2.0 LITERATURE REVIEW

2.1 Thyroid gland

The thyroid gland is located in anterior part of the lower neck, below the larynx (voice box). It is the first glandular tissue to appear during embryological development in mammals. It arises from two regions of the endodermal pharynx which later develops into a bi-lobed structure (Park & Chatterjee, 2005). In adults, the normal thyroid gland is presented by two lobes joined across the trachea by the isthmus. Each lobe pre-lateral surface is covered by the infrahyoid muscles (Summers, 1950). The thyroid gland consists of three major types of epithelial cells. These include follicular cells, which line the follicles and secrete thyroxine and triiodothyronine, calcitonin-producing parafollicular cells (C cells) which secrete calcitonin, and the solid cell nest (SCN), which are remnants of the ultimobranchial body. The mean weight of the thyroid gland for an adult is about 20 gram (Hoyes & Kershaw, 1985; Kalina *et al.*, 1971; Takasu *et al.*, 1992).

2.2 Thyroid hormones

Thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) are produced from the thyroid gland and are involved in the coordination of a multitude of physiological responses and developmental processes in all vertebrates (Yen, 2001). In general, thyroid hormone synthesis involves the following steps: 1) thyroid stimulating hormone (TSH) binds to thyroid stimulation hormone receptor (TSH-R) and stimulates the iodide (I^-) transport from the blood plasma into the thyroid gland by NIS; (2) I^- uptake by the follicular cells: (3) oxidation of I^- and iodination of tyrosine; (4) coupling of two

iodotyrosine to form hormonally active iodothyronines, 3, 5, 3'-triiodothyronine (T_3) and 3, 5, 3', 5'-tetraiodothyronine or T_4. The steps involved in thyroid hormone synthesis are shown in Figure 2.1. T_3 is structurally similar to T_4 and has three atoms of iodine instead of four. Most T_3 are produced by phenolic ring 5'- monodeiodination of T_4 in extrathyroidal tissues (Granner, 1999). About 90 % of the thyroid hormone released from the thyroid gland is in the form of T_4. However, most of the secreted T_4 are converted into T_3 which is 10 times more potent in its biological activity (Sherwood & Cengage Learning (Firm), 2010). These two hormones play an important role in the regulation of skeletal development and growth. Deficiencies of these hormones result in hypothyroidism. Hypothyroidism during childhood causes developmental delay, growth retardation, delayed bone age and epiphyseal dysgenesis (O'Shea *et al.*, 2003). In contrast, excessive amount of thyroid hormones leads to hyperthyroidism, which causes an increase in the metabolic rate, weight loss despite good appetite, excessive sweating, irritability, anxiety, tremulousness, palpitations, goitre and proximal muscle weakness (Sapini Y, 2010; Whitley, 1999).

2.3 Hypothyroidism

Hypothyroidism is a condition in which the thyroid gland does not produce enough functionally active thyroid hormones (Roberts & Ladenson, 2004). It is a relatively common disorder and arising more often in women than men (Eugene *et al.*, 2005). Hypothyroidism can be divided into primary, secondary and tertiary subcategories. Primary hypothyroidism is characterised by a decreased production of thyroid hormones as a consequence of thyroid diseases. Common causes of primary hypothyroidism include functional problems within the thyroid gland, infiltrative

