*) familial and young onset diabetes, and (*iv*) diabetes with extrapancreatic features. These categories provide more helpful guidance for clinical managements and new terminologies of different forms of monogenic β-cell diabetes also suggested.

Maturity-onset diabetes of the young

Definition and diagnostic criteria of MODY

Maturity-onset diabetes of the young (MODY) is originally described as an autosomal dominant inheritance of non-insulin dependent diabetes, now known as T2D, which is diagnosed before 25 years (Fajans *et al.*, 1960). However, according to a better understanding in its molecular etiology, MODY is now classified as a form of "other specific types of DM" which demonstrates monogenic defects in β-cell functions (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). The diagnostic criteria of MODY are as follows (Hattersley, 2003): *(i)* early onset of diabetes (usually less than 25 years), *(ii)* autosomal dominant

inheritance, *(iii)* rarely obese and non-ketotic diabetes, and *(iv)* diabetes results from β-cell dysfunction. The actual prevalence of MODY in general population is difficult to determine because its clinical phenotype is very heterogeneous and it is sometimes misdiagnosed as other types of DM. However, the studies in selected cohorts showed that it accounts for 1-5% of DM cases.

Genetic and clinical heterogeneity of MODY

Molecular genetics of MODY has been more progressively studied than other forms of DM. The autosomal dominant model of inheritance and the ability to collect large multi-generation pedigrees due to an early onset greatly advanced genetic study by linkage analysis. The studies by both candidate gene approach and positional cloning led to the identification of six different genes responsible for MODY (Table 1). These genes encode glucokinase enzyme (associated with MODY2) (Pearson *et al.*, 2001) and transcription factors expressed in pancreatic β-cells, including hepatocyte nuclear factor-4α (HNF-4α, MODY1) (Yamagata *et al.*, 1996a), hepatocyte nuclear factor-1α (HNF-1α, MODY3) (Yamagata *et al.*, 1996b), insulin promoter factor-1 (IPF-1, MODY4) (Stoffers *et al.*, 1997), hepatocyte nuclear factor-1β (HNF-1β, MODY5), and NeuroD1/BETA2 (MODY6) (Kristinsson *et al.*, 2001). The prevalence of each MODY subtype varies among ethnic groups. MODY2 are the most common cause of MODY in France, accounting for more than 60% of studied families, whereas its prevalence in United Kingdom and Germany were 11% and 8%, respectively. In general, MODY3 is most common in Caucasians but its prevalence varies from 21% to 64%. The other four types of MODY are rare. MODY with unknown genetic etiology (MODY-X) represents 16-45% of MODY cases in Caucasians and more than 90% of the cases in Asian populations.

Clinical phenotypes associated with defects of the six genes are distinct, requiring different treatments. Mild hyperglycemia in patients with *glucokinase* mutations (MODY2) is presented at birth and the patients are often asymptomatic at diagnosis. In addition, obesity, hypertension, dyslipidaemia and diabetic-associated complications

Table 1 Molecular genetics and clinical presentations of the different MODY subtypes (modified from Fajan *et al.*, 2000).

	MODY1	**MODY2**	**MODY3**	**MODY4**	**MODY5**	**MODY6**	**MODYX**
Gene	*HNF-4α*	*GCK*	*HNF-1α*	*IPF1*	*HNF-1β*	*NeuroD1/Beta2*	-
Gene locus	20q12-q13.1	7p15.3-p15.1	12q24.3	13q12.1	17cenq21.3	2q32	Heterogeneous?
Function	Orphan nuclear receptor	Glucose-phosphorylation enzyme	Homeodomain transcription factor	Homeodomain transcription factor	Homeodomain transcription factor	Homeodomain transcription factor	-
Distribution							
- Caucasians	2-4%	8-63%	21-64%	rare	0-1%	Rare	16-45%
- Asians	2%	2.5%	8%	0%	0.8-2%	0%	>90%
Diagnosis age	Post-pubertal	Childhood	Post-pubertal	Post-pubertal	Post-pubertal	Post-pubertal	Heterogeneous?
Primary defect	Pancreas/ liver	Pancreas/ liver	Pancreas/ Liver/kidney/	Pancreas/ others	Pancreas/liver/ Kidney/genitals	Pancreas/others	Pancreas/ Heterogeneous?
Associated phenotype	-	Low birth weight	Diminished renal glucose threshold	-	Renal morphologic abnormalities, renal insufficiency, pancreatic atrophy, genital abnormalities	-	-
Severity	Severe	Moderate	Severe	Moderate?	Severe	Severe?	Moderate/ Heterogenous?
Chronic complications	Frequent	Rare	Frequent	Rare	?	?	?

are uncommon. Patients carrying *HNF-4α* mutations (MODY1) exhibit a severe impairment in insulin secretion but may present mild diabetes. However, hyperglycemia tends to increase over time. Patients carrying *HNF-4α* mutations may exhibit the extra-pancreatic phenotypes; for instance, low serum levels of triglycerides, apoAII, apoCIII, and lipoprotein a (Shih *et al.*, 2000). Hyperglycemia in patients associated with HNF-1α (MODY3) is often symptomatic and progressive. Clinical features of MODY3 patients are highly variable from one family to another, or even within the same family. Both MODY1 and MODY3 patients are sensitive to hypoglycemic effect of sulfonylurea and insulin is required for treatment. Diabetic complications are frequently observed in MODY3 patients and some of them exhibited the extra-pancreatic phenotypes such as kidney dysfunction, renal tubulopathy, low serum concentration of apoM level, decreased renal reabsorption of glucose and glycosuria. Patients carried *HNF-1β* mutations (MODY5) is usually associated with disorder in other organs. These include renal dysfunction, pancreatic atrophy, abnormal liver function tests, familial glomerulocystic kidney disease, renal cysts, and genital malformations (Edghill *et al.*, 2006). The most common extra-pancreatic feature of *HNF-1β* mutations is renal cysts that, leads to a novel syndrome, namely "renal cysts and diabetes, RCAD". Clinical phenotypes of patients carrying *IPF1* (MODY4) and *NeuroD1* (MODY6) mutations are similar to other transcription factor-associated MODYs.

Pathophysiology of MODY

Five of six MODY subtypes are caused by mutations in the genes encoding transcription factors which are enriched in pancreatic β-cells (Fig. 1). The studies in knockout mice and humans indicated that these transcription factors coordinately play roles in embryonic development of pancreas and final differentiation to β-cells. In addition, they are involved in normal β-cell functions regulating gene expression in fully differentiated β-cells. *Insulin* and *glucose-transporter protein* (*GLUT2*) genes are important targets of their regulation. Insulin - a key hormone in maintaining glucose homeostasis is exclusively synthesized and secreted from pancreatic β-cells in response to glucose and other nutrient sensing. Once glucose is transported into the β-cell via a specific glucose-transporter protein (GLUT2) on β-cell cell membrane, it is catalyzed into glucose-6-phosphate by glucokinase, a rate-limiting enzyme in glycolysis pathway and associated with MODY2, before passing through the sequential steps of energy production. In turn, increasing of ATP and ADP ratio inhibits and closes the ATP-sensitive potassium channels, leading to depolarization of plasma membrane. As a result, membrane depolarization opens the voltage-dependent calcium channels. Increased intracellular calcium elicits movement of insulin-containing secretory vesicles to the plasma membrane and insulin is then secreted into the circulation (Fig. 1).

Hyperglycemia in MODY2 patients appears to result from a reduction in the activity of glucokinase which leads to decreased β-cell sensitivity to glucose. Since HNF-4α (MODY1) regulates genes involved in glucose transport and glycolysis (Stoffel *et al.*, 1997), the pathophysiology underlying MODY1 patients is described as an impairment of glucose-stimulated insulin secretion, similar to that of *glucokinase* mutations (MODY2). Because *HNF-1α* expression is regulated by HNF-4α, pathophysiology associated with *HNF-1α* mutations (MODY3) is occurred in the same manner. Not only in pancreas, but also in liver and kidney that HNFs play a role in tissue-specific gene expression. Therefore, mutations in *HNF-1α* (MODY3), *HNF-4α* (MODY1) and *HNF-1β* (MODY5) are associated with abnormalities in liver and kidney functions. The understanding of pathophysiology associated with *IPF-1* (MODY4) is based on the information from a single family whose the proband was an infant with neonatal diabetes and exocrine pancreatic insufficiency resulting from pancreatic agenesis (Wright *et al.*, 1993). Due to its seldom occurrence, the molecular pathogenesis

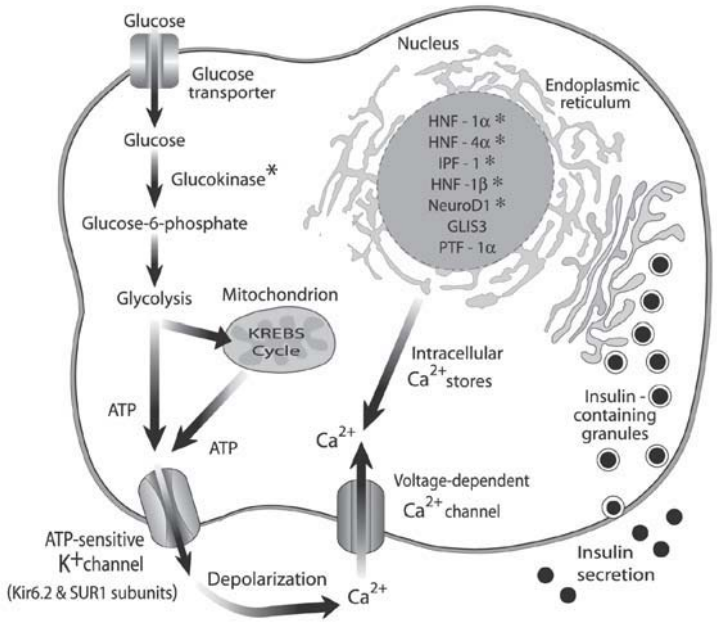


Figure 1 Schematic representation of the pancreatic β-cell and the proteins implicated in monogenic β-cell diabetes (modified from Fajan *et al.*, 2001). Proteins associated with MODY are marked with asterisk (*).

underlying mutation in *NeuroD1* (MODY6) is rarely examined.

Molecular pathology of MODY genes

MODY1 – *hepatocyte nuclear factor-4α* mutations

Hepatocyte nuclear factor-4α (*HNF-4A* or gene symbol *TCF14*) gene is located on chromosome 20q12-q13.1. This gene contains 13 exons with alternatively spliced exons 1A, 1B, 1C, and 1D to join with the sequence of exons 2-10 in RNA transcripts. Nine isoforms of HNF-4α are generated by two alternate P1 and P2 promoters, and by exonic splicing. The liver-specific P1 promoter drives the expression of transcripts HNF-4α1 to 6, while the pancreatic-specific P2 promoter regulates HNF-4α7 to 9. HNF-4α protein is composed of six domains, including A through F: A/B domain (amino acids 1-50), C domain (amino acids 50-116), D domain (amino acids 116-174), E domain (amino acids 174-370), and F domain (amino acids 370-465). HNF-4α is a liver-enriched transcription factor belongs to the nuclear receptor superfamily which normally expressed in liver, kidney, intestine, and pancreatic islets. It binds to DNA as a homodimer and activates the transcription of various target genes involved in embryogenesis, glucose and lipid metabolisms and glucose-stimulated insulin secretion (Lehto *et al.*, 1999). Its target genes include *insulin*, *GLUT2*, *aldolase B*, *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), *pyruvate kinase* (*PK*), *fatty acid binding proteins* (*Fabp*), and *cellular retinol binding protein* (*CRBP*).

Heterozygous mutations in *HNF-4α* gene are responsible for MODY1 (Yamagata *et al.*, 1996a), a rare MODY subtype accounting for 2-5% of MODY cases. Up to now, more than 40 mutations have been reported (Fig. 2) and genetic variations near the P1 and P2 promoters may be susceptible to late-onset T2D. *HNF-4α* mutations including missense, nonsense, insertion/deletions are found throughout the gene and balanced translocation of *HNF-4α* gene is also

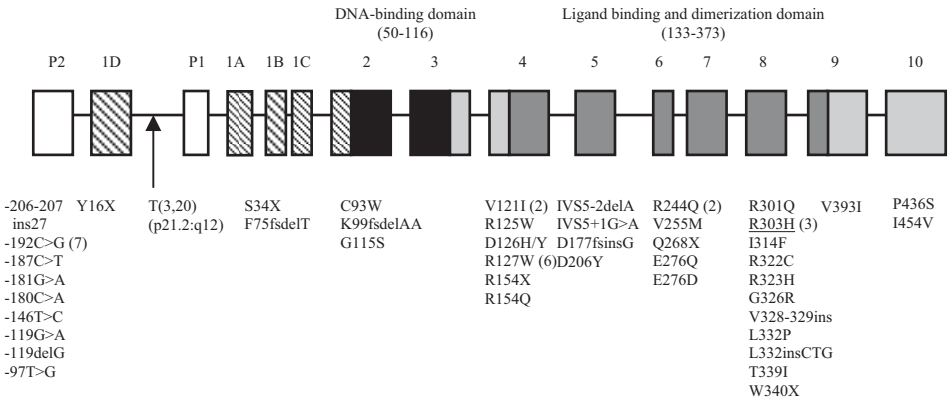


Figure 2 Mutations in *HNF-4α* gene. Location of *HNF-4α* mutations within the 10 exons, promoter, and splice sites of the gene. The functional domains of the HNF-4α protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets. Mutations with underlined were identified in Thai patients (modified from Yamagata *et al.*, 2003).

reported to cause MODY1. *HNF-4α* missense mutations are predominantly located in exon 8, encoding transactivation domain of the protein and affected the regions that are well conserved among species. Moreover, single nucleotide substitutions of P2, pancreatic β-cell promoter, were reported to be responsible for developing of late-onset T2D (Ek *et al.*, 2006).

MODY2 – *glucokinase* mutations

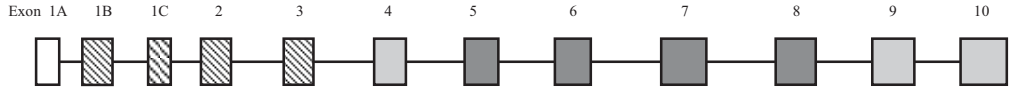
Glucokinase (*GCK*) gene is located on chromosome 7p15.3-p15.1, comprising 12 exons (spaning ~48,168 bp) and encoding glucokinase (or hexokinase IV) – a protein with 465 amino acids. GCK is expressed in pancreas, liver and brain. Three tissue-specific GCK isoforms are generated by using alternative promoters and transcription start sites. The isoforms in pancreatic β-cells and hepatocytes differ in their N-terminal sequences. GCK is a glycolytic enzyme that acts as a glucose sensor in pancreatic β-cells and plays important role in the regulation of insulin secretion. In turn, insulin can up-regulate the GCK expression in hepatocyte. Thus, β-cells can control glucose utilization in hepatocytes through the action of insulin that increases hepatic GCK concentrations.

Glucokinase was the first gene to be identified in MODY. MODY2 is the most common subtype in European Caucasians, particularly in French, Spanish, Italian, and is also common worldwide. In contrast, less than 5% of MODY2 were reported in Asian populations. Up to now, more than 210 different *GCK*-inactivating mutations causing MODY have been reported (Fig. 3). Missense, nonsense, frameshift, and splice site mutations have been identified and are distributed throughout the gene.

Homozygous *GCK* mutations result in a more severe phenotype, a complete deficiency of glucokinase, and are associated with permanent neonatal diabetes mellitus (PNDM). Moreover, heterozygous *GCK*-activating mutations cause familial hyperinsulinism and hypoglycemia (Glaser *et al.*, 1998).

MODY3 – *hepatocyte nuclear factor-1α* mutations

Hepatocyte nuclear factor-1α (*HNF-1α* or gene symbol *TCF1*) gene is located on chromosome 12q24.3, containing 10 exons, and encodes a protein with 631 amino acids. Using alternative splicing and polyadenylation sites, three isoforms (A, B and C) of HNF-1α protein are generated, differing in their



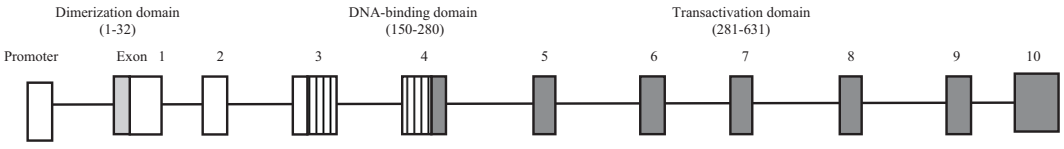
Exon 1A	1B	1C	2	3	4	5	6	7	8	9	10
IVS1A+1G>T	IVS1b+12	IVS2-7G>A (2)	IVS3+1G>A	IVS4+7C>T	IVS5-1G>A	IVS6+1G>A (2)	IVS7+5G>T	IVS8+1G>A	IVS9+1G>T	K420E
IVS1-2A>G	A>T	IVS2-8G>A	IVS3+1G>T	IVS4+2del5	G162D	IVS6+5G>A	IVS7-1G>A (2)	IVS8-2del29	IVS9+1G>C	S433_1436del
IVS1+1G>T		V16E	IVS3-2A>G	L122P (2)	L164F	IVS6+2T>A	G227-2A>T	IVS8-1G>C	IVS9-1G>C	C434Y
		E17fsX161	IVS3-1G>T	L122V	T168P	IVS6-2A>T	G227S	IVS8-31_-3del	S418-7del1	S441W (3)
		I19N	L77P	L122-1G>T	T168A	G193+1del33	G227C	E290X	S433_I436del	S445fsdel11
		L20P	D78H	F123L	K169N	G193R	T228A	G294D	E339X	G446R
		Q26X	D78E (2)	D128N	F171L	G193_D194ins	T228R	G295fsdelG	T342F	R447Q
		L30fsdelT	G80S	C129Y	A173S	-DKSFCASWQ	T228M (2)	Y297X	R358X	S453L
		L30P	G80A	I130T	G175R	V200L (2)	C233R	M298K (2)	P359L	S453X (2)
		R36W (3)	G80D	S131P	G175E	M202T	Y234X	G299R	S360X	A454V
		M37fsX7	G81S	V133A	G175V	V203A	M235T	E300Q	C364X	K459fsX610
		Q38P	T82I	L134P	A176S	T206M (5)	E237K	E300K	V367M	G464fsdelG
		K39fsX6 (2)	L88del10	H137R	G178E	T206R (2)	M238fsdelT (4)	R303W	R369Y	
		E40K	Q98X	H137D	N180K	T209M	Q239R	L304P	R369P	
		E40dup21	W99R	E138P	V181A	M210K	G246E	R308W (2)	R377H	
		R43H	W99X	H141fsdelC	V182M (2)	M210T	E248K	L309P	A378T	
		R43S (3)	V101M	L144P (2)	V182L (3)	S212F	E248X	V310fsdelGT	A379V (2)	
		G44S	T103N	T149P	R186X (5)	C213R (2)	M251I	L314fsdelC (2)	H380Q	
		G44D	Q106P	T149I	R186Q	Y214C	C252Y	L314P	H380D	
		G44fsdelG	Q106_M107	F150L	D187Y (3)	Y215X	C252R	G318R	C382Y	
		H50Y	-delinsL	F150S (2)	A188T (3)	E216X	C252G	G318A	S383L (3)	
		H50R	Y108H (5)	S151fsdelT	A188E	C220X	T255A	L324P	S383X	
		A53S	Y108C	CC	R191W (4)	E221K	E256K (3)	F334fsdel19	A384T	
		T60I	Y108X	H156Y	R191Q	C220X	E256D	S336L	G385V	
		Y61S	Y108F	E157K		E221K	W256R		A387V	
		Y61X	I110T	D158A		G223S (2)	W257fsinsT		A387T	
		V62M	P111L	K161N		M224T	G258C		V389A	
		V62A	T116P	K161+2del1		M224R	A259T		R392C	
		T65	A119D	0		I225M	G261R (5)		R392X	
		E70K (4)		K161+2del1		V226M	G261E		E395X	
		E70D		5		V226fsdelT	S263P (2)		E399X (2)	
		G72R (5)				-insAA	G264S		V401del1	
		D73G					E265K (3)		R403fsdelC	
							E265X		I404fsdelAT (2)	
							E268X		I404S	
							R275C		G407S	
							D278E		S411F (2)	
							E279X			
							E279Q			
							S281F			

Figure 3 Mutations in *GCK* gene. Location of *GCK* mutations within the 10 exons, and functional domains of the GCK protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets (modified from Gloyn *et al.*, 2003).

tissue distribution patterns. HNF-1α protein is composed of three functional domains: N-terminal dimerization domain (amino acids 1-32), DNA-binding domain (amino acids 150-280) and C-terminal transactivation domain (amino acids 281-631). It is a liver-enriched transcription factor belongs to homeobox gene family and expressed in liver, kidney and pancreatic islets. It normally forms homodimers or heterodimer with HNF-1β and controls multiple genes implicating in pancreatic β-cell function, notably in metabolism-secretion coupling. Its target genes include *amylin*, *insulin*, *GLUT2* and *L-type pyruvate kinase* (*L-PK*), *HMG-CoA reductase*, *mitochondrial 2-oxoglutarate dehydrogenase* (*OGDH*) *E1*.

Over 300 different mutations in *HNF-1α* associated with MODY, T1D and T2D have been described so far. The prevalence of *HNF-1α* mutations (MODY3) is different among various ethnic groups. It is most common in Caucasian, but less frequent in Asian populations. The *HNF-1α* mutations including missense, nonsense, frameshift insertions/deletions, duplications, promoter region mutations, and splice site mutations are located throughout the gene (Fig. 4). Among these, missense mutations are most common, spreading throughout the entire gene, and are concentrated in the dimerization and DNA-binding domains (Bellanne-Chantelot *et al.*, 2007). The truncated HNF-1α proteins are generated by nonsense mutation, or more

frequently a nucleotide deletion/insertion resulting in a frameshift encoding an altered amino acid sequence downstream of the mutational event and an introduction of a new stop codon. About 62% of truncating mutations are found in the C-terminal transactivation domain (Bellanne-Chantelot *et al.*, 2007). There is a *HNF-1α* mutational hotspot at codon 291 (P291fsinsC) resulted from insertion of C nucleotide in the poly-C tract of exon 4 which has been reported in more than 70 MODY families worldwide. A few cases of MODY3 patients have mutations outside the protein coding region, such as in promoter region and



Promoter	Exon 1	2	3	4	5	6	7	8	9	10
+102G>C	IVS1-1G>A	IVS2+1G>A	IVS3+1G>A	IVS4+2T>C	IVS5+2T>A (2)	A373G (2)	IVS7-6G>A	IVS8+1G>A	IVS9-1G>A	S608fsdelAG
-8G>A	M1L	IVS2+1G>C (4)	IVS3-1G>A	IVS4-2A>G	IVS5+4.5insA	G375fsdelG	IVS7-2A>G	IVS8+2T>C	IVS9+1G>A	D614fsdelAA
-62C>G	Q7X	IVS2+5G>A	G191D	A239T	IVS5-2A>G	P379T (5)	IVS7-2A>T	Y503X (3)	D546G	V617I
-97T>G	L8M (2)	IVS2+21G>A	T196A	A239V	IVS5-1delTAG	P379A (8)	Q442X	H505N (3)	D546fsdel	I618M
-119G>A	Q9P	(2)	T196fsdelCCAA	E240Q	R321H	P379S (2)	R442fsX456	E508K	-GACA	I618fsdelTC
-119delG	T10M	P112L (6)	R200W (4)	C241R (3)	Y322C (2)	P379R	A443fsdelCA (3)	T513S	E548fsdelTG	E619K
-128T>G	L12F	W113X	R200G	C241G (5)	Y322fs	P379H	S445fsdelAG	R514H (2)	G554fsX556	T620I (2)
-187C>T	L12H	V115L	R200Q (4)	C241Y	Y322X	P379fsdelCC	P447L (5)	T515K	L555F	
-207, -206	A14V (2)	A116T	R203C (2)	Q243E	Q324X	P379fsdelCT (12)	T457I	L518fsdel	A559fsinsC	
fsin27	L17V	A116V (2)	R203H (8)	R244S	S328R	P379fsdelC (2)	V462fs	-TCCinsA (3)	A559fsinsA	
-218T>C	L17Q	K117E (2)	K205Q	V246L	L341X	P379fsdelT (4)	Q463X	P519L	V567I	
-233-234	G20R (2)	S121fsdelC (3)	K205fsdelA (2)	V246G	T343fsdelCA	P379fsinsT	S465F	T521I	R583G (2)	
delAT	G20A	Y122C (2)	G207D (2)	S246fsdelGT	V351fsdelG	P379fsinsC	Q466X	M522V	R583Q (3)	
-283A>G	E24fsdelGA	L123R	G207fsinsG	S247fsdelAG (2)	T354M	L383fsinsA	P467L (2)	T525S	S585fsinsTC	
-462G>A	A25P	Q124X	A209fsins10	Q250X	T354fsinsC	L389V (5)	Q474X	T525I	P588S	
-527C>T	L26P (2)	N127delAAC	S210fsinsGGG	Q250fsinsC	P353delACGG	T392fsdelA	P475L	D526N	V590fsinsA	
	I27T	I128N	-GCCAGC	G255S	-GCCTGGAGC	P393fsdelC	P475fsomsA	D526Y		
	I27S	P129T	A217G	S256T	S361fs	Q399X	S482fsdelAG (2)	T528I		
	Q28X	Q130H	Y218C (3)	V259F		Q401fsdelC	Q486X (2)	D529fsinsA		
	G31D (6)	R131W (11)	P224S	V259D (2)		P409H	P488fsdelC	S531T		
	P33L	R131Q (9)	S225fsdelC	T260M		M412T	F489V	T537R		
	L44fsdelCCinsA	I132K	K226E	E261K		I414G415;	T492I	P538fs		
	L44fsdelC (2)	E132D	E228K	R263C (2)		ATCG>CCA (2)	S498R	T539fsdelC		
	G47E	V133M (5)	E228fsinsG	R263fsinsT		G415R	P499S			
	E48K (2)	V133L	R229Q (7)	R263L		P424L	H500N			
	E48fsdelG	V133fsdel	R229X (6)	R263H		S432C	A501T			
	R54X	-GGTCGAT	R229P (2)	V264F		T433I				
	R55G56fsdel	D135N	E230fsdelGA	N266K						
	-GAGGG	D135fsdelA	V233L	W267R						
	N62S	T137S	V233fsinsA	W267X						
	E66fsinsG	G138S	E234X	F268S						
	R68W (2)	S142F	C236X	R271W (7)						
	G69fsdelG	H143Y (3)	C236fsinsA	R271G (3)						
	E73X	H143fsdelC	R228fs	R271Q						
	D75E	S145F		R271fsdelC						
	T82M	Q146K;H147R		R272fsdelGC						
	K87X	Q146X		R272S (2)						
	E95X	L148I		R272C (3)						
	L107I (2)	N149K		R272H (11)						
	L107V	K150N		K273N						
	L107R (2)	M154V		E275fsdelGAAG						
	Q109fsdelA (2)	M154T		A276G						
		M154R		A276D						
		K158N		P289fsinsG						
		K158X		L281P (2)						
		R159W (4)		G288C						
		R159Q (8)		P289S						
		A161T		P291T						
		R168P		P291fsinsC (76)						
		R171X (8)		P291fsdelC (5)						
		V173M		P291fsdelA						
		A174V		G292fsdelG						
		Q175X		A301T						
		Q176X		G307fsdelC						
				P308L						
				A311D						
				S315fsinsA						

Figure 4 Mutations in *HNF-1α* gene. Location of *HNF-1α* mutations within the 10 exons, promoter, and splice sites of the gene. The functional domains of the HNF-1α protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets. Mutations with underlined were identified in Thai patients (modified from Yamagata *et al.*, 2003).

5′-UTR. The mutations in promoter region may affect binding sites of other transcription factors including HNF-4α (Gragnoli *et al.*, 1997), NF-Y, C/EBP, HNF-3, and AP-1. In addition, deletions of partial and whole *HNF-1α* gene have also been identified in some MODY cases (Pearson *et al.*, 2001).

MODY4 – *insulin promoter factor-1* mutations

Insulin promoter factor-1 (*IPF-1*, also known as *PDX-1*, *IDX-1* and *STF-1*) gene is located on chromosome 13q12.1. It contains 2 exons, spaning about 6 kb, and encodes a protein with 283 amino acids. IPF-1 protein has two functional domains: transactivation domain (1-38 amino acids) and DNA-binding domain (146-206 amino acids). It is expressed in pancreas, duodenum, and pylorus and is a homeodomain-containing transcription factor that plays crucial roles in pancreatic development and in regulation of various target genes including *GLUT2*, *GCK*, *insulin*, *somatostatin* and *islet amyloid polypeptide* (*IAPP*).

Mutations of IPF-1 cause MODY4 (Fig. 5) which is a rare form of MODY in various ethnic groups. Homozygous *IPF-1* mutation results in pancreatic agenesis while its heterozygous mutations are responsible for MODY4 phenotype (Stoffers *et al.*, 1997) and may contribute to susceptibility in late-onset T2D. A previous report has described a family which a proband carried homozygous mutation of *IPF-1* (P63fsdelC) who had pancreatic agenesis. Both parents who had diabetes were heterozygous for the same mutation. Moreover, *IPF-1* mutations have also been reported in gestational diabetes and predisposition to T2D (Gragnoli *et al.*, 2005).

