1.0 INTRODUCTION

1.1 Background

Congenital hypothyroidism (CH) occurs in babies who are born without the ability to produce adequate amount of thyroid hormones. CH is reported to affect 1 in 3000 - 4000 life birth (Amar-Singh, 2010; Delange, 1979; Fisher *et al.*, 1979). Most cases of CH (80 - 90 %) result from dysembryogenesis of the thyroid gland. The remaining 10 - 20 % of cases are due to thyroid dyshormonogenesis or defects in intermediary steps of thyroid hormone synthesis (Gruters, 1992). A number of studies have shown that thyroid dyshormonogenesis is associated with defects in gene encoding proteins that are involved in thyroid hormone synthesis such as dual oxidase 2 (DUOX2) (Varela *et al.*, 2006), solute carrier family 5 or sodium iodide symporter (SLC5A/NIS) (Pohlenz & Refetoff, 1999), thyroglobulin (Tg) (Ieiri *et al.*, 1991), thyroid peroxidase (TPO) (Deladoey *et al.*, 2008), and thyroid stimulating hormone, beta (TSHB) (Deladoey *et al.*, 2003).

Among the candidate genes, mutations in the *TPO* are amongst the most common causes of dyshormonogenetic CH (Mangklabruks *et al.*, 1991). The single gene for *TPO* is located on chromosome 2p25 which spans at least 150 kb and contains 17 exons (Endo *et al.*, 1995). The full-length 3152 bp mRNA transcript TPO1 (GenBank accession number NM_000547.5) encodes 933 amino acids which consists a large extracellular fragment, single short transmembrane segment and short cytoplasmic tail. TPO protein plays important roles in the biosynthesis of thyroid hormone (Kimura *et al.*, 1987; Magnusson *et al.*, 1987). It catalyses the iodination of Tg and is involved in

the coupling of some of the iodotyrosyl residues to generate active iodothyronines, T_3 and T_4 (Cetani *et al.*, 1995; Gardas *et al.*, 1997).

The TPO enzyme activity depends on two conditions: (1) a proper protein folding and membrane insertion and (2) an intact catalytic region (Ruf & Carayon, 2006). Therefore, mutations that occur in the catalytic region or cause the production of premature TPO protein could lead to a decrease in TPO activity which subsequently causes dyshormonogenetic CH and/or hypothyroid goitre. To date, more than 60 mutations in the *TPO* gene have been described. The majority of the mutations are localised in exons 8, 9 and 10 that encode the catalytic heme-binding domain of the protein. The reported mutations include frame shift, missense, alternative-splicing, nonsense mutations and total gene deletion (Coleman & Tsongalis, 2009; Ris-Stalpers & Bikker, 2010). *TPO* defects are commonly inherited in an autosomal recessive mode (Park & Chatterjee, 2005) but autosomal dominant inheritance had also been reported (Fugazzola *et al.*, 2003).

Over the years, many cases of thyroid carcinoma associated with dyshormonogenetic goitre have been reported (Alzahrani *et al.*, 2006; Camargo *et al.*, 2001; Chertok Shacham *et al.*, 2012; Cooper *et al.*, 1981; Medeiros-Neto *et al.*, 1998). However, the influence of the *TPO* mutation on malignant transformation has rarely been discussed and remains unclear. Since a recent study had reported that alterations of the *TPO* gene was found in patients with Hashimoto's thyroiditis and papillary thyroid cancer (Liu *et al.*, 2010), defects in *TPO* gene might not only cause thyroid dyshormonogenesis but possibly involve in other thyroid diseases like thyroid carcinoma and autoimmune thyroid diseases.

1.2 Objectives of the study

Hot-spot mutations in the *TPO* gene in a cohort of 17 unrelated patients with CH due to thyroid dyshormonogenesis had previously been screened (Kang, 2010). A c.1159G>A mutation in exon 8 was identified in a patient (CHP41). The cohort size of unrelated patients had since increased. Therefore, the aims of the study were;

I. To screen for the c.1159G>A mutation in CHP41's family members and other unrelated patients with dyshormonogenetic CH.
II. To further characterise for other nucleotide alterations in the entire exons of the *TPO* gene in unrelated patients with thyroid dyshormonogenesis and their family members.
III. To predict and study the effect(s) of the alteration(s) on the TPO activity/function.