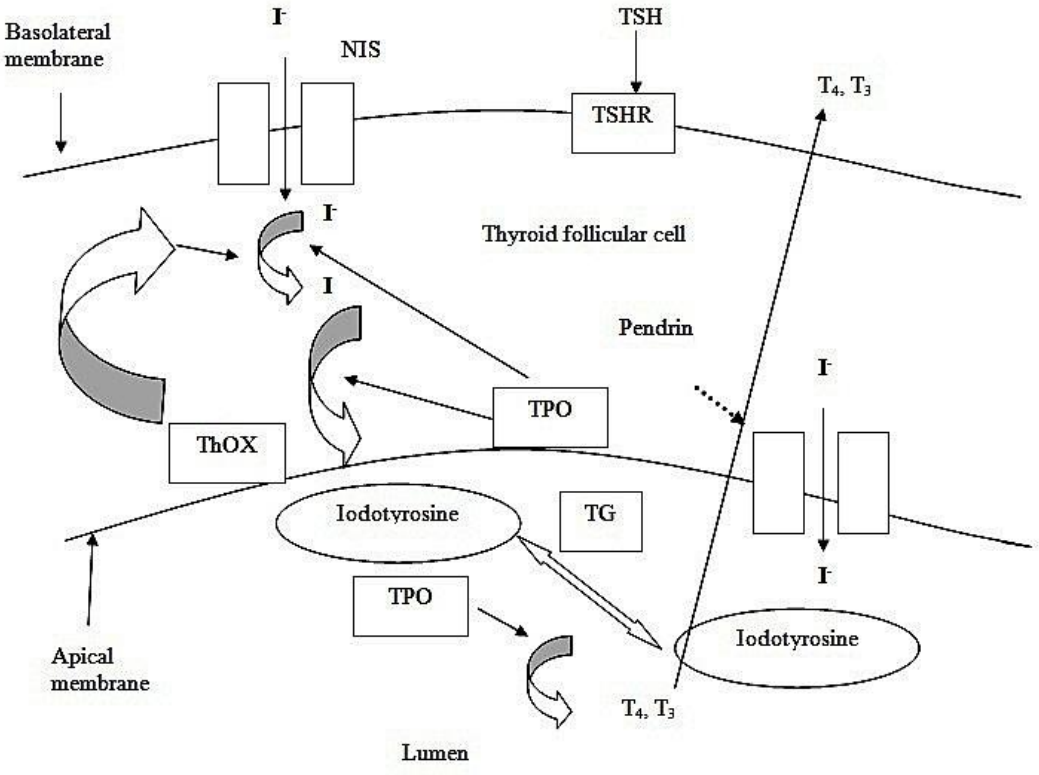

Figure 2.1 Schematic diagram of a follicular cell, illustrating the steps involved in thyroid hormone synthesis (Park & Chatterjee, 2005).

disease of the thyroid, silent or subacute thyroiditis, chronic autoimmune thyroiditis, radioiodine therapy, and postoperative hypothyroidism. External radiation to the neck of children or defects in any steps of thyroid hormone synthesis might also result in hypothyroidism. Secondary or tertiary hypothyroidism occurs when the problem lies within the pituitary or hypothalamus. The thyroid gland is intrinsically normal but it does not receive stimulation from the pituitary or brain to synthesise thyroid hormones. Secondary hypothyroidism can be acquired when pituitary gland does not produce and release adequate amount of TSH that is needed by the body. In tertiary hypothyroidism, problems within the hypothalamus cause decreased synthesis of thyroid releasing hormone (TRH) and subsequent decreased stimulation of the pituitary (Whitley, 1999). Hypothyroidism that is present at birth is known as CH whereas hypothyroidism in later life is called acquired or clinical hypothyroidism. Severe shortage of thyroid hormones after birth can lead to mental retardation and motor handicaps (de Vijlder, 2003). Meanwhile, hypothyroidism in later childhood and adults can result in a slowing of metabolic processes and is reversible with treatment. Severe untreated hypothyroidism can result in myxedema coma (Bonert & Friedman, 2007). Other common symptoms and signs of hypothyroidism are shown in Table 2.1.

2.4 Neonatal transient hypothyroidism

Neonatal transient hypothyroidism (NTH) or transient neonatal hypothyroidism (TNH) is a temporary form of hypothyroidism which can last from several days to several months after birth. The incident of NTH varies from 1 in 5845 in Iran to 1 in 700 in Belgium (Delange *et al.*, 1978; Ordookhani *et al.*, 2007). The causes of NTH remain unclear but factors including environmental, maternal, neonatal and genetic are

Table 2.1 Clinical features of hypothyroidism (modified from Bonert & Friedman, 2007)

Neonates and children	Adults
Poor feeding	Fatigue
Inactivity	Cold intolerance
Prolonged jaundice	Weakness
Tongue protrusion	Lethargy
Umbilical hernia	Weight gain
Constipation	Constipation
Learning difficulties	Myalgias
Mental retardation	Arthralgias
Short stature	Menstrual irregularities
Delayed bone age	Hair loss
Delayed puberty	Dry, coarse, cold skin
	Coarse, thin hair
	Hoarse voice
	Brittle nails
	Periorbital, peripheral edema
	Delayed reflexes
	Slow reaction time
	Orange skin hue
	Bradycardia
	Pleural, pericardial effusions

believed to be linked to NTH (Cheron *et al.*, 1981; Delange, 1998; Lin *et al.*, 1994; Niu *et al.*, 2005).

2.5 Permanent congenital hypothyroidism

Permanent CH occurs in babies who are born without the ability to produce adequate amount of thyroid hormones. It is one of the world's most common endocrine problems that affecting 1 / 3000 - 4000 newborn baby (Delange, 1979; Fisher *et al.*, 1979). Permanent CH is mainly due to the primary hypothyroidism instead of central hypothyroidism (Van Vliet, 2003). About 80 to 90 % of CH cases are the result of dysembryogenesis of the thyroid gland. The remaining 10 - 20 % cases are due to thyroid dyshormonogenesis (Gruters, 1992). Symptoms and signs of CH in neonates include poor feeding, jaundice, hypothermia, bradycardia, an enlarged posterior fontanelle and umbilical hernia. However, most neonates with CH do not show significant symptoms and signs in the first few months after birth (Roberts & Ladenson, 2004). As a result, delay in diagnosis and treatment of CH cause these neonates to suffer from permanent neurologic abnormalities, growth failure and irreversible mental retardation (Gruters *et al.*, 2004).