MODY5 – *hepatocyte nuclear factor-1β* mutations

Hepatocyte nuclear factor-1β (*HNF-1β* or gene symbol *TCF2*) gene is located on chromosome 17q12-q21. It contains 9 exons and encodes a protein with 557 amino acids. HNF-1β protein comprises three functional domains: dimerization domain (1−32 amino acids), DNA binding domain (90−311 amino acids), and transactivation domain (312−557 amino acids). It is a homeodomain transcription factor that shares structural homology with HNF-1β in their dimerization and DNA-binding domains. It is expressed in liver, kidney, stomach, uterus and pancreas and plays crucial roles in the embryonic development of these organs. It can form homodimer or a heterodimer with HNF-1β (Rey-Campos *et al.*, 1991) which recognizes the same binding site on target promoters. HNF-1β acts as transcriptional activator of the target genes including: *insulin*, *albumin*, *glucose transporter-2*, *L-type pyruvate kinase* and *α-fetoprotein*.

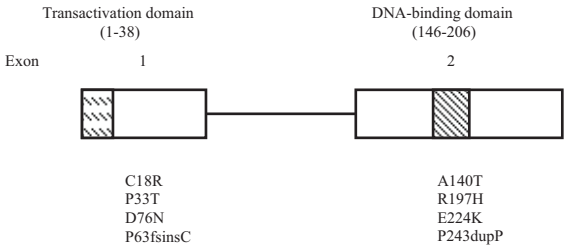


Figure 5 Mutations in *IPF-1* gene. Location of *IPF-1* mutations within the 2 exons, and functional domains of the IPF-1 protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets (modified from Winter *et al.*, 2000).

Mutations in *HNF-1β* are associated with MODY5 (Horikawa *et al.*, 1997), an uncommon subtype of MODY accounting for less than 1% of MODY cases. More than 40 different *HNF-1β* mutations have been reported in 46 families (Edghill *et al.*, 2006). These mutations, including missense, nonsense, frameshift insertion/deletions, and splice site mutations, were identified throughout the gene (Fig. 6). The majority of these mutations are private but there is a hotspot of mutation at the intron 2 splice donor site. The mutations are predominantly clustered in the first four exons, encoding for the dimerization and DNA-binding domain (Edghill *et al.*, 2006). The high proportion of whole gene deletions (one third of adult MODY5 patients), single exonic deletions (Bellanne-Chantelot *et al.*, 2005), and duplication of *HNF-1β* were also reported (Carette *et al.*, 2007). Several molecular pathologies of *HNF-1β* causing MODY5 were identified but the explanation for their different mechanisms was unclear. It was proposed, however, that the duplication might lead to genomic instability due to an unusual genomic architecture.

MODY6 – *NeuroD1* mutations

NeuroD1 (also known as *BETA2*) gene is located on chromosome 2q32 (Tamimi *et al.*, 1996). It contains two exons; exon 1 encodes part of the 5′-UTR of mRNA and exon 2 encodes for 11 nucleotides of the 5′-UTR and the protein with 356 amino acids. NeuroD1 protein is expressed in pancreatic islets, intestine, and brain. It belongs to the basic helix-loop-helix (bHLH) family of transcription factor and functions as transcriptional activators by forming heterodimer with the ubiquitous HLH protein E47. NeuroD1 regulates *insulin* gene transcription by binding to an E-box motif in the *insulin* promoter (Naya *et al.*, 1995; Sharma *et al.*, 1999).

Mutations in *NeuroD1* cause MODY6 – a rare MODY subtype. Only 4 mutations in *NeuroD1* were described in 4 MODY families (Fig. 7). The R111L and P260fsinsC mutations were firstly identified as *NeuroD1* mutations-associated with MODY6. The E110K and S159P mutation were identified in an Iceland MODY family (Kristinsson *et al.*, 2001) and a Chinese proband with early-onset type 2 diabetes, respectively (Liu *et al.*, 2007).

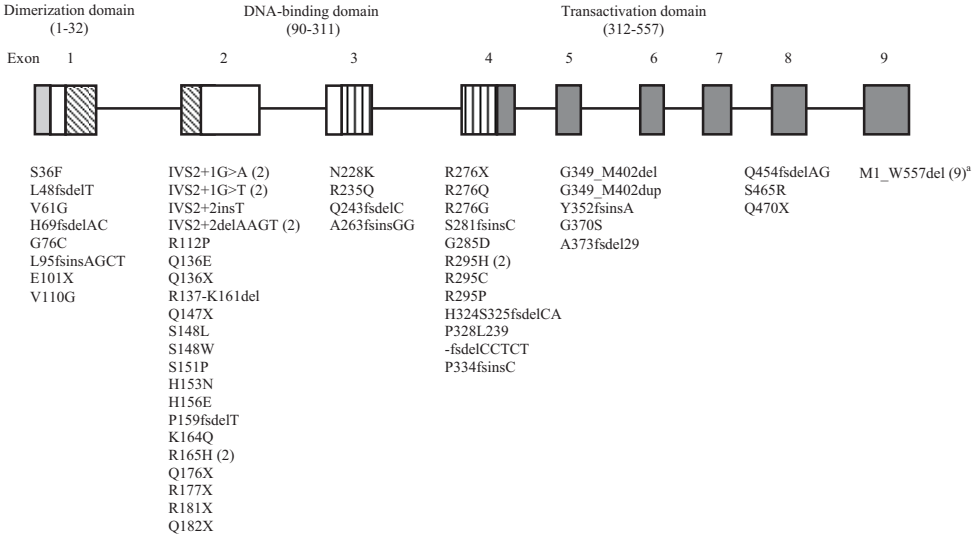


Figure 6 Mutations in *HNF-1β* gene. Location of *HNF-1β* mutations within the 9 exons, and functional domains of the HNF-1β protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets. [a] indicated whole gene deletions of *HNF-1β* (modified from Yamagata *et al.*, 2003).

MODY-X

MODY-X is denominated for MODY with unknown genetic etiology. It accounts for 20-25% of MODY cases in Caucasians and as many as 60-80% in Chinese, Japanese and Korean families. In Thai ethnic origin, Siriraj Diabetes Research Group (SiDRG) investigated genetic variations in the six known MODY genes in patients with MODY and early-onset T2D and found that the six known MODY genes account for a small proportion of both classic MODY (19%) and early-onset T2D patients (10%), suggesting that the majority of cases are MODY-X (Plengvidhya *et al.*, 2008), which is similar to the reports of other Asian ethnic groups.

Attempts have been made to identify unknown MODY genes. The results of genome-wide scan in European (Pearson *et al.*, 2001) and American. (Kim *et al.*, 2004) families with MODY-X suggested the existence of MODY-X loci on several chromosomes. A number of candidate genes involved in pancreatic β-cell transcription network as well as insulin secretion process have been examined in MODY-X families but none has been conclusively shown to cause MODY in the studied families. Recently, SiDRG investigated the role of *PAX4*, encoding transcription factor that plays a crucial role for β-cell development, in Thai patients with MODY-X (Plengvidhya *et al.*, 2007). A novel missense mutation, R164W, has been identified and found to be segregated with diabetes in the affected family. The mutant Pax 4 protein showed reduced repressor activities on insulin and glucagon promoters as compared to the wild-type protein. Therefore, mutation in *Pax4* could be a cause of MODY in the patients studied.

Functional studies of mutant genes responsible for MODY

***Glucokinase* mutations**

A majority of *glucokinase* mutations results in alteration of enzyme kinetics. The overall effect of inactivating mutations is the reduction of phosphorylation potential of the enzyme, which may lead to reduce glucose consumption in the β–cells and reduce insulin secretion, finally resulting in hyperglycemia. However, in more details, different *glucokinase* mutations impair enzymatic function through different mechanisms such as enzymatic activity, protein stability, and increased interaction with glucokinase regulator (GCKR). These data promote the understanding of relationship between glucokinase structure and function (Garcia-Herrero *et al.*, 2007). For examples, an insertion of asparagine residue N161 fully inactivates glucokinase whereas M235V and R308W mutations only partially impair enzymatic activity. However, glucokinase kinetics was almost unaffected by R397L mutation (Garcia-Herrero *et al.*, 2007).

Mutations of transcription factor-encoding genes

The transcription factors play roles in the regulation of β-cell function, insulin production, and glucose-stimulated insulin secretion. The functions of the wild-type and mutant transcription factors are

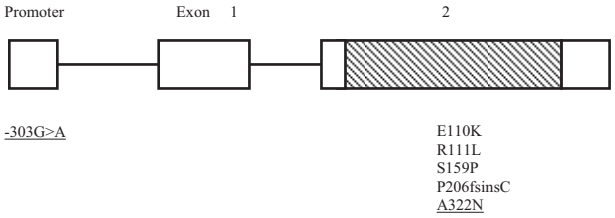


Figure 7 Mutations in *NeuroD1/BETA2* gene. Location of *NeuroD1* mutations within the 2 exons, and the coding region of NeuroD1 protein is entirely localized to exon 2 as shown in dotted area. Mutations found in Thais are shown with underlined and the numbers in brackets refer to codons.

extensively investigated, particularly in regulation of β-cell function using *in vivo* and *in vitro* models. The most simple and popular technique for studying mutant transcription factor proteins is *in vitro* promoter assay. In general, the promoter assay requires creations of three different constructs in plasmid vectors including protein expression construct, reporter construct, and internal control construct. The gene encoding transcription factor is cloned into the protein expression construct and the promoter region of a target gene of interest is combined with a reporter gene in the reporter construct. These two recombinant plasmid constructs are introduced with the internal control construct into an appropriate cell line. If the reporter system is well chosen, then the level of reporter gene expression will correlate with the transcriptional activity of the introduced transcription factor.

The transcriptional activity on target promoters may be used as a criteria for classification of the mutant proteins into several groups, for examples, reduced transcriptional activity, completed loss-of-function with or without dominant-negative effects, and gain-of-function. *In vitro* functional studies of *HNF-4α*, *HNF-1α*, *IPF-1*, *HNF-1β*, and *NeuroD1* mutations revealed that most of mutations may cause the defects through the mechanism of haploinsufficiency associated with loss-of-function and/or gene dosage effect. Loss-of-function mutations may occur in the regions encoding dimerization domain, DNA-binding domain, transactivation domain of the protein, and also occur in the promoter region of the gene. The effects of loss-of-function mutations were variable, ranging from absolute aberration of transactivation potential caused by nonsense mutations to partial loss of transactivation potential caused by missense mutations. Mutations in the promoter region may result in reduced protein expression levels, in turn, reduction of genes relevant to insulin secretory pathway. The reduction in transactivation activity of mutant proteins may be due to several reasons, for instances, reduction in protein stability and/or DNA-binding ability, impairment of nuclear import and defect in cooperative transactivation with its heterodimeric partner HNF-1β or coactivator p300 (Yang *et al.*, 1999; Kim *et al.*, 2003).

Dominant-negative effect was reported in some of the HNF-1α, HNF-1β, and also IPF-1 mutant proteins. Since HNF-1α can form a dimer, it is not surprising that mutant proteins may possess dominant-negative effect but their significance is not yet clear. These mutant proteins with intact dimerization domains but which are unable to bind DNA exhibited a much more drastic effect; the mutation not only abolished transactivation but also may form nonproductive dimers with wild-type protein thereby inhibiting the wild-type activity.

Gain-of-function mutants are defined as protein with enhanced activity to transactivate expression of target genes. It is a possible mechanism to cause some cases of MODY3 and MODY5. The HNF-1α gain-of-function mutants showed the differential effects in enhancing the wild-type activity. The downstream molecular mechanism of HNF regulatory network is important in determining pancreatic β-cell function. Thus, mutations in any MODY genes resulting in breakdown and/or disruption of this regulatory network may lead to impaired insulin secretion and hyperglycemia. Studying of functional properties of mutant proteins may provide a better understanding in pathogenesis of MODY and other types of DM.

Neonatal diabetes mellitus

Neonatal diabetes mellitus (NDM) is a rare form of DM occurred at an early infancy. The disease can be transiently developed (transient neonatal diabetes, TND) or permanent throughout the life (permanent neonatal diabetes, PND). NDM is caused by mutations of the genes involving in β-cell development and function. The most common causes of NDM are mutations of *ATP binding cassette, subfamily C, member 8* (*ABCC8*) and *potassium inwardly rectifying channel, subfamily J, member 11* (*KCNJ11*) genes, which encode sulfonylurea receptor

(SUR1) and $K_{ir}6.2$, respectively. Both SUR1 and $K_{ir}6.2$ are essential subunits of pancreatic ATP-dependent potassium channel (Fig. 1). Mutations in these two genes lead to the abnormalities of SUR1 and $K_{ir}6.2$ and reduction of response of potassium channel to ATP. Patients carried mutations in *ABCC8* and *KCNJ11* showed similar clinical features, in which marked hyperglycemia and ketoacidosis are presented. However, mutations in *KCNJ11* are more frequently found in PND and 20% of PND express neurological features, while mutations in *ABCC8* are common among TND. Mutations of *glucokinase* gene are involved in β-cell dysfunction. Homozygous *glucokinase* mutations completely abolish the enzyme activity. Therefore, the patients with neonatal diabetes due to homozygous *glucokinase* mutation require insulin treatment while the patients with MODY2 resulted from heterozygous *glucokinase* mutations do not. Mutations in *IPF1* (MODY4) and *HNF-1β* (MODY5) can also cause NDM. However, mutations in other genes encoding transcription factors, including *pancreas specific transcription factor-1α* (*PTF-1α*) and *GLIS family zinc finger 3* (*GLIS3*) (Fig. 1) are more frequently found to result in NDM (Hattersley *et al.*, 2006).

Mitochondrial diabetes mellitus

Mitochondrial diabetes or maternally inherited-diabetes with deafness (MIDD) is a specific maternally inherited form of DM, accounting for approximately 1% of DM cases (Kobayashi *et al.*, 1997). The presences of maternal transmission with bilateral hearing impairment allow discrimination of MIDD from other monogenic β-cell diabetes. The most common cause of MIDD occurred from A3243G mutation in the mitochondrial gene encoding $tRNA^{Leu(UUR)}$ (Kadowaki *et al.*, 1994). Pathophysiology underlying the disease is probably due to a depletion of ATP level in β-cell cytoplasm supporting a crucial role of β-cell respiratory-chain in maintenance of glucose homeostasis.

CONCLUSIONS

Molecular genetic studies of monogenic β-cell diabetes have provided an invaluable insight into molecular pathogenesis and mechanisms for this and other types of DM. The knowledge of molecular defects in monogenic β-cell diabetes is useful not only for selection of effective treatment, but also for further development of new drugs. The successful treatment of MODY3 and PND patients with sulfonylurea is a well-illustrated example. The evidence that defects in only one point of a particular gene is sufficient to produce clinical phenotypes indicate a central role of this particular gene in pancreatic β-cell development and function. Defects in several genes that have these roles have been identified as a cause of MODY, NDM, and MIDD. However, there are a number of patients with monogenic β-cell diabetes, especially in the Asian ethnic origins, that the causative genes are still unknown. Further study to identify these unknown causative genes and other genes that play interactive roles will provide a better understanding of a complex biological network underlying glucose homeostasis and pathogenesis of DM.

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OriginalArticle

Construction of a Mutation due to a Fourteen Base-Pair Insertion in *HNF-1α* Gene Causing Maturity-Onset Diabetes of the Young (MODY) in Thai Patients

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ABSTRACT

Objective: The aim of this study is to generate a mutation causing maturity-onset diabetes of the young (MODY) in Thai patients by insertion of a fourteen base-pair (bp) into a *HNF-1α* gene using a modified site-directed ligase-independent mutagenesis (SLIM) method.

Methods: Two pairs of long- and short-tailed primers were designed to amplify a plasmid construct containing a *HNF-1α* and to insert a 14-bp at a desired position. Long-tailed primers contained the overhanging 14-nucleotide (nt) insert at their termini which were complementary to each other. Polymerase chain reactions (PCR) were performed in two separated tubes using different pairs of primers. After amplifications, PCR products from both tubes were pooled together, denatured and then re-annealed to allow formation of double stranded DNA molecules containing the 14-bp insert within *HNF-1α*. The pooled and reannealed PCR products without ligation were transformed into competent *E.coli* cells to generate a ligated recombinant plasmid with a 14-bp insertion.

Results: Five of 14 bacterial colonies contained the desired recombinant plasmid with a 14-bp insertion within *HNF-1α*. The efficiency of the method for generation of recombinant plasmid was about 36 percent.

Conclusion: This method is simple and rapid to insert a long stretch of nucleotides into a plasmid construct containing a gene of interest at a desired position. A recombinant plasmid containing an insertion mutation in a *HNF-1α* gene was successfully generated, allowing an opportunity to perform functional study of the mutated gene.

Keywords: *HNF-1α*, insertion, MODY, PCR, site-directed mutagenesis

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Maturity-onset diabetes of the young (MODY) is a monogenic form of type 2 diabetes. It is characterized by an autosomal dominant mode of inheritance, early age at onset (usually less than 25 years), non-ketotic diabetes and defective pancreatic β-cell function.[1,2] Recently, the Siriraj Diabetic Mellitus Research (SiDMR) Group identified a novel *HNF-1α* (G554fsX556) mutation in a family of Thai MODY patients (unpublished data). The G554fsX556 mutation results from an insertion of 14 base-pair (bp) at the positions between 1659 and 1660 of the wild-type sequence. This mutation generates a truncated protein comprising of 555 amino acids, instead of the full-length 631 amino acids, which still contains intact dimerization and DNA-binding domains but lacks some part of the C-terminal transactivation domain, necessary for properly controlling transcription of its target genes. Importantly, this novel mutation was found in the patients from one MODY family but it was not observed in 200 normal healthy controls, indicating it to be a pathogenic mutation. However, the impact on protein function of this mutation is still unknown. To study the function of this mutant protein, a plasmid construct containing a 14-bp insertion at the nucleotide positions between 1659 and

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1660 of *HNF-1α* is required to be generated by site-direct mutagenesis to imitate the natural mutation.

Site-direct mutagenesis is a powerful tool to generate a mutant gene of interest for analysis of protein structure and function.[3] This method aims to introduce specific amino acid change at predetermined sites of protein encoded by the gene of interest.[4,5] Sometimes, it is required to introduce insertion or deletion of several nucleotides instead of single nucleotide changes.[6-8] Formerly, these experiments were difficult, time consuming and involved several steps including polymerase chain reaction (PCR) and ligation.[9,10] A number of new methods were developed to reduce the number of steps such as splicing overlap extension,[11] megaprimer approach,[12] and domain swapping.[13] However, these methods still have some technical difficulties including failure to completely remove the original DNA template, generation of incorrect transformation-competent cells and fragments derived from PCR mis-priming events.[14] The latter is problematic since the generated products are smaller but they usually contain the origin of replication and antibiotic resistance cassettes. Thus, they are more easily transformed into competent bacterial cells than the desired plasmids. To overcome this problem, the site-directed ligase-independent mutagenesis (SLIM) method has recently been developed.[14] It is claimed that this method is easy and has a high efficiency to produce an insertion mutation.

At first, we attempted to use the megaprimer approach to introduced a 14-bp insertion into a construct of *HNF-1α.* However, this was not successful. In this report, the SLIM method is modified to insert a 14-bp into *HNF-1α* for generation of a plasmid construct containing the gene with an insertion mutation for further functional studies.

MATERIALS AND METHODS

Plasmid construct containing human *HNF-1α*

The pcDNA3.1 expression vector containing human *HNF-1α* cDNA was kindly provided by Associate Professor Dr. Hiroto Furuta from the First Department of Medicine, Wakayama University, Wakayama, Japan. This vector was created to contain a FLAG epitope at the 3 end of the wild-type *HNF-1α* sequence. The size of this plasmid was 7.4 kb.

Oligonucleotide primers

Two pairs of primers including forward long-tailed primer (FL), forward short-tailed primer (Fs), reverse long-tailed primer (RL), and reverse short-tailed primer (Rs) were designed for using in the SLIM method. The sequences of primers are shown in Table 1.

PCR amplifications

Two PCR reactions were performed in separated tubes. In the first tube, the PCR reaction contained FL and Rs primers while in the second tube it consisted of Fs and RL primers. Both PCR reactions were performed in the reaction volume of 50 µl containing 2 µl of *HNF-1α* construct, 5 µl of 10x *Pfu* buffer, 0.2 mM dNTPs, 15 pmole each of primers, 1 µl of 3 U of *Pfu* DNA polymerase (Promega, Madison, USA) and sterile water up to 50 µl. The PCR profile was 95°c for 30 s for the first round of reaction, followed by 18 cycles of 95°c for 30 s, 55°c for 1 min, 68°c for 8 min, and a final extension at 68°c for 10 minutes.

PCR product hybridization

PCR products (12.5 µl) from each tube were mixe d and added with 5 µl of D-buffer (20 mM $MgCl_2$, 20 mM Tris, pH 8.0 and 5 mM DTT) containing 20 U DpnI enzyme to digest the methylated parental template. This reaction mixture was incubated at 37°c for 60 minutes. Thirty µl of H-buffer (300 mM NaCl, 50 mM Tris, pH 9.0 and 20 mM EDTA, pH 8.0) were added into the reaction mixture to stop DpnI digestion. Then, this reaction was denatured at 99°c for 3 minutes and hybridized by using two cycles of 65°c for 5 minutes and 30°c for 15 minutes to generate a double stranded DNA molecule with a complementary single-stranded 14-nucleotide (nt) overhang at its 5' and 3' termini, allowing spontaneous circularization to form the plasmid with the 14-bp insert. An aliquot of 5 µl from the reaction was used to transform into competent TOP10' *E. coli* cells by the heat-shock method[14].

Screening of recombinant plasmid by PCR

After growing the transformed *E. coli* overnight, colonies were picked and subjected to plasmid extraction by using Aurum plasmid mini kit (Biorad). Briefly, *E. coli* containing recombinant plasmid was grown in 2 ml LB broth containing 100 µg/ml ampicillin for 12-16 hrs. Then, the cultured media was transferred into a capped tube and centrifuged at 10,000 rpm for 5 minutes. Cell pellets were diluted in 250 µl of resuspen sion solution. Two hundred and fifty µl of lyses solution were added into the suspension and gently mixed. Then, 350 µl of neutralization solution were added and mixed. Cell debris was removed by centrifuging at 10,000 rpm for 5 minutes. The supernatant was transferred to the spin column and centrifuged for 1 minute and the column was washed twice with 750 µl of washing solution. Then, DNA was eluted into a fresh tube. An aliquot of 2 µl of DNA was used as a template to screen for the presence of inserted sequence by using the PCR method. The PCR reaction was performed in the reaction volume of 50 µl containing 5 µl of 10x *Taq* polymerase buffer, 0.2 mM dNTPs, 15 pmole of HNF-insF and BGH-reverse primers (Table 1), 1 µl of *Taq* DNA polymerase (Promega, Madison, USA) and sterile water up to 50 µl. The PCR profile was 95°c

TABLE 1. Details of primers for modified site-directed ligase-independent mutagenesis (SLIM) method for screening of 14-nucleotide insert with *HNF-1α*

Primer name	Nucleotide sequences (5' → 3')	Annealing Tm (°c)
Forward long (FL)	5'-AGTGAGTGAAGCCCGGGCTTCACACGCCGGCATCT-3	55
Forward short (Fs)	5'-GGGCTTCACACGCCGGCATCTCAG-3'	55
Reverse long (RL)	5'-GGGCTTCACTCACTGGACTCACTGGAAGCTTCAGT-3'	55
Reverse short (Rs)	5'-GGACTCACTGGAAGCTTCAGTGTC-3'	55
HNF-ins-F	5'-GTCCAGTGAGTGAAGCCC-3'	55
BGH-R	5'-TAGAAGGCACAGTCGAGG-3'	55

Notes: Underlined sequences are 14 nucleotides required to be inserted into *HNF-1α* gene. The 14 nucleotides at the termini of FL and RL primers are complementary.

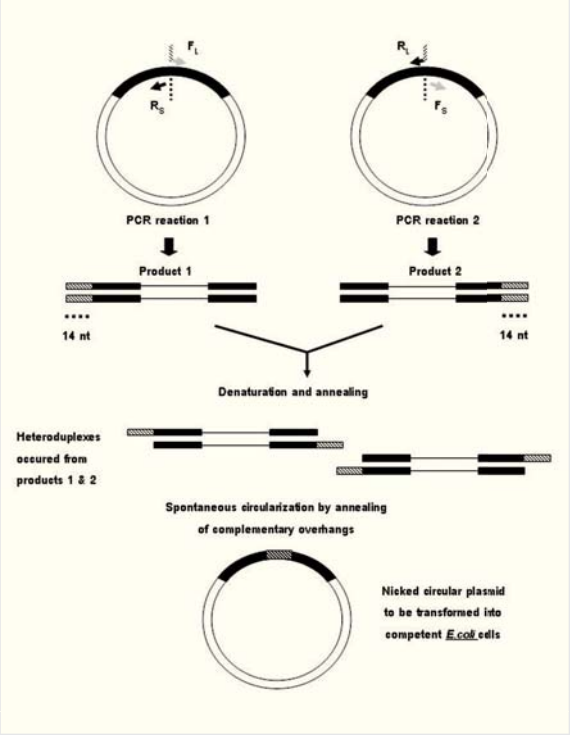

Fig 1. The principle of modified site-directed ligase-independent mutagenesis (SLIM) method.

Two long-tailed (FL and RL) and short-tailed (Fs and Rs) primers were designed for amplification and insertion of a long stretch of nucleotides into a gene of interest in a recombinant plasmid. The black arrows represent the primers that are complementary to sequences of the gene at the site to be inserted with the nucleotides. The two long-tailed primers contain 14-nucleotide (nt) overhangs that are complementary to each other (indicated by hash lines). PCR reactions were performed in two separate tubes by using FL/Rs and RL/Fs primers. The two PCR products (Products 1&2) were mixed, denatured, and annealed to generate heteroduplexes containing complementary 14-nt overhangs at their 5' and 3' termini, which are able to form nicked circular plasmids to be transformed into competent *E. coli* cells. "Modified from Chiu J, et al. Site-directed, Ligase-Independent Mutagenesis (SLIM): a single-tube methodology approaching 100% efficiency in 4 h. Nucleic Acids Res 2004;32:174" (reference no 14)

for 30 s for the first round of reaction, followed by 18 cycles of 95°c for 30 s, 55°c for 1 min, 68°c for 8 min, and final extension at 68°c for 10 minutes. PCR products were examined by electrophoresis on 1% agarose gel.

DNA sequencing

The recombinant plasmid containing the 14-bp insertion was confirmed by DNA sequencing using BigDye Terminator Cycle Sequencing Ready Reaction Kits. Each reaction contained 700 ng of recombinant plasmid DNA, 4 µl of BigDye buffer, 4 µl of BigDye master mix enzyme, 0.32 pmol of sequencing primer and sterile water to a final volume of 20 µl. Seq uencing profiles are as follows: 96°c for 1 minute, and then 96°c for 15 seconds, 50°c for 15 seconds and 60°c for 4 minutes for 25 cycles. The sequencing product was precipitated. The dried sequencing sample was sent to the BioService Unit (BSU, BIOTEC, Thailand) to analyze the DNA sequence.

RESULTS

Principle and primer design

The principle of the SLIM method is shown in Fig 1. Both long-tailed primers, FL and RL, contained 14 nucleotides that would be inserted into *HNF-1α* at the desired position in forward and reverse directions in addition to the sequences complementary to that of the plasmid (Table 1). Short primers contained only nucleotide sequences derived from the plasmid. PCRs were performed in two separated tubes instead of one tube as was done in the original SLIM method. FL and Rs primers were used in one reaction and RL and Fs primers in the other. In the reactions, the entire vector and the insert sequences (in FL and RL primers) were amplified. The two amplified PCR products were identical, except for the sequence of 14-nt insert which was located on the opposite termini. Denaturation and annealing of both PCR products generated 2 species of heteroduplex DNA molecules containing complementary 14-nt overhangs at their 5' and 3' termini. Annealing of these 14-nt overhangs created a stable form of circular plasmids containing the 14-bp insert. The circular DNA molecules were transformed into E .coli cells where they were ligated, giving rise to E .coli colonies on selective media.

Screening and efficiency of the method

A total of 14 colonies were obtained and subjected to plasmid extraction. All of the 14 DNA samples were screened by PCR using specific primers: HNF-insF, a forward primer containing a 14-nt insert, and BGH-reverse primer (Table 1). Plasmid containing 14-bp insertions produced a 343-bp PCR product while plasmid without 14-bp insertion could not be amplified and generated no product. There were 5 colonies from 14 colonies that gave 343-bp PCR products (Fig 2). Thus, this method had the efficiency of 36 percent. A recombinant plasmid that gave a positive result for the PCR screening was examined by DNA sequencing. The result revealed that it contained the 14-bp insertion as expected (Fig 3).

