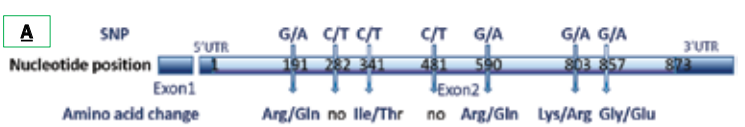
Performance of Novel Direct NAT2 haplotyping in Thai populations

Introduction

- N-acetyltransferase 2 (NAT2)
  - Encoding an enzyme acting primarily in liver cells to metabolize arylamine and hydrazine moieties found in many drugs, chemicals and carcinogens
  - High genetic diversity, 38 nucleotides positions and 108 haplotypes reported.
  - NAT2*4 haplotype is a wildtype with high enzymatic activity. The slow haplotypes (e.g.*5B, *6A, and *7B) are low enzymatic activity, Some haplotypes are high activities (e.g. *12A and 13A)
  - NAT2-diplotypes classify acetylator phenotype of human into
    - rapid (e.g. *4/*4, *4/12A, *4/*13A)
    - intermediate (e.g. *4/*5B, *4/*6A, *4/*7B)
    - slow (e.g. *5B/*5B, *5B/*6A,*5B/*7B)
    - ultra-slow (e.g. *6A/*6A, *6A/*7B, *7B/*7B)
- Slow/ultra-slow acetylator has strong risk for isoniazid induced hepatitis
- Conventional method for NAT2 haplotyping
  - Sequencing or SNP genotyping requiring sophisticated instruments (sequencer or real-time PCR)
  - PCR-RFLP and other SNP genotyping are extensive laboratory techniques
  - Minimum of 4 SNPs are needed for haplotype inference, which is prone to errors and difficult for non-statistician
- Unmet need for clinical application in low and middle income countries where TB are burdens to the population
  - Simple, short turn around time and low-cost NAT2 diplotyping without haplotype inference step
  - Could be deployed in the low and middle income countries at regional laboratories

Materials & Methods

- 520 DNA samples, which NAT2 diplotype were determined by conventional method (NAT2-exon2 sequencing plus haplotype inference by PHASE)
- Haplotype specific PCR-based method (HS-PCR) for NAT2-diplotyping
  - 6 reaction tubes containing specific primers for each NAT2 haplotype (NAT2*4, *5B, *6A, *7B, *12A, and *13A).
  - A primer pair to amplify TIMP1 on Chromosome-X utilized as an internal control
  - NAT2 haplotypes were directly determined by specific size of PCR products presented in an electrophoresed agarose gel



B

Rxn. No.	NAT2 haplotype	191 rs1801279	282 rs1041983	341 rs1801280	481 rs1799929	590 rs1799930	803 rs1208	857 rs1799931	Amplicon size (bp)
1	*NAT2*4(wild)*	G	C	T	C	G	A	G	588
	*NAT2*11A*	G	C	T	T	G	A	G	
2	*NAT2*5B*	G	C	C	T	G	G	G	583
	*NAT2*5C*	G	C	C	C	G	G	G	
3	*NAT2*6A*	G	T	T	C	A	A	G	300
4	*NAT2*7B*	G	T	T	C	G	A	A	431
	*NAT2*7C*	G	T	T	C	G	G	A	
5	*NAT2*12A*	G	C	T	C	G	G	G	387
	*NAT2*12C*	G	C	T	T	G	G	G	
6	*NAT2*13A*	G	T	T	C	G	A	G	366 / 641
	Internal Control *(TRIMP1)*								817

C

Figure. 1 A: Schematic of NAT2 gene with seven most common SNPs, B: Selected SNPs for the last nucleotide at 3' end of primers specific to amplify each of NAT2-haplotypes, and C: gel photo of electrophoresed amplicons

Results

Concordance rate of diplotyping between the novel HS-PCR vs. the conventional method were 99.04% (515/520 samples).

The discordant results (5/520 samples) were due to rare NAT2 haplotypes: *5C (n=3), *7C (n=1) and *11A (n=1)

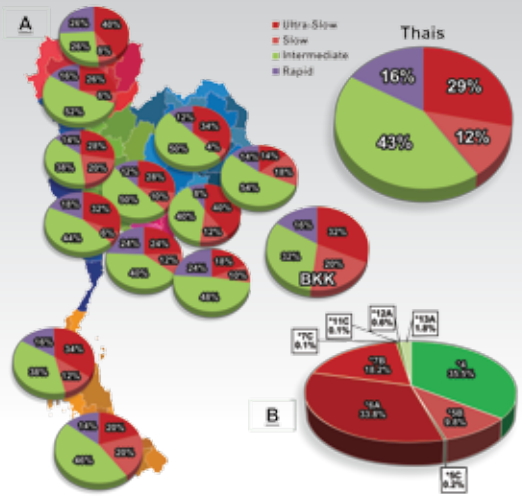


Figure. 2 Distribution of NAT2 variants., A:: Acetylator phenotypes distribution in Thai population stratified by 13 national health service areas and B: NAT2-haplotype frequency in Thais population

Table 1. The 5 discordant haplotypes identified in 1140 haplotypes

Number of discordant haplotypes	Conventional Method	Acetylation activity	Novel Method (HS-PCR)	Acetylation activity
3	*5C	Low	*5B	Low
1	*7C	Low	*7B	Low
1	*11A	High	*4	High

Discussion & Conclusion

- The HS-PCR method for NAT2 diplotyping**
  - Provided a complete concordant interpretation of acetylator status (89 rapid, 229 intermediate, and 202 slow) compared with conventional method
  - A novel affordable direct NAT2-diplotyping, with no risk of errors from statistical inference
  - Can be implemented in a simple molecular laboratory
- This novel HS-PCR for NAT2 diplotyping is the first step to enable the routine use of NAT2 acetylator status in clinical practice
- This work had been presented in ICHG conference in Kyoto 2016

**The primer set described in this work is in the process of patent filing in the Kingdom of Thailand (No. 1601001130)

References

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