REVIEW

Congenital Thyrotropin Deficiency

—From Discovery to Molecular Biology, Postgenome and Preventive Medicine—

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IT is well known that the human glycoprotein hormone family is comprised of four members: thyrotropin (TSH) and gonadotropin (Gn), which includes follicle stimulating hormone (FSH), luteinizing hormone (LH) and chorionic gonadotropin (CG) [1, 2]. As shown in Fig. 1, synthesis and secretion of TSH, FSH and LH from the pituitary gland are regulated by the hypothalamic releasing hormones, thyrotropin releasing hormone (TRH) and Gn releasing hormone (GnRH) (luteinizing hormone releasing hormone (LHRH)) as well as by the negative feedback mechanism involving the peripheral hormones, thyroid hormones and sex steroid hormones. The CG synthesized in the placenta is functionally most like LH and essential for the maintenance of pregnancy.

The glycoprotein hormones are composed of two non-covalently linked α - and β -subunits (chains) as a heterodimer. The amino acid sequence of α (common α) subunits are identical in all glycoprotein hormones, while those of β subunits are homologous to each other yet each is unique and these differences confer the specific biological activities. Complex carbohydrate side chains are attached to the α - and β -subunits. Isolation, cloning and sequence analysis of the DNAs for glycoprotein hormones and their transcription factors have been performed extensively.

Congenital glycoprotein hormone deficiency is classified into three types: isolated deficiency with lack of a single hormone, combined (multiple) deficiency (partial hypopituitarism) associated with lack of other pituitary hormones, and panhypopituitarism. On the

other hand, hypothyroidism and hypogonadism are classified into four types according to the site of impairment in the hypothalamic- pituitary- thyroidal and gonadal axis (Fig. 1). In the case of hypothyroidism, three types are known: central including hypothalamic (tertiary) and pituitary (secondary), primary (impairment of the thyroid gland itself) and peripheral (resistance to thyroid hormone).

In 1971, the first case of congenital glycoprotein hormone deficiency was reported by Miyai *et al.* [3] as a familial isolated TSH deficiency with congenital hypothyroidism (cretinism). The case was well documented by sophisticated clinical laboratory tests such as immunoassay and provocation tests. About two decades later, introduction of molecular biology techniques paved the way for the gene analyses of the patient. We performed molecular cloning of hTSH β subunit gene [4, 5] and demonstrated that a single base substitution in the CAGYC region of the TSH β -subunit was responsible for this disorder [6, 7]. Based on our subsequent studies on wild and mutant types of glycoprotein hormones with site directed mutagenesis of the CAGYC region in the β -subunits and CMGCC region in the α -subunit [6, 8–10], we hypothesized that the CXGXC motif plays an important role in the biosynthesis of glycoprotein hormones [11]. Recently, postgenome medicine has attracted special interest and we studied the three dimensional structure of the CXGXC motif of wild glycoprotein hormones as well as mutant types that simulate the isolated TSH deficiency [12]. Furthermore, in 1992, we cloned a transcription factor, human Pit-1 (*PIT1*) gene [13], and discovered the first case of combined deficiency of TSH, growth hormone (GH) and prolactin (PRL),

manifestations of nongoitrous cretinism, and 2) assuming that the disorder is inherited, familial occurrence would be expected. We therefore focused our approach on searching for nongoitrous cretinism appearing in siblings and/or offspring of a consanguineous marriage, and finally identified the first case in Japan. The *proposita* were 2 sisters with congenital TSH deficiency and their parents' marriage was consanguineous.