2.6 Biochemical screening and clinical diagnosis of CH

Most countries worldwide including Malaysia have newborn screening program to allow early diagnosis of CH. Screening of CH in newborns are carried out through the measurement of TSH levels in their cord blood or at 3 - 5 days of life and the results are further confirmed with serum thyroid function test (TFT), which measures the TSH, free T_4 (FT_4) or total T_4 levels. Normal serum TSH levels in the disease-free individuals

varies with age during the neonatal period and are as follows: less than 25.0 μIU/ml in cord blood; 0.4 - 10.0 μIU/ml after 5 days of life; 0.5 - 8.7 μIU/ml at a month of age; and 0.4 - 5.5 μIU/ml at 3 months of age and adult. The reference values of FT_4 are as follows: 20.0 - 68.0 pmol/L in cord blood and after 5 days of life; 23.0 - 54.0 pmol/L at a month of age; and 11.5 - 23.2 pmol/L at 3 months of age and adult (Musa *et al.*, 2006). The reference value of total T_4 is between 60 - 140 nmol/L for both children and adults. CH is diagnosed when the neonate has elevated serum TSH level with low level of T_4 or abnormal total T_4 level. In Malaysia babies with cord blood TSH >25 μIU/ml are recalled to confirm the diagnosis of CH by repeating the TSH and FT4. If confirmed, they are given treatment with L-thyroxine as soon as possible and are regularly followed up for growth and developmental monitoring and dose adjustment of thyroxine as the children grow. However, neonatal screening using TSH alone can be insensitive in diagnosing CH cases which are due to hypothalamic-pituitary defect (Ross, 2001; Spencer *et al.*, 1996). The status of the thyroid gland including size and location can be determined using thyroid ultrasonography or Technetium-99m (^{99}Tm) scintigraphy. Thyroid scintigraphy and ultrasonography are usually carried out before treatment is instituted. If this is not possible, most screening centres perform these imaging studies when the patients reached the age of 3 years, when it is safe to stop the T_4 replacement therapy temporarily before scanning (Rose *et al.*, 2006).

2.7 Treatment of CH

The objective of the treatment is to maintain the thyroid hormones levels within the normal reference range required for metabolic processes throughout the body. Patients with CH can be treated with synthetic levothyroxine (L-T_4) or liothyronine (L-T_3) or a combination of the two synthetic hormones. Levothyroxine sodium is preferred

in thyroid hormone replacement therapy since liothyronine treatment results in fluctuations in thyroid hormone levels in the blood. The optimum dose of levothyroxine for hypothyroid patients is related to bodyweight and age. The starting replacement dose for CH patients is 25 - 50 µg per day or 10 - 15 mcg/kg of body weight per day for the first six months and is then readjusted according to clinical evaluation and serum FT_4 and TSH levels in follow up examination. Patients with CH are advised to avoid soy products during the course of treatment as genistein in soy products can interfere with the absorption of the two synthetic hormones (Fruzza *et al.*, 2012). Appropriate treatment of these patients will result in suppressed serum TSH levels and a normal FT4 or total T4 using the reference normal range for age (Bonert & Friedman, 2007; Toft, 1994).

2.8 Thyroid dyshormonogenesis

Defects in any of the steps during the thyroid hormone biosynthesis lead to dyshormonogenesis which typically manifests as CH and goitre (Gruters, 1992). Thyroid dyshormonogenesis is an uncommon cause of CH which only accounts for 10 - 15 % of total CH cases (de Vijlder *et al.*, 1997). An autosomal recessive mode of inheritance has been noted in most reported cases with dyshormonogenesis. Majority of the patients with thyroid dyshormonogenesis have developed either a diffuse goitre or nodular gland at various phases of their lifetime in an attempt to compensate for the diminished capacity of the thyroid gland to produce thyroid hormones (Hulse, 1984). Most cases of goitrous CH with iodide organification defect are attributable to thyroid peroxidase (TPO) deficiency (Avbelj *et al.*, 2007; Bakker *et al.*, 2000; Deladoey *et al.*, 2008). Thyroid dyshormonogenesis is also associated with defects in other genes 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), and *thyroid stimulating hormone, beta* (*TSHB*) (Deladoey *et al.*, 2003).

