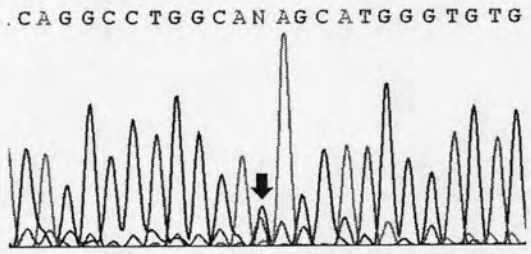
CHAPTER V
DISCUSSION AND CONCLUSION

We described two unrelated Thai patients with different clinical severity of MPS I. PCR-sequencing the entire coding region of *IDUA* successfully identified mutations in both patients. One novel mutation was identified.

Patient 1 with clinical features consistent with Hurler syndrome harbored the known c.252insC mutation. This mutation has been previously described in several populations including Thai[51, 52] and found to be associated with a severe phenotype. Sequence analysis of parental genomic DNA confirmed that both parents were heterozygous for the c.252insC mutation. Patient 2 with Scheie syndrome harbored a single base transition, c.826G>A in exon 7, leading to a glutamic acid to lysine substitution (p.E276K) at codon 276. Parental DNA was not available for analysis.

Other heterozygous sequence variants were also detected in patient 2 (Figure 19), suggesting that the patient did not have a large or whole deletion of the *IDUA* gene.

A. The heterozygous mutation in intron 3



B. The heterozygous mutation in intron 9

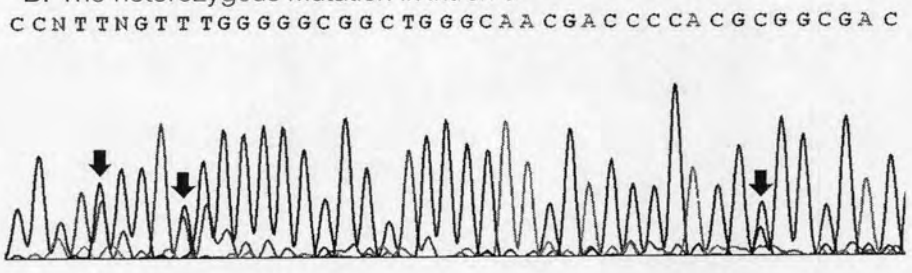


Figure 19 An electropherogram of the patient with Scheie syndrome showing some heterozygous variants.

The presence of the c.826G>A mutations was confirmed by restriction enzyme digestion of the PCR products (Figure 17). Even though only the mutant sequence was detected, the homozygous nature of the c.826G>A mutation could not be definitely concluded. The presence of the exon 7 deletion remains another possibility.

Testing for protein function was performed by measuring the alpha-L-iduronidase activity in a patient's leukocytes as well as in cells transiently transfected with the expression constructs by fluorometric assay using 4-Methylumbelliferone (4MU) as a substrate. The p.E276K mutation identified in the patient with Scheie syndrome caused a significant reduction of alpha-L-iduronidase activity (0.60±0.06 nmol/h/mg) in the patient's leukocytes when compared with that in Thai unaffected controls. The mean control alpha-L-iduronidase activity in the leukocytes of 8 normal Thai unaffected individuals was 23.10±8.80 nmol/h/mg.

The effect of p.E276K mutation was also tested in a transient transfection study in COS-7 cells. The p.E276K mutant construct had a significant reduction of alpha-L-iduronidase activity when compared with that of the wild-type IDUA construct and a similar level of the enzyme activity to the p.W402X mutant. The p.W402X is among the most common mutation found in caucasian patients with MPS I. The p.W402X mutation has been shown to have a significant reduction of alpha-L-iduronidase activity in a transfection study[50].

In summary, we reported two unrelated Thai patients with MPS I with different clinical severity caused by a deficiency of alpha-L-iduronidase. A novel pathogenic mutation of the *IDUA* gene, c.826G>A (p. E276K), was found. In addition, the previously identified c.252insC mutation was also detected in our patient. This study expands the genotypic spectrum of *IDUA* and emphasizes an important role of genetic testing for definite diagnosis as well as genetic counseling.