1.2 Clinical features and laboratory findings

Severe cases such as those we reported showed typical signs and symptoms of nongoitrous cretinism. Thyroidal radioactive iodine uptake was low but substantially increased after administration of TSH. Serum concentrations of (free) thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) were low and those of TSH were consistently undetectable, which did not increase after administration of TRH in either hypothyroid or euthyroid states. The detectable free common α -subunit in serum was increased by TRH and was decreased by thyroid hormone administration, suggesting that the common α -subunit gene is intact [18]. Plasma TRH was detectable by radioimmunoassay [19], although the technique has not yet to be established. That the TSH deficiency was isolated was confirmed by provocation tests of other pituitary hormones; that is, significant increase of serum PRL to TRH administration, of FSH and LH to GnRH, of GH to growth hormone releasing hormone (GRH) and of adrenocorticotrophic hormone (ACTH) to corticotropin releasing hormone (CRH) or insulin induced hypoglycemia were observed [3, 19]. In the first cases, unexpected abnormal increases in serum GH were observed after intravenous injection of TRH, GnRH and CRH, while serum GH level did not change after saline injection as a control experiment [19, 20]. There were no confirmatory studies on the peculiar phenomenon and the mechanism remains unknown. The parents of the first cases were phenotypically normal with normal responses to the provocation tests.

1.3 Genetic analysis of TSH β -subunit gene

In 1985 we first performed molecular cloning of hTSH β subunit gene [4, 5]. Later on we analyzed the TSH β -subunit gene in the first case of congenital isolated TSH deficiency and found a single base substitution from guanine (G) to adenine (A) at position 85 which altered *GGA* encoding glycine (Gly) (G) to

AGA encoding arginine (Arg) (R) at the 29th codon ([G29R]) [6, 7, 21]. The missense mutation was found to be homozygous in two patients in siblings and heterozygous in their parents whose marriage was consanguineous indicating that the mutation was responsible for the disease, and that the inheritance was recessive. The same mutation was demonstrated in 6 affected members in 5 Japanese families so far examined [6, 7, 21, 22]. Although mutual blood relationships among the families were not clear from the family history, all families originated from a small area in the western part of Shikoku Island in Japan and an identical polymorphism in intron 2 was associated with the mutation in 3 patients in different families. These findings suggest that the mutant gene originated from a common ancestor.

To date, different mutations have been reported for the TSH β -subunit gene in congenital isolated TSH deficiency, as shown in Table 1 [6, 7, 21–29]. The mutations were homozygous or compound heterozygous. For instance, in the case reported by Karges *et al.* [30], mutations were found at codon 49 in one allele and 105 in the other allele in which cytosine (C) to thymine (T) transition at codon 49 resulted in alteration of glutamine (Gln) (Q) to stop (termination) codon (X) and deletion of one base pair T at codon 105 resulted in alteration of cysteine (Cys) (C) to valine (Val) (V) followed by frameshift (fs) and stop codon (X) at 114. These were described as [Q49X]/[C105delT (fs 114X)]. There were some differences in their clinical features. For instance, in patients with a mutation [Q49X], the circulating TSH was found to be immunologically active (detectable by immunoassay) but biologically inactive (recombinant TSH harboring this mutation had no biological activity that could be determined by bioassay) [25].

2. Congenital TSH deficiency associated with combined pituitary hormone deficiency caused by mutation in the transcription factor gene

2.1. Discovery of the first case of *PIT1* deficiency ([f] in Fig. 1)

In 1980, in the course of searching for congenital isolated TSH deficiency, we encountered a case report describing congenital TSH deficiency and we contacted both the author and the patient. Reevaluation of the patient demonstrated that the disorder was not isolated TSH deficiency but combined deficiency of

Table 1. Reported mutations in genes related to congenital TSH deficiency. Data were obtained from figures [38, 42, 46] and original articles [6, 7, 14, 21–29, 34–52, 58]. Refer to Fig. 1 [] and abbreviations.