2.9 Iodide organification defects

Under normal condition, I^- will be oxidised and bind to the tyrosine residues in Tg, where this process is commonly referred to as iodide organification. However, in some cases, I^- in the thyroid gland cannot be oxidised and/or bound to the Tg protein and these defects can lead to thyroid problems like hypothyroidism. Iodide organification defects can be partial or total depending on the degree to which I^- can be organified. Partial organification defect (PIOD) is characterised by a discharge of more than 10 % of the radio-iodine taken up by the gland within one hour during perchlorate discharge test. In contrast, total iodide organification defect (TIOD) is more critical where this condition usually happens in patients with severe CH. These patients have shown a discharge of more than 90 % of radio-iodine after administration of sodium perchlorate within an hour. Iodide organification defects are usually due to either a shortage of any of these elements like hydrogen peroxide (H_2O_2), I^- and Tg during iodination process or disorders in *TPO* (de Vijlder, 2003; Perez-Cuvit *et al.*, 1977).

2.10 Goitre

Goitre refers to the enlargement of the thyroid gland. Goitres are classified into three groups according to the functional status of the gland ie: euthyroid (simple goitre), hyperthyroid (toxic nodular goitrem or Grave's disease) or hypothyroid (iodine

deficiency, CH or Hashimoto's thyroiditis). Goitre due to Grave's disease characteristically has symmetrically enlarged gland with a smooth surface and often with a bruit. A diffuse, smooth goitre with negative anti-thyroid may be due to iodine deficiency, where the thyroid gland enlarges as it attempts to comply with the pituitary's demand. However, other thyroid problems like CH and other inflammatory conditions of the thyroid gland are also responsible for the formation of goitre. Meanwhile, a nodular thyroid gland with hypothyroidism and positive anti-thyroid antibodies is suggestive of Hashimoto's thyroiditis (Bonert & Friedman, 2007). A large goitre often can cause swallowing and breathing problems. Even though the majority of goiters are benign, it is important to ensure that they are not due to underlying neoplasm. It must be recognised that 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).

2.11 Thyroid nodules

Thyroid nodules are common especially among women in area of iodine deficiency. Nuclear medicine thyroid scan on nodular function is used to differentiate "cold" and "hot" nodules. During the examination, the radioactive iodine acts like non-radioactive iodine and will be absorbed by the thyroid gland to be used in the production of thyroid hormones. Functional thyroid tissue will absorb the radioactive iodine and become radioactive. A nodule is hot or hyperfunctioning if it picks up more radioactive iodine than a normal tissue. In contrast, a cold nodule is less functional and usually will pick up less or none of the radioactive iodine. "Hot" nodules are almost always benign, whereas cold nodules may harbor cancer in a small proportion (< 10 %) of patients (Belfiore *et al.*, 1992; Mackenzie & Mortimer, 2004; Sarkar & Becker,

2001). Thyroid nodules are usually presented in benign form including follicular adenoma, colloid nodules, benign cysts or nodular thyroiditis. However, it is important to determine the status of the nodules as about 5 % of them have shown to be malignant. Moreover, most thyroid cancers are of low grade malignancy and share pathologic features with benign nodules. Therefore, routine evaluation of the status of these nodules by fine needle aspiration biopsy (FNAB) and cytological examination are needed (Bonert & Friedman, 2007).

2.12 Follicular adenoma

Follicular adenoma is the most common benign tumor of the thyroid gland (Stevens *et al.*, 2011). It is characterised by a firm, homogeneous, round or oval tumor that is surrounded by a thin fibrous capsule (Bisi *et al.*, 1989; Silverberg & Vidone, 1966). It cannot be distinguished from follicular carcinoma without histological examination as both tumors share similar microscopic features. However, if compared to follicular carcinoma, follicular adenoma tends to be less cellular, has thinner capsule and lacks invasiveness (D'Avanzo *et al.*, 2004). The ratio of follicular adenoma to follicular carcinoma in surgical specimen is approximately 5:1 (Rosai *et al.*, 1991). Patients with follicular neoplasm (including follicular adenoma and follicular carcinoma) usually will be advised to undergo surgery to remove the thyroid nodule unless there is clear evidence that the nodule is hyperfunctioning (Mackenzie & Mortimer, 2004).

2.13 *Thyroid peroxidase* (*TPO*) gene

Thyroid peroxidase (*TPO*) gene is also known as *tyroperoxidase* (*TPX*), *thyroid microsomal antigen* (*MSA*) or *thyroid dyshormonogenesis 2A* (*TDH2A*), which is located on the short arm of chromosome 2, locus 2p25 (Endo *et al.*, 1995). It consists of 17 exons and 16 introns which spans about 150 kbp in genomic DNA (Kimura *et al.*, 1989; McLachlan & Rapoport, 1992). The expression of *TPO* is under the control of transcription factors such as thyroid transcription factor 1 (TTF-1), thyroid transcription factor 2 (TTF-2) and paired box gene 8 (PAX-8) (Damante & Di Lauro, 1994) .