DISCUSSION

Several reports have shown that mutations of MODY genes are different in different ethnic groups. For example, mutations of *HNF-1α* or MODY3 is a common type of MODY in the European population while mutations of *glucokinase* or MODY2 is a common type of MODY in the French population.[15] Thus, the mutation of MODY genes in the Thai population could be different from other populations. Study of six known MODY genes was carried out in Thai MODY patients. Screening for mutations in these MODY genes was performed by using single strand conformation polymorphism (SSCP) and DNA sequencing. Both reported that novel mutations of MODY genes were identified in the Thai population. A novel variation could be polymorphism or pathogenic mutation. Generally, polymorphism does not have any effect on the protein function while pathogenic mutation does. Thus, a potentially novel mutation requires functional study to prove its role in causing the disease.

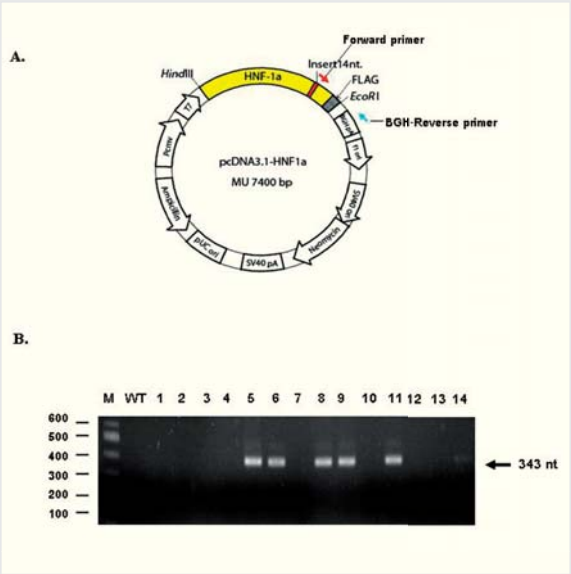

Fig 2. The screening strategy and screening results of *E. coli* colonies by PCR.

A. The plasmid construct containing *HNF-1α* with 14-base pair (bp) insert. The arrows are HNF-ins-Forward and BGH-Reverse primers for screening of the plasmid construct with the 14-bp insert. The HNF-ins-Forward includes 14 nucleotides complementary to the insert sequence in addition to some part of the wild-type sequence. The BGH-Reverse primer is complementary to the plasmid sequence.

B. The results of screening *E. coli* colonies by PCR. The recombinant plasmids prepared from *E. coli* colonies were amplified by PCR using HNF-ins-Forward and BGH-Reverse primers. The plasmid containing *HNF-1α* with 14-bp insert generated PCR product with the size of 343 bp while the plasmid containing wild-type *HNF-1α* showed no PCR product. The plasmids from 5 of 14 *E. coli* colonies (lanes 5, 6, 8, 9, and 11) contained the mutated *HNF-1α.*

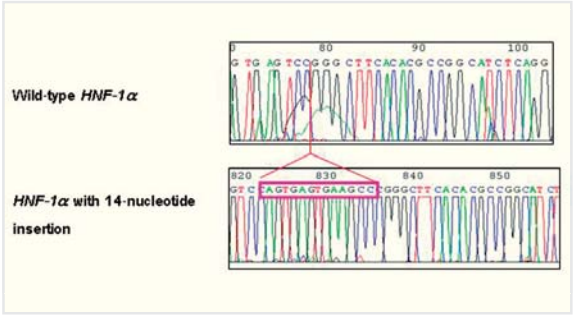

Fig 3. The result of DNA sequencing.

A recombinant plasmid showing the positive screening result by PCR as demonstrated in Fig 3 was subjected to DNA sequencing. The result in the bottom panel shows that it contained 14-bp insertion in the *HNF-1α* at the correct site. The top panel is the sequence of wild-type *HNF-1α* in the corresponding region.

We have used a megaprimer method to generate a 14-bp insertion into *HNF-1α* without a successful result although several attempts had been made to optimize the PCR conditions. We then changed to use the site-directed ligase-independent mutagenesis (SLIM) technique. SLIM has been demonstrated to overcome technical difficulties for site-direct mutagenesis by introducing a long stretch of nucleotide sequence. This method was claimed to be simple and efficient to produce an insertion at the desired position of a gene cloned into a plasmid. However, we found some technical difficulty of the original SLIM method that performing PCR for in one tube generated the products containing either wild-type *HNF-1α* or a mutant *HNF-1α* and other non-specific products. To solve this problem, PCRs were therefore modified to be performed in two separate tubes. This approach generated a successful result in producing a 14-bp insertion into *HNF-1α.* However, efficiency of this method was less than that of the original SLIM method (36% vs 95%). This might be explained by insufficient *DpnI* digestion which is an important step to eliminate the parental plasmids containing the wild-type *HNF-1α.* This was confirmed by analysis of the plasmids prepared from the colonies with negative PCR results by restriction enzyme digestion and gel electrophoresis that all of them were the plasmids containing the wild-type *HNF-1α* (data not shown).

The availability of plasmid constructs containing mutated *HNF-1α* with a 14-bp insertion will provide us with an opportunity to perform functional analysis on the truncated *HNF-1α* on its target genes in cultured cell lines which will give further information on the molecular and cellular mechanism of MODY in Thai patients.

CONCLUSION

The generation of a plasmid construct containing mutated *HNF-1α* with a 14-bp insertion causing MODY in Thai patients by the modified site-directed ligase-independent mutagenesis (SLIM) method was successful. This method is simple and rapid to introduce an insertion of a long stretch of nucleotides into a gene of interest in a recombinant plasmid. Availability of the plasmid construct containing mutant *HNF-1α* allows us to perform further analysis into the effect on this mutation on the function of the encoded protein.

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Molecular genetics of diabetes mellitus

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ABSTRACT

Diabetes mellitus (DM) is a disease that causes major public health problem worldwide. In Thailand, it was estimated that 9.6% or 2.4 million adults were affected with DM and the prevalence is increasing. The unawareness of having the disease leads to delayed treatment and development of chronic complications. The cost for management of DM and its complications is increasing enormously, causing a great economic and healthcare burden. DM is caused by both environmental and genetic factors. Although type 1 diabetes (T1D) is not a genetically predestined disease, an increased susceptibility to the disease can be inherited. Genetic factor plays a crucial role in pathogenesis and complications of type 2 diabetes (T2D) while environmental factors are also required for the disease development. Even if modifications of life-style are important for controlling T2D, the identification of susceptibility genes will lead to understanding of its complex pathogenesis and development of more effective treatment. Up to date, a number of diabetic susceptible genes are identified in Western populations. It is now the time to identify the causative and susceptibility genes of diabetes in Thai. This review aims to provide a current overview of molecular genetics of DM and some available information in Thais.

Keywords: diabetes mellitus, hyperglycemia, genetic susceptibility, pathogenesis, diabetic complication

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous metabolic disorder characterized by chronic hyperglycemia, resulting from deficiency or failure in maintenance of normal glucose homeostasis. The interaction between susceptible genetics and environmental factors is known to trigger the disease. Most patients are suffered from its complication, including retinopathy, nephropathy, coronary and peripheral vascular diseases. The vascular complications, such as ischemic heart and renal diseases are attributed to excess mortality of the disease. The cost of treatments of the disease and its complications is immense. The prevalence of DM has increased sharply in recent decades. The number of affected individual worldwide is currently estimated to be approximately 150 million and predicted to reach 220 million by 2010 and 300 million by 2025 (Zimmet *et al.*, 2001). It was estimated in 2000 that 9.6% (2.4 millions) of Thai adults were affected with DM and 5.4% (1.4 millions) had impaired fasting glucose (Aekplakorn *et al.*, 2003). These have indicated that DM is an

enormous global public health problem (Wolford and Vozarova de Courten, 2004) and also an increasingly significant public health problem in Thailand. Since the disease is very heterogeneous, abnormality at different biological pathways can lead to hyperglycemia and subtyping of the disease requires precise diagnostic criteria. A lot of research studies have concentrated on identifications of diabetic susceptibility genes. These efforts will facilitate a better understanding in the pathogenesis underlying each DM subtype, thereby leading to the development of effective and appropriate therapeutic approaches.

Classification and pathogenesis

DM generally presents in two major forms, type 1 diabetes (T1D) and type 2 diabetes (T2D). The former is less common while the latter is more common, accounting for approximately 10% and 90% of diabetic cases, respectively. The onset of T1D is in childhood while that of T2D is predominantly after 40 years of age and generally occurs in obese people. T1D is more severe and rapidly progressive, due to a great impairment or absolute deficiency of insulin secretion caused by an autoimmune destruction of pancreatic β-cells (Fig. 1). This destruction is mediated by both humoral (auto-antibodies) and cellular (infiltrated lymphocytes) autoimmunities which are chronic but potent to produce symptoms in childhood (Petrovsky and Schatz, 2003). However, the exact mechanism involved in the initiation and progression of β-cell destruction in T1D is still unclear. While T1D results from absolute insulin deficiency, T2D usually presents the relative insufficiency. Most often, this relative insulin insufficiency is attributable to an inability of β-cells to adequately compensate for insulin resistance (Reaven *et al.*, 1976) (Fig. 2). Generally, exogenous insulin is not required for the survival of T2D patients. This form of diabetes frequently goes undiagnosed for many years because the gradually developed hyperglycemia and at the earlier stage is often not severe enough to cause

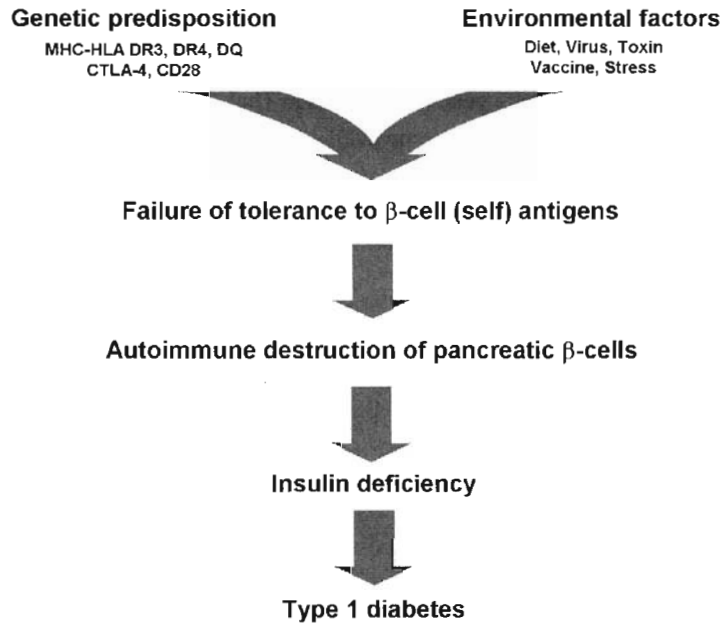

Figure 1 Pathogenesis of type 1 diabetes (T1D). Genetic predisposition and environmental factors are involved in failure of tolerance to β-cell (self) antigens and autoimmune destruction of pancreatic β-cells leading to insulin deficiency and T1D.

noticeable symptoms. Obesity is clearly demonstrated as the most common etiology of insulin resistance, although structural abnormalities of insulin and defects in insulin signaling pathway can also contribute to the resistance (Tager *et al.*, 1979; Bogardus *et al.*, 1985).

Other specific types of DM, including a monogenic form as well as non-genetic form, account for approximately 1%-2% of cases. Maturity-onset diabetes of the young (MODY) is one of monogenic form that seems to be intermediacy between T1D and T2D. Similar to T1D, it is caused by genetic abnormality which affects β-cell function but autoimmunity is not involved. In addition, diabetic symptoms are usually presented at a young age, usually less than 25 years old. However, MODY is classified as T2D subtype due to its decreased severity and the presence of insulin production. Moreover, exogenous insulin is not necessary for survival of MODY patients (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). Other specific types of DM are very rare.

Genetic bases of DM

The evidences from family and twin studies clearly indicated the contribution of genetic components to diabetes susceptibility although the influence of environments could not be excluded. T1D shows a strong aggregation of disease within family with the risk to sibling (λ_s) of 15 folds as compared to unrelated individuals (Field, 2002). For T2D, the risk for developing disease in sibling is 4 to 6 folds higher than those of unrelated individuals. The concordance rate in monozygotic twins is much higher as compared to dizygotic twins for both T1D and T2D. For T1D, the concordance rate in monozygotic twins was estimated to range from 21%-70%, higher than 0%-13% reported in dizygotic twins (Redondo *et al.*, 2001). For T2D, the concordance rate in monozygotic twins was ranged from 34%-83% whereas it was 16%-40% in dizygotic twins (Kaprio *et al.*, 1992; Poulsen *et al.*, 1999). Majority of DM

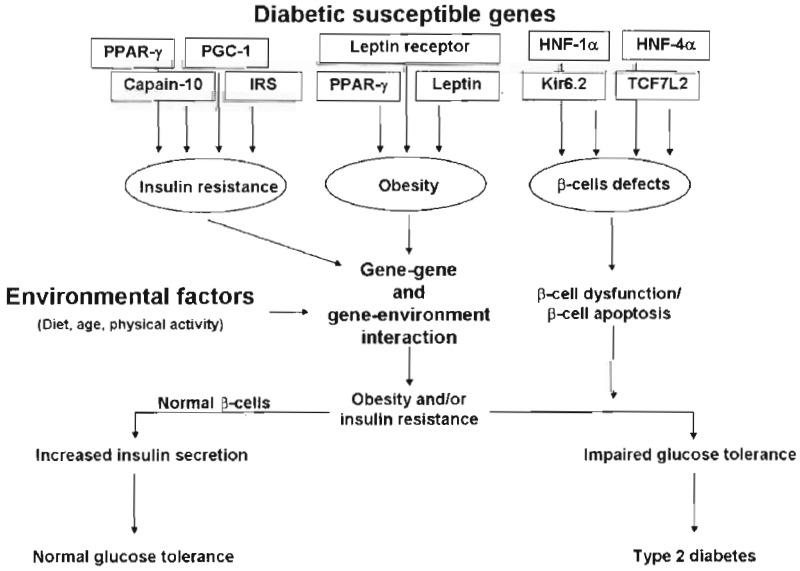

Figure 2 Pathogenesis of type 2 diabetes (T2D). The interaction of diabetic susceptible genes (causing insulin resistance, obesity, and β-cell defects) and environmental factors leads to relative insulin insufficiency. Impaired glucose tolerance and T2D are developed when pancreatic β-cells are unable to adequately compensate for the requirement of insulin in obesity and/or insulin resistance.

demonstrates familial clustering but does not show any clear pattern of Mendelian inheritance, a characteristic of multifactorial disorder. The simultaneous presence of several abnormal genes or polymorphisms together with environmental triggers, including age, sex, diet, obesity, infection, as well as physical activity, is required for the development of disease (Iselius *et al.*, 1985; Bogardus *et al.*, 1989; Martin *et al.*, 1992). However, approximately 10% of T2D appears to segregate in a Medelian fashion, as a monogenic form with autosomal dominant inheritance pattern, known as MODY (Fajans, 1989).

Identification of diabetic susceptibility genes is said to be geneticists' nightmare (Feingold, 1976), because of its complications with a number of problems. First of all is its genetic heterogeneity. Alleles at more than one locus can individually trigger the same phenotype and such alleles may be identified in one population but not be replicated in the others. Other problems are a reduced penetrance as well as a phenocopy. The variable of onset age, leading to difficulty in defining of affected state at a particular time, is an especial problem of T2D. Studying the monogenic form of DM (i.e. MODY), which is the early-onset disease, was easier and thereby used as a clue for identifying the multifactorial form of T2D. It was believed that variants causing less functional destruction in genes responsible for MODY would be contributed to the more common and multifactorial form of T2D. For example, the G319S variant of *HNF-1a*, one of six known genes responsible for MODY, is a major susceptible gene for the common form of T2D in the Oji-Cree Native Canadian population (Triggs-Raine *et al.*, 2002). This finding demonstrates that heterogeneous phenotypic of T2D might at least depend on the degree of functional impairment caused by variants in a particular gene.

To date, several diabetic susceptible genes have been identified by linkage and association approaches either in a genomic scale (genome-wide scan) or in a smaller scale by selection of only candidate chromosomal regions or candidate genes arisen from their biological function (candidate gene analysis). Recently, improvement in genotyping technology together with development in the field of bioinformatics have facilitated genome-wide scan in an easier manner. Consequently, a number of DM susceptible genes have recently been identified by genome-wide association (GWA) approach.

Candidate genes for T1D

The first T1D susceptible locus, designated as *IDDM1*, was identified by association approach (Table 1) (Florez *et al.*, 2003). It is located on *human leukocyte antigen* (*HLA*) genes region, which encodes major histocompatibility complex (MHC). Fine mapping indicated that HLA-DQB1 and HLA-DRB1 are the most important alleles associated with DM. *HLA* region was later found to be a major genetic determinant of disease risk, accounting for 42% of inherited T1D. The variable number of tandem repeats (VNTR) upstream of *Insulin* (*INS*) gene was subsequently identified, designated as *IDDM2*. This locus contributes about 10% toward T1D susceptibility (Bennett *et al.*, 1995). It was found that a variation of VNTR associated with the levels of insulin protein expressed in the thymus, involving in central tolerance to insulin. Both *IDDM1* and *IDDM2* were identified by the investigation of suspected genes. Afterwards, a series of chromosomal regions that may contain susceptibility genes for T1D, including *IDDM3-IDDM18* (Table 1), were identified mainly by genome-wide linkage analysis of affected sibling pairs. These regions contain several genes that encode proteins implicated with their biological functions, including pancreatic transcription factors, apoptotic proteins, and specific enzymes expressed in pancreas. Several T-cell co-stimulatory receptor encoding genes were identified in *IDDM12*, including genes encode CTLA4, CD28 and ICOS (Raffel and Rotter, 2002;

Triggs-Raine *et al.*, 2002; Bain *et al.*, 2003). None of suspected gene was identified in some *IDDM* regions, including *IDDM3* and *IDDM8*, even though these regions have been replicated in different data sets.

A recent GWA study has confirmed six genes/regions that previously shown a strong statistically significant association with T1D. These included genes encoding MHC, insulin, CTLA-4, and protein tyrosine phosphatase, non-receptor type 22, (PTPN22) and the regions around the interleukin 2 receptor alpha (IL2RA/CD25) and interferon-induced helicase 1 (IFIH1/MDA5) genes (The Wellcome Trust Case Control Consortium, 2007). In addition, this study showed three novel regions significantly associated with T1D including chromosomes 12q13, 12q24, and 16p13. The two regions on chromosome 12 contain several candidate genes involving in immune signaling. These included genes encoding receptor tyrosine-protein kinase erbB-3 precursor (ERBB3), SH2B adaptor protein 3 (SH2B3/LNK), TRAF-type zinc finger domain containing 1 (TRAFD1), and protein tyrosine phosphatase, non-receptor type 11 (PTPN11). In contrast, the chromosome 16p13 region consists of only two genes whose functions are unknown, i.e., KIAA0350 and dexamethasone-induced transcript. Among these novel loci, gene encoding PTPN11 is the most attractive candidate involving the major role in insulin and immune signaling (Mustelin *et al.*, 2005).

Candidate genes for T2D

Chromosome 2q37.3, designated as *NIDDM1*, was the firstly identified region with a significant linkage to T2D (Hanis *et al.*, 1996). Positional cloning of this region led to identification of gene encoding calpain-10, a cysteine protease

Table 1 T1D-susceptible loci identified by genome-wide linkage studies (modified from Florez *et al.*, 2003).

Locus	Location	Marker/candidate gene	LOD	Population
*IDDM1**	6p21.3	*HLA*	7.3-65.8	UK,US, France, North Africa,
*IDDM2**	11p15	*INS* VNTR	2.1-4.3	UK,US
IDDM3	15q26.2	D15S107	□	□
IDDM4	11q13.3	*FGF3*	3.4	UK,US
IDDM5	6q25.1	*ESR1*	11.5-2.0	UK,US
IDDM6	18q21.2	*JK*, D18S487	1.1, 1.2	UK,US
IDDM7	2q31	*HOXD8*, D2S152	2.6	US,UK
IDDM8	6q27	D6S264	1.8-5.0	US,UK
IDDM9	3q21	D3S1576	2.4	UK
IDDM10	10p11	D10S193	1.9-4.7	UK,US
IDDM11	14q24.3	D14S67	□	□
IDDM12	2q33	*CTLA4, CD28, ICOS*	2.6	US,UK
IDDM13	2q35	D2S164	2.6	US,UK
IDDM15	6q21	D6S283	2.3, 2.4	US,UK
IDDM16	14q32.3	D14S542	□	□
IDDM17	10q25	D10S554	4.9	Bedouin
IDDM18	5q33	*IL12B*		

* Initially found by association, *HLA*; human leukocyte antigen, *INS*; insulin, *FGF3*; fibroblast growth factor 3, *ESR1*; estrogen receptor 1, *JK;* surface antigen, *HOXD3*; homeobox D3, *CTLA4*; cytotoxic T lymphocyte-associated 4, *ICOS*; inducible costimulator, *IL12B*; interleukin 12B

that plays a role in the regulation of both insulin secretion and insulin action. Further analysis in Mexican-American and European populations indicated that the disease susceptibility is best described by a combination of risk haplotypes. The second report was the linkage at chromosome 12q24.31, designated as *NIDDM2*, in Finnish Caucasian (Mahtani *et al.*, 1996). This region contains *HNF1A* gene, one of six genes known to be responsible for MODY. Then, several research groups have studied the linkage of various genetic loci to T2D (Table 2) (Florez *et al.*, 2003) but only few regions have been shown to have significant evidences of linkage (LOD>3.6) which could be replicated in multiple studies, including chromosomes 1q25.3, 3p24.1, 3q26-28, 10q26.13, and 18p11.22. In addition, several candidate genes which are involved in insulin sensitivity, β-cell function and obesity were investigated by association approach. More than 40 different genes have been reported to be associated with T2D but few associations have been replicated in additional populations (Florez *et al.*, 2003) (Table 3). Among them, amino acid substitution of Pro12Ala of *peroxisome proliferators-activated receptor-gamma (PPARγ)*, which encodes

Table 2 Chromosomal regions and candidate genes with significant and suggestive linkage with T2D identified by genome-wide linkage studies (modified from Florez *et al.*, 2003).

Location	Marker/Candidate gene	LOD	Population
1q25.3	D1S2127/ *PKLR, LMX1*	1.5-4.3	Pima India, US, France, UK
1q42.2	D1S3462	2.4	Finn
2p21	D2S2259	2.3	France
2q24.3	D2S2345	1.2, 3.0	Australia aborigines, France
2q37.3	D2S125/ *CAPN-10*	2.1-4.1	Mexican- Americans, US
3p24.1	D3S2432	1.1-3.9	Mexican-Americans, Finn
3q28	D3S1580	1.4-4.7	France, Japan, Australia Aborigines
4q34.1	D4S1539	1.3, 2.1	Ashkenazi Jews, France
5q13.3	D5S1404	1.2, 2.8	UK, Caucasians
5q31.1	D5S816	1.2, 2.4	Finn, UK
7q32.3	D7S1804-D7S500	2.0	Pima India
8p21.3	D8S258	1.3, 2.6	UK, US
9p24.2	D9S288-D9S295	2.4	Mexican-Americans
9q21.12	D9S166	2.9, 3.3	Finn, Chinese
10p14	D10S1412	2.0, 2.4	African-Americans, Chinese
10q26.13	D10S587	2.0,3.8	UK, Mexican- □Americans
11p13	D11S935	1.9, 3.1	Japan, US
12q15	D12S375	1.5, 3.1	US
12q21.32	D12S853	1.5, 2.8	Caucasians, France
12q24.31	D12S1349/*HNF-1a*	1.5-3.7	Finn, US, Pacific Islanders
18p11.22	D18S843	2.4, 4.2	US, Finn
20p12.3	D20S905	0.9, 2.0	Finn, Ashkenazi Jews
20q13.12	D20S886	2.2, 2.9	Finn, Chinese
Xq23	GATA172D05	1.7, 3.0	Japanese, Caucasians

PKLR; pyruvate kinase liver and red blood cell, *CALPN-10*; calpain-10, *HNF-4α*; hepatonuclear factor-4α

transcription factor that plays a central role in adipocyte development, is the most widely reproduced association (Altshuler *et al.*, 2000; Hara *et al.*, 2000; Douglas *et al.*, 2001; Mori *et al.*, 2001; Ardlie *et al.*, 2002). Another strong candidate gene for T2D is *an ATP-binding cassette, sub-family C, member 8 (ABCC8)* gene, which encodes the sulfonylurea receptor (SUR1), a drug target for an oral hypoglycemic agent, sulfonylurea (Thomas *et al.*, 1995). In addition, *potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11)* gene which encodes $K_{ir}6.2$, an essential subunit of pancreatic ATP-dependent potassium channel (K_{ATP}), and *PPAR-γcoactivator 1 (PGC1)* gene are loci that also reproducibly associated with T2D (Hani *et al.*, 1998; Ek *et al.*, 2001; Gloyn *et al.*, 2001; Gloyn *et al.*, 2003). Other genes, which were found to be associated with T2D in more than one population, include *glucagon receptor (GCGR)* (Bennett *et al.*, 1995; Hager *et al.*, 1995), *glucokinase (GCK)* (Chiu *et al.*, 1992; McCarthy *et al.*, 1994; Takekawa *et al.*, 1994) and *solute carrier family 2 , member 1 (SLC2A1)* (Li *et al.*, 1988; Tao *et al.*, 1995; Pontiroli *et al.*, 1996).

From the combined data of traditional candidate gene approach together with recent information from six GWA studies (Frayling *et al.*, 2007; Saxena *et al.*, 2007; Scott *et al.*, 2007;

Table 3 Candidate genes and their polymorphisms significantly associated with T2D (modified from Florez *et al.*, 2003).

Gene	Encoded protein	Polymorphism	Risk allele	*p*-value	Population
ABCC8	Sulfonylurea receptor	759C>T	T	0.0008	US/UK
				0.03	France
				0.03	Denmark
				0.01	Scandinavia
Adiponectin	Adiponectin	-11377C>G	G	0.04	France
		-11377C>G	C	0.002	Japan
		-11377C>G	G	0.04	Sweden
GCGR	Glucagon receptor	G50S	Ser	0.0001	France
				0.008	UK
GCK	Glucokinase	GCK 3'	Z+2	0.008	Mauritius-Creole
				0.0016	Finland
				0.014	Japan
KCNJ11	Potassium inward rectifier channel $K_{ir}6.2$	G23K	Lys	0.015	France
				0.024	UK
				0.01	UK
PPAR-g	Peroxisome proliferators-activated receptor-g	Pro12Ala	Pro	0.03	US Japanese-American
				0.003	Japan
				0.000054	Japan
				0.0002	Scandinavia, Quebec
SCL2A1	GLUT1 glucose transporter protein	*Xba*I	6.2 kb band	0.05	Europe and Japan
				0.0008	Japan
				0.017	Italy

Candidate genes and their polymorphisms are included in the table following the criteria of (*i*) three significant ($P<0.05$) independent studies, (*ii*) two independent studies with $P < 0.01$, or (*iii*) a single study replicating the first positive result with $P < 0.001$.

Sladek *et al.*, 2007; Steinthorsdottir *et al.*, 2007; Zeggini *et al.*, 2007), there are now 11 regions that associated with T2D at the levels of statistical confidence required for genetic association studies (Table 4) (Frayling, 2007). Seven of them were not identified from the candidate gene approach. Common variants in *transcription factor 7-like 2* (*TCF7L*) appeared as one of the top signals in the GWA studies (Sladek *et al.*, 2007). This gene encodes a transcription factor that is expressed in fetal pancreas and is involved in the WNT signaling pathway. The other six novel genes include *haematopoietically expressed homeobox* (*HHEX*), *cyclin-dependent kinase inhibitor 2A* (*CDKN2A-B*), *CDK5 regulatory subunit associated protein 1-like 1* (*CDKAL1*), *solute carrier family 30 (zinc transporter), member 8* (*SLC30A8*), *insulin-like growth factor 2 mRNA binding, protein 2* (*IGF2BP2*) and *fat mass and obesityassociated* (*FTO*) (Table 4). However, chromosome regions, probably with small effects, remain to be identified. The sample sizes of more than 10,000 cases and controls are required for finding such additional genes (Frayling, 2007).

Table 4 Single nucleotide polymorphisms (SNPs) and closet genes associated with T2D identified by genetic association studies (modified from Frayling, 2007).