TRH receptor [b] • SIT 115 116 117 del ^[58] • A118T ^[58]	PROP1 (Prophet of Pit-1) [g] • S38 del 13 bp (112–114 del bp) ^[42] • G50 del GA (149 del GA) ^[40] • R53 del A (147 del A) ^[42] • R71C ^[41] • R71H ^[41] • R73C ^[42] • R73H ^[42] • Q83X ^[42] • F88S ^[42] • R99X ^[42] • R99Q ^[42] • S101 del AG (301 del AG) ^[39,42] • IVS2(-2) A>T ^[42] • F117 ^[39,42] • R120C ^[39,42] • S156 ins T (467 ins T) ^[44] • W194X ^[43]
TSH-β subunit [i] • E12X ^[23] • G29R ^[6,7,21,22] • Q49X ^[25,26] • F57 del T ^[29] (266 del T) • C85R ^[27] • C105 del T ^[24] (410 del T) • IVS2(+5) G>A ^[28]	HESX1 [c] • Q6H ^[46] • I26T ^[47] • R53C ^[45] • 306 307 ins AG ^[47] • R160C ^[46] • S170L ^[46] • N178 del G ^[48] • T181A ^[46]
<i>PIT1</i> (Human Pit-1) (GHF1) (POU1F1) [f] • Q4X ^[38] • P14L ^[38] • P24L ^[36,38] • F135C ^[38] • R143Q ^[36,38] • R143L ^[37] • R145X ^[38] • A158P ^[38] • Q167X ^[38] • R172Q ^[38] • R172X ^[14,38] • E174G ^[38] • S179R ^[38] • W193R ^[38] • L194Q ^[37] • K216E ^[38] • E230K ^[38] • F233L ^[38] • P239S ^[38] • E249 del A (747del A) ^[38] • E250X ^[38] • V260 ins A (778ins A) ^[38] • R265W ^[38] • R271W ^[34,38] • V272X ^[38] • del <i>PIT1</i> ^[35]	LHX3 [h] • Y116C ^[49] • I59 del T ^[50] • del LHX3 ^[49]
	LEX4 [d] • IVS4(-1) G>C ^[51]
	SOX3 [e] • A240 ins 21 bp ^[52]

Abbreviations.

Nucleotides, A: adenine; C: cytosine; G: guanine; T: thymine; bp: base pair of nucleotide; number: nucleotide number; IVS: intervening sequence

Amino acids, A, Ala: alanine; C, Cys: cysteine; D, Asp: aspartic acid; E, Glu: glutamic acid; F, Phe: phenylalanine; G, Gly: glycine; H, His: histidine; I, Ile: isoleucine; K, Lys: lysine; L, Leu: leucine; M, Met: methionine; N, Asn: asparagine; P, Pro: proline; Q, Gln: glutamine; R, Arg: arginine; S, Ser: serine; T, Thr: threonine; V, Val: valine; W, Trp: tryptophane; Y, Tyr: tyrosine; number: codon number;

Mutations, del, del: deletion; ins: insertion; >: transition; X: stop (termination) codon

Examples, SIT 115 116 117 del: serine, isoleucine and threonine at codon 115,116 and 117 are deleted; E12X: glutamic acid at codon 12 changes to stop codon; G29R: glycine at codon 29 changes to arginine; F57 del T (266 del T): 266th nucleotide, thymine is deleted at codon 57 (phenylalanine); IVS2(+5) G>A: guanine to adenine transition at position +5 in the 2nd intervening sequence; V260 ins A (778 ins A): adenine is inserted at codon 260 (valine) (778th nucleotide)

TSH, GH, and PRL which seemed to be a common pituitary dwarfism associated with other pituitary hormone failure. However, since the patient was one of two affected siblings and the offspring of a consanguineous marriage, we hypothesized that the disorder was caused by a mutation in an unknown gene that controls synthesis and/or secretion of the three hormones. We then took blood samples from the patient and her family with their permission. In 1988, rat Pit-1 (GHF-1) (current nomenclature: POU1F1) was cloned as a transcription factor for GH and PRL [31]. As dwarf mice (*dw* and *df*) had apparently similar hormone deficiencies of TSH and GH [32], we started to conduct studies on mouse and human Pit-1 genes as a candidate gene for the disorder. In 1990, Li *et al.* [33] reported that the *dw* mouse showed disruptions in the Pit-1 gene. In 1992, we cloned the human Pit-1 (*PIT1*) gene [13]. At that time we were unable to reestablish contact with the patient's family, but we could analyze the *PIT1* gene using the stored blood samples [14].