The full-length of the human TPO transcript (TPO1) is 3152 bp in size (GenBank accession number NM_000547.5), consists of 17 exons and codes for 933 amino acids (Kimura *et al.*, 1987; Libert *et al.*, 1987; Magnusson *et al.*, 1987; Seto *et al.*, 1987). Apart from TPO1, seven different TPO transcripts which are generated through alternative splicing had also been discovered. Differential splicing generates shorter transcripts: TPO2, TPO3, TPO4 and TPO5 lacking exons 10, 16, 14 and 8 respectively (Elisei *et al.*, 1991; Ferrand *et al.*, 2003; Zanelli *et al.*, 1990). In addition, three other multi-spliced transcripts such as TPO2/3 (lacking exons 10 and 16), TPO2/4 (lacking exon 10 and 14), and TPO6 that lacks exons 10, 12 - 14 and 16 had also been described (Ferrand *et al.*, 2003). A schematic diagram of these transcripts is shown in Figure 2.2. Among these eight types of TPO transcripts, only TPO1, TPO3 and TPO4 are expected to produce enzymatically active TPO protein and can therefore be expected to play important roles in thyroid hormone synthesis. TPO2, which is lacking in exon 10, leads to a production of TPO protein which does not have any enzymatic activity and is rapidly degraded after synthesis. TPO5 lacks exon 8 which has been suggested to code for a site that participates in catalytic mechanism. The protein

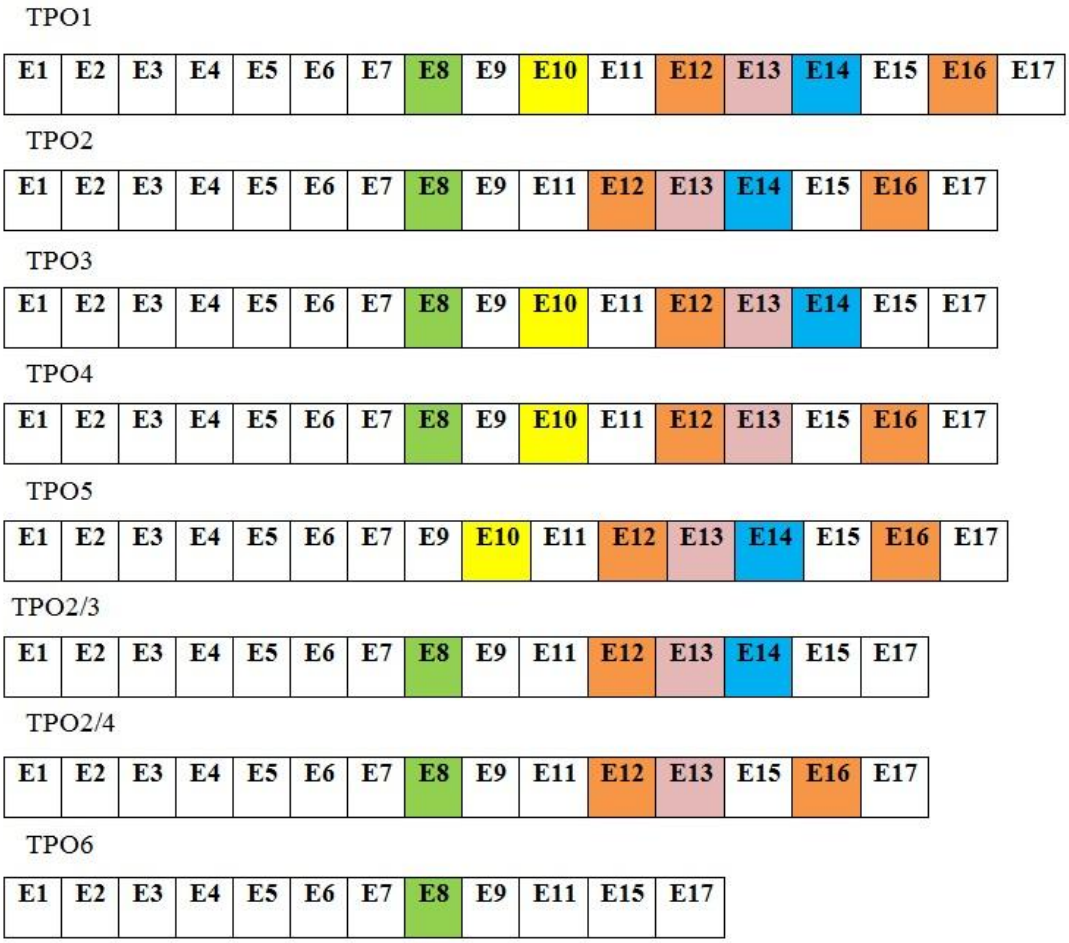

Figure 2.2 Human TPO mRNA transcripts. TPO1 is the full-length form of TPO mRNA. Variants lacking single exons are TPO2 (lacking exon 10); TPO3 (lacking exon 16); TPO4 (lacking exon 14) and TPO5 (lacking exon 8). Multi-spliced variants are TPO2/3 (exons 10 and 16 deleted); TPO2/4 (exons 10 and 14 deleted) and TPO6 (exons 10, 12, 13, 14 and 16 deleted).

translated from TPO5 is also unable to acquire a proper three dimensional configuration and reach the cell surface. In contrast, TPO4 which is lacking in exon 14 is able to fold correctly and reach the cell surface. However, this isoform has a shorter half life compared to TPO1 (Elisei *et al.*, 1991; Ferrand *et al.*, 2003; Zanelli *et al.*, 1990).