Example variant	Closet gene	Previous evidence	*p*-value (Meta-analysis)	Additional evidence from human physiology	N*
rs1801282 (P12A)	*PPARG*[a]	Monogenic +drug target	2×10^{-6}	Nothing consistent	>20,000
rs5215 (E23K)	*KCNJ11*[a]	Monogenic +drug target	5×10^{-11}	Alters insulin secretion in general population	15,600
rs7901695	*TCF7L2*[b]	None	1×10^{-48}	Alters insulin secretion in general population	2,760
rs4430796	*TCF2*[a]	Monogenic	8×10^{-10}	Nothing consistent	>20,000
rs10010131	*WFS1*[a]	Monogenic	1×10^{-7}	Nothing consistent	>20,000
rs1111875	*HHEX–IDE*[c]	Some, e.g. HHEX KO mouse has disrupted pancreaticdevelopment	7×10^{-17}	Early studies indicate altered insulin secretion in general population	12,800
rs13266634	*SLC30A8*[c]	None	1×10^{-19}	Early studies indicate altered insulin secretion in general population	14,400
rs10946398	*CDKAL1*[c]	None	2×10^{-18}	Early studies indicate altered insulin secretion in general population	16,200
rs10811661	*CDKN2A–2B*[c]	Some –CDKN2A KO mouse has reduced isletproliferation	8×10^{-15}	Nothing consistent	12,400
rs4402960	*IGF2BP2*[c]	Some –binds insulin like growthFactor mRNA	9×10^{-16}	Nothing consistent	16,200
rs8050136	*FTO*[c]	None	1×10^{-12}	Alters BMI in general Population	10,400

* Total number of cases and controls needed in a 1:1 ratio to provide 80% power to detect an effect at $p = 5\times10^{-7}$, [a] gene identified by candidate approach, [b] gene identified by region-wide association, [c] gene identified by genome-wide association, *BMI, body mass index; CDKAL; CDK5 regulatory subunit associated protein 1-like 1; CDKN2, cyclin-dependent kinase inhibitor 2A; FTO, fat mass and obesityassociated; HHEX, haematopoietically expressed homeobox; IDE, insulin-degrading enzyme; IGF2BP2, insulin-like growth factor 2 mRNA binding, protein 2; KCNJ11, potassium inwardly-rectifying channel, subfamily J, member 11*; KO, knockout; N/C, not captured; *PPARG , peroxisome proliferator-activated receptor-γ gene*; *SLC30A8, solute carrier family 30 (zinc transporter), member 8; TCF2, transcription factor 2, hepatic; LF-B3, variant hepatic nuclear factor; TCF7L2, transcription factor 7-like 2 (T-cell specific, HMG-box); WFS1, Wolfram syndrome 1*.

Candidate genes for MODY

To date, six different genes are known to cause MODY including: *hepatocytenuclear factor-4α (HNF-4α)*, *glucokinase (GCK)*, *hepatocytenuclear factor-1α (HNF-1α)*, *insulin promoting factor-1 (IPF-1)*, *hepatcyteonuclearfactor-1β, (HNF-1β)*, and *neurogenic differentiation 1/β-cell E-box transactivator 2 (NeuroD 1/β 2)*. However, there are a number of MODY families that have no mutations in these six known genes responsible for MODY, which are referred to as MODY-X. The estimated prevalence of MODY-X is 15-20% of European families (Chevre *et al.*, 1998), and 60-80% of Chinese (Plengvidhya *et al.*, 2007) and Japanese families (Nishigori *et al.*, 1998). Analysis of genetic variability of MODY genes in Thai diabetic patients performed by Siriraj Diabetes Research Group (SiDRG) showed that sequence variation of the six known MODY genes accounts for a small proportion of both classic MODY (19%) and early-onset type 2 diabetes patients (10%) suggesting that MODY-X is also frequent in Thai population. Recently, SiDRG has investigated the role of *PAX4*, encoding transcription factor that plays a crucial role for β-cell development, in Thai patient with MODY-X. A novel mutation, R164W, has been identified. It was segregated with diabetes in the affected family. The mutant Pax 4 protein has less repressor activity on insulin and glucagon promoters as compared to the wild type one (Plengvidhya *et al.*, 2007).

Genetics of diabetic complications

In diabetes, long-term exposure to hyperglycemia leads to serious and frequently disabling or fatal complications. Diabetic complications are often categorized as microvascular (retinopathy, nephropathy), neuropathy, and macrovascular (cardiovascular and cerebrovascular). These complications can result in potential loss of vision, renal failure, foot ulcers, amputation, and Charcot joints, and autonomic neuropathy may be present with gastrointestinal and genitourinary.

Not only diabetes (T1D and T2D) but also their complications are influenced by genetic factors. There is mounting evidence for the role of genetic factors in several diabetic complications, particularly diabetic nephropathy (DN) and cardiovascular defects. The strongest evidence from epidemiological observations and family studies for the role of genetic background has been found for DN which is the most common and rapidly increasing cause of end-stage renal disease (ESRD) in the populations of developed countries. The familial clustering of overt DN and diabetic ESRD has been observed widely in multiple racial and ethnic groups, with the earliest reports of familial aggregation of diabetic kidney disease in patients with T1D. Family members with diabetes, even in the absence of clinical nephropathy, demonstrate similar patterns of glomerular involvement. The majority of data come from the studies in T1D (Seaquist *et al.*, 1989; Quinn *et al.*, 1996) but some were obtained from the analyses in T2D (Imperatore *et al.*, 2000). Unlike for nephropathy, the epidemiological studies do not provide strong support for the role of genes in diabetic retinopathy (DR) (Leslie and Pyke, 1982). However, some clinical observations and genetic analyses in T2D (Leslie and Pyke, 1982; Imperatore *et al.*, 2000) suggest that genetic influences are also involved in this microvascular complication. A study showed significant ethnic differences in the incidence of cardiovascular diseases (CAD) in T2D patients that were very likely the result of the heterogeneity of their genetic background (U.K. Prospective Diabetes Study Group, 1998).

Hundreds of loci have been studied so far in order to explain genetic susceptibility to diabetic complications. Most loci identified to date have not been replicated probably due to the complex etiologies of all diabetic complications resulting from interaction between plural genetic and clinical factors. Recent information indicating that, the most intriguing genes for further genetic studies are those encoding aldose receptor, advanced

glycation end products receptor, vascular endothelial growth factor, intercellular adhesion molecule 1, β3-adrenergic receptor gene, hemochromatosis, and α2β1 integrin. Pathways involving these gene products may represent a fruitful area for further studies aimed at investigating the genetics and pathophysiology of DN and DR. One gene that should be mentioned is that encodes aldose reductase in the polyol pathway, which is associated with DN and DR in T1D and T2D in several studies (Demaine *et al.*, 2000; Moczulski *et al.*, 2000; Neamat-Allah *et al.*, 2001; Wang *et al.*, 2003; D *et al.*, 2004). Another good example is haptoglobin which is a protein in the group of antioxidant proteins that was linked to cardiovascular complications in different populations (Hochberg *et al.*, 2002; Levy *et al.*, 2002). Recently, the role of genetic variability of *A20/TNFAIP3* has been show to modulate CAD risk in T2D, which was mediated by allelic differences in *A20* expression (Boonyasrisawat *et al.*, 2007).

Recent studies suggested that inflammation would be an essential component of T2D and its complications. An increased systemic and/or intra-renal inflammation in high glucose milieu is important in the pathogenesis of nephropathy in patients with T2D. The impact of inflammation on DN were studied by investigating polymorphisms in several genes encoding inflammatory cytokines and chemokines such as IL-1β, IL-1Ra, and TNF-α (Levy *et al.*, 2002). The understanding of genetic factors predisposing diabetic complications would help to unveil their pathogenesis.

CONCLUSION

DM and its complications are a global health problem. Every effort must be made to minimize the development of the disease and its complications. The effective means to identify the disease at an early stage, changes of life-style, and dietary behavior are important for prevention and control of DM. Characterization of genetic factors involving in the development of DM and its complications will lead to the understanding of their pathogenesis and to develop novel therapeutic approaches. A limitation of progression in this aspect is attributable to the complicated molecular genetics of DM *per se*. Linkage analysis and association study are the traditional techniques employed to identify diabetic susceptible genes. Several candidate genes have been identified but a few genes were reproducible in additional studies in different populations. The genome-wide association (GWA) analysis has recently been carried out to identify several novel diabetic susceptible genes with small contributing effects, the roles of which are being studied. The diabetic susceptible genes in Thai population are likely to be distinct and required to be characterized.

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Appendix III

Abstracts in international proceeding

JUNE 2009 VOLUME 58 SUPPLEMENT 1

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ABSTRACT BOOK

69th Scientific Sessions

Friday, June 5–Tuesday, June 9, 2009

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HLA-Cw1, Cw2, Cw4, and Cw14. All these associations were of borderline statistical significance, requiring reconfirmation on a much larger dataset of Romanian T1DM families. To conclude, our results indicate that some loci from the class I HLA region, namely HLA-Cw, could be involved in the genetic susceptibility for T1DM in the Romanian population.

2292-PO

The Influence of Single Nucleotide Polymorphisms (SNPs) of Chosen Candidate Genes Located at the Long Arm of Chromosome 7 (7q31-q35) on Development of Diabetic Nephropathy in Type 1 Diabetes

MIROSLAW ŚNIT, JANUSZ GUMPRECHT, KATARZYNA NABRDALIK, WŁADYSLAW GRZESZCZAK, *Zabrze, Poland*

The worldwide growing burden of end-stage renal disease (ESRD) mostly due to increasing number of people with diabetes become the reason for research looking for a single marker of development and progression of chronic kidney disease (CKD) that could be found at early stages of the disease when preventive action delaying the destructive process could be performed. We conducted the association study of SNPs of chosen candidate genes located at the long arm of chromosome 7 (7q31-q35) in parent-offspring trios. 102 subjects, 34 patients affected by type 1 diabetes with diabetic nephropathy and their 68 biological parents, had the SNPs of C2023G ABP1(amilorid binding protein), C(-106)T AKR1B (aldolase reductase), A(-579)G CALD1 (caldesmon 1), G934T CPA4 (carboxypeptidase A4), A19G LEP (leptin) and A831G PODXL1 (podocalyxin-like protein 1) genes genotyping with the use of the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) performed. To evaluate the allele transmission from heterozygous parents to affected individuals there was transmission disequilibrium test (TDT) accomplished. The G allele frequencies of A(-579)G CALD1 gene were significantly higher than expected (Table). There were 55 % of AG genotype and 15 % of GG genotype in diabetic patients observed. The other candidate genes polimorphisms lack evidence for association with diabetic nephropathy. Our findings suggest that DNA variations in the A(-579)G CALD1 gene encoding protein responsible for podocyte cytoskeleton and glomerular filtration membrane functioning may play role in the genetic predisposition to development of diabetic nephropathy.

Table.TDT for transmission frequency of allele A and G of A(-579)G CALD1 gene.

	Allele A transmitted	Allele A not transmitted	Allele G transmitted	Allele G not transmitted	Total number of transmitted allels	χ^2	p value
Observed	8	18	18	8	26	3.12	0.049
Expected	13	13	13	13			

GENETICS—TYPE 2 DIABETES

2293-PO

A Single Nucleotide Polymorphism (SNP) in the Adiponutrin Gene and Obesity or Type 2 Diabetes: Lack of Association in a Chinese Population

HUA LIANG, MENGYIN CAI, YAN BI, MING LI, YANHUA ZHU, JINHUA YAN, JIANPING WENG, *Guangzhou, China*

Adiponutrin is a non-secreted protein derived from adipose tissue. It possesses mainly acylglycerol transacylase activity which may facilitate lipid storage in WAT. It is possible, therefore, that adiponutrin is involved in the pathogenesis of common obesity. Recently, adiponutrin gene (ADPN) SNP rs2072907 was reported to be associated with obesity, suggesting ADPN as a candidate gene for obesity. In addition, obesity is a main risk factor for type 2 diabetes, we therefore aimed to assess whether the ADPN SNP rs2072907 is associated with obesity or type 2 diabetes in Chinese population. We genotyped SNP rs2072907 using multiplex Ligase Detection Reaction assay in 500 type 2 diabetics and 331 nondiabetic subjects. No significant difference was found in distribution of the SNP rs2072907 genotypes between type 2 diabetics and nondiabetic subjects. Among the 331 nondiabetic subjects, the distribution of the SNP rs2072907 genotypes was similar in nondiabetic overweight/obese (BMI ≥24kg/m2) and nonobese groups (BMI <24kg/m2), and SNP rs2072907 genotypes were not associated with differences in BMI, glucose or insulin. When this nondiabetic sample population was stratified according to sex, we found significantly greater fasting insulin, 2h insulin, the integrated area under the curve of plasma insulin levels and HOMA-IR in CC compared with GG+GC in men only. Based on these findings, we concluded that adiponutrin gene was not a susceptibility gene for obesity or type 2 diabetes in Chinese population, but ADPN SNP rs2072907 might confer an increased risk of hyperinsulinaemia to nondiabetic Chinese men.

2294-PO

A Study of *Peroxisome Proliferators-Activated Receptor-g (PPARg), Adiponectin,* and *Calpain-10 (CAPN10)* in Thais with Type 2 Diabetes

NATTACHET PLENGVIDHYA, WANISA SALAEMAE, KANJANA LEEJINDA, PRAPAPORN JUNGTRAKOON, NALINEE CHONGJAROEN, WATIP TANGJITTIPOKIN, NAPATAWN BANCHUIN, PA-THAI YENCHITSOMANUS, *Bangkoknoi, Bangkok, Thailand*

Genetic variations of *PPARg, adiponectin,* and *CAPN10* are associated with T2D in several populations. The aim of our study is to investigate whether SNPs of these three genes were associated with T2D in Thais. Pro12Ala and other four tagSNPs (rs9817428, rs1373640, rs4135275, and rs3856806) of *PPARg,* 3 SNPs (-11377C>G, 45T>G, 276G>T) and 1 variant (-11154_-11155delinsCA) of *adiponectin,* and 3 SNPs (SNP43, SNP63, SNP110) and 1 variant (Indel19) of *CAPN10* were genotyped in 272 T2D patients and 210 controls by using PCR-RFLP, PCR-SSCP, or sizing PCR methods. There was no association amid the studied variations and T2D. However, using linear regression analysis, associations among different variations and certain clinical characteristics were observed. Patients who carried minor allele (T) of *PPARg* rs3856806 had higher FPG (CC, 189.73±78.35 mg/dl; CT, 208.51±79.77 mg/dl; TT, 218.22±104.83 mg/dl; p=0.027), using sex, age, and treatment with anti-hyperglycemic drug as covariates. Patients who carried minor allele (8R) of *adiponectin* -11154_-11155delinsCA had lower BMI (7R7R, 28.13±5.06 kg/m2; 7R8R, 27.12±4.37 kg/m2; 8R8R, 23.98±4.87 kg/m2; p=0.013), waist circumference (7R7R, 88.76±10.87 cm; 7R8R, 86.05±10.56 cm; 8R8R, 78.75±12.09 cm; p=0.025), and waist/hip ratio (7R7R, 0.90±0.07 ; 7R8R, 0.88±0.06; 8R8R, 0.83±0.08; p=0.018) using sex, age, and treatment with anti-obesity drug as covariates. Patients who carried minor allele (G) of *CAPN10* SNP110 had lower HDL level (AA, 49.74±11.85 mg/dl; AG, 45.31±9.56 mg/dl; GG, 42.00±19.61 mg/dl; p=0.005), using sex, age, BMI, and treatment with anti-hyperlipidemic drug as covariates. Non-diabetic controls who carried minor allele (G) of *CAPN10* SNP110 had higher systolic BP (AA, 114.02± 3.65 mmHg; AG, 118.83±14.61 mmHg; GG, 126.80±11.88 mmHg; p=0.004) and higher diastolic BP (AA, 70.23±9.12 mmHg; AG, 72.26± 9.33mmHg; GG, 82.00±8.37 mmHg; p=0.015), using sex and age as covariates. We concluded that variations of these three genes, although were not associated with T2D, may influence different clinical parameters in studied subjects.

2295-PO

Association of *Paired Box 4 (Pax4)* Polymorphisms with Type 2 Diabetes in Thais

NATTACHET PLENGVIDHYA, JATUPORN SUJJITJOON, NONGLUCKSANAWAN RITTHISUNTHORN, NALINEE CHONGJAROEN, WATIP TANGJITTIPOKIN, NAPATAWN BANCHUIN, PA-THAI YENCHITSOMANUS, *Bangkoknoi, Bangkok, Thailand*

Paired Box 4 (Pax4) encodes a transcription factor that plays important roles in development, differentiation, and proliferation of pancreatic β-cell. Genetic variations of *Pax4* were reported to be associated with type 2 diabetes (T2D) in various ethnic groups. This study aimed to investigate whether two non-synonymous single nucleotide polymorphisms (SNPs) of *Pax4* were associated with T2D in Thais. The *Pax4* SNP rs2233580 (781G>A; R192H) and rs712701 (1168C>A; P321H) were genotyped in 270 T2D patients and 212 non-diabetic controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). We found that homozygous (AA) and heterozygous (GA) genotypes of rs2233580 were more frequent in T2D patients (p=0.037). There was no significant difference in genotype frequency of rs712701 between two groups. In addition, the A allele of rs2233580 (9.4% vs 5.7%; p=0.029) and the A allele of rs712701 (62.6% vs 56.1%; p=0.042) were more common in T2D patients than that in the controls and the A-A haplotype of these two SNPs was also more frequent in T2D patients (8.9% vs 5.6%; p=0.027). T2D patients who carried either AA or GA genotype of rs2233580 were diagnosed with diabetes at early age compared to those who carried GG genotype (46 yrs vs 49 yrs; p=0.010). Likewise, patients who carried AA genotype of rs712701 had diabetes at earlier age than those who carried CC genotype (48 yrs vs 56 yrs, p=0.001). The A-A haplotype associated with earlier age at diagnosis of T2D than the most frequent G-A haplotype (coefficient -2.433; p=0.034). In conclusion, these two non-synonymous SNPs of *Pax4* may influence the risk of developing and age at onset of T2D in Thais.

For author disclosure information, see page A713.

2296-PO

Association of the Peroxisome Proliferator Activated Receptor Gamma-2 Gene Pro12Ala Polymorphism and Birth Weight in Newborns—Preliminary Communication

AKSANA KRAUCHUK, EDYTA SIMOŃSKA-CICHOCKA, JOANNA ŻYWIEC, JANUSZ GUMPRECHT, KATARZYNA NABRDALIK, WŁADYSLAW GRZESZCZAK, *Zabrze, Poland*

Type 2 diabetes has a strong genetic background and also the association with decreasing body size at birth is observed. The polymorphism of the peroxisome proliferator–activated receptor gamma (PPARγ)-2 gene is one of the genetic factors which is thought to induce an insulin resistance or predispose to type 2 diabetes. The purpose of this study was to analyze whether the PPARγ-2 gene polymorphism Pro12Ala is associated with decreased birth weight. We studied 100 newborn children with adequate gestational age (39,12 ± 0,88 weeks), whose mother presented no disorders during pregnancy (gestational diabetes, hypertension) and were not cigarette smokers.The genomic DNA was extracted from umbilical cord blood leukocytes with the use of standard techniques. Statistics were done using SPSS for Windows v7. The newborns were divided into the three genotype groups: homozygote CC, homozygote GG, heterozygote CG. Genotype frequencies were as follows: CC-74%; CG-25%, GG-1%. There were no significant differences between groups in birth weight (3338,01g ± 478,97 vs 3491,53 ± 411,35 vs 3491,53 ± 478,91, respectively), birth length (54,14cm ± 3,25 vs 54,0cm ± 2,88 vs 54,0cm ± 2,88, respectively) and head circumference (33,98cm ± 1,39 vs 34,07cm ± 1,41 vs 34,07cm ± 1,41, respectively). However a trend between birth weight and male sex of newborns carrying Pro12Pro PPARγ genotype comparing to other genotypes carriers (3342,568g ± 407,37 vs 3626,250g ± 503,56 respectively, p = 0,06) was observed. In regression analysis (ANCOVA) there was no influence of mother BMI, mother age, newborn genotype and sex on birth weight.

2297-PO

Genetic Case-Control Association Study of *Vaspin Visfatin* in Thais with Type 2 Diabetes Mellitus

NATTACHET PLENGVIDHYA, NONGLUCKSANAWAN RITTHISUNTHORN, NALINEE CHONGJAROEN, KANJANA LEEJINDA, WATIP TANGJITTIPOKIN, NAPATAWN BANCHUIN, PA-THAI YENCHITSOMANUS, *Bangkoknoi, Bangkok, Thailand*

Vaspin and *visfatin* encodes adipokines that may link to obesity, insulin resistance, and glucose metabolism. Thus, their genetic variations may result in type 2 diabetes (T2D). The aim of our study was to investigate the association between sequence variations of *vaspin* or *visfatin* and T2D in Thais. Ten tagSNPs of *vaspin* and eight tagSNPs of *visfatin* were genotyped in 270 T2D and 212 non-diabetic subjects by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The *vaspin* rs3736804 (C>A) minor A allele was more common in controls than in T2D subjects (19% vs 14%, p=0.031) while CC genotype was more frequent in T2D patients than in controls (74.82% vs 66.04%, p=0.035). Haplotype analysis showed that *vaspin* haplotype rs3818258G/rs3736804A was more frequent in controls than in T2D subjects (18.6% vs 13.5%, p=0.018) and was significantly associated with the levels of triglyceride (coefficient 133.11, p=0.005) and low density lipoprotein (coefficient 141.68, p=0.001) in controls. This haplotype was also significantly associated with systolic and diastolic blood pressure (BP) in T2D subjects (coefficient 98.55, p=0.019 and coefficient 65.26, p=0.016, respectively). Although there was no association between tagSNPs of *visfatin* and T2D, a haplotype composed of minor alleles of eight tagSNPs (TCGGCCTA) showed a significant association with diastolic BP in controls (coefficient 15.09, p=0.048). Our findings suggested that variants of *vaspin* and *visfatin* may influence risks of developing T2D and may affect certain metabolic parameters in the studied population.

2298-PO

Genetic Variations of *Adiponectin* in Thais with Type 2 Diabetes

NATTACHET PLENGVIDHYA, PRAPAPORN JUNGTRAKOON, WANISA SALAEMAE, SARIN CHIMNARONK, NALINEE CHONGJAROEN, KANJANA LEEJINDA, WATIP TANGJITTIPOKIN, NAPATAWN BANCHUIN, PA-THAI YENCHITSOMANUS, *Bangkoknoi, Bangkok, Thailand, Phutthamonthon, Nakorn Pathom, Thailand*

Adiponectin is an insulin-sensitizing hormone exclusively secreted from adipose tissue. Hypoadiponectinemia is associated with metabolic features including obesity, insulin resistance and type 2 diabetes (T2D). The impact of genetic variations of *adiponectin* on the development of T2D was extensively studied in several populations. In the present study, we examined genetic variations of *adiponectin* which might exert the pathogenic effect in Thai patients with T2D. A number of 272 T2D patients and 210 non-diabetic controls were screened for genetic variations in *adiponectin* coding regions by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP). Samples with mobility shift revealed in the PCR-SSCP analysis were analyzed for nucleotide changes by direct sequencing. Five rare non-synonymous polymorphisms including R55H, R112H, R131H, R221S, and H241P were identified; three of which (R55H, R112H, and R131H) are novel. Moreover, R55H, R131H, and H241P were not identified in 210 non-diabetic controls suggesting the role of these polymorphisms in the development of T2D. R55 located in collageneous domain and responsible for triple helix formation of adiponectin protein is evolutionary conserved in eight different species including human, chimpanzee, canine, cow, mouse, rat, chicken and zebra fish. Thus, R55H may cause a structural change and have a pathogenic effect. R131 and H241 located in globular domain are also conserved among several species. Molecular modeling and *in silico* R131H mutagenesis revealed a change of hydrogen-bond forming between R131H and neighboring residues which may cause structural and functional changes. We concluded that these novel non-synonymous SNPs of *adiponectin* identified in Thais might influence the risk of T2D development.

2299-PO

Relationship between Vitamin D Receptor Gene Polymorphism and Post Transplantation Diabetes Mellitus

YAO BIN, *Guangzhou, Guangdong, China*

Objective: Study here aimed to investigate the relationship between FokI VDR gene polymorphism and post transplantation diabetes (PTDM).

Methods: The study was performed to all patients who received their first kidney transplantation and were followed up in our transplant center. The following criteria were used to determine what kind of patients was excluded: less than 18 years old, history of diabetes, hyperglycemia prior to transplantation, multiple organs transplantation. All patients underwent fasting plasma glucose (FPG) tests. Then oral glucose tolerance test (OGTT) were performed in non-diabetic recipients. Every recipient underwent FokI VDR polymorphism analysis by PCR-RFLP.

Results: Extensive survey was performed among 105 patients who received kidney grafting between February 2004 and December 2006. Of all the patients,16 (15.24%) were diagnosed as PTDM during following up. The genotypes of FokI VDR were analyzed by PCR-RFLP. The frequency of VDR FokI FF genotype was 36.2%, Ff genotype 44.8%, ff genotype 19.0% in 105 patients. Frequencies of F and f were 58.6% and 41.4%, respectively. The distributions of genotypes and alleles of VDR FokI in study subjects are as follow: $\chi 2$ test of heterogeneity between PTDM patients and control subjects in the group of FF ,Ff and ff. $\chi 2$=6.417,P =0.040.in the group of F and f. $\chi 2$=6.908, P=0.009.

The results show the frequencies of three genotypes (FF/Ff/ff) and two alleles (F/f) differed between PTDM and Non-PTDM.

Conclusions: High prevalence of abnormal glucose metabolism in renal allograft recipients during following up was observed. FokI VDR polymorphism might be a genetic mark for predicting risk of PTDM.

2300-PO

Systems Biology Refutes Rodent Adipocentric Model of Human Diabetes

IVAN NAGAEV, *Gothenburg, Sweden*

Obesity is a key risk factor of diabetes in mammals. As relative to man, rodent models suggest that adipocytes may secret abnormal set of adipokines in obesity. It was tested by a systemic analysis of genetic, evolution, biology and development aspects of leptin, adiponectin, resistin, IL6, TNF, RBP4, and insulin as a true hormone. As to only pancreatic insulin, none of adipokines is truly adipose-specific. Resistin is produced by fat in mice but immune cells in man, dog and pig. Not rodent but man and pig placenta is a main source of leptin. RBP4 fat/liver ratio is 0.1 in men but 0.26 in mice. Only adiponectin mRNA in fat predicts serum protein levels. TNF is not released by human but rodent fat cells. Nonfat cells are the main origin of TNF, IL6 and resistin in human fat. Diverse fat depots have specific profiles, differ by anatomy and vary in a life. Brown fat is a lifelong organ in rodents but brief in human infants. Impact of obesity on fat tissue itself also diverged in species. Visceral adiponectin and RBP4 levels in obesity are stable in men but reduced in mice. Leptin is boosted in obese mice but modestly increased in men. Muscle of obese humans may slowly raise adiponectin level that is radically enhanced by weight loss. Cancers activate insulin in insulinoma, resistin and TNF in leukemia, leptin in chondrosarcoma and RBP4 in liver tumors but usually repress adiponectin. In contrast to firmly controlled insulin in all species, adipokines vary across species and models of obesity. If endoderm forms endocrine system, leptin and adiponectin evolved with a mesoderm that forms adipose tissue. Though all genes deviated in species,

For author disclosure information, see page A713.

Appendix IV

Abstracts of international oral presentations

The 11th Symposium on Molecular Diabetology in Asia

(The Study Group of Molecular Diabetology in Asia)

December 19, 2009

Venue : Splendor Kaohsiung Hotel, Kaohsiung , TAIWAN

President: Lee-Ming Chuang, M.D., Ph.D.