2.2 Genetic analysis of *PIT1*, *PROP1*, *HESX1*, *LHX3* *LHX4* and *SOX3* genes

In the first case of combined deficiencies of TSH, GH and PRL caused by a homozygous nonsense mutation in the *PIT1* gene, one base alteration, a C to A in exon 4 converted the 172nd codon for Arg to a stop codon (X) ([R172X]) [14]. To date different mutations in the *PIT1* gene have been reported as shown in Table 1 [14, 34–38].

Since then, the mutations of other transcription factor genes in cases of combined pituitary hormone deficiency such as PROP1 (Prophet of Pit 1) (TSH, GH, PRL, Gn deficiency) ([g] in Fig. 1) [39–44], HESX1 (pituitary hypoplasia and septo-optic dysplasia) ([c] in Fig. 1) [45–48], LHX3 (TSH, GH, PRL, Gn deficiency and rigid cervical spine) ([h] in Fig. 1) [49, 50], LHX4 (TSH, GH, ACTH deficiency) ([d] in Fig. 1) [51], and SOX3 (panhypopituitarism and infundibular hypoplasia) ([e] in Fig. 1) [52] have been reported as shown in Table 1. These mutations involved homozygosity, heterozygosity (dominant negative mutation/haploinsufficiency) or compound heterozygosity for the genes. The degree of deficiencies of pituitary hormones as well as clinical manifestations in these cases were varied.

3. Congenital TSH deficiency due to TRH deficiency and other etiology

In 1975, Illig *et al.* [53] reported that circulating TSH determined by immunoassay was elevated in children with hypothalamic hypopituitarism and suggested that TSH in the patients was immunologically active but biologically inactive. Subsequent studies on hypothalamic hypothyroidism demonstrated that serum TSH was detectable by immunoassay but low when determined by cytochemical bioassay with decreased receptor binding activity [54]. In 1981 Spitz *et al.* [55] reported euthyroid patients with increased serum levels of TSH with high molecular weight and low biological activity but subsequent studies have not appeared.

In 1979 Smail *et al.* [56] reported a case of congenital mild hypothyroidism with a low level of serum free T_4 and detectable immunoreactive TSH, which increased slightly after administration of TRH. Since the patient did not demonstrate any other pituitary hormone deficiency, it was suggested that the hypothyroidism was due to isolated TRH deficiency, and it was demonstrated that serum TRH was undetectable in a similar case even after administration of anti-thyroid drug [57].

In 1997, Collu *et al.* [58] reported a case of congenital central hypothyroidism with complete absence of TSH and PRL responses to TRH administration but without other pituitary hormone deficiencies. Genetic analysis of the TRH receptor gene in the patient demonstrated a compound heterozygosity for two mutations [SIT 115, 116, 117 del] / [A118T] as shown in Fig. 1 ([b]) and Table 1.

Many cases of congenital TSH deficiency with unknown etiology have been reported and further studies are required.