2.14 Thyroid peroxidase enzyme

TPO is a 110 kDa membrane-bound, glycosylated, heme-containing protein. 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. TPO is mainly expressed in the thyroid gland and is localised on the apical membranes of the thyrocytes (Cetani *et al.*, 1995; Chedrese, 2009; Gardas *et al.*, 1997). A study has reported that TPO is also detected in orbital tissues (Lai *et al.*, 2006). The three-dimensional (3-D) structure of this protein remains unknown although some low resolution crystals have been obtained (Gardas *et al.*, 1997; Hendry *et al.*, 1999). TPO shares 42 % sequence identity with myeloperoxidase (MPO) where it's 3-D structure is known. Therefore, the high homology of these two proteins has allowed the prediction of the secondary structure and organisation of the TPO to be made. The structure of TPO that was deduced from alignments studies and structural homologies have shown that human TPO comprises three distinct modules: MPO-like, complement control protein-like and epidermal growth factor-like domains (Figure 2.3) (Banga *et al.*, 1990; Bresson *et al.*, 2005; Hobby *et al.*, 2000; Libert *et al.*, 1987).

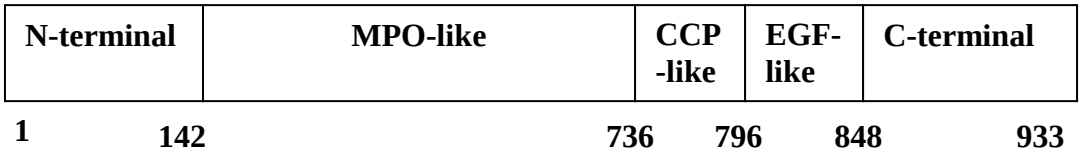

Figure 2.3 Schematic diagram of the structure of human TPO. Human TPO is formed by myeloperoxidase (MPO)-like, a complement control protein (CCP)-like, and an epidermal growth factor (EGF)-like domains, from the N- to the C-terminal extremities (Bresson *et al.*, 2005).

2.15 *TPO* gene mutations and polymorphisms

The most prevalent cause of congenital dyshormonogenetic hypothyroidism is believed to be TPO deficiency (Mangklabruks *et al.*, 1991). The TPO enzyme activity depends on two conditions: (1) proper folding and membrane insertion, (2) an intact catalytic site region (Ruf & Carayon, 2006). To date, at least 67 different types of mutation in the *TPO* gene have been described worldwide. Majority of the mutations are localised in exons 8, 9 and 10 that encode the catalytic heme-binding domain of the protein (Coleman & Tsongalis, 2009; Ris-Stalpers & Bikker, 2010). The reported mutations range included frameshift by single or multiple insertion or deletion of nucleotides, missense, alternative-splicing, nonsense mutations and total gene deletion. *TPO* defects are commonly inherited in an autosomal recessive mode (Park & Chatterjee, 2005) even though there was a reported case of a single *TPO* mutated allele in TIOD patients (Fugazzola *et al.*, 2003). The complete list of *TPO* gene mutations is shown in Figure 2.4.

Single nucleotide polymorphism (SNP) is classically defined as a normal gene variants accounting for more than 1 % of alleles in a population. To date, 43 polymorphisms (31 non-synonymous coding and 12 synonymous coding polymorphisms) in the coding region of *TPO* gene have been documented. The summary of the published *TPO* gene polymorphisms is shown in Table 2.2.