Professor

Department of Internal Medicine &

Graduate Institute of Clinical Medicine

National Taiwan University School of Medicine

Taipei, TAIWAN

Friday, 18th December, 2009

19:00 **Welcome Party (Palace Club, 77F)**

20:30 **Committee Meeting (Amber Room, 42F)**

Saturday, 19th December, 2009

Place: 41F Diamond II

8:30 – 8:40 **Opening Remarks**
Lee-Ming Chuang (Taiwan)
Tong-Yuan Tai (Taiwan)
Kishio Nanjo (Japan)

8:40 – 9:20 **President Address/Lecture**
Chairperson: Tong-Yuan Tai (Taiwan)
Topic: Genetic association of diabetes and its clinical traits in Chinese population living in Taiwan
Speaker: Lee-Ming Chuang (Taiwan)

9:20– 9:45 **Educational Lecture 1:** Post-GWA era of type 2 diabetes
Speaker: Hiroto Furuta (Japan)
Chairperson: Masahiro Nishi (Japan)

9:45 – 10:10 **Educational Lecture 2:** Mitochondria gene polymorphism and diabetes
Speaker: Hong Kyu Lee (Korea)
Chairperson: Kyong Soo Park (Korea)

10:10 – 10:30 **Coffee break**

10:30 – 10:55 **Educational Lecture 3:** Molecular pathogenesis of diabetic nephropathy
Speaker: Shyi-Jang Shin (Taiwan)
Chairperson: Pei-Wen Wang (Taiwan)

10:55 – 11:20 **Educational Lecture 4:** Phenotype-genotype interactions on renal function in type 2 diabetes
Speaker: Juliana Chan (HongKong)
Chairperson: Hong Kyu Lee (Korea)

11:20 – 12:00 **Invited Lecture I:** Genome-wide association study of Type 2 Diabetes in Taiwan
Speaker: Jer-Yuarn Wu (Taiwan)
Chairperson: Lo-Tone Ho (Taiwan)

12:00 – 12:40 **Lunch**

Poster Discussion

12:40 – 13:20 Chairperson: **Ching-Chu Chen (Taiwan)**

P-1: Altered mitochondrial biogenesis and ER stress in visceral adipose tissue of obese insulin resistant C57BL/6J mice

Speaker: Pei-Wen Wang (Taiwan)

P-2: Serum vascular adhesion protein-1 predicts 10-year survival in type 2 diabetes
Speaker: Hung-Yuan Li (Taiwan)

P-3: Heritability of insulin sensitivity in adolescent twin/sibling: OGTT-based versus fasting-based indices
Speaker: Pi-Hua Liu (Taiwan)

P-4: Promoter gene polymorphisms of erythropoietin is associated with anemia in patients with type 2 diabetes mellitus
Speaker: TT Chiou (Taiwan)

P-5: FoxO1/PGC-1α inhibition by berberine are associated with reduced hepatic gluconeogenesis in high fat diet and streptozotocin induced diabetic rats
Speaker: Xuan Xia (China)

13:30 – 14:00 **Invited Lecture II:** Protective effect of PPARγ and PPARδ agonists agonist on ischemic -reperfusion organ injury
Speaker: Kenneth K. Wu (Taiwan)
Chairperson: Lee-Ming Chuang (Taiwan)

14:00 – 14:25 **Educational Lecture 5:** Characterization of genetic factors associated to type 2 diabetes in the Chinese
Speaker: Chen Hu (China)
Chairperson: Jianping Weng (China)

14:25 –15:10 **Oral Presentations I**
Chairperson: Yjin-Shing Jap (Taiwan),

OP-1
Magnetic resonance image of transplanted mouse islets labeled with chitosan-coated superparamanetic iron oxide nanoparticles
Jyuhn-Huarng Juang (Taiwan)

OP-2
Transplantation of mesenchymal stem cells mildly ameliorate diabetic nephropathy of streptomyzin inducing diabetic rats
Jianwei Li (China)

OP-3
Autophagy in pancreatic beta cells is essential for islet homeostasis and adaptation of beta cell mass in response to high-fat diet
Toyoyoshi Uchida (Japan)

15:10 – 15:30 **Coffee Break**

15:30 – 16:15 **Oral Presentations II**
Chairperson: **Juliana Chan (Hong Kong)**

OP-4
Association study of genetic polymorphisms of transcription factor 7-like 2 (*TCF7L2*) gene and type 2 diabetes in the Thai population
Watip Tangjittipokin (Thailand)

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OP-5
TCF7L2 genetic variants and progression to diabetes in the Chinese population: pleiotropic effects on insulin secretion and insulin resistance
Yi-Cheng Chang (Taiwan)

OP-6
Association of the insulin-like growth factor binding protein-3 (*IGFBP3*) -202A/C polymorphism with type 2 diabetes
Heung-Man Lee (Hong Kong)

16:15 – 17:00 **Oral Presentations III**
Chairperson: **Katsuya Tanabe (Japan), Jyuhn-Huarng Juang (Taiwan)**

OP-7
Long-term administration of exendin-4 improves glucose tolerance in Wfs1-deficient mice
Manabu Kondo (Japan)

OP-8
Wolfram syndrome 1 gene (*WFS1*) product localizes to secretory granule and determines acidification of granule in pancreatic beta cells.

OP-9
Ghrelin inhibits insulin secretion dependent on the AMPK-UCP2 Pathway
Ying Wang (Japan)

17:00-17:45 **Oral Presentations IV**
Chairperson: **Hiroto Furuta (Japan)**, **Wyne. H.H. Sheu (Taiwan)**

OP-10
Polymorphisms in *KCNQ1* are associated with gestational diabetes in a Korean population
Soo Heon Kwak (Korea)

OP-11
A P1198L mutation in *ABCC8* gene decreases ATP sensitivity of the K_{ATP} channel and causes permanent neonatal diabetes
Tomoyuki Takagi (Japan)

OP-12
IL4 gene and type1 diabetes mellitus in children
Yann-Jinn Lee (Taiwan)

17:45 – 17:55 **Closing Remarks from Chairman of the Study Group on Molecular Diabetology in Asia**
Kishio Nanjo (Japan)

Association Study of Genetic Polymorphisms of Transcription Factor 7-Like 2 (*TCF7L2*) Gene and Type 2 Diabetes in the Thai Population

Watip Tangjittipokin[1], Nalinee Chongjaroen[1], Nattachet Plengvidhya[2], Pa-thai Yenchitsomanus[3]

[1]Department of Immunology, [2]Department of Medicine, [3]Division of Medical Molecular Biology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Transcription factor 7-like 2 (TCF7L2) is a key element of the Wnt signaling pathway. Genome-wide association studies have showed that *TCF7L2* has been the most important locus predisposing to type 2 diabetes. Genetic polymorphism of the *TCF7L2* is one of the few validated variants with large effects on the risk of type 2 diabetes in the populations of European ancestry.

OBJECTIVE— We aim to investigate whether the noncoding variants in *TCF7L2* are associated with type 2 diabetes in Thai population.

RESEARCH DESIGN AND METHODS— Five single nucleotide polymorphisms, (rs7896340, rs7901695, rs7903146, rs12255372 and rs11196205) within the LD block were genotyped in type 2 diabetes patients (n=202) and ethnically matched control subjects (n=205) by high resolution melting (HRM) analysis using simple probe format. The associations of SNPs, haplotypes with type 2 diabetes and clinical characteristics of the patients were analyzed.

RESULTS—SNPs rs7896340 and rs11196205 of the *TCF7L2* were associated with type 2 diabetes in Thai population (p=0.023, dominant inheritance). The odds ratios (ORs) was 1.89 for the minor allele (95% CI 1.08-3.3) compared with the major allele. The haplotypes composed of GG minor allele of these two SNPs were also significantly associated with type 2 diabetes with ORs 1.89 (95% CI 1.12-3.19, p=0.018 (global p-value=0.013). Moreover, patients who carried minor allele of these two SNPS had earlier age onset of diabetes (AA=50.32± 10.78 year, AG+GG=45.51±10.52 year, p=0.012).

CONCLUSIONS—These data suggested that the *TCF7L2* polymorphisms are associated with type 2 diabetes and earlier age onset of the disease in the Thai population.

The 15th Congress of the ASEAN Federation of Endocrine Societies (AFES2009)

"The Art and Science of Endocrinology: From Evidence to Practice"

Congress Venue:

The AFES2009 Congress will be held at ***Queen Sirikit National Convention Center (QSNCC)***
Address: 60 New Rachadapisek Road, Klongtoey, Bangkok 10110, Thailand
Tel: (662) 229-3000
Fax: (662) 229-3001

"COPY NUMBER VARIATION (CNV) GENOTYPING OF CAPN10 GENE IN THAIS WITH TYPE 2 DIABETES BY DENATURING HIGH PRESSURE LIQUID CHROMATOGRAPHY (DHPLC)" (OP1-04) has been selected for oral presentation

Date: 29 November 2009
Time: 14.06-14.18 hr. (Present: 10 minutes, Q&A: 2 minutes)
Venue: Boardroom 2

Copy Number Variation (CNV) Genotyping of *CAPN10* Gene in Thais with type 2 diabetes by Denaturing High Pressure Liquid Chromatography (dHPLC)

Kanjana Chanprasert[1], Watip Tangjittipokin[1], Wanna Tongnoppakhun[2], Nattachet Plengvidhya[3], Pa-thai Yenchitsomanus[4]

[1]Department of Immunology, [2]Departments of Research and Development, [3]Department of Medicine, [4]Division of Medical Molecular Biology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

Type 2 Diabetes Mellitus (T2D) is a multifactorial disorder. Positional cloning studies had mapped T2D susceptibility to *CAPN10* encoding the intracellular cysteine protease that plays role in insulin-mediated glucose metabolism, insulin production and release from pancreatic beta cells. However, Siriraj Diabetes Research Group (SiDRG) had not found association between variations in *CAPN10* and T2D in Thais and deviations from Hardy Weinberg Equilibrium (HWE) were observed in genotype distribution.

Copy number variations (CNVs) are 1 kb or larger in size and exist in variable copy numbers. CNVs can cause genomic disorders, or confer risk to complex disease. CNVs comprised of deletion, insertion and duplication that may cause the genotype deviate from HWE. Also the deviation from HWE in *CAPN10* gene has been reported in various ethic groups. Hence, we aim to detect existence of CNVs in *CAPN10* and investigate its impact on HWE and the risk of T2D in our population.

Indel19, a variant showing frequency deviation from HWE, was studied in 262 cases and 230 controls by multiplex PCR combining dHPLC method. We were success to detect CNVs in *CAPN10*. Indel19 was well-suited for HWE after correction ($p>0.05$).

The existence of CNV in *CAPN10* has been identified in Thais. This CNVs may lead to deviation from HWE. Although *CAPN10* was not associated with T2D, the method and information that obtained from our study would be helpful for accurate genotyping the variation in CNV region which could be useful for precise association analysis of variation within CNV region and complex diseases.

Appendix V

Abstracts of national oral presentations

RGJ - Ph.D. Congress X

การประชุมวิชาการ
โครงการปริญญาเอกกาญจนาภิเษก ครั้งที่ 10

April 3-5, 2009
Jomtien Palm Beach Resort
Pattaya, Chonburi

สกว
TRF

The Royal Golden Jubilee Ph.D. Program
The Thailand Research Fund

ISBN 978-611-12-0004-1

16.10 – 16.25	S3A-O1	The Effects of Culture Media and Embryo Density on Developmental Competence of Cat Embryos	น.ส. ธนิดา สนั่นเมือง	Ms. Thanida Sananmuang
16.25 – 16.40	S3A-O2	Functional Studies of Double Non-Synonymous Single Nucleotide Polymorphisms of *Paired Box 4* in Thais with Type 2 Diabetes	น.ส. จตุพร สุจจิตร์จูล	Ms. Jatuporn Sujjitjoon
16.40 – 16.55	S3A-O3	Effect of Translationally Controlled Tumor Protein against Cell Death from 2-Hydroxyethyl Methacrylate in Human Dental Pulp Cells	น.ส. ณัฐพร วนโชติตระกูล	Ms. Nattaporn Wanachoattrakul
16.55 – 17.10	S3A-O4	Human Breast Cancer Susceptibility Gene 1 (*BRCA1*) Damaged by Ruthenium(II)-Arene PTA (RAPTA) Complexes	นาย พรวิชัย เต็มบุตร	Mr. Pornvichai Temboot
17.10 – 17.25	S3A-O5	Identification of Susceptibility Loci in the Northeast Thai Families with Kidney Stone Disease by Genome-Wide Linkage Analysis	น.ส. อรนุช ประดิษฐ์ทรัพย์	Ms. Oranud Praditsap
	S3B: Cancer ***(Venue: Marine I)***			
	Chairpersons: Prof. Apiwat Mutirangura / Assoc. Prof. Puangrat Yongvanit			
15.30 – 15.50	Invited Lecture S3B-L1: ***Prof. Wim E. Hennink***, "*In Situ* Forming Hydrogels for the Controlled Release of Proteins"			
15.50 – 16.10	Invited Lecture S3B-L2: ***Prof. Seiji Okada***, "Application of Immunodeficient Mice for Biomedical Research"			
16.10 – 16.25	S3B-O1	Potential of Using 22-Oxa-1α, 25-dihydroxyvitamin D_5 as Supplementary Therapy for Cholangiocarcinoma: A Preclinical Study	นาย วันชนะ สืบไวย	Mr. Wunchana Seubwai
16.25 – 16.40	S3B-O2	MARCKS Phosphorylation Promotes Cholangiocarcinoma Cell Migration and Metastasis via PKC-Dependent Pathway upon TPA Stimulation	น.ส. อัญชลี เดชะเสน	Ms. Anchalee Techasen
16.40 – 16.55	S3B-O3	Inflammation Driven Cholangiocarcinogenesis: Role of Lipid Peroxidation Inducing DNA Damage	น.ส. สมคิด เดชะคำภู	Ms. Somkid Dechakhamphu
16.55 – 17.10	S3B-O4	Differential Expression Profile of Peripheral Blood Leukocytes Related to Poor Survival of Cholangiocarcinoma Patients	น.ส. ชุติมา ทวัพย์อิ่มเอิบ	Ms. Chutima Subimerb
17.10 – 17.25	S3B-O5	Chemopreventive Effects of Fermented Brown Rice and Rice Bran (FBRA) Against Tobacco-Specific Nitrosamines (NNK)-Induced Lung Tumorigenesis in Female A/J Mice	นาย สุพจน์ พุทธผดุง	Mr. Suphot Phutthaphadoong
4 April 2009	**S3A: Developmental Biology** ***(Venue: Marine II)***			
	Chairperson: Assoc. Prof. Janenuj Wongtavatchai			
08.30 – 08.50	Invited Lecture S3A-L3: ***Prof. Michael S. McGrath***, "Role of Macrophages in Cancer Metastasis: AIDS Lymphoma as a Model"			
08.50 – 09.10	Invited Lecture S3A-L4: ***Prof. Jean-Christophe Avarre***, "Real-Time Microarrays: a New Technology With Great Potential"			
09.10 – 09.25	S3A-O6	Characterization of Phosphorylated Proteins in Viral-infected Hemocytes of *Peneaus monodon*	นาย ศุภรัตน์ แดงชัยภูมิ	Mr. Suparat Taengchaiyaphum
09.25 – 09.40	S3A-O7	Identification and Characterization of the Novel Gene Implicated in Hemocyte Homeostasis in *Penaeus monodon*	นาย อดิศักดิ์ ประภาวรรัตน์	Mr. Adisak Prapavorarat
09.40 – 09.55	S3A-O8	RNAi-mediated Functional Characterization of Two Prophenoloxidases from Black Tiger Shrimp *Penaeus monodon*	น.ส. วลัยพร เจริญทรัพย์ศรี	Ms. Walaiporn Charoensapsri
09.55 – 10.10	S3A-O9	Pathobiological Characteristic of Streptococcosis in Farmed Tilapia *Oreochromis nilotica* in Thailand	น.ส. หทัยรัตน์ ไม้สัก	Ms. Hathairat Maisak
10.10 – 10.30	**Coffee Break**			

16.40 – 16.55	S3C-O10	A One-Step Rapid Immunochromatographic (IC) Test for Diagnosis of Fasciolosis Caused by *Fasciola gigantica*	นาย ปณัฐ อนุรักษ์ปรีดา	Mr. Panat Anuracpreeda
	S3C: Biological Science ***(Marine II)***			
	Chairperson: Assoc. Prof. Chanan Angsuthanasombat			
15.00 – 15.20	Invited Lecture S3C-L4: ***Prof. John Mekalanos***, "Characterization of the Type III Secretion System of *Vibrio cholerae* non-O1, non-O139 strain AM-19226"			
15.20 – 15.40	Invited Lecture S3C-L5: ***Prof. Timothy S. Wiedmann***, "Aerosol Delivery to the Retina During Pars Plana Vitrectomy"			
15.40 – 15.55	S3C-O11	Effective Dystrophin Induction in the Muscles of *mdx* Mice by a Morpholino Oligomer	นาย นที เจียรวิริยะไพศาล	Mr. Natee Jearawiriyapaisarn
15.55 – 16.10	S3C-O12	Changes in Vasoactive Intestinal Peptide Immunoreactivity in the Brain of Nest-Deprived Native Thai Hens	น.ส. นัตติยา ประกอบแสง	Ms. Nattiya Prakobsaeng
16.10 – 16.25	S3C-O13	Changes in the Levels of Serotonin and Dopamine in the Central Nervous System and Ovary, and Their Possible Roles in the Ovarian Development in the Giant Freshwater Prawn, *Macrobrachium rosenbergii*	นาย ยสวันต์ ตินิกุล	Mr. Yotsawan Tinikul
16.25 – 16.40	S3C-O14	Prenatal Diagnosis of α- and β-Thalassemias by Analysis of Fetal Blood Using Capillary Electrophoresis System	น.ส. หทัยชนก ศรีวรกุล	Ms. Hataichanok Srivorakun
16.40 – 16.55	S3C-O15	A Functional Role for Calpain and Proteolysis in the Erythrocyte as Modulators of Disease Severity in β-Thalassemia	นาย สุริยัน สุขติ	Mr. Suriyan Sukati
16.55 – 17.10	S3C-O16	Genetic Variations of *Adiponectin* in Thais with Type 2 Diabetes	น.ส. ประภาพร จึงตระกูล	Ms. Prapaporn Jungtrakoon
17.10 – 17.25	S3C-O17	Study on Pharmacokinetics of *Kaempferia parviflora* in Rats after Intravenous Administration	น.ส. คัทลียา เมฆจรัสกุล	Ms. Catheleeya Mekjaruskul

S4: วิทยาศาสตร์การเกษตร และวิทยาศาสตร์สิ่งแวดล้อม (Agricultural Science and Environmental Science)

3 April 2009	**Agricultural Science and Environmental Science** ***(Venue: Oriental Palm I)***			
	Chairperson: Prof. Aran Patanothai			
15.30 – 15.50	Invited Lecture S4-L1: ***Prof. Henrik Balslev***, "Neotropical Ethnobotany"			
15.50 – 16.10	Invited Lecture S4-L2: ***Prof. Timothy D. Leathers***, "Alternan Research at the National Center for Agricultural Utilization Research"			
16.10 – 16.25	S4-O1	Characteristics and Implication of Residual Layers in Natraqualfs, Northeast Plateau, Thailand	น.ส. จุฑามาศ แก้วมโน	Ms. Chutharmard Kaewmano
16.25 – 16.40	S4-O2	The Influence of Rainfall Events on Soil Respiration in Wheat Field	น.ส. ชมภูนุช ฉายาเวช	Ms. Chompunut Chayawat
16.40 – 16.55	S4-O3	Determination of Target Areas for Breeding Sugarcane for Specific Adaptation and Identification of Traits Determining Specific Adaptation in Sugarcane	นาง พีรญา กลมสอาด	Mrs. Peeraya Klomsa-ard
16.55 – 17.10	S4-O4	*In Situ* Measurements of Root and Soil Respirations in Dry Dipterocarp Forest	นายพงษ์เทพ หาญพัฒนากิจ (MAG)	Mr. Phongthep Hanpattanakit (MAG)
17.10 – 17.25	S4-O5	Optimal Conditions for H_2 Production in *Synechocystis* sp. PCC 6803	น.ส. วิภาวี แบบประเสริฐ	Ms. Wipawee Baebprasert
4 April 2009	**Agricultural Science** ***(Venue: Oriental Palm I)***			
	Chairperson: Prof. Peerasak Srinives			
08.30 – 08.50	Invited Lecture S4-L3: ***Prof. Julian J. Eaton-Rye***, "The Role of the Low-Molecular-Weight Polypeptides at the Monomer-Monomer Interface of Photosystem II in the Cyanobacterium *Synechocystis* sp. PCC 6803"			
08.50 – 09.10	Invited Lecture S4-L4: ***Prof. Hidetaka Hori***, "A Novel Immune System in Insects Through Midgut Membrane Protein – Roles of Radical Formed by 252-kDa Chlorophyllide-Binding Protein"			

S3A-O2

Functional Studies of Double Non-Synonymous Single Nucleotide Polymorphisms of *Paired Box 4* in Thais with Type 2 Diabetes

Jatuporn Sujjitjoon,[a] Suwattanee Kooptiwut,[b] Nattachet Plengvidhya,[c] Malika Churintaraphan,[b] Namoiy Semprasert,[b] Nonglucksanawan Ritthisuntorn,[a] Nalinee Chongjaroen,[a] Watip Tangjitipokin,[a] Napatawn Banchuin,[a] and Pa-thai Yenchitsomanus[d]

Departments of [a]Immunology, [b]Physiology, [c]Medicine, [d]Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Introduction and Objective

Variations of *Paired box 4 (Pax4)* gene were reported to be associated with diabetes in several ethnic groups (1, 2) including Thais (3). Two non-synonymous SNPs rs2233580 (781G>A; R192H) and rs712701 (1168C>A; P321H) in *Pax4* were found to be associated with early-age at onset of type 2 diabetes (T2D) in Thais. We therefore investigated transcriptional repressor activity of the Pax4 protein containing these two variants on its target-gene promoters by luciferase reporter assay.

Methods

pcDNA3.1/HisB vectors containing the SNPs encoding either 321H alone or combined 192H and 321H mutants were generated by PCR-mediated site-directed mutagenesis. The entire coding sequences of all constructs were verified by automated DNA sequencing. αTC1.9 cells were transiently transfected with pcDNA3.1/HisB empty vector or *Pax4* wild-type (WT) or each of *Pax4* mutants together with pGL3-human *Glucagon* promoter and pRL-SV40 using Fugene 6 reagent. After 24 hrs, luciferase activities were measured by Dual-Luciferase Reporter assay. Normalized luciferase activities from two independent experiments were presented as mean ± SEM and then analysed by one-way ANOVA followed by *post-hoc* test. The p-value <0.05 was considered to have statistically significant difference.

Results

The normalized luciferase activities of Pax4 WT and Pax4 double-mutants, 192H and 321H, on human *glucagon* promoter were 0.66±0.33 and 1.37±0.36, respectively (p=0.001). Similar to Pax4 double-mutants, normalized luciferase activity of Pax4 192H was 1.38±0.49, exhibited a significantly decreased repressor activity compared to that of Pax4 WT (p=0.001). There was no statistically significant difference between activities of Pax4 321H (0.68±0.24) and Pax4 WT. These data suggested that a reduction of repressor activity observed from Pax4 double-mutants may be caused by 192H alone. However, Pax4 P321H had been previously reported to have a functional defect leading to T1D (1). This result is likely due to different target-gene promoters and/or cell lines that were used in the experiments.

Conclusion

Transcriptional repressor activity of Pax4 192H was reduced but no functional defect of Pax4 321H on human *glucagon* promoter in αTC1.9 cells was observed. However, functional study of these two variants using other target-gene promoters and/or cell lines will further be investigated for better understanding the role of Pax4 in pathogenesis of diabetes mellitus.

Keywords: Paired box 4, type 2 diabetes, functional study, luciferease reporter assay

Selected References:

1. Biason-Lauber A, Boehm B, Lang-Muritano M, Gauthier BR, Brun T, Wollheim CB, et al. Diabetologia 2005; 48: 900-5.
2. Shimajiri Y, Sanke T, Furuta H, Hanabusa T, Nakagawa T, Fujitani Y, et al. Diabetes 2001; 50: 2864-9.
3. Plengvidhya N, Kooptiwut S, Songtawee N, Doi A, Furuta H, Nishi M, et al. J Clin Endocrinol Metab 2007; 92: 2821-6.

S3A-O5

Identification of Susceptibility Loci in the Northeast Thai Families with Kidney Stone Disease by Genome-Wide Linkage Analysis

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Introduction and Objective

Kidney stone disease (nephrolithiasis) is a relatively common public health problem in the northeastern (NE) Thai population. Previous studies have shown that many risk factors might influence pathogenesis of the disease and genetic factors may also play roles. However, the genetic basis of kidney stone in NE Thai population is still unclear and it may be different from that reported in other ethnic groups because of its distinct biochemical characteristics. The aim of this study is thus to identify the genes that are involved in pathogenesis of kidney stone in NE Thai families.

Methods

Genome-wide linkage analysis was performed in 2 extended families with kidney stone disease diagnosed by clinical and radiographical investigations. Twenty seven subjects were subjected to a genome-wide scan using Affymetrix GeneChip® Mapping 10K 2.0 Array. Two-point, multipoint parametric and non-parametric analyses were conducted with the software package, easyLINKAGE, using both dominant and recessive models.

Results

A positive result of suggestive linkages (LOD>2) on chromosomes 18, 12, and 11 was observed in one family by all analyses using the dominant model. In addition, significant linkages (LOD>3.5) on chromosomes 2, 4, and 18 were found in the other family by non-parametric analysis using the dominant model.

Conclusion

This study reports linkage loci that may contain susceptibility gene(s) associated with kidney stone in the NE Thai families. The significant chromosome interval reported here needs further confirmation in other families. Fine mapping and identification of candidate genes within these regions are in progress.

Keywords: kidney stone disease, nephrolithiasis, genetics, genome-wide linkage

Selected References:

1. Sritippayawan S, Borwornpadungkitti S, Paemanee A, Predanon C, Susaengrat W, Chuawattana D, Sawasdee N, Nakjang S, Pongtepaditep S, Nettuwakul C, Rungroj N, Vasuvattakul S, Malasit P, Yenchitsomanus P. Urol Res 2009; revised.
2. Park S, Pearle MS. Urol Clin N Am 2007; Aug; 34(3): 323-34.
3. Curhan GC. Urol Clin N Am 2007; Aug; 34(3): 287-93.
4. Miller NL, Evan AP, Lingeman JE. Urol Clin N Am 2007; Aug; 34(3): 295-313.

S3C-O16

Genetic Variations of *Adiponectin* in Thais with Type 2 Diabetes

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Introduction and Objective

Adiponectin is an insulin-sensitizing hormone exclusively secreted from adipose tissue. Hypoadiponectinemia is associated with metabolic features including obesity, insulin resistance and type 2 diabetes (T2D). The impact of genetic variations of *adiponectin* on the development of T2D was extensively studied in several populations. In the present study, we examined genetic variations of *adiponectin* which might exert the pathogenic effect in Thai patients with T2D.

Methods

A number of 272 T2D patients and 210 non-diabetic controls were screened for genetic variations in *adiponectin* coding regions by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP). Samples with mobility shift revealed in the PCR-SSCP analysis were analyzed for nucleotide changes by direct sequencing. The molecular remodeling and *in silico* mutagenesis were performed by PyMOL and Swiss-Pdb Viewer 4.0.1 programs.

Results

Five rare non-synonymous polymorphisms including R55H, R112H, R131H, R221S, and H241P were identified; three of which (R55H, R112H, and R131H) are novel. Moreover, R55H, R131H, and H241P were not found in 210 non-diabetic controls suggesting the role of these polymorphisms in the development of T2D. R55 located in collageneous domain and responsible for triple helix formation of adiponectin protein is evolutionary conserved in eight different species including human, chimpanzee, canine, cow, mouse, rat, chicken and zebra fish. Thus, R55H may cause a structural change and have a pathogenic effect. R131 and H241 located in globular domain are also conserved among several species. Molecular modeling and *in silico* R131H mutagenesis revealed a change of hydrogen-bond forming between R131H and neighboring residues which may cause structural and functional changes.

Conclusion

The novel non-synonymous alterations, R55H and R131H, identified in Thais might influence the risk of T2D development. However, the functional effects of these two amino acid changes should be further confirmed by biochemical studies.

Keywords: Adiponectin, genetic variation, type 2 diabetes, Thai

Selected References:

1. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. J Biol Chem 2003; 278: 40352-463.
2. Okamoto Y, Kihara S, Funahashi T, Matsuzawa Y, Libby P. Clin Sci (Lond) 2006; 110: 267-78.

การประชุมวิชาการ

Human Genomics and Molecular Biology 2009

พิธีเปิดโดย ศ.ดร.วิชัย บุญแสง ผู้อำนวยการฝ่ายวิชาการ สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) และ ศ.ประดิษฐ์ พงศ์ทองคำ นายกสมาคมพันธุศาสตร์แห่งประเทศไทย

หัวข้อการบรรยาย

1. Human Genomics and Molecular Biology - ศ.ดร.เพทาย เย็นจิตโสมนัส
2. Genomic Analysis and Bioinformatics - อ.ดร.ประพัฒน์ สุริยผล
3. Genetic and Genomic Analysis in Kidney Stone - อ.ดร.นัญวรรณ รุ่งโรจน์
4. Genetic and Genomic Analysis in Diabetes - ดร.วทิพย์ ตั้งจิตติโภคิน
5. Transcription Factor and Promoter Analysis in Human Diseases - รศ.ดร.พญ.สุวัฒณี คุปติวุฒิ
6. Transcriptomic Analysis and Human Diseases - อ.นพ.ชัชวาล ศรีสวัสดิ์
7. Proteomic Analysis in Human Cancers - ดร.มุทิตา จุลทิ่ง
8. Protein-Protein Interaction Analysis and Human Diseases - พศ.ดร.นพ.ถาวรชัย ลิ้มจินดาพร

วันจันทร์ที่ 14 ธันวาคม 2552

เวลา 08.30 ถึง 16.30 น. ณ ห้องอัมรินทร์ โรงแรมเอส.ดี. อเวนิว (S.D. Avenue Hotel) กรุงเทพฯ

ค่าลงทะเบียน : ผู้สนใจทั่วไป 200 บาท นิสิต นักศึกษา 100 บาท

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http://www.si.mahidol.ac.th/th/department/research/hgmb.asp
หมดเขตลงทะเบียนวันที่ 30 พฤศจิกายน 2552

จัดโดย : โครงการเมธีวิจัยอาวุโส สกว. - สกอ. (ศ.ดร.เพทาย เย็นจิตโสมนัส และคณะ)

สนับสนุนโดย : สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) สำนักงานคณะกรรมการการอุดมศึกษาแห่งชาติ (สกอ.) สมาคมพันธุศาสตร์แห่งประเทศไทย และสถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

ติดต่อสอบถาม : คุณฉัตรชัย ภูน้ำค้าง หน่วยอณูชีววิทยาการแพทย์ สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล ถนนพรานนก แขวงศิริราช เขตบางกอกน้อย กรุงเทพฯ 10700 โทรศัพท์ 02-419 7000 ต่อ 6666-70 โทรสาร 02-418 4793. อีเมล์ : aochatchai@gmail.com

กำหนดการประชุมวิชาการ

Human Genomics and Molecular Biology 2009

จัดโดย กลุ่มเมธีวิจัยอาวุโส สกว.-สกอ. (ศ. ดร. เพทาย เย็นจิตโสมนัส) และ

สมาคมพันธุศาสตร์แห่งประเทศไทย

วันจันทร์ ที่ 14 ธันวาคม พ.ศ. 2552 เวลา 08.30 น. ถึง 16.30 น.