4. Neonatal screening for central hypothyroidism

Since growth and irreversible mental retardation due to congenital hypothyroidism can be prevented by early diagnosis and treatment, neonatal mass screening has been widely performed. Measurement of decreased T_4 in dried blood samples on filter paper taken from neonates by radioimmunoassay (RIA) [59] and non-isotopic enzyme immunoassay (EIA) [60] can detect all but peripheral types; however, this test gives false positive results for subjects with T_4 binding glob-

ulin (TBG) deficiency who do not require treatment. Then we developed RIA [61] and EIA [62] for measuring free T_4 which was not influenced by TBG deficiency but these T_4 methods risk overlooking mild cases. Since measurement of elevated TSH is useful for detecting even mild cases of primary type, which are the most common, RIA [63–66] and EIA [67–69] for measuring TSH in dried blood samples have been developed. However, the sensitivity of conventional enzyme linked immunosorbent assay (ELISA) for TSH in currently use is too low to detect decreased TSH in central type. Therefore measurements of both TSH and free T_4 (or T_4 plus TBG) have been used for screening central type [70]. Recently we developed a highly sensitive bioluminescence ELISA for TSH, which is expected to be useful for detecting both primary and central types with a single assay [15]. The incidence of the primary type has been estimated to be as high as 1:3,000 [71], whereas that of central type was estimated as low as 1:20,000 [70]. However, since case reports of central type have increased and the incidence seems to be higher in some areas such as Shikoku Island in Japan as described above, neonatal mass screening for central type of congenital hypothyroidism should be promoted in the future.

Significance of the CXGXC motif

1. CXGXC motif in patients with congenital glycoprotein hormone deficiency

As indicated above, 6 Japanese patients with congenital isolated TSH deficiency caused by a missense mutation in the TSH β -subunit gene had a Gly (G) to Arg (R) transition ([G29R]) in the CAGYC region [6, 7, 21, 22]. The amino acid sequence of this region is composed of Cys (C)-alanine (Ala) (A)-Gly (G)-tyrosine (Tyr) (Y)-Cys (C), and described as $C_1A_2G_3Y_4C_5$ in normal subjects and $C_1A_2R_3Y_4C_5$ in the patients. Recently Valdes-Socin *et al.* [72] reported a case of hypogonadism due to isolated LH deficiency caused by a missense mutation in the LH β -subunit gene which changed Gly (G) to aspartic acid (Asp) (D) ([G36D]) in the same CAGYC region described as $C_1A_2D_3Y_4C_5$ (Table 2).

Table 2. Relationship between amino acid sequence of CXGXC motif and the immunological and/or biological activities of wild and mutant types of glycoprotein hormones (boldface: active, (italic): inactive). The sequences were obtained from Database of the National Center for Biotechnology Information [73], the Table [10] and original articles [6–10,21, 22, 72, 74]. Abbreviations of amino acids: see Table 1.

	Common α -subunit					β -subunit				
Wild type										
human (h)	C	M	G	C	C	C	A	G	Y	C
other species	—	—	—	—	—	—	—	—	—	—
	—	A	—	—	—	—	—	—	F	—
	—	K	—	—	—	—	—	—	H	—
	—	S	—	—	— ⁷⁴⁾	—	—	—	L	—
	—	T	—	—	—	—	E	—	—	—
	—	V	—	—	—	—	E	—	L	—
	—	V	—	H	—	—	E	—	Q	—
						—	G	—	—	—
						—	M	—	—	—
						—	S	—	—	—
						—	S	—	H	—
						—	T	—	—	—
						—	T	—	L	—
						—	W	—	R	—
Site directed mutagenesis										
control	C	M	G	C	C	C	A	G	Y	C
mutagenesis	(Y)	—	—	—	— ^{9,10)}	—	D	—	—	— ⁸⁾
(hTSH and/or hCG)	—	R	—	—	— ¹⁰⁾	—	—	(D)	—	— ⁸⁾
	—	—	(A)	—	— ^{9,10)}	—	—	(R)	—	— ^{6,8)}
	—	—	(D)	—	— ^{9,10)}					
	—	—	(R)	—	— ^{9,10)}					
Diseases										
Isolated LH deficiency ⁷²⁾						—	—	(D)	—	—
Isolated TSH deficiency ^{6,7,21,22)}						—	—	(R)	—	—

2. CXGXC motif in wild type glycoprotein hormones

As shown in Table 2, the same $C_1A_2G_3Y_4C_5$ motif is found in β -subunits of all glycoprotein hormones in humans as well as in certain species. The amino acids, Ala, glutamic acid (Glu) (E), Gly, methionine (Met) (M), serine (Ser) (S), threonine (Thr) (T), tryptophan (Trp) (W) at site X_2 and phenylalanine (Phe) (F), histidine (His) (H), leucine (Leu) (L), Gln, Arg, Tyr at X_4 have been identified and examined to date [10, 73]. Therefore at least the $C_1X_2G_3X_4C_5$ motif is highly conserved among species, where amino acids at X are variable.

