CHAPTER V
DISCUSSION AND CONCLUSION

Genetic disorder is a disease caused by abnormalities in an individual's genetic material (genome). There are over 6000 genetic disorders that can be passed down through generations. In this study, we described some genetic disorders commonly found at Pediatric Clinic of the King Chulalongkorn Memorial Hospital.

X-ALD is a rare genetic disease with symptoms normally starting between the ages of 4 and 10 years. We identified 9 unrelated Thai patients clinically suspected of X-ALD. Mutation screening in the *ABCD1* gene by PCR-sequencing revealed 5 different mutant alleles including 2 novel ones. (A646P, E609K, R401W, L392P, A247D). Restriction enzyme analysis was also used to confirm and screen for the identified mutation in at-risk family members or suspected carriers. The results, revealing the mutations as either *de novo* or inherited, have proved to be helpful for more accurate genetic counseling as well as prenatal diagnosis.

In this study, a mutation in the *ABCD1* gene could not be identified in 4 X-ALD patients, it remains possible that mutations could be in the promoter or intron region which was not included in our method.

In Pompe disease, there are some populations in which particular mutations are more common due to founder effects. For example, the p.R854X mutation has been found in many African American and African cases; the p.D645E has been seen in many Chinese infantile cases; and the p.G925A mutation has been seen in many European cases.[28] In this study, we analyzed 5 unrelated Thai families with Pompe and found the p.D645E mutation in all families (100%). It might be the most commonly found mutation in Thai people caused by a founder effect similar to previous studies in the Chinese and Taiwan populations [28]. For a cheap, rapid and reliable test, genetic testing using restriction enzyme analysis for the D645E mutation can be performed in Thai patients with Pompe. If this mutation is not detected, further analysis by PCR-sequencing to identify other mutations in the *GAA* gene should be performed. Genetic analysis remains very useful for genetic counseling and prenatal diagnosis of this disease.

Mutations in *STAT3* have been recently identified in a disorder causing profound, multisystem inflammatory disorders.[11] STAT3 is an essential mediator of the immune suppressive action of interleukin-10, and the loss of responsiveness of this interleukin may underlie much of the proinflammatory nature of the hyper-IgE syndrome (HIE). The heterozygous missense mutations have been found only in the DNA-binding or SH2 domain. Mutations within the DNA binding domain resulted in a protein with impaired ability to target the promoter of the target genes and mutations in the SH domain caused reductions in target-gene expression.[10,11] From these previous findings, we performed targeted mutation analysis by designing primers only around the DNA binding and SH domains for direct sequencing analysis. We identified the known mutation, c.1144C>T (p.R382W) in our Thai patient. This is the first molecularly-confirmed HiE Thai patient.

Holt-Oram syndrome (HOS) is a rare genetic disorder. It is characterized by malformations of upper limbs and variable cardiac defects. The diagnosis can be confirmed by mutation analysis of the *TBX5* gene. At present, mutations in *TBX5* can be found throughout in the coding region of the gene as shown in figure 6.[16] In this study, we analyzed all exons of the *TBX5* but could not identify the potential disease causing mutation. However, some previous studies found another gene responsible for HOS. It has been suggested that if the patients were diagnosed with Holt-Oram syndrome and *TBX5* analysis failed to show a mutation, *SALL4* analysis should be considered. [22] Therefore, we analyzed all coding regions of *SALL4* in our HOS patients without mutations in *TBX5* and could not identify the potential disease causing mutation. From this result, there could be other unidentified genes responsible for Holt-Oram syndrome in these Thai patients.

Systemic lupus erythematosus (SLE) is a severe autoimmune disease inflicting damage to multiple organs. The exact etiology of SLE has not been clear, but it is obvious that genetic factors, gender and environment are involved its pathogenesis. It has been shown that there are multiple genomic loci containing SLE susceptibility genes in humans and mice.[30] In 2007, studies in mice found that overexpression of human DcR3 in mice resulted in an SLE-like syndrome. In addition, some previous studies have shown elevated DcR3 levels in active SLE patients when compared with inactive SLE

patients and healthy controls, although the reasons of this association remain elusive. [31-33] Therefore, DcR3 is a potential new candidate gene for SLE.

Our study showed that DcR3 levels were not different between SLE patients and unaffected controls. Our results were not similar to the previous studies. The possibilities remain that the controls that we used in this study were individuals with other diseases but not autoimmune diseases, unlike the controls used in previous studies. Most of our samples were from inactive SLE patients. However, we found two severe SLE patients (with SLEDAI score 24 and 29) who had significantly increased levels of DcR3 (1,299 pg/µl and 961.7 pg/µl). There was one patient who we received two serum samples during inactive stage (score 0) with the DcR3 level of 255.3 pg/µl and during active stage (score 8) with up the DcR3 level to 711.01 pg/µl.

We identified one SLE patient with the c.364C>T mutation on exon1 of the *DcR3* gene. The mutation is expected to result in a histidine to tyrosine substitution at codon 122. The mutation is located on the Fas binding site. It is a region for binding with the Fas receptor to protect apoptosis. We tested whether this mutation was a potential disease-causing mutation by screening for its presence by PCR-RFLP in 500 unaffected controls. We found 1 in 500 unaffected controls carrying this mutation. We also measured the DcR3 level in this patient. The patient did not have an increased DcR3 levels. It remains possible that this mutation might result in gain of function leading to an increased ability of DcR3 in binding with the Fas receptor. The DcR3 level could be normal in the SLE patient with the mutation. Of note, although there have been many genome wide association studies for SLE, none have shown a positive association tc a polymorphism nearby the *DCR3* locus. This does not absolutely contradict our hypothesis; *DCR3* could still be a gene underlying SLE. If the DNA change found in our patient is proved to be a *de novo* pathogenic mutation, then association studies are correctly unable to detect it.

The practical techniques to detect each mutation in this study can be concluded in Table 16.

Table16. Practical techniques to detect mutations in this study.

Mutation	Practical technique	Example disease
Distribute throughout the gene	Analysis of all exons or coding regions	X-linked ALD
Common /hotspot mutation	PCR-RFLP	Pompe disease
Some regions	Analysis of specific regions	HIES
Not found mutation	Maybe mutation in other genes	HOS

In conclusion, genetic testing can be developed for each genetic disease to provide the most accurate and affordable method for disease diagnosis. Developing such methods depend on characteristics of genes that associate with the diseases, for example, gene size, gene complexity, gene expression, previously reported mutations in the gene. These factors need to be taken into consideration for successfully developing practical genetic techniques.