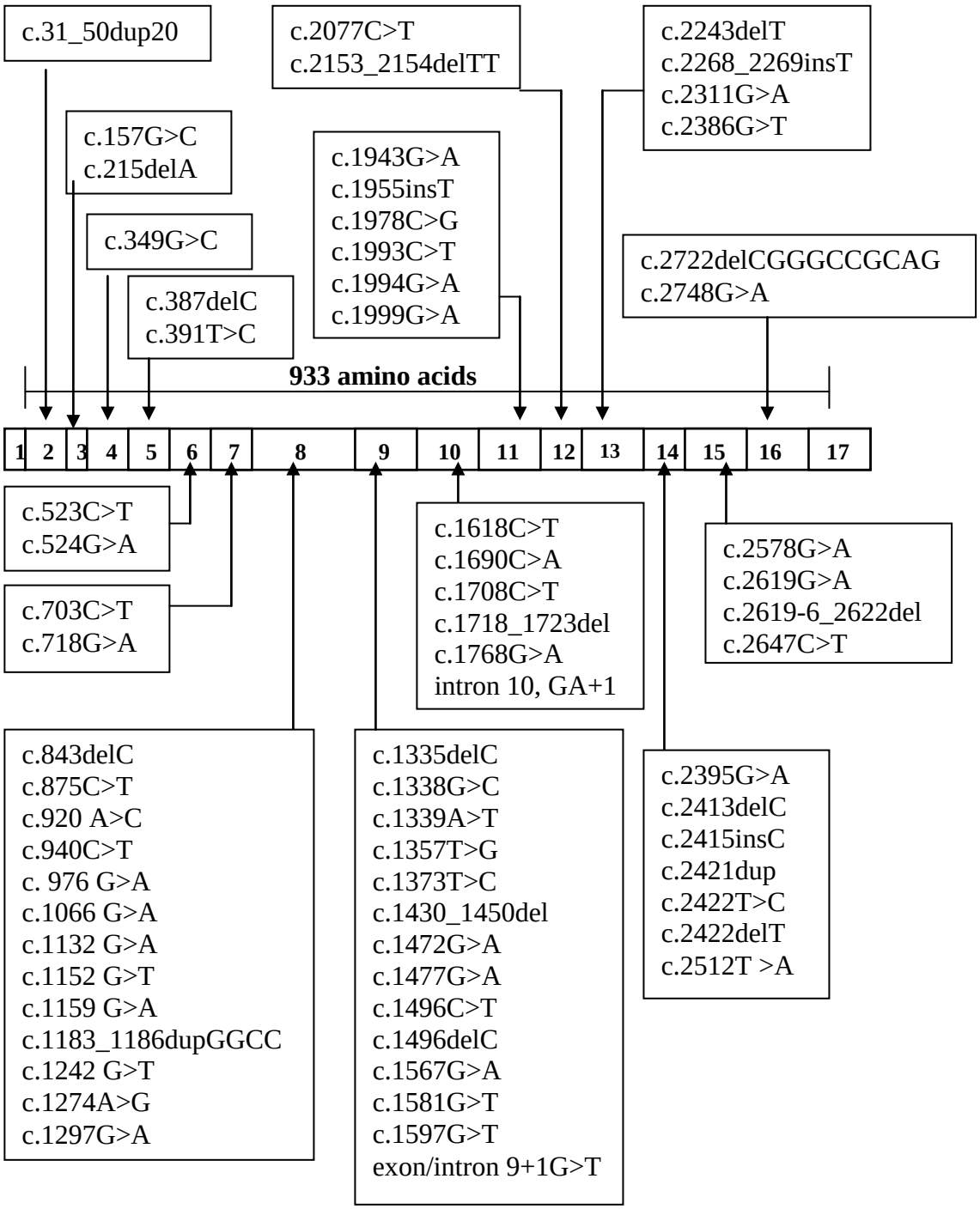

Figure 2.4 A schematic diagram showing inactivating mutations reported in the coding region of the human *TPO* gene (Modified from Kang, 2010; Coleman & Tsongalis, 2009; Ris-Stalpers & Bikker, 2010).

Table 2.2 A summary of published *TPO* polymorphisms. Refer to the Emsembl genome annotation database for complete details, gene reference code: ENSG00000115705.

Amino acid residue	Variation type	Codons	Residues
3	Synonymous coding	GCG, GCA	Ala
4	Synonymous coding	CTC, CTT	Leu
10	Synonymous coding	ACG, ACA	Thr
70	Non-synonymous coding	CCA, GCA	Pro, Ala
77	Non-synonymous coding	TCC, CCC	Ser, Pro
106	Non-synonymous coding	AAC, AAA	Asn, Lys
135	Non-synonymous coding	CCT, CAT	Pro, His
204	Synonymous coding	CCG, CCA	Pro
257	Non-synonymous coding	GCT, TCT	Ala, Ser
319	Non-synonymous coding	GGG, GTG	Gly, Val
344	Synonymous coding	ACC, ACG	Thr
373	Non-synonymous coding	GCG, GTG	Gly, Val
398	Non-synonymous coding	AGC, ACC	Ser, Thr
426	Non-synonymous coding	GCG, GGG	Ala, Gly
439	Synonymous coding	AAG, AAA	Lys
447	Non-synonymous coding	ATC, TTC	Ile, Phe
453	Non-synonymous coding	TAC, GAC	Tyr, Asp
495	Non-synonymous coding	GCC, ACC	Ala, Thr
522	Synonymous coding	GCT, GCC	Ala
529	Non-synonymous coding	TTA, CTA	Leu, Phe
529	Synonymous coding	TTA, TTC	Leu
576	Synonymous coding	GCG, GCA	Ala
590	Non-synonymous coding	GGT, AGT	Gly, Ser
605	Non-synonymous coding	ACC, TCC	Thr, Ser
606	Non-synonymous coding	CCC, TCC	Pro, Ser
618	Non-synonymous coding	GTG, ATG	Val, Met
648	Non-synonymous coding	CGG, CAG	Arg, Gln

Table 2.2 Continued.