On the other hand, a similar amino acid sequence Cys (C)-Met(M)-Gly (G)-Cys (C)-Cys (C) ($C_1M_2G_3C_4C_5$) is found in the human common α subunit. Again, the motif is highly conserved among species and described as $C_1X_2G_3X_4C_5$, where the amino acids, Ala, lysine (Lys) (K), Met, Ser, Thr, Val at

site X_2 and Cys, His at site X_4 have been identified and examined to dates [10, 73, 74].

3. Site directed mutagenesis in the CMGCC and CAGYC regions of the α and β -subunits of glycoprotein hormones

We studied the effect of site directed mutagenesis in the $C_1M_2G_3C_4C_5$ [9, 10] and $C_1A_2G_3Y_4C_5$ [6, 8] regions of the human TSH and/or CG in terms of the immunological and/or biological activities. As shown in Table 2, there were no detectable hormone activities in mutant TSH and CG in which the middle Gly (G_3) in the $C_1M_2G_3C_4C_5$ region of the α -subunit were altered to Ala, Asp and Arg ($C_1M_2A_3C_4C_5$, $C_1M_2D_3C_4C_5$ and $C_1M_2R_3C_4C_5$), and Cys at site C_1 was altered to Tyr ($Y_1M_2G_3C_4C_5$). Similarly, the mutant TSH and/or CG lost hormone activity when Gly (G_3) in the $C_1A_2G_3Y_4C_5$ region of the β -subunit was altered to

Asp (D) and Arg (R) as patients with isolated LH deficiency ($C_1A_2D_3Y_4C_5$) and TSH deficiency ($C_1A_2R_3Y_4C_5$), respectively. However, hormone activity was retained when X_2 was changed to Arg as $C_1R_2G_3C_4C_5$ in the TSH α -subunit and to Asp as $C_1D_2G_3Y_4C_5$ in the CG β -subunit.

Based on findings obtained from the wild type and mutant type glycoprotein hormones in patients as well as site directed mutagenesis experiments, we hypothesized that the CXGXC motif in both α and β -subunits play important roles in the biosynthesis of glycoprotein hormones [11, 12].

4. Three dimensional structure of the CXGXC motif

An early study on the secondary structure of the $C_1M_2G_3C_4C_5$ and $C_1A_2G_3Y_4C_5$ regions by the Chou-Fasman prediction suggested that these regions have type II turns in which Gly (G_3) is a candidate for the structure [11]. In 1994, however, Laphorn *et al.* [75] and Wu *et al.* [76] reported the three dimensional crystal structure of hCG indicating that the regions do not have turn structures but β -strands. The study demonstrated that a common disulfide bridge (bond) (C_s) and a unique disulfide bridge (cystine knot (C_k)) are located in the regions ($C_{k1}M_2G_3C_{s4}C_{k5}$ and $C_{k1}A_2G_3Y_4C_{k5}$) suggesting that two disulfide bridges at both ends (C_{k1} and C_{k5}) are essential in the regions.

To assess the importance of Gly (G_3) in the regions, we analyzed the three dimensional structure of the regions by the conformational energy calculation using a computational procedure [12]. We performed the main chain conformational calculation for our models of five residues in which the positions of Cys residues at both ends (C_{k1} and C_{k5}) were fixed in space, and bond lengths and bond angles of the peptides were maintained at standard values by the loop closure algorithm. Calculating all the combinations of 8 dihedral angles (Φ and Ψ) corresponds to exploring all the main chain conformations and we selected only the conformations where the peptide chain atoms do not collide with surrounding atoms.

