6.0 CONCLUSION, LIMITATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

6.1 Conclusion

The findings in this study revealed a total of six mutations and 12 polymorphisms of the *TPO* gene in a cohort of 21 unrelated dyshormonogenetic patients. Three out of these six *TPO* mutations (c.670_672del in exon 7, c.1186C>T in exon 8 and c.1502T>G in exon 9) are novel which had expanded the mutation spectrum of *TPO* associated with dyshormonogenetic CH. *In silico* functional analyses indicated that all of the six mutations and a novel polymorphism, c.180-6C>A, either affect normal activity of TPO protein or/and leading to the alternative splicing. It is also believed that another novel polymorphism, c.1-192C>A, in the GC box might alter the expression levels of *TPO* gene in an individual. The c.2268dup, which is also known as a founder mutation, is a common disease allele amongst Malaysian-Chinese patients with dyshormonogenetic CH but it was not seen in Malay and Indian subjects. Functional analyses in this study illustrated for the first time, the devastating effects of the c.2268dup mutation on the TPO protein function. This study also revealed that patients who were homozygotes for the c.1159G>C, c.1502T>G or c.2268dup mutations were associated with goitre development not at birth but later in their life. Previous reported cases had postulated the association of goitre malignant transformation with *TPO* gene mutations, hence, it is important to have a careful surveillance for potential thyroid neoplasm in these patients. This study also showed suppression of TPO expression in follicular adenoma in two sisters with c.2268dup, observed more commonly in carcinoma cases. Finally, our studies conclude that

mutation in the *TPO* gene is one of the underlying genetic causes of CH with dyshormonogenesis in this cohort of patients.

6.2 Limitations and suggestions for future research

This study has demonstrated the potential devastating effects of the *TPO* alterations. Future studies are therefore required to confirm the effects of these alterations. Since the *TPO* gene is only expressed specifically in the thyroid gland and full length of TPO1 mRNA transcript cannot be directly obtained from blood or thyroid tissue through RT-PCR, other techniques such as recombinant DNA or PCR fusion can be used to overcome this problem. Nonetheless, the *in silico* findings about the effect of the alteration to the *TPO* pre-mRNA splicing pattern can be validated through *in vivo* splicing assay. Finally, in order to validate the possibility and mechanism of malignant transformation of goitrous thyroid lesion, more subjects associated with c.2268dup mutation need to be studied.