Amino acid residue	Variation type	Codons	Residues
660	Non-synonymous coding	CAG, GAG	Gln, Glu
666	Non-synonymous coding	GAC, GAA	Asp, Glu
693	Non-synonymous coding	CGG, TGG	Arg, Trp
706	Non-synonymous coding	ATG, GTG	Met, Val
715	Synonymous coding	CCC, CCT	Pro
725	Non-synonymous coding	ACT, CCT	Thr, Pro
748	Non-synonymous coding	GTG, ATG	Val, Met
762	Synonymous coding	CGC, CGT	Arg
769	Non-synonymous coding	CGG,TGG	Arg, Trp
791	Non-synonymous coding	CCT, TCT	Pro, Ser
793	Non-synonymous coding	CTC, CCC	Leu, Pro
799	Non-synonymous coding	GAG, AAG	Glu, Lys
846	Non-synonymous coding	CGG, TGG	Arg, Trp
847	Non-synonymous coding	GTG, GCG	Val, Ala
847	Synonymous coding	GTG, GTA	Val
923	Non-synonymous coding	GGC, GAC	Gly, Asp

2.16 Expression of *TPO, Tg, TSH-R* and *NIS* in thyroid nodules

Thyroid nodules are extremely common. Only about 5 % of thyroid nodules have been shown to be malignant (Cooper *et al.*, 2009). Therefore, it is important to determine the status of the nodules. Currently, it is achieved by FNAB as an initial test for diagnosing malignancy. However, FNAB does not give a conclusive result in some cases. As a result, many expression profiling studies have been carried out with the hopes of identifying new diagnostic markers.

Lazar *et al.* (1999) reported that later stages of thyroid cancer were associated with lower TPO mRNA expression. This finding was confirmed by other studies where a lower expression of TPO was commonly seen in thyroid carcinoma cases (Di Cristofaro *et al.*, 2006; Finley *et al.*, 2004; Hawthorn *et al.*, 2004; Huang *et al.*, 2001; Pauws *et al.*, 2004; Prasad *et al.*, 2008).

Tg is a large homodimeric glycoprotein that comprises 2768 amino acids (van de Graaf *et al.*, 2001). It is responsible for the storage of the inactive form of thyroid hormones and iodine (Marino & McCluskey, 2000; Medeiros-Neto *et al.*, 1993). The levels of Tg mRNA were significantly lower in adenoma and carcinoma tissues as compared to normal tissues in 1991 (Ohta *et al.*, 1991). Contrary to that, other studies have shown that only thyroid carcinoma have lower expression of Tg (Karger *et al.*, 2006; Ringel *et al.*, 2001).

TSH-R was categorised under the superfamily of G-protein-coupled receptors that comprises 764 amino acids. It serves as a customised binding site for TSH. Binding of TSH to TSH-R stimulates thyroid epithelial cell proliferation. It also

regulates the expression of other thyroid-related genes such as *Tg, TPO* and *NIS*, which are required for the synthesis of thyroid hormones. Most of the well-differentiated carcinomas have different levels of TSH-R mRNA ranging from normal to markedly deceased. In contrast, majority of the undifferentiated anaplastic carcinomas are resulted in a loss of mRNA expression (Brabant *et al.*, 1991; Bronnegard *et al.*, 1994; Macchia, 2000; Sheils & Sweeney, 1999).

NIS is a transmembrane glycoprotein that consists of 643 amino acids. It plays an important role in regulating the level of I^- in the thyroid gland by actively transporting the I^- into thyroid epithelial cells (Levy *et al.*, 1997). The expression of NIS is largely modulated by the expression and functional activity of TSH-R (Caillou *et al.*, 1998). An increased expression of NIS was observed in papillary thyroid cancer specimens (Saito *et al.*, 1998). Contrary to Seito results, other studies have demonstrated a downregulation of NIS expression in both benign and carcinomas tumors (Lazar *et al.*, 1999; Ringel *et al.*, 2001; Sodre *et al.*, 2008). In contrast, increased expression of NIS has been demonstrated in patients with Grave's disease and in autonomously functioning thyroid nodules (Castro *et al.*, 1999; Jhiang *et al.*, 1998; Joba *et al.*, 1999; Sodre *et al.*, 2008).