ณ ห้องอัมรินทร์ โรงแรมเอส.ดี. อเวนิว (S.D. Avenue Hotel) กรุงเทพฯ

07.30 – 08.30 น. ลงทะเบียน

08.30 – 09.00 น. พิธีเปิดการประชุม
โดย ศ. ดร. วิชัย บุญแสง ผู้อำนวยการฝ่ายวิชาการ สำนักงานกองทุนสนับสนุนการวิจัย
และ ศ. ประดิษฐ์ พงศ์ทองคำ นายกสมาคมพันธุศาสตร์แห่งประเทศไทย

การบรรยายภาคเช้า*

09.00 – 09.40 น. **Human Genomics and Molecular Biology**
ศ. ดร. เพทาย เย็นจิตโสมนัส
สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

09.40 – 10.20 น. **Genomic Analysis and Bioinformatics**
อ. ดร. ประพัฒน์ สุริยผล
สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

10.20 – 10.40 น. พักรับประทานอาหารว่างและชา-กาแฟ*

10.40 – 11.20 น. **Genetic and Genomic Analysis in Kidney Stone**
อ. ดร. นัญวรรณ รุ่งโรจน์
สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

11.20 – 12.00 น. **Genetic and Genomic Analysis in Diabetes**
ดร. วทิพย์ ตั้งจิตติโภคิน
ภาควิชาวิทยาภูมิคุ้มกัน คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

12.00 – 13.00 น. พักรับประทานอาหารกลางวัน

การบรรยายภาคบ่าย*

13.00 – 13.40 น. **Transcription Factor and Promoter Analysis in Human Diseases**
รศ. ดร. พญ. สุวัฒณี คุปติวุฒิ
ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

13.40 – 14.20 น. **Transcriptomic Analysis and Human Diseases**
ผศ. ดร. นพ. ชัชวาล ศรีสวัสดิ์
ภาควิชาชีวเคมี คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

14.20 – 14.40 น. พักรับประทานอาหารว่างและชา-กาแฟ*

14.40 – 15.20 น. **Proteomic Analysis in Human Cancers**
ดร. มุทิตา จุลกิ่ง
สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

15.20 – 16.00 น. **Protein-Protein Interaction Analysis and Human Diseases**
ผศ. ดร. นพ. ถาวรชัย ลิ้มจินดาพร
ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

16.00 – 16.15 น. **สรุปและปิดการประชุม**
ศ. ดร .เพทาย เย็นจิตโสมนัส
สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

***หมายเหตุ**
- จบการบรรยาย เปิดโอกาสให้มีการถาม-ตอบ และแสดงความคิดเห็น ตามความเหมาะสมของเวลา
- หากมีความจำเป็นเรื่องเวลา อาจจะมีการเสริฟอาหารว่างและชา-กาแฟ ระหว่างการบรรยาย

บทคัดย่อ
การเสนอผลงานแบบบรรยาย

การประชุมนักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. ครั้งที่ 9

วันที่ 15-17 ตุลาคม 2552
โรงแรมฮอลิเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ
จังหวัดเพชรบุรี

สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) สำนักงานคณะกรรมการการอุดมศึกษา (สกอ.)

Functional defect of truncated hepatocyte nuclear factor-1α (G554fsX556) associated with maturity-onset diabetes of the young

Kooptiwut, S.[1*], Sujjitjoon, J.[2], Plengvidhya, N.[2,3], Boonyasrisawat, W.[2], Chongjaroen, N.[2], Jungtrakoon, P.[2], Semprasert, N.[1], Furuta, H.[4], Nanjo, K.[4], Banchuin, N.[2], Yenchitsomanus, P.[5]

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[5]*Division of Medical Molecular Biology, of Medicine Department of Research and Development, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*

Abstract

A novel frameshift mutation attributable to 14-nucleotide insertion in *hepatocyte nuclear factor-1α* (*HNF-1α*) encoding a truncated HNF-1α (G554fsX556) with 76-amino acid deletion at its carboxyl terminus was identified in a Thai family with maturity-onset diabetes of the young (MODY). The wild-type and mutant HNF-1α proteins were expressed by *in vitro* transcription and translation (TNT) assay and by transfection in HeLa cells. The wild-type and mutant HNF-1α could similarly bind to human glucose-transporter 2 (*GLUT2*) promoter examined by electrophoretic mobility shift assay (EMSA). However, the transactivation activities of mutant HNF-1α on human *GLUT2* and rat *L-type pyruvate kinase* (*L-PK*) promoters in HeLa cells determined by luciferase reporter assay were reduced to approximately 55-60% of the wild-type protein. These results suggested that the functional defect of novel truncated HNF-1α (G554fsX556) on the transactivation of its target-gene promoters would account for the β-cell dysfunction associated with the pathogenesis of MODY.

Keywords: diabetes, maturity-onset diabetes of the young, MODY, *HNF-1α*, frameshift mutation, dual-luciferase assay, Thai

Outputs

1. Kooptiwut S, Sujjitjoon J, Plengvidhya N, Boonyasrisawat W, Chongjaroen N, Jungtrakoon P, Semprasert N, Furuta H, Nanjo K, Banchuin N, Yenchitsomanus P.T. *Functional defect of truncated hepatocyte nuclear factor-1alpha (G554fsX (556associated with maturity-onset diabetes of the young*. Biochem Biophys Res Commun **2009**; 383 (1): 68-72.

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Prothrombin Haplotypes Associated with Kidney Stone Risks in Northeastern Thai Population

Rungroj, N.[1,2], Sritippayawan, S.[3], Thongnoppakhun, W.[2], Paemanee, A.[4], Sawasdee, N.[1], Nettuwakul, C.[1], Sudtachat, N.[4], Ungsupravate, D.[1], Praihirunkit, P.[5], Chuawattana, D.[3], Akkarapatumwong, V.[5], Borwornpadungkitti, S.[6], Susaengrat, W.[6], Vasuvattakul, S.[3], Malasit, P.[1,4], Yenchitsomanus, P.[1,4*]

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Abstract

Kidney stone is a common public health problem in northeastern Thai population. Genetic and environmental factors may involve in its pathogenesis. To determine genetic variations associated with the disease, we performed a case-control association study using 112 subjects each of patient and control groups by genotyping 67 single nucleotide polymorphisms (SNPs) within 8 genes including *TFF1*, *S100A8*, *S100A9*, *S100A12*, *AMBP*, *SPP1*, *UMOD*, and *F2*, encoding urinary stone-inhibitor proteins; trefoil factor 1, calgranulin (A, B, and C), bikunin, osteopontin, Tamm-Horsfall protein, and urinary prothrombin fragment 1, respectively. Significant differences between the case and control groups of allele and genotype frequencies of 8 SNPs in *F2* were found while those in the remaining 7 genes were not. Interestingly, frequencies of two *F2* haplotypes were significantly different between the case and control groups, one haplotype (TGCCGCCGCG) associated with increased kidney stone risk (P = 0.0013, OR 1.612, 95% CI 1.203-2.160) and the other (CGTTCCGCTA) with reduced disease risk (P = 0.0007, OR 0.464, 95% CI 0.296- 0.727); these significant differences were maintained after correction for multiple testing. These findings indicate that *F2* haplotypes associated with risks of kidney stone disease in the population studied.

Keywords: kidney stone, nephrolithiasis, association study, single nucleotide polymorphisms, SNPs, urinary prothrombin fragment 1

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การประชุมวิชาการประจำปี
คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
ครั้งที่ 25 ประจำปี 2552

การแพทย์หัวใจประชาชน ในภาวะวิกฤตเศรษฐกิจ
(People - Centered Health Care in Economic Crisis Era)

ระหว่างวันที่ 13 - 16 ตุลาคม 2552
ณ ห้องบรรยาย 1 - 4
อาคารเตรียมวิทยาศาสตร์คลินิก
คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

ผู้เข้าร่วมประชุมจะได้รับหน่วยการศึกษาต่อเนื่อง CME 25.5, CPE 23.75, CNEU 10*

Determination of Genetic Variation of Galectin-3 in Cholangiocarcinoma

Mutita Junking, Sopit Wongkham, Banchob Sripa, Pa-thai Yenchitsomanus

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Background and Objective: Cholangiocarcinoma (CCA) is a malignancy of bile duct epithelia and the most common liver cancer in Northeast Thailand. It is a highly invasive/metastatic malignancy that is difficult to be diagnosed until the advanced stage, resulting in poor prognosis. Our previous report on galectin-3 (Gal-3) in *Opisthorchis viverrini*-related CCA tissues showed that down regulation of Gal-3 expression associated with poorly differentiated CCA and lymphatic invasion which are known to result in a poor prognosis for CCA patients. Regulation of Gal-3 expression is a complex process which depends on cell types, external stimuli and environmental conditions. However, the mechanisms which regulate Gal-3 expression are still unclear.

Methods: In this study, PCR amplicons were designed for PCR and High Resolution Melting (HRM) mutation scanning of *LGALS3* promoter and exons and tested them with DNA from 7 CCA cell lines compared with normal genomic DNA. Variation of each region was confirmed by DNA sequencing.

Results: Promoters and exons of *LGALS3* were screened for its genetic variation. KKU-M055, which has low level Gal-3 expression, mutation at exon 1 was detected by PCR and HRM. Genetic variation in each region of exon was also found in all 7 CCA cell lines by HRM analysis.

Conclusions: Here, mutation of exon1 of Gal-3 which contain transcriptional-binding sequence was found. Genetic variations at other region of each exon were also found. This may be the one mechanism involve with regulation of Gal-3 expression in CCA.

Keywords: Galectin-3, LGALS3, bile duct, cholangiocarcinoma, mutation, HRM

รวมบทคัดย่อการประชุมเสนอผลงานวิจัย ระดับบัณฑิตศึกษาแห่งชาติ ครั้งที่ ๑๔

ณ มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าพระนครเหนือ

๑๐ - ๑๑ กันยายน ๒๕๕๒

The 14th National Graduate Research Conference

co-organized by
CGAU and King Mongkut's University of Technology North Bangkok (KMUTNB)

September 10-11, 2009

บัณฑิตวิทยาลัย มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าพระนครเหนือ
Graduate College of King Mongkut's University of Technology North Bangkok (KMUTNB)

ร่วมกับ ที่ประชุมคณะผู้บริหารบัณฑิตศึกษามหาวิทยาลัยของรัฐ และมหาวิทยาลัยในกำกับของรัฐ (ทคบร.)
Council of the Graduate Studies Administrators of Public and Autonomous Universities (CGAU)

สภาคณะผู้บริหารบัณฑิตศึกษาแห่งประเทศไทย (สคบท.)
Council of the Graduate Studies Administrators of Thailand (CGAT)

The 14th National Graduate Research Conference
King Mongkut's University of Technology North Bangkok
September 10–11, 2009

การแสดงออกของยีนที่เกี่ยวข้องกับกระบวนการออโตเฟจีในเซลล์ตับที่ติดเชื้อไวรัสเด็งกี่

Autophagic Gene Expression Profiling in Dengue Virus-Infected Liver Cell Line

อำภา ยาสมุทร์[1], เพทาย เย็นจิตโสมนัส[2] และ ถาวรชัย ลิ้มจินดาพร[2]

บทคัดย่อ

การศึกษาการตอบสนองของเซลล์เจ้าบ้านต่อการติดเชื้อไวรัสเด็งกี่สามารถนำไปสู่ความเข้าใจในพยาธิกำเนิดของโรคได้ ในงานวิจัยนี้มีจุดประสงค์เพื่อดูการแสดงออกของยีนของเซลล์ตับที่ตอบสนองต่อการติดเชื้อไวรัสเด็งกี่ กลุ่มยีนที่สนใจศึกษาเกี่ยวข้องกับกระบวนการออโตเฟจีที่มีรายงานว่ามีบทบาทในการเพิ่มจำนวนของไวรัส แต่กลไกในการเหนี่ยวนำและควบคุมยังไม่ทราบแน่ชัด การวิจัยนี้จึงได้นำเทคโนโลยี Human Autophagy RT^2 Profiler^{TM} PCR Array มาใช้ศึกษายีนที่เกี่ยวข้องกับออโตเฟจี จำนวน 84 ยีน โดยเทคนิค real-time reverse transcription polymerase chain reaction (real-time RT-PCR) พบว่ามียีนที่มีการแสดงออกเพิ่มขึ้นมากกว่า 2 เท่า จำนวน 19 ยีน เมื่อเทียบกับเซลล์ที่ไม่ติดเชื้อ จากผลการทดลองผู้วิจัยคาดว่ายีนที่มีการแสดงออกเพิ่มขึ้นน่าจะเกี่ยวข้องกับกลไกในการเหนี่ยวนำและควบคุมกระบวนการออโตเฟจีในเซลล์ตับที่ติดเชื้อไวรัสเด็งกี่ องค์ความรู้ที่ได้จากการทดลองนี้จะนำไปสู่ความเข้าใจในกระบวนการออโตเฟจีที่ถูกกระตุ้นด้วยเชื้อไวรัสเด็งกี่มากขึ้น

Abstract

The study of host response to dengue virus infection contributes to the understanding of pathogenesis. This study aims to determine autophagic gene expression profiling of dengue virus-infected liver cell line. Autophagy is a cellular degradation process which responses to various stimuli including virus infection. Autophagy supports dengue virus replication but the cellular mechanism remains elusive. Human Autophagy RT^2 Profiler^{TM} PCR Array technology is a pathway-focused gene expression profiling using real-time reverse transcription polymerase chain reaction (real-time RT-PCR). Among 84 autophagic genes, 19 genes were up-regulated in dengue virus-infected cells more than 2 folded comparing with those of uninfected cells. The up-regulated genes may involve in the induction or regulation of autophagy in dengue virus-infected liver cell line. The knowledge from this study leads to better understanding of the autophagic pathway stimulated by dengue virus.

คำสำคัญ : ออโตเฟจี, เชื้อไวรัสเด็งกี่, การแสดงออกของยีน

Keywords : autophagy, dengue virus, gene expression

1 นักศึกษาสาขาวิชาวิทยาภูมิคุ้มกัน (หลักสูตรนานาชาติ) หลักสูตรวิทยาศาสตรมหาบัณฑิต คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

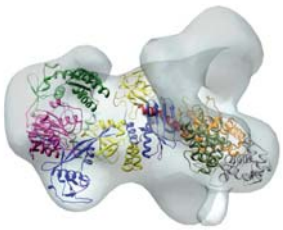
2 คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

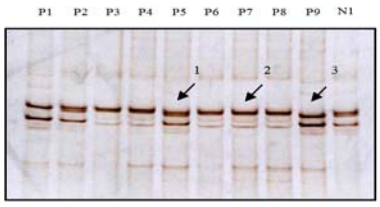
เอกสารประกอบภาคบรรยาย

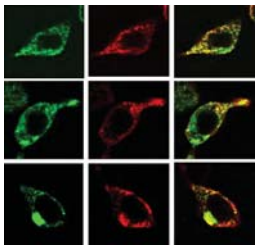
Molecular and Cellular Biology Workshop 2009

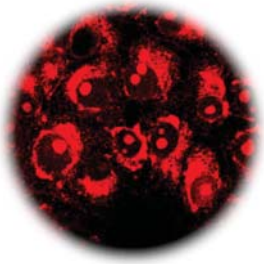
วันที่ 25 พฤษภาคม 2552

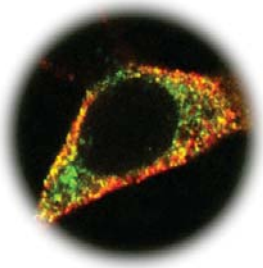
ห้องประชุมวีกิจ วีรานุวัตี์ ตีกอัษฎางค์ ชั้น 4











คณะกรรมการบัณฑิตศึกษา และ
งานบัณฑิตศึกษาและการศึกษาต่อเนื่อง
คณะแพทยศาสตร์ศิริราชพยาบาล
มหาวิทยาลัยมหิดล

โครงการบรรยายและฝึกอบรมภาคปฏิบัติ

"Molecular and Cellular Biology Workshop 2009"

วันที่ 25 – 29 พฤษภาคม 2552

ณ ห้องประชุมวีกิจ วีรานุวัติ์ ตึกอัษฎางค์ ชั้น 4

คณะแพทยศาสตร์ศิริราชพยาบาล

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1. หลักการและเหตุผล

ในปัจจุบัน เทคโนโลยีทางด้านอณูและเซลล์ชีววิทยาได้พัฒนาและเจริญก้าวหน้าอย่างรวดเร็ว เทคโนโลยีทางด้านนี้ ได้มีการนำมาใช้ในงานวิจัยและพัฒนาด้านวิทยาศาสตร์ชีวภาพการแพทย์และวิทยาศาสตร์สุขภาพ นอกจากนี้ยังมีการนำมาประยุกต์ในทางการแพทย์ เพื่อพัฒนาการตรวจวินิจฉัย การควบคุมป้องกัน และการรักษาโรค ดังนั้น นักศึกษาบัณฑิตศึกษา นักวิจัย และอาจารย์ ที่กำลังปฏิบัติงานวิจัยและพัฒนาทางด้านชีวภาพการแพทย์และวิทยาศาสตร์สุขภาพ จำเป็นต้องมีความรู้และทักษะในการใช้เทคโนโลยีด้านนี้ เพื่อนำมาใช้และประยุกต์ให้เหมาะสมกับงานที่กำลังปฏิบัติอยู่

คณะแพทยศาสตร์ศิริราชพยาบาล ได้ตระหนักถึงการพัฒนาและความเจริญก้าวหน้าในเทคโนโลยีดังกล่าว จึงได้จัดโครงการบรรยายและภาคปฏิบัติ "Molecular and Cellular Biology Workshop 2009" ขึ้น สำหรับนักศึกษาในระดับบัณฑิตศึกษา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล รวมทั้งนักวิจัย อาจารย์ และผู้สนใจทั่วไป ทั้งในระดับเริ่มต้นใหม่ และผู้ที่ต้องการหาความรู้เพิ่มเติม

2. วัตถุประสงค์

1. เพื่อเผยแพร่ความรู้ทางด้านอณูและเซลล์ชีววิทยา
2. เพื่อฝึกอบรมภาคปฏิบัติในการใช้เทคโนโลยีด้านอณูและเซลล์ชีววิทยา

3. คณะกรรมการดำเนินการ

คณะกรรมการบัณฑิตศึกษา

คณะกรรมการจัดการฝึกอบรม Molecular and Cellular Biology Workshop 2009

คณะกรรมการจัดการฝึกอบรมภาคปฏิบัติการ Molecular and Cellular Biology Workshop 2009

4. ผู้รับผิดชอบโครงการ

1. งานบัณฑิตศึกษาและการศึกษาต่อเนื่อง สำนักงานคณบดี คณะแพทยศาสตร์ศิริราชพยาบาล
2. หน่วยอณูชีววิทยาการแพทย์ สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล
3. หน่วยปฏิบัติการเทคโนโลยีชีวภาพทางการแพทย์ ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ

5. ระยะเวลา

ภาคบรรยาย 1 วัน วันจันทร์ที่ 25 พฤษภาคม 2552

ภาคปฏิบัติการ 4 วัน วันที่ 26 – 29 พฤษภาคม 2552

โดยฝึกภาคปฏิบัติวันละ 1 Demonstration และ 1 workshop ได้แก่

Demonstration I Genetic Mutation/Polymorphism Analysis

Demonstration II Bioinformatics

Demonstration III Protein Purification and Detection

Demonstration IV Genetic Qualitative and Quantitative Analysis

Workshop I Genetic Analysis

Workshop II Molecular Cloning

Workshop III Protein Analysis

Workshop IV Cell Culture and Analysis

6. สถานที่

ภาคบรรยาย

วันที่ 25 พฤษภาคม 2552 ณ ห้องประชุมวีกิจ วีรานุวัติ์ ตึกอัษฎางค์ ชั้น 4 คณะแพทยศาสตร์-ศิริราชพยาบาล

ภาคปฏิบัติการ

วันที่ 26-29 พฤษภาคม 2552 ณ หน่วยอณูชีววิทยาการแพทย์ หน่วยอณูพันธุศาสตร์ และหน่วยเครื่องมือพิเศษเพื่อการวิจัย สถานส่งเสริมการวิจัย ตึกอดุลยเดชวิกรม ชั้น 10 และ 12 คณะแพทยศาสตร์-ศิริราชพยาบาล

7. ผู้เข้าประชุม

- นักศึกษาระดับบัณฑิตศึกษา คณะแพทยศาสตร์ศิริราชพยาบาล
- นักศึกษาระดับบัณฑิตศึกษา มหาวิทยาลัยมหิดล
- นักวิจัย อาจารย์ และผู้สนใจทั่วไป

ผู้เข้าร่วมประชุมภาคบรรยาย จำนวนรวม 250 คน

- บุคคลภายนอก ค่าลงทะเบียน 500 บาท / คน
- นักศึกษาบัณฑิตศึกษา ค่าลงทะเบียน 300 บาท/คน

ผู้เข้าร่วมประชุมภาคปฏิบัติการ จำนวนรวม 40 คน*

- บุคคลภายนอก ค่าลงทะเบียน 2,000 บาท/คน (* ต้องลงทะเบียนฝึกอบรมภาคบรรยายด้วย)

นักศึกษาบัณฑิตศึกษา ค่าลงทะเบียน 1,000 บาท/คน/4วัน

8. การประเมิน

1. จำนวนผู้เข้าร่วมโครงการ
2. ผลสำรวจความพึงพอใจของผู้เข้าร่วมประชุม และร่วมโครงการ
3. ผลสำรวจความพึงพอใจของผู้ให้บริการ

9. ประโยชน์ที่คาดว่าจะได้รับ

ผู้เข้าฝึกอบรมบรรลุตามวัตถุประสงค์ใน ข้อ 2. เมื่อสิ้นสุดการฝึกอบรม

10. การรับสมัคร

ผู้ที่สนใจเข้าร่วมโครงการ สามารถติดต่อสมัคร และลงทะเบียน ได้ที่งานบัณฑิตศึกษาและการศึกษาต่อเนื่อง ตึกอดุลยเดชวิกรมชั้น 6 โทร. 02-419-6431 **ภายในวันที่ 15 พฤษภาคม 2552**

คณะกรรมการจัดการฝึกอบรม

Molecular and Cellular Biology Workshop 2009

1. ศ. นพ. ชัยรัตน์ ฉายากุล	ประธาน
2. ศ. ดร. เพทาย เย็นจิตโสมนัส	รองประธาน
3. ศ. ดร. โกวิท พัฒนาปัญญาสัตย์	กรรมการ
4. รศ. ดร. รัชนีกร กัลล์ประวิทย์	กรรมการ
5. ศ. นพ. สัญญา สุขพณิชนันท์	กรรมการ
6. ศ. ดร. นพ. ประเสริฐ เอื้อวรากุล	กรรมการ
7. รศ.ดร.พญ.พัชรีย์ เลิศฤทธิ์	กรรมการ
8. ผศ.พญ.ศันสนีย์ เสนะวงษ์	กรรมการ
9. ผศ. ดร. พญ. วัฒนา วัฒนาภา	กรรมการ
10. ผศ. ดร. นพ. ถาวรชัย ลิ้มจินดาพร	กรรมการ
11. นาง อรุณี ลีศิริชัยกุล	กรรมการและเลขานุการ
12. น.ส. สุภาภรณ์ เฉลิมวุฒิ	กรรมการและผู้ช่วยเลขานุการ
13. น.ส. ปิยพร สุทธิทรัพย์	กรรมการและผู้ช่วยเลขานุการ

คณะกรรมการจัดฝึกอบรมภาคปฏิบัติการ

Molecular and Cellular Biology Workshop 2009

1. นพ. ปรีดา มาลาสิทธิ์	ที่ปรึกษา
2. ศ. ดร. โกวิท พัฒนาปัญญาสัตย์	ที่ปรึกษา
3. ศ. ดร. เพทาย เย็นจิตโสมนัส	ประธาน
4. อ. ดร. ชัญญา พุทธิขันธ์	รองประธาน
5. อ. ดร. ประพัฒน์ สุริยผล	กรรมการ
6. อ. ดร. วรรณา ทองนพคุณ	กรรมการ
7. อ. ดร. นัญวรรณ รุ่งโรจน์	กรรมการ
8. ดร. สุธา เสงี่ยมบุตร	กรรมการ
9. ดร. บรรพต ศิริเดชาดิลก	กรรมการ
10. ดร. วทิพย์ บุณยศรีสวัสดิ์	กรรมการ
11. ดร. มุทิตา จุลกิ่ง	กรรมการ
12. ดร. ฐนียา ดวงจินดา	กรรมการและเลขานุการ
13. น.ส. หนึ่งหทัย สวัสดี	กรรมการและผู้ช่วยเลขานุการ
14. น.ส. สุกัลยา กรรณสมบัติ	กรรมการและผู้ช่วยเลขานุการ
15. นาย ฉัตรชัย ภูน้ำค้าง	กรรมการและผู้ช่วยเลขานุการ

รายชื่อคณะทำงานจัดฝึกอบรม

Molecular and Cellular Biology Workshop 2009

ผู้ช่วยสอนด้านห้องปฏิบัติการ

1. นาย ชูชัย เนตรธุวกุล	หน่วยอณูชีววิทยาการแพทย์
2. น.ส. ดวงพร อังศุประเวศ	หน่วยอณูชีววิทยาการแพทย์
3. น.ส. ณัฐกานต์ สุโกมล	หน่วยอณูชีววิทยาการแพทย์
4. นาย สมชาย เทียมเมฆา	หน่วยอณูชีววิทยาการแพทย์
5. น.ส. ชุติธร ทวิเลิศ	หน่วยอณูชีววิทยาการแพทย์
6. น.ส.สรรเพชุดา สุภาษา	หน่วยอณูชีววิทยาการแพทย์
7. น.ส. พัชรี ทรงประโคน	หน่วยเครื่องมือพิเศษเพื่อการวิจัย
8. น.ส. ขวัญฤทัย ชิ้นอินมนู	หน่วยชีวสารสนเทศและจัดการข้อมูลวิจัย
9. น.ส. ยุพาพร โอศิริพันธุ์	หน่วยชีวสารสนเทศและจัดการข้อมูลวิจัย
10. น.ส. ธนพรรณ พร้อมมูล	หน่วยปฏิบัติการเทคโนโลยีชีวภาพทางการแพทย์
11. น.ส. นิรินทร์ยา สุดตาชาติ	หน่วยปฏิบัติการเทคโนโลยีชีวภาพทางการแพทย์
12. นางนลินี จงเจริญ	ภาควิชาวิทยาภูมิคุ้มกัน
13. นาย ธีระพงศ์ โพธิ์เอี่ยม	เครือข่ายโรคพันธุกรรมระบบประสาท หน่วยวิจัยและพัฒนาบริการสุขภาพ

ประสานงานห้องปฏิบัติการ

1. อ. ดร. วรรณา ทองนพคุณ	หน่วยอณูพันธุศาสตร์
2. ดร. วทิพย์ บุณยศรีสวัสดิ์	ภาควิชาวิทยาภูมิคุ้มกัน
3. น.ส. หนึ่งหทัย สวัสดี	หน่วยอณูชีววิทยาการแพทย์
4. นาย ชูชัย เนตรธุวกุล	หน่วยอณูชีววิทยาการแพทย์
5. นาย สมชาย เทียมเมฆา	หน่วยอณูชีววิทยาการแพทย์

ประสานงานธุรการ

1. น.ส. สุกัลยา กรรณสมบัติ	หน่วยปฏิบัติการเทคโนโลยีชีวภาพทางการแพทย์
2. นาย ฉัตรชัย ภูน้ำค้าง	หน่วยอณูชีววิทยาการแพทย์
3. น.ส. สุพาภรณ์ เฉลิมวุฒิ	งานบัณฑิตศึกษาและการศึกษาต่อเนื่อง
4. น.ส. ปิยพร สุทธิทรัพย์	งานบัณฑิตศึกษาและการศึกษาต่อเนื่อง

กำหนดการบรรยายและฝึกอบรมภาคปฏิบัติ

Molecular and Cellular Biology Workshop 2009

วันที่ 25-29 พฤษภาคม 2552

คณะแพทยศาสตร์ศิริราชพยาบาล

ภาคบรรยาย ณ ห้องประชุมวีกิจ วีรานุวัติ์ ตึกอัษฎางค์ ชั้น 4 วันที่ 25 พฤษภาคม 2552

เวลา 08:30 – 08:50 น.	ลงทะเบียน	
เวลา 08:50 – 09:00 น.	พิธีเปิด	
เวลา 09:00 – 09:15 น.	Opening Address	*ศ.ดร.นพ.พรชัย โอเจริญรัตน์*
เวลา 09:15 – 10:00 น.	Introduction to Molecular and Cellular Biology	*ศ.ดร.เพทาย เย็นจิตโสมนัส*
เวลา 10:00 – 10:30 น.	**น้ำชา -อาหารว่าง**	
เวลา 10:30 – 11:15 น.	Genetic Analysis and Engineering	*รศ.ดร.วราภรณ์ อัครปทุมวงศ์*
เวลา 11:15 – 12:00 น.	RNA Analysis and Manipulation	*ผศ.ดร.นพ.ชัชวาลย์ ศรีสวัสดิ์*
เวลา 12:00 – 13:00 น.	**พักรับประทานอาหารกลางวัน**	
เวลา 13:00 – 13:45 น.	Protein Analysis and Engineering	*ดร.บรรพต ศิริเดชาดิลก*
เวลา 13:45 – 14:30 น.	Protein-Protein Interaction Analysis	*ผศ.ดร.นพ.ถาวรชัย ลิ้มจินดาพร*
เวลา 14:30 – 15:00 น.	**น้ำชา -อาหารว่าง**	
เวลา 15:00 – 15:45 น.	Cell Analysis by Immunofluorescence and Flow Cytometry	*ดร.ฐนียา ดวงจินดา*
เวลา 15:45 – 16:30 น.	Biohazard and Biosafety in Molecular and Cellular Laboratory	*รศ.ดร. มธุรส พงษ์ลิขิตมงคล*

ภาคปฏิบัติ ณ หน่วยอณูชีววิทยาการแพทย์ หน่วยอณูพันธุศาสตร์ และหน่วยเครื่องมือพิเศษเพื่อการวิจัย สถานส่งเสริมการวิจัย ตึกอดุลยเดชวิกรม ชั้น 10 และ 12 วันที่ 26-29 พฤษภาคม 2552

<u>Demonstration</u> 26 -29 พ.ค. 52 (08:30-10:00 น.)