Fig. 2 (a) illustrates the stick model of the peptide chain of the $C_1M_2G_3C_4C_5$ motif in the α -chain showing a stable structure. Ramachandran plot analysis which shows the allowed area of the dihedral angles (Φ and Ψ) also demonstrated that only Gly was allowed at site X_3 (G_3). In the stick model of the $C_1A_2G_3Y_4C_5$ motif in the β -chain, a small diversity was

(a) CMGCC (α -chain)

(b) CAGYC (β -chain)

Fig. 2. Stick models of the peptide chains of CMGCC motif in the α -chain (a) and the CAGYC motif in the β -chain (b) for the crystal structure. The stick models in black and white are computer generated averaged models and correspond to the conformations with the middle Gly having positive and negative Φ values, respectively.

demonstrated as shown in Fig. 2 (b). Ramachandran plot analysis showed that a part of the areas for X_3 in the $C_1A_2X_3$ (G_3) Y_4C_5 motif was located in the allowed areas for amino acids other than Gly. Then we generated a C_β atom at X_3 in the $C_1A_2G_3Y_4C_5$ motif. As shown in Fig. 3, this atom is in a position to collide with two Cys residues in the β -chain (β Cys-9 and β Cys-57) which are important for cystine knot formation so that residues other than Gly and Ala would not be accommodated considering the C_β direction. Furthermore a mutant of $C_1A_2A_3Y_4C_5$ (Ala in-

Fig. 3. A view of structures and relationships of G_3 in the $C_1A_2G_3Y_4C_5$ region of the β -chain (β -Gly 36) to Cys 9 (β -Cys 9) and Cys 57 (β -Cys 57) which forms a cystine knot in the β -chain, and to Ser 34 of the α -chain (α -Ser 34) which forms hydrogen bonds with β -Gly 36 (---). A large gray circle shows C_β of Ala 36 (A_3) (a mutation of the $C_1A_2X_3(A_3)Y_4C_5$ motif) which is close to the cystine knot and interferes with the hydrogen bond formation.

stead of Gly at site X_3) could not form a heterodimer with the α -chain because an inter molecular hydrogen bond between Gly36 in the $C_1A_2G_3Y_4C_5$ region of the β -chain and Ser34 of the α -chain could not be bridged. These findings indicate that the Gly residue at site X_3 (G_3) in the $C_1X_2X_3(G_3)X_4C_5$ motif in the α - and β -chains is essential for heterodimer formation of glycoprotein hormones.

Hypothetical disorders related to glycoprotein hormones

Based on the previous studies, the existence of hypothetical disorders related to glycoprotein hormones is suggested as shown in Fig. 1({}). Some of these disorders along with strategies and trials for their discovery are described below.

1. "Congenital isolated TSH deficiency due to mutation in the TRH gene" ({a} in Fig. 1)

The patients may have clinical features similar to congenital central hypothyroidism due to mutation in the TRH receptor gene [58] except that serum TSH

and PRL may show increased responses after administration of TRH.

2. "Total glycoprotein hormone deficiency due to the mutation in common α -subunit gene" ({j} in Fig. 1)

As shown in the animal model (alpha SU null mice), the common α -subunit deficiency may induce combined TSH, LH, FSH and CG deficiency presenting congenital secondary hypothyroidism and infertility in humans. We have been looking for such a disorder but have not yet discovered any cases. However, chromosome assignment of the gene encoding the human α -subunit showed that the gene was located on chromosome 6q [77]. Therefore, we attempted to assess pituitary thyroid functions in 5 patients with abnormalities of chromosome 6, but these functions were within normal limits on examinations performed thus far [Miyai K, Horigome H, Kameda N, Shiraishi H and Yoshimoto M *et al.*, unpublished data].