26 พ.ค. 52

Demonstration I – Genetic Mutation/Polymorphism Analysis	อ.ดร.วรรณา ทองนพคุณ
- Mutation/polymorphism screening and analysis by denaturing high-performance liquid chromatography (DHPLC)	นายธีระพงศ์ โพธิ์เอี่ยม
- DNA sequencing	

27 พ.ค. 52

Demonstration II – Bioinformatics	อ.ดร.ประพัฒน์ สุริยผล
- Genetic and molecular biology databases	น.ส.ขวัญฤทัย ชิ้นอินมนู
- Web-based programs for genetic and molecular biology analysis	น.ส. ยุพาพร โอศิริพันธุ์

28 พ.ค. 52

Demonstration III – Protein Purification and Detection	อ.ดร.ชัญญา พุทธิขันธ์
- Immobilized - Metal Affinity Chromatography (IMAC) for His tag protein purification	น.ส.ธนพรรณ พร้อมมูล
- Detection of purified protein	

29 พ.ค. 52

Demonstration IV – Genetic Qualitative and Quantitative Analysis	ดร.วทิพย์ บุณยศรีสวัสดิ์
- Genetic qualitative and quantitative analysis by real-time polymerase chain reaction (real-time PCR)	นายชูชัย เนตรธุวกุล นายธีระพงศ์ โพธิ์เอี่ยม

<u>Workshop I-IV</u> 26 -29 พ.ค. 52 (10:00-12:00 น.)/ (13:00-16:30 น.)

Workshop I – Genetic Analysis	อ.ดร.นัญวรรณ รุ่งโรจน์
- Human genomic DNA preparation	นางนลินี จงเจริญ
- Polymerase chain reaction (PCR)	น.ส.นิรินทร์ยา สุดตาชาติ
- Mutation/polymorphism analysis by restriction endonuclease digestion	นายชูชัย เนตรธุวกุล
- Agarose-gel electrophoresis	

Workshop II – Molecular Cloning	อ.ดร.ชัญญา พุทธิขันธ์
- Plasmid and PCR product preparation	ดร.มุทิตา จุลกิ่ง
- Restriction enzyme digestion	น.ส.ธนพรรณ พร้อมมูล
- DNA ligation	น.ส.ดวงพร อังศุประเวศ
- Bacterial transformation and screening	
- Agarose-gel electrophoresis	
Workshop III – Sample preparation and protein analysis	ดร.บรรพต ศิริเดชาดิลก
- Protein extraction	นายสมชาย เทียมเมฆา
- Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)	น.ส.ณัฐกานต์ สุโกมล
- Coomassie Blue staining	
Workshop IV – Cell Analysis	ดร.ฐนียา ดวงจินดา
- Immunofluorescent staining	ดร.สุธา เสงี่ยมบุตร
- Confocal microscopy	น.ส.พัชรี ทรงประโคน
- Flow cytometry	น.ส.ชุติธร ทวิเลิศ
	น.ส.สรรเพชุดา สุภาษา

หมายเหตุ: *น้ำชา –อาหารว่าง 10:00 น./ 14:30 น.*
อาหารกลางวัน 12:00 – 13:00 น. (เฉพาะภาคบรรยายเท่านั้น)

วิทยากรบรรยาย

1. ชื่อ - นามสกุล : ศ. ดร. เพทาย เย็นจิตโสมนัส

หน่วยงาน : หน่วยอณูชีววิทยาการแพทย์ สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

ประวัติการศึกษา :

- B.Sc. in Medical Technology, Chiangmai University
- M.Sc. in Biochemistry, Mahidol University
- Ph.D. in Human Genetics, Australian National University, Australian
- Other Molecular Genetic Research Training,
  UNESCO/TWAS Fellowship
  St Mary Hospital, London, UK

ความเชี่ยวชาญ : Human Genetics, Medical Molecular Biology

งานวิจัยที่กำลังทำ/สนใจ :

- งานวิจัยด้านอณูพันธุศาสตร์โรคพันธุกรรม
- งานวิจัยด้านอณูชีววิทยาของการเกิดโรคไตผิดปกติในการขับกรดในผู้ป่วยเด็กไทย
- งานวิจัยด้านปฏิสัมพันธ์ระหว่างโปรตีน
- งานวิจัย พัฒนา และประยุกต์เทคโนโลยีทางด้านอณูพันธุศาสตร์และจีโนมิกส์ ในการตรวจวินิจฉัยและศึกษาวิจัยโรคพันธุกรรมที่พบในคนไทย

2. ชื่อ - นามสกุล : รศ.ดร. วราภรณ์ อัครปทุมวงศ์

หน่วยงาน : สถาบันอณูชีววิทยาและพันธุศาสตร์ มหาวิทยาลัยมหิดล ศาลายา

ประวัติการศึกษา :

- วิทยาศาสตรบัณฑิต (เกียรตินิยมอันดับ 1) สาขาเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่
- วิทยาศาสตรมหาบัณฑิต สาขาชีวเคมี มหาวิทยาลัยมหิดล
- Ph.D. in Science, University of Adelaide, Australian

ความเชี่ยวชาญ : Molecular Biology, Human Molecular Genetics

งานวิจัยที่กำลังทำ/สนใจ : Molecular Biology, Human Molecular Genetics

3. ชื่อ - นามสกุล : ผศ. ดร. นพ. ชัชวาลย์ ศรีสวัสดิ์

หน่วยงาน : ภาควิชาชีวเคมี คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

ประวัติการศึกษา :

- แพทยศาสตรบัณฑิต (เกียรตินิยมอันดับ1) คณะแพทยศาสตร์ศิริราชพยาบาลมหาวิทยาลัยมหิดล
- Ph.D. in Biological Chemistry, University of Michigan (Ann Arbor), USA

งานวิจัย :

- Developing RNA aptamers for research and diagnostics of human diseases (e.g. dengue viral infection, hemoglobin diseases)
- Using the RNA interference technology for therapeutics. Currently, studying the suppression of collagen production in keloid fibroblasts with small-interfering RNAs against collagen genes.

4. ชื่อ - นามสกุล : ดร. บรรพต ศิริเดชาดิลก

หน่วยงาน : หน่วยปฏิบัติการเทคโนโลยีชีวภาพทางการแพทย์ ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ

ประวัติการศึกษา :

- B.Sc. (Biochemistry, Honors) Brown University, USA
- Ph.D. (Molecular & Cell Biology) University of California, Berkeley, USA

ความเชี่ยวชาญ : Molecular and Structural Biology, Biochemistry

งานวิจัยที่กำลังทำ/สนใจ : ศึกษาการเพิ่มจำนวนของ dengue virus ใน cells โดยมุ่งเน้นไปที่การศึกษากลไกการทำงานของ replicating complex ของ dengue virus ซึ่งทำหน้าที่ในการเพิ่มจำนวนของ genome ของ virus

5. ชื่อ - นามสกุล : ผศ. ดร. นพ. ถาวรชัย ลิ้มจินดาพร

หน่วยงาน : ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

ประวัติการศึกษา :

- M.D. in Medicine, Mahidol University
- Ph.D. in Microbiology and Immunology, Georgetown University
- Post-doctoral training at Center for Molecular Medicine and Genetics, Wayne State University, Detroit, USA
- Diplomate Thai Board of Family Medicine

ความเชี่ยวชาญ : Genetics and molecular biology, Protein protein interactions

งานวิจัยที่กำลังทำ/สนใจ : Protein- protein interactions, especially in apoptosis of dengue virus-infected cells

6. ชื่อ - นามสกุล : ดร. ฐนียา ดวงจินดา

หน่วยงาน : หน่วยปฏิบัติการเทคโนโลยีชีวภาพทางการแพทย์ ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ

ประวัติการศึกษา :

- B.Sc. Biochemistry, faculty of Science, Chulalongkorn University, Bangkok, Thailand
- M.Sc. Biochemistry, faculty of Science, Mahidol University, Bangkok, Thailand
- DPhil (Oxon) Clinical Medicine, University College, University of Oxford, Oxford, UK

ความเชี่ยวชาญ : T cell responses in virus infection, Pathogenesis of Dengue Haemorrhagic Fever (DHF)

งานวิจัยที่กำลังทำ/สนใจ : T cell responses in virus infection, Pathogenesis of Dengue Haemorrhagic Fever (DHF)

7. ชื่อ - นามสกุล : รศ. ดร. มธุรส พงษ์ลิขิตมงคล

หน่วยงาน : ภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

ประวัติการศึกษา :

- วิทยาศาสตรบัณฑิต(เกียรตินิยม) สาขาจุลชีววิทยา จุฬาลงกรณ์มหาวิทยาลัย
- วิทยาศาสตรมหาบัณฑิต สาขาจุลชีววิทยา มหาวิทยาลัยมหิดล
- Ph.D. in Molecular Biology, Universite Louis Pasteur France
- Post doc. Visiting Professor, Cancer Genetics, Osaka University, Japan

งานวิจัยที่กำลังทำ/สนใจ : บทบาทของเชื้อไวรัส HPV และอณูชีววิทยาของมะเร็งปากมดลูก

การประชุมวิชาการ
พันธุศาสตร์แห่งชาติ ครั้งที่ 16

NGS 2009

พันธุศาสตร์...แก้วิกฤตพลังงานชาติ
Genetics for National Energy Crisis

25-27 มีนาคม 2552

จัดโดย
ภาควิชาเทคโนโลยีชีวภาพ มหาวิทยาลัยธรรมศาสตร์
ร่วมกับ สมาคมพันธุศาสตร์แห่งประเทศไทย

ณ อาคารเรียนและปฏิบัติการ คณะวิทยาศาสตร์และเทคโนโลยี
มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต จ.ปทุมธานี

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ผู้ดำเนินการอภิปราย : ศาสตราจารย์ ดร. สาวิตรี ลิ่มทอง
คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์

10:45-11:00 น. พัก-รับประทานอาหารว่าง

11:00-12:30 น. บรรยายพิเศษ *เรื่อง* "ความก้าวหน้าพันธุศาสตร์ทางการประมงและสัตวบาล"
ผู้บรรยาย : 1. ศาสตราจารย์ ดร. อุทัยรัตน์ ณ นคร
นักวิจัยดีเด่นแห่งชาติสาขาเกษตรศาสตร์และชีววิทยา
ประจำปี 2550 ของสภาวิจัยแห่งชาติ
คณะประมง มหาวิทยาลัยเกษตรศาสตร์
2. รองศาสตราจารย์ ดร. วรวิทย์ สิริพลวัฒน์
คณะเกษตรกำแพงแสน มหาวิทยาลัยเกษตรศาสตร์

12:30-13:30 น. พัก-รับประทานอาหารกลางวัน

13:30-16:00 น. เสนอผลงานภาคบรรยาย
พัก-รับประทานอาหารว่าง

16:00-17:30 น. สัมมนา *เรื่อง* "การเรียนการสอนพันธุศาสตร์ระดับมัธยมศึกษาและอุดมศึกษา"
ผู้นำสัมมนา : 1. ศาสตราจารย์ประดิษฐ์ พงศ์ทองคำ
นายกสมาคมพันธุศาสตร์แห่งประเทศไทย
2. อาจารย์ชูศิลป์ อัตชู
ที่ปรึกษาสมาคมพันธุศาสตร์แห่งประเทศไทย

17:30-18:00 น. การประชุมสามัญของสมาคมพันธุศาสตร์แห่งประเทศไทย

18:00-21:00 น. งานเลี้ยงรับรอง

27 มีนาคม 2552

09:00-10:45 น. อภิปราย *เรื่อง* "ความก้าวหน้าพันธุศาสตร์ทางการแพทย์ : กลุ่มโรคเมตาบอลิค"
ผู้อภิปราย : 1. ผู้ช่วยศาสตราจารย์ พญ. มยุรี หอมสนิท
คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
2. อาจารย์ นพ. มานพ พิทักษ์ภากร
คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
3. อาจารย์ นพ. ณัฐเชษฐ์ เปล่งวิทยา
คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
ผู้ดำเนินการอภิปราย : ศาสตราจารย์ ดร. เพทาย เย็นจิตโสมมนัส
คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

10:45-11:00 น. พัก-รับประทานอาหารว่าง

OH01

การใช้มัลติเพล็กซ์พีซีอาร์เชิงกึ่งปริมาณเพื่อตรวจหาการขาดหายไปของยีนวอนฮิพเพิลลินดาว (*VHL*)

Semi-quantitative multiplex PCR for the deletion analysis of von Hippel-Lindau (*VHL*) gene

อรนุช ประดิษฐ์ทรัพย์[1] จินตนา ศิรินาวิน[1,2] มานพ พิทักษ์ภากร[1,2] วรรณา ทองนพคุณ[1] อนันต์ชัย อัศวเมฆิน[1] นัญวรรณ รุ่งโรจน์[1] เพทาย เย็นจิตโสมนัส[1,3] และ ชนินทร์ ลิ่มวงศ์[1,2]*

Oranud Praditsap[1], Chintana Sirinavin[1,2], Manop Pithukpakorn[1,2], Wanna Thongnoppakhun[1], Anunchai Assawamakin[1], Nunyawan rongroj[1], Pa-thai Yenchitsomanus[1,3] and Chanin Limwongse[1,2]*

[1]หน่วยอณูพันธุศาสตร์ สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล 2 ถนนพรานนก บางกอกน้อย กรุงเทพฯ 10700

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บทคัดย่อ

มิวเตชั่นในยีนวอนฮิพเพิลลินดาว (*VHL*) เป็นสาเหตุของโรควอนฮิพเพิลลินดาว (VHL) ซึ่งเป็นกลุ่มอาการมะเร็งที่ถ่ายทอดทางพันธุกรรมซึ่งพบได้น้อย ผู้ป่วยโรคนี้มีความเสี่ยงต่อการเกิดเนื้องอกในหลายอวัยวะ เช่น ตา ระบบประสาทส่วนกลาง ต่อมหมวกไต ตับอ่อน และไต มิวเตชั่นในยีนนี้ส่วนใหญ่เป็นชนิดมิวเตชั่นเฉพาะจุด แต่ยังพบการขาดหายไปของยีนบางส่วนหรือหมดทั้งยีนได้ถึงร้อยละ 40 มีการพัฒนาการตรวจวิเคราะห์การขาดหายไปของยีนเพื่อวินิจฉัยผู้ป่วย VHL หลายวิธี แต่วิธีเหล่านี้ค่อนข้างยุ่งยากซับซ้อน

เสียเวลาและแรงงานมากในการตรวจ ในรายงานนี้คณะผู้วิจัยจึงได้นำเสนอเทคนิคที่ง่ายและรวดเร็วกว่าในการตรวจหาการขาดหายไปของยีน *VHL* โดยใช้วิธีมัลติเพล็กซ์พีซีอาร์เชิงกึ่งปริมาณผ่านเครื่องวิเคราะห์สารพันธุกรรมแบบโครมาโตกราฟีในสภาพของเหลวภายใต้ความดันสูง (DHPLC) โดยทำมัลติเพล็กซ์พีซีอาร์แยกเป็น 3 ชุด เพื่อใช้ตรวจการขาดหายไปของยีนทั้งหมด ซึ่งรวมโปรโมเตอร์ส่วนของยีนทั้งหมดที่ใช้ในการถอดรหัส (เอ็กซอน 1-3) และส่วนที่เป็น putative poly A ของยีน *VHL* ด้วย การเพิ่มปริมาณดีเอ็นเอในมัลติเพล็กซ์พีซีอาร์ของยีน *VHL* แต่ละชุดมีตัวควบคุมภายในปฏิกิริยาเป็น X-chromosome หรือ autosome อยู่ด้วย โดยใช้ปริมาณดีเอ็นเอเริ่มต้นที่แน่นอนและจำนวนรอบที่เหมาะสมในการทำพีซีอาร์ที่อยู่ในช่วง log-linear phase การวิเคราะห์ PCR product แต่ละชุดที่ได้จากผู้ป่วย VHL ใช้เครื่อง DHPLC สำหรับแยกขนาดดีเอ็นเอ แล้วประเมินความสูงของโครมาโตแกรมของ PCR product แต่ละชิ้นส่วน และนำไปเปรียบเทียบกับผลที่อ่านได้จากคนปกติในชุดปฏิกิริยาแบบเดียวกัน การใช้เทคนิคนี้สามารถตรวจพบการขาดหายไปขนาดใหญ่ของยีน *VHL* ในผู้ป่วย VHL จำนวน 6 ราย ที่ตรวจไม่พบมิวเตชั่นเฉพาะจุดมาก่อนหน้านี้ วิธีของคณะผู้วิจัยนี้จึงเป็นวิธีที่ง่าย แม่นยำ ประหยัด และรวดเร็วในการตรวจกรองการขาดหายไปของยีน มีความเหมาะสมที่จะนำมาใช้เป็นการตรวจกรองขั้นแรกของการตรวจมิวเตชั่นในยีน *VHL*

ABSTRACT

Germline mutations in von Hippel-Lindau (*VHL*) gene are responsible for von Hippel-Lindau (VHL) syndrome, the rare hereditary cancer syndrome predisposing to tumors in multiple organs including retina, cerebellum, spinal cord, adrenal gland, pancreas and kidney. Although mutations analyzed in *VHL* gene are found to be point mutation as a majority, the partial or complete gene deletions are identified up to 40%. Several deletion detection methodologies have been developed to confirm VHL patients. However, the methods are considerably sophisticated and laborious. In this report, we therefore present a simpler and faster technique to detect *VHL* deletion using denaturing high performance liquid chromatography (DHPLC)-based semi-quantitative multiplex PCR method. Three sets of multiplex PCRs were established for testing whole gene deletions including the promoter, entire coding regions (exons 1-3) and putative poly A site of the *VHL* gene. Each multiplex-PCR set of *VHL* gene was amplified with either X-chromosome or autosome internal control using quantitated DNA templates and appropriate PCR cycle number for the log-linear phase. The PCR-product sets of VHL patients were individually analyzed by DHPLC in the sizing mode to evaluate the peak heights of each fragment and compared with corresponding set of normal individuals. Using this technique, large deletions in the *VHL* gene could be identified in six point-mutation negative VHL patients. Our method is simple, accurate, inexpensive, and rapid for deletion screening that facilitates its use as a first screening tool for *VHL* mutation detection.

คำสำคัญ: วอนฮิพเพิลลินดาว, การขาดหายไปของยีน, มัลติเพล็กซ์พีซีอาร์, ดีเอชพีแอลซี
Keywords: von Hippel-Lindau, deletion, multiplex-PCR, DHPLC

บทนำ

โรค von Hippel-Lindau syndrome (VHL) เป็นกลุ่มอาการมะเร็งของอวัยวะหลายระบบที่มีการถ่ายทอดแบบลักษณะเด่น ชนิดของมะเร็งที่พบ ได้แก่ angiomata ของ retina และ hemangioblastomas (HB) ของ central nervous system, renal cell carcinoma (RCC) และ renal cysts, pheochromocytoma (PH), และ pancreatic cysts (VHL, MIM#199330) อุบัติการของโรค VHL มีประมาณ 1/36,000 คน ยีนที่เป็นสาเหตุของโรคคือยีน *VHL* ซึ่งเป็น tumor suppressor gene (GenBank accession no. NC_000003) อยู่บนแขนข้างสั้นของโครโมโซมที่ 3 (3p25-26) มี genomic DNA ขนาด 10,444 bp และ mRNA ขนาด 2,968 bp ประกอบด้วย 3 exon ที่กำหนดการสร้างโปรตีนชื่อ VHL ซึ่งมี 2 ขนาด โดยใช้ start codon ต่างที่กันคือ $pVHL_{30}$ (213 amino acids; 30 kDa) และ $pVHL_{19}$ (160 amino acids; 19 kDa) (Clark, 2008) กลไกการเกิดโรคเป็นไปตามทฤษฎีการเกิดมะเร็งของ Knudson กล่าวคือเกิดจากมิวเตชั่นที่เป็น first hit ซึ่งอาจเป็นชนิดที่ได้รับการถ่ายทอดทางพันธุกรรม (germline mutation) หรือเป็นชนิดที่เกิดขึ้นในเซลล์ร่างกายผู้ป่วยเอง (somatic mutation) แล้วตามด้วยมิวเตชั่นที่เป็น second hit ในเซลล์ของอวัยวะที่เกิดมะเร็ง มิวเตชั่นที่พบได้บ่อยในยีน *VHL* เป็นชนิดมิวเตชั่นเฉพาะจุด (point mutation) แต่ก็พบการขาดหายไป (deletion) ของยีนในอัตราส่วนที่สูงถึงร้อยละ 40 การตรวจวิเคราะห์หาการขาดหายไปขนาดใหญ่ของยีนสามารถทำได้หลายวิธี เช่น Southern blot analysis, pulsed field gel electrophoresis, long range polymerase chain reaction (PCR), fluorescent in situ hybridization (FISH), quantitative real time PCR, และ multiplex ligation-dependent probe amplification (MLPA) แต่ข้อเสียของวิธีการเหล่านี้คือมีขั้นตอนยุ่งยาก เสียเวลา แรงงาน และค่าใช้จ่ายมาก รวมทั้งสารเคมีที่ใช้มีเวลาจำกัดและต้องอาศัยผู้ปฏิบัติงานที่มีความชำนาญสูง จึงอาจไม่เหมาะที่จะใช้เป็นงานประจำ

ในงานวิจัยนี้คณะผู้วิจัยได้พัฒนาวิธีการตรวจหา germline mutation ชนิดการขาดหายไปของยีนในผู้ป่วยโรค VHL โดยการทำมัลติเพล็กซ์พีซีอาร์ที่ครอบคลุมส่วนของยีนหมดทั้ง 3 exon รวมทั้งส่วนของ promoter และ poly A site ร่วมกับการวิเคราะห์ด้วยเครื่อง denaturing high performance liquid chromatography (DHPLC) เรียกชื่อวิธีว่า DHPLC-based semi-quantitative multiplex PCR ซึ่งเป็นวิธีที่ง่าย แม่นยำ ประหยัด และรวดเร็ว นอกจากนี้ยังเหมาะที่จะใช้ในงานประจำสำหรับตรวจกรองมิวเตชั่นในยีน *VHL* ขั้นแรก

อุปกรณ์และวิธีการ

ผู้ป่วย

ผู้ป่วยที่ทำการศึกษาในงานวิจัยนี้เป็นผู้ป่วยจากโรงพยาบาลศิริราช 6 ครอบครัว ที่ได้รับการวินิจฉัยว่า

เป็นโรค VHL แต่ตรวจไม่พบมิวเตชั่นเฉพาะจุดที่ใดในยีน *VHL* ผู้ป่วยและสมาชิกในครอบครัวได้แสดงเจตนายินยอม (informed consent) ให้เก็บเลือดแล้ว จึงนำเลือดที่ได้มาเตรียมดีเอ็นเอโดยการสกัดเซลล์เม็ดเลือดขาวด้วยวิธี phenol/chloroform extraction

วิธีมัลติเพล็กซ์พีซีอาร์เชิงกึ่งปริมาณผ่านเครื่องวิเคราะห์สารพันธุกรรมแบบโครมาโตกราฟีในสภาพของเหลวภายใต้ความดันสูง (DHPLC-based semi-quantitative multiplex PCR)

วิธี DHPLC-based semi-quantitative multiplex PCR เป็นการเพิ่มปริมาณของชิ้นส่วนดีเอ็นเอหลายท่อนในปฏิกิริยาเดียวกันเพื่อเปรียบเทียบปริมาณของ PCR product ในช่วง log-linear phase โดยใช้ดีเอ็นเอเริ่มต้นเท่ากัน ถ้าผู้ป่วยมีอัลลีลที่มีการขาดหายไปของดีเอ็นเอบริเวณหนึ่งไป จำนวนชุดของดีเอ็นเอเริ่มต้นบริเวณนั้นจะลดลงเป็นครึ่งหนึ่งของคนที่มีอัลลีลทั้งสองเป็นปกติ ในช่วง log-linear phase ของปฏิกิริยาพีซีอาร์จะได้ปริมาณ PCR product เป็นสัดส่วนโดยตรงกับปริมาณดีเอ็นเอเริ่มต้น ดังนั้นการเปรียบเทียบความสูงของโครมาโตแกรมของ PCR product จากการวิเคราะห์ด้วยเครื่อง DHPLC จึงสามารถบอกได้ว่าผู้ป่วยมีการขาดหายไปของดีเอ็นเอบริเวณใดในยีน *VHL* (Su YN, 2005)

งานวิจัยนี้ได้แบ่งมัลติเพล็กซ์พีซีอาร์ออกเป็น 3 ชุดครอบคลุมยีน *VHL* ทั้ง 3 exon รวมทั้งส่วนของ promoter และ putative poly A site ในปฏิกิริยามัลติเพล็กซ์พีซีอาร์แต่ละชุดมีการใช้ยีน DMD เป็น internal control สำหรับ X chromosome และอาจใช้ยีน SPP1 เป็น autosome internal control ร่วมด้วยในปฏิกิริยาบางชุด ส่วนการวิเคราะห์ด้วยเครื่อง DHPLC เป็นการทำงานในลักษณะโครมาโตกราฟีในสภาพของเหลวภายใต้ความดันสูงที่ใช้แยกดีเอ็นเอตามขนาด โดยที่ดีเอ็นเอขนาดเล็กจะถูกชะออกมาก่อนดีเอ็นเอขนาดใหญ่ตามลำดับเวลา และความสูงของโครมาโตแกรมจะเป็นสัดส่วนโดยตรงกับปริมาณ PCR product จากตัวอย่างตรวจที่ฉีดเข้าไปในระบบ

ผลการทดลองและวิจารณ์

การใช้ DHPLC-based semi-quantitative multiplex PCR เพื่อวิเคราะห์หาการขาดหายไปของยีนในผู้ป่วยที่ไม่พบมิวเตชั่นชนิดจุด 6 ครอบครัว พบการขาดหายไปของยีนบางส่วน โดยดูจากความสูงของโครมาโตแกรมที่ลดลงเป็นครึ่งหนึ่งเมื่อเทียบกับคนปกติในทั้ง 6 ครอบครัว โดยครอบครัวที่ 1, 2, 3 และ 5 (VHL002, VHL004, VHL007, และ VHL017) มี exon 3 และบริเวณรอบ putative poly A site หายไป ครอบครัวที่ 4 (VHL012) มีเฉพาะส่วนของ exon 3 ที่หายไป ส่วนครอบครัวที่ 6 (VHL027) มีการขาดหายไปของ exon 2 และ 3

เมื่อนำดีเอ็นเอของผู้ป่วยทั้ง 6 ครอบครัว ไปตรวจยืนยันการขาดหายไปของยีนบางส่วนด้วยการทำ long-range PCR พบว่าใน 4 ครอบครัว (ครอบครัวที่ 1, 4, 5 และ 6) ได้ PCR product ที่มีขนาดสั้นลงเมื่อเทียบกับคนปกติ ซึ่งแสดงว่าบริเวณที่ขาดหายไปอยู่ระหว่างบริเวณที่ primer คร่อมอยู่จริง สอดคล้องกับผลที่ได้จากการทำ DHPLC-based semi-quantitative multiplex PCR อย่างไรก็ตามการทำ long-range PCR ร่วมกับ restriction map analysis เพิ่มเติมจะช่วยให้ทราบขอบเขตของการขาดหายไปในยีนได้ชัดเจนขึ้น แต่ไม่ได้เปลี่ยนแปลงผลต่อการวินิจฉัยโรคในผู้ป่วย

ใน 2 ครอบครัว (ครอบครัวที่ 2 และ 3) การทำ long-range PCR ไม่พบความผิดปกติของขนาดดีเอ็นเอบริเวณรอบ putative poly A site เหมือนที่พบจากการทำ DHPLC-based semi-quantitative multiplex PCR ซึ่งอธิบายได้ว่า primer ที่ใช้ในการทำ long-range PCR ไม่ได้คร่อมบริเวณที่มีการขาดหายไป โดยเป็นไปได้ว่าบริเวณที่ขาดหายไปมีขนาดใหญ่เลยออกไปจากตำแหน่งของ primer จึงทำให้ไม่มี PCR product ขนาดสั้นให้เห็น (มีแต่ขนาดปกติ) ส่วนอีก 2 ครอบครัว (ครอบครัวที่ 1 และ 5) primer ที่ใช้ในการทำ multiplex PCR ของส่วน putative poly A site อยู่ถัดจาก exon 3 ของยีน *VHL* ด้าน 3' ไป แต่ยังไม่ถึงตำแหน่งของ putative poly A site จริง (กล่าวคือตำแหน่ง putative poly A site ยังคงอยู่) สรุปได้ว่า 2 ครอบครัวนี้มีการขาดหายไปของ exon 3 เท่านั้น ไม่ได้รวม putative poly A site ไปด้วย ดังนั้นการแปลผลการขาดหายไปของดีเอ็นเอบริเวณรอบ putative poly A site จากการทำ DHPLC-based semi-quantitative multiplex PCR ต้องมีความระมัดระวังเป็นอย่างยิ่ง และควรยืนยันด้วย long-range PCR อย่างไรก็ตามถ้าพบการขาดหายไปของ exon 3 อยู่ด้วยอาจไม่จำเป็นต้องทำ เนื่องจากสามารถวินิจฉัยโรคได้แล้ว

สรุปผลการทดลอง

งานวิจัยนี้พบว่าการวิเคราะห์หาการขาดหายไปของยีน *VHL* ด้วยวิธี DHPLC-based semi-quantitative multiplex PCR ซึ่งเป็นวิธีที่สะดวก รวดเร็ว ประหยัด และผลการตรวจเชื่อถือได้ เมื่อใช้ร่วมกับการตรวจมิวเตชั่นเฉพาะจุดสามารถตรวจหา germline mutation ของยีน *VHL* ในผู้ป่วยและสมาชิกในครอบครัวได้ครบทุกชนิด ทำให้วินิจฉัยโรคได้แม่นยำยิ่งขึ้น อีกทั้งยังสามารถประเมินความเสี่ยงต่อการเกิดโรคได้แต่เนิ่นๆ ซึ่งช่วยให้แพทย์วางแผนการป้องกันและดูแลผู้ป่วยโรค VHL ได้อย่างมีประสิทธิภาพยิ่งขึ้น

กิตติกรรมประกาศ

ขอขอบคุณผู้ป่วยโรค VHL และครอบครัว และทุนวิจัยมุ่งเป้าของมหาวิทยาลัยมหิดล (อ.นพ. ชนินทร์ ลิ่มวงศ์)

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วันที่ 22-24 มิถุนายน 2552

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เซ็นทรัลเวิลด์ กรุงเทพมหานคร

Oral Presentation by Postgraduate Students

วันที่ 24 มิถุนายน 2552: [Session Y35-Y36]

ประธานร่วม รศ.ดร.พญ.วิไล ชินธเนศ (CU), ศ.ดร.นพ.ประเสริฐ เอื้อวรากุล (SI)

คณะผู้ให้ความเห็น ผศ.ดร.พญ.กนิษฐา ภัทรกุล (CU), อ.ดร.พญ.กัญญา ศุภปิติพร (CU), ศ.นพ.ชัยรัตน์ ฉายากุล (SI), ผศ.ดร.นพ.ถาวรชัย ลิ้มจินดาพร (SI), ศ.พญ. ธาดา สืบหลินวงศ์ (CU), ศ.ดร.นพ.ประเสริฐ เอื้อวรากูล (SI), ศ.นพ.พรชัย โอเจริญรัตน์ (SI), รศ.ดร. พูลลาภ ชีพสุนทร (CU), รศ.ดร. รัชนีกร กัลล์ประวิทธ์ (SI), ผศ.ดร.พญ.วัฒนา วัฒนาภา (SI), ศ.ดร.นพ. อภิวัฒน์ มุทิรางกูร (CU)

13.15 - 13.27 **Blood Transfusion Reduction with Parenteral Iron in Gynecologic Cancer Patients Receiving Chemotherapy**
Penkae Dangsuwan, Tarinee Manchana
Faculty of Medicine, Chulalongkorn University [Poster: CP- 11]

13.27 - 13.39 **Effects of Music Therapy on Self- Esteem and Depression among Female Adolescents in Rajvithi Home.**
Panida Yomaboot, Thienchai Ngamthipwatthana, Sucheera Phattharayuttawat, Woraphat Ratta-apha
Faculty of Medicine Siriraj Hospital, Mahidol University [Poster:SP- 07]

13.39 - 13.51 **Involoement of Pro- nociceptive 5- HT2A Receptor in the Pathogenesis of Medication Overuse Headache**
Weera Supornsilpchai, Supang Maneesri le Grand, Anan Srikiatkhachorn
Faculty of Medicine, Chulalongkorn University [Poster: CP- 02]

13.51 - 14.03 **Interaction between Human Kidney Anion Exchanger 1 (KAE1) and Kinesin Family Member 3B (KIF3B) In Human Kidney Cells**
Natapol Duangtum, Thawornchai Limjindaporn, Nunghathai Sawasdee, Piengpaga Ngaojanlar, Pa-thai Yenchitsomanus
Faculty of Medicine Siriraj Hospital, Mahidol University [Poster:SP- 08]

14.03 - 14.15 **The Promoter Methylation of COX- 2 and ID-4 Genes in Psoriasis**
Phantipa Protjaroen1, Dr.Kriangsak Ruchusatsawat2, Dr.Jongkonnee Wongpiyabovorn3
Faculty of Medicine, Chulalongkorn University [Poster: CP- 06]

Coffee Break

14.30 - 14.42 **The Inhibitory Effect of Siriraj Wattana Recipe and Gallic Acid on UVA-Mediated Melanogenesis by Modulation of Cellular Glutathione**
Vanida Tangsupa-a-nan, Kamolratana Kongtaphan, Tassanee Onkoksoong, Saipan Klumklomjit, Pravit Akarasereenont, Adisak Wongkajornsilp and Uraiwan Panich
Faculty of Medicine Siriraj Hospital, Mahidol University [Poster:SP- 09]

SP-08

Interaction between Human Kidney Anion Exchanger 1 (kAE1) and Kinesin Family Member 3B (KIF3B) in Human Kidney Cells

Natapol Duangtum[1], Thawornchai Limjindaporn[1], Nunghathai Sawasdee[2], Piengpaga Ngaojanlar[2], Pa-thai Yenchitsomanus[2]

[1]Department of Anatomy and [2]Division of Medical Molecular Biology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

Objective: The kAE1 protein plays a role in acid-base homeostasis by regulating bicarbonate reabsorption across the basolateral membrane of ?-intercalated cells. AE1 mutations showed impaired trafficking and retention of kAE1-mutant proteins in subcellular compartments thereby leading to distal renal tubular acidosis. Expression of kAE1 at the appropriate site likely requires interactions with other cellular proteins to facilitate its trafficking to the correct destination. KIF3B interacted with C-terminus of kAE1 in our yeast two-hybrid screening, suggesting a potential involvement of KIF3B in kAE1 trafficking. However, this interaction in mammalian cells has not been established. This study aims to confirm interaction between kAE1 and KIF3B in kidney HEK-293T cell line.

Materials and Methods: Amplification of KIF3B-HA was performed by PCR and the PCR product was sub cloned into pcDNA3.1 plasmid. pcDNA-KIF3B-HA was co-transfected into HEK-293T cells with pcDNA-kAE1-His and studied the interaction by co-immunoprecipitation (Co-IP). The effect of KIF3B in kAE1 sub-cellular localization was studied by double immunofluorescence staining. In addition, KIF3B and kAE1 were separately fused with fragment of yellow fluorescent protein (YFP) for yellow fluorescent protein-protein fragment complementation assay (YFP-PCA), which is a mammalian two-hybrid system.

Results: The results from the co-immunoprecipitation study demonstrated that kAE1 was co-precipitated with KIF3B. By YFP-PCA assay using KIF3B and kAE1 as a bait and a prey reconstituted YFP in human kidney cells observed by yellow light in confocal microscopy. Furthermore, kAE1 and KIF3B was co-localized in the cytoplasm and at the cell surface of transfected HEK-293T cells.

Conclusion: KIF3B and kAE1 physically interacts in human kidney cells. KIF3B may involve in kAE1 trafficking. Further studies will be directly toward the role of KIF3B in kAE1 trafficking in both physiologic and pathological conditions.

งานการเจ้าหน้าที่
07921
วันที่ 17 มี.ค. 2552
เวลา 9.20

23 มี.ค. 2552

11050
16 มี.ค. 2552

ที่ ตง 51007/ 2909

องค์การบริหารส่วนจังหวัดตรัง
ถนนพัทลุง ตง 92000

11 มีนาคม 2552

หน่วยอณูพันธุศาสตร์
เลขที่รับ.........
วันที่ 24 มี.ค. 2552
เวลา 16.00 น.

เรื่อง ขอเชิญเป็นวิทยากรฝึกอบรมเชิงปฏิบัติการ เรื่อง “หลักพันธุศาสตร์สำหรับครูผู้สอน”

เรียน คณบดีคณะแพทยศาสตร์ ศิริราชพยาบาล

ด้วยองค์การบริหารส่วนจังหวัดตรังร่วมกับวิทยาลัยเกษตรและเทคโนโลยีตรัง และสมาคมพันธุศาสตร์แห่งประเทศไทย กำหนดจัดโครงการฝึกอบรมเชิงปฏิบัติการ เรื่อง“หลักพันธุศาสตร์สำหรับครูผู้สอน” ระหว่างวันที่ 19-20 มีนาคม 2552 ณ โรงแรมเดอะแกรนด์เอ็ม.พี.รีสอร์ท จังหวัดตรัง โดยมีวัตถุประสงค์เพื่อพัฒนาและส่งเสริมความรู้ทางวิชาการให้กับครูที่สอนกลุ่มสาระการเรียนรู้เกี่ยวกับเรื่องพันธุศาสตร์ ชีววิทยา เกษตร ฯลฯ ในระดับชั้นประถมศึกษาและมัธยมศึกษา ทำให้ครูสามารถพัฒนาคุณภาพการเรียนการสอนเกี่ยวกับเนื้อหาสาระของหลักพันธุศาสตร์ในโรงเรียนให้มีประสิทธิภาพสูงขึ้น

งานการเจ้าหน้าที่
16 มี.ค. 2552

คณะผู้จัดโครงการได้พิจารณาแล้วว่า ดร.วรรณา ทองนพคุณ เป็นผู้มีความรู้ความสามารถทางด้านพันธุศาสตร์เป็นอย่างดี จึงใคร่ขอเชิญเป็นวิทยากรประจำโครงการ

จึงเรียนมาเพื่อโปรดพิจารณาให้ความอนุเคราะห์ และโปรดแจ้งให้ทราบด้วย จักเป็นพระคุณยิ่ง

เรียน หัวหน้าสถานส่งเสริมการวิจัย
เพื่อพิจารณา

18 มี.ค. 52

ขอแสดงความนับถือ

(นายกิจ หลีกภัย)
นายกองค์การบริหารส่วนจังหวัดตรัง

เรียน หัวหน้าหน่วย อณูพันธุศาสตร์
เพื่อ ☐ อนุมัติ
☑ ทราบ/พิจารณา
☑ ... เกี่ยวข้อง เพื่อตอบกลับ
... แจ้ง ดร.วรรณา
เพื่อ ☐ โปรดทราบ
☐ โปรดดำเนินการ
สถานส่งเสริมการวิจัย
24 มี.ค. 52

กองการศึกษา ศาสนาและวัฒนธรรม
โทรศัพท์ 0-7521-8262 ต่อ 344-347
โทรสาร 0-7521-8262 ต่อ 201

กำหนดการฝึกอบรมเชิงปฏิบัติการ เรื่อง "หลักพันธุ์ศาสตร์สำหรับครูผู้สอน"
ระดับประถมศึกษา

วันที่ 19 มีนาคม 2552

08.00 – 08.45 น. ลงทะเบียน

08.45 – 09.30 น. พิธีเปิดและปาฐกถาพิเศษ โดย นายกิจ หลีกภัย นายกองค์การบริหารส่วนจังหวัดตรัง เรื่อง สนับสนุนการศึกษาวิจัยวิทยาศาสตร์พื้นฐานเพื่อการพัฒนาวิทยาศาสตร์และเทคโนโลยีของประเทศไทย

09.30 – 10.30 น. บรรยายพิเศษ การกลายพันธุ์กับความหลากหลายทางชีวภาพ โดย ดร.สุมินทร์ สมุทคุปต์

10.30 – 10.40 น. รับประทานอาหารว่าง

10.40 – 12.40 น. สิ่งมีชีวิต – สิ่งไม่มีชีวิต, การเจริญเติบโตของพืช – สัตว์, วงจรชีวิตของพืช – สัตว์ สิ่งมีชีวิตกับการดำรงพันธุ์, โครงสร้างของอวัยวะสืบพันธุ์ โดย อ.ชูศิลป์ อัตชู

12.40 – 13.20 น. รับประทานอาหาร

13.20 – 15.20 น. การเจริญเติบโตของสิ่งมีชีวิต เน้นมนุษย์ การถ่ายทอดทางพันธุกรรม Mendel ลักษณะที่ปรากฏ – ไม่ปรากฏ การกลาย การแปรผัน หมู่เลือด โรคพันธุธรรม ยีน โครโมโซม โดย รศ.ดร.บุษบา ฤกษ์อำนวยโชค และ รศ.ดร.สมศักดิ์ อภิสิทธิวานิช

15.20 – 15.30 น. รับประทานอาหารว่าง

15.30 – 17.30 น. กิจกรรม (โจทย์ปัญหา, ข้อสอบ) โดย รศ.ดร.สมศักดิ์ และ อ.คณิศร์

วันที่ 20 มีนาคม 2552

08.30 – 10.30 น. เทคโนโลยีชีวภาพ โคลนนิ่ง GMO การแพทย์ อุตสาหกรรม ปรับปรุงพันธุ์ DNA fingerprint โดย รศ.ดร.บุษบา ฤกษ์อำนวยโชค และ รศ.ดร.สมศักดิ์ อภิสิทธิวานิช

10.30 – 10.40 น. รับประทานอาหารว่าง

10.40 – 12.40 น. กิจกรรม (แยกกลุ่ม)

กลุ่ม 1 แมลงหวี่หลากพันธุ์ โดย รศ.ดร.สมศักดิ์ และ ผศ.ดร.นฤมล

กลุ่ม 2 วงจรชีวิต พืช สัตว์ คน โดย อ.ชูศิลป์ และ อ.ดำรงค์

กลุ่ม 3 Fingerprint และการถ่ายทอดพันธุกรรมมนุษย์ โดย รศ.ดร.บุษบา และ รศ.ดร.อรินทิพย์

กลุ่ม 4 ความหลากหลาย โดย ศ.ประดิษฐ์ และ อ.คณิศร์

กลุ่ม 5 การถ่ายทอดทางพันธุกรรม Mendel ผศ.เตือนใจ และ รศ.สุภาพร

12.40 – 13.20 น. รับประทานอาหาร

13.20 – 15.20 น. กิจกรรม (แยกกลุ่ม)

กลุ่ม 1 แมลงหวี่หลากพันธุ์ โดย รศ.ดร.สมศักดิ์ และ ผศ.ดร.นฤมล

กลุ่ม 2 วงจรชีวิต พืช สัตว์ คน โดย อ.ชูศิลป์ และ อ.ดำรงค์

กลุ่ม 3 Fingerprint และการถ่ายทอดพันธุกรรมมนุษย์ โดย รศ.ดร.บุษบา และ รศ.ดร.อรินทิพย์

กลุ่ม 4 ความหลากหลาย โดย ศ.ประดิษฐ์ และ อ.คณิศร์

กลุ่ม 5 การถ่ายทอดทางพันธุกรรม Mendel โดย ผศ.เตือนใจ และ รศ.สุภาพร

15.20 – 15.30 น. รับประทานอาหารว่าง

15.30 – 16.30 น. ตอบปัญหา – สรุป โดย ศ.ดร.สุมิทร์ และ รศ.ดร.สุรินทร์

16.30 – 17.00 น. มอบวุฒิบัตรและปิดการอบรม

กำหนดการฝึกอบรมเชิงปฏิบัติการ เรื่อง "หลักพันธุ์ศาสตร์สำหรับครูผู้สอน"
ระดับมัธยมศึกษา

วันที่ 19 มีนาคม 2552

08.00 – 08.45 น. ลงทะเบียน

08.45 – 09.30 น. พิธีเปิดและปาฐกถาพิเศษ โดย นายกิจ หลีกภัย นายกองค์การบริหารส่วนจังหวัดตรัง เรื่อง สนับสนุนการศึกษาวิจัยวิทยาศาสตร์พื้นฐานเพื่อการพัฒนาวิทยาศาสตร์และเทคโนโลยีของประเทศไทย

09.30 – 10.30 น. บรรยายพิเศษ การกลายพันธุ์กับความหลากหลายทางชีวภาพ โดย ดร.สุมินทร์ สมุทคุปต์

10.30 – 10.40 น. รับประทานอาหารว่าง

10.40 – 12.40 น. การสืบพันธุ์ การถ่ายทอดลักษณะพันธุกรรม การระบุเพศ Mendel ยีนเด่น – ยีนด้อย Mitosis Meiosis โดย ศ.ประดิษฐ์ พงศ์ทองคำ

12.40 – 13.20 น. รับประทานอาหาร

13.20 – 14.20 น. ความผิดปกติของยีนและโครโมโซม โดย ดร.วรรณา ทองนพคุณ

14.20 – 15.20 น. กิจกรรม (แยกกลุ่ม สาธิต – ปฏิบัติ)
กลุ่ม 1 โครโมโซม แบ่งเซลล์ โดย รศ.ดร.สุรินทร์ และ ผศ.ดร.นฤมล
กลุ่ม 2 แยกสกัด DNA โดย ผศ.เตือนใจ และ ผศ.ดร.ปิยะศักดิ์
กลุ่ม 3 Probability โดย ศ.ประดิษฐ์ และ รศ.สุภาพร
กลุ่ม 4 Fingerprint โดย ดร.วรรณา และ รศ.ดร.บุษบา
กลุ่ม 5 ต่อ Nucleotide โดย รศ.ดร.อรินทิพย์ และ ศ.ดร.สุมินทร์

15.20 – 15.30 น. รับประทานอาหารว่าง

15.30 – 17.30 น. กิจกรรม (แยกกลุ่ม สาธิต – ปฏิบัติ)
กลุ่ม 1 โครโมโซม แบ่งเซลล์ โดย รศ.ดร.สุรินทร์ และ ผศ.ดร.นฤมล
กลุ่ม 2 แยกสกัด DNA โดย ผศ.เตือนใจ และ ผศ.ดร.ปิยะศักดิ์
กลุ่ม 3 Probability โดย ศ.ประดิษฐ์ และ รศ.สุภาพร
กลุ่ม 4 Fingerprint โดย ดร.วรรณา และ รศ.ดร.บุษบา
กลุ่ม 5 ต่อ Nucleotide โดย รศ.ดร.อรินทิพย์ และ ศ.ดร.สุมินทร์

วันที่ 20 มีนาคม 2552

08.30 – 10.30 น. Protein, DNA, โครงสร้างและหน้าที่ โดย รศ.ดร.อรินทิพย์ ธรรมชัยพิเนตร

10.30 – 10.40 น. รับประทานอาหารว่าง

10.40 – 12.40 น. เทคโนโลยีชีวภาพ โดย รศ.ดร.สุรินทร์ ปิยะโชคณากุล

12.40 – 13.20 น. รับประทานอาหาร

13.20 – 14.20 น. ประยุกต์ทางเกษตร โดย อ.ดำรงค์ สินไชย

14.20 – 15.20 น. ประยุกต์ทางการแพทย์ โดย ดร.วรรณา ทองนพคุณ

15.20 – 15.30 น. รับประทานอาหารว่าง

15.30 – 16.30 น. ตอบปัญหาน่ารู้ทางพันธุศาสตร์ – สรุป โดย ผศ.ดร.นฤมล และ รศ.ดร.บุษบา

16.30 – 17.00 น. มอบวุฒิบัตรและปิดการอบรม

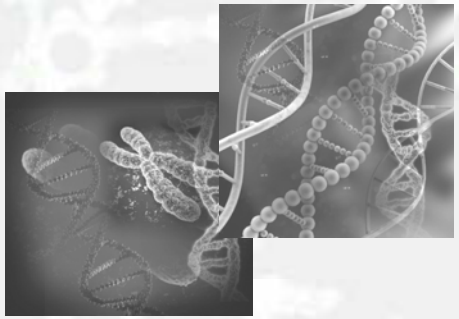
ผลประโยชน์ที่คาดว่าจะได้รับ

(1) ผู้เข้าร่วมได้รับความรู้ที่ก้าวหน้าทันสมัยด้านอณูพันธุศาสตร์ของโรคพันธุกรรมที่ซับซ้อนและพบบ่อย

(2) ผู้เข้าร่วมประชุมทราบถึงพัฒนาการของเทคโนโลยีใหม่ด้านอณูพันธุศาสตร์และจีโนมิกส์และวิธีศึกษาโรคพันธุกรรมที่ซับซ้อนและพบบ่อย

(3) ทำให้เกิดบรรยากาศทางวิชาการ การแลกเปลี่ยนความรู้ และความคิดเห็น

(4) ทำให้เกิดความตื่นตัวในการทำงานวิจัยความร่วมมือ และเครือข่ายประชาคมวิจัย



หน่วยงานที่ให้การสนับสนุน

ติดต่อสอบถาม : คุณฉัตรชัย ภูน้ำค้าง หน่วยอณูชีววิทยาการแพทย์ สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล ถนนพรานนก แขวงศิริราช เขตบางกอกน้อย กรุงเทพฯ 10700 โทรศัพท์ 02-419 7000 ต่อ 6666-70 โทรสาร 02-418 4793.

การประชุมวิชาการอณูพันธุศาสตร์ของมนุษย์

(Human Molecular Genetics Conference)

“อณูพันธุศาสตร์ของโรคพันธุกรรมที่ซับซ้อนและพบบ่อย”

(Molecular Genetics of Complex and Common Genetic Diseases)

จัดโดย โครงการเมธีวิจัยอาวุโส สกว.-สกอ.

ศ. ดร. เพทาย เย็นจิตโสมนัส และคณะ

วันจันทร์ ที่ 29 เดือนกันยายน พ.ศ. 2551

เวลา 08.00 น. ถึง 17.00 น.

ณ ห้องกรุงธนบอลล์รูม โรงแรมรอยัลริเวอร์

(เชิงสะพานกรุงธนบุรี) กรุงเทพฯ

หลักการและเหตุผล

เมื่อโครงการจีโนมของมนุษย์ (Human Genome Project) สำเร็จลงในปี ค. ศ. 2003 ได้เกิดโครงการจัดทำแผนที่แสดงตำแหน่งความหลากหลายของนิวคลีโอไทด์เดี่ยวบนโครโมโซม (single nucleotide polymorphism or SNP haplotype mapping) ในจีโนมมนุษย์ และการพัฒนาเทคโนโลยีในการตรวจที่มีประสิทธิภาพและให้ผลเร็ว (high-throughput technology) เพื่อตรวจความผันแปรของนิวคลีโอไทด์ในจีโนมมนุษย์ครั้งเดียวได้หลายตำแหน่ง และศึกษาการแสดงออกของยีน (gene expression) จำนวนมากพร้อมกัน และมีการนำเทคโนโลยีเหล่านี้ มาใช้ในงานวิจัยด้านอณูพันธุศาสตร์ของโรคพันธุกรรมที่ซับซ้อนและพบบ่อย (complex and common genetic diseases) ของมนุษย์ ซึ่งในอดีตศึกษาได้ยาก ทำให้เกิดองค์ความรู้ ที่จะนำไปสู่การประยุกต์และพัฒนาในด้านการตรวจวินิจฉัย การรักษา และควบคุมป้องกัน โรคกลุ่มนี้ได้อย่างมีประสิทธิภาพมากขึ้น

ความรู้เหล่านี้ก้าวหน้าอย่างรวดเร็ว มีผลการวิจัยและบทความทางวิชาการตีพิมพ์เป็นจำนวนมาก จนยากที่ผู้มิได้อยู่ในวงการและทำวิจัยในเรื่องนั้นๆ จะติดตามได้ทัน นอกจากนี้ งานวิจัยด้านโรคพันธุกรรมที่ซับซ้อนและพบบ่อยในคนไทย ยังมีน้อย ทำให้ขาดฐานข้อมูลและองค์ความรู้เรื่องนี้ ในคนไทย จึงควรจะมีการสนับสนุนและส่งเสริม ให้เพิ่มขึ้น

เพื่อนำความรู้ที่ก้าวหน้าทันสมัยในด้านนี้มาเผยแพร่แก่วงการวิชาและประชาคมวิจัยของประเทศไทย สร้างบรรยากาศทางวิชาการ การแลกเปลี่ยนเรียนรู้ และกระตุ้นให้เกิดการวิจัยและพัฒนาในด้านนี้ในประเทศไทยต่อไป จึงได้จัดการประชุมวิชาการครั้งนี้ขึ้น

วัตถุประสงค์

(1) เพื่อเผยแพร่ความรู้ด้านอณูพันธุศาสตร์ของโรคพันธุกรรมที่ซับซ้อนและพบบ่อย

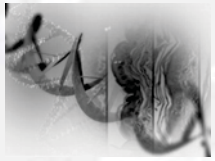
(2) เพื่อสร้างบรรยากาศทางวิชาการ การแลกเปลี่ยนเรียนรู้ และความร่วมมือของประชาคมวิจัย

(3) เพื่อกระตุ้นให้เกิดการวิจัยและพัฒนาด้านอณูพันธุศาสตร์ของโรคพันธุกรรมที่ซับซ้อนและพบบ่อยในคนไทย

รูปแบบการประชุม

เป็นการบรรยาย พร้อมมีเอกสารประกอบ การถามตอบและแสดงความคิดเห็น โดยที่ผู้เข้าร่วมประชุมไม่เสียค่าใช้จ่าย โดยรับเฉพาะผู้ได้รับเชิญและผู้ลงทะเบียนล่วงหน้าเพื่อสำรองที่นั่ง การจัดเตรียมเอกสาร อาหารกลางวัน และอาหารว่างไม่รับลงทะเบียนหน้าห้องประชุม

หมดเขตลงทะเบียนวันศุกร์ที่ 5 กันยายน 2551 และจะได้การตอบรับการลงทะเบียนเพื่อยืนยันการเข้าร่วมประชุมภายในวันที่ 8 กันยายน 2551



กำหนดการประชุม

08.00 – 09.00 น. ลงทะเบียน

09.00 – 09.30 น. พิธีเปิดการประชุม
โดย ศ. ดร. วิชัย บุญแสง ผู้อำนวยการฝ่ายวิชาการ สำนักงานกองทุนสนับสนุนการวิจัย และ ศ. ประดิษฐ์ พงศ์ทองคำ นายกสมาคมพันธุศาสตร์แห่งประเทศไทย

การบรรยายภาคเช้า

09.30 – 10.00 น. Molecular genetics of complex and common genetic diseases ศ. ดร. เพทาย เย็นจิตโสมนัส สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

10.00 – 10.40 น. Bioinformatics in human genetics and genomics
ดร. อภิชาติ อินทรพานิชย์
ศูนย์เทคโนโลยีอิเล็กทรอนิกส์และคอมพิวเตอร์แห่งชาติ

10.40 – 11.00 น. พักรับประทานอาหารว่างและชา - กาแฟ

11.00 – 11.30 น. Molecular genetics of diabetes mellitus
อ. นพ. ณัฐเชษฐ์ เปล่งวิทยา ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

11.30 – 12.00 น. Molecular genetics of diabetic complications
ดร. วทิพย์ บุณยศรีสวัสดิ์ ภาควิชาวิทยาภูมิคุ้มกัน คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

12.00 – 13.00 น. พักรับประทานอาหารกลางวัน

การบรรยายภาคบ่าย

13.00 – 13.40 น. Molecular genetics of systemic lupus erythrematosus
รศ. พญ. ดร.ณัฏฐิยา หิรัญกาญจน์ ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

13.40 – 14.20 น. Molecular genetics of autism
รศ. นพ. ดร. พรพรต ลิ้มประเสริฐ ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

14.20 – 14.50 น. Molecular genetics of stroke
อ. นพ. มานพ พิทักษ์ภากร ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

14.50 – 15.10 น. พักรับประทานอาหารว่างและชา-กาแฟ

15.10 – 15.40 น. Molecular genetics of cancers (I)
ศ. นพ. พรชัย โอเจริญรัตน์ ภาควิชาศัลยศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

15.40 – 16.10 น. Molecular genetics of cancers (II)
รศ. ดร. พิมพิชญา ปัทมสิริวัฒน์ ภาควิชาจุลทรรศน์ศาสตร์คลินิก คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล

16.10 – 16.40 น. Molecular genetics of susceptibility to infectious diseases
อ. ดร. ประพัฒน์ สุริยผล สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

16.40 – 16.50 น. สรุปและปิดการประชุม ศ. ดร .เพทาย เย็นจิตโสมนัส