3. "Congenital glycoprotein hormone deficiency due to impairment of glycosylation" ({k} in Fig. 1)

Since glycosylation is known to be essential for synthesis of glycoprotein hormones, its impairment may induce loss of biological and/or immunological activities of the hormones. However, Macchia *et al.* [78] reported that serum total T_4 , free T_4 index and T_3 were below normal, and that TSH was in the midnormal range in patients with congenital disorders of glycosylation. A recent report by Ferrari *et al.* [79] also demonstrated that thyroid functions in the disorder were normal although TSH showed severe impairment of lectin binding.

4. "Isolated CG deficiency due to the mutation in the CG β -subunit gene" (Fig. 1)

To date, congenital isolated deficiencies of all glycoprotein hormones except CG (TSH, LH and FSH) due to mutation in the β -subunit gene have been reported. Hypothetical patients with congenital CG deficiency may become pregnant but are not able to maintain the pregnancy (infertility). However, the patients may be diagnosed as having sterility because the pregnancy would not be detectable by common pregnant tests using the determination of CG. Recently we developed an ultra sensitive ELISA for CG which can detect a

very low concentration of basal CG in the blood of non pregnant women. As a preliminary experiment, we determined circulating CG in 3 women with sterility of unknown etiology, but the basal CG level was detected and this level increased after administration of GnRH, indicating that these women did not have CG deficiency [Miyai K *et al.*, unpublished data].

5. "CXGXC motif disorders"

As described above, the $C_1X_2G_3X_4C_5$ motif is present in all human glycoprotein hormones ($C_1M_2G_3C_4C_5$ in α -subunits and $C_1A_2G_3Y_4C_5$ in β -subunits) where the Cys residues at both ends (C_1 and C_5) and the middle Gly (G_3) are essential for exhibiting their hormone activities. A novel glycoprotein hormone, thyrostimulin, which is similar to TSH, also has the $C_1X_2G_3X_4C_5$ motif as $C_1V_2G_3H_4C_5$ in α_2 -(GPA2) and $C_1W_2G_3R_4C_5$ in β_5 -(GPB5) subunits [80, 81].

To date, two cases of congenital glycoprotein hormone deficiency caused by mutations in the $C_1X_2G_3X_4C_5$ motif in β -subunits have been reported; these were TSH ($C_1A_2R_3Y_4C_5$) and LH ($C_1A_2D_3Y_4C_5$) deficiencies as described above. It seems reasonable to assume that replacements of the G_3 , C_1 and C_5 residues with any other residue would result in isolated deficiencies of TSH and LH as well as FSH and CG. As for the $C_1M_2G_3C_4C_5$ motif in the α -subunits, alteration of G_3 , C_1 and C_5 to any other residue may induce combined TSH, LH, FSH and CG deficiency, although such deficiencies have not yet been reported.

Furthermore, a number of similar CXGXC motifs have been found in various human proteins such as transforming growth factor- β (CAGAC), phosphoinositide 3-kinase- β (CAGCC), inhibin β A chain

(CEGEC) *etc.* [12]. The motif ($C_{k1}G_2G_3C_{s4}C_{k5}$) in endothelial growth factor [82] is similar to that ($C_{k1}M_2G_3C_{s4}C_{k5}$) in α -subunits of glycoprotein hormones. It would have been interesting to assume that any mutations in the motifs in these proteins could result in a loss of biological activities and induce unknown disorders.

Finally I would like to conclude this review with some of my favorite words.

"The discovery of a new disease is possible not only by accidental but also by creative powers that render these inevitable."

"Measure what is measurable and make measurable what is not so." (Galileo Galilei)

"An ounce of prevention has always been better than a pound of cure." (E. B. Michel)

"Nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature, by the careful investigation of cases of rarer forms of disease." (William Harvey)

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