Growth Hormone Deficiency and Pit-1 Transcription Factor

**Hormones** are the chemicals that carry messages from one cell to another via the blood stream.

**What is growth hormone deficiency?**

Growth hormone deficiency, hypopituitary dwarfism, or hypopituitarism, is the absence or deficiency of growth hormone produced by the pituitary gland to stimulate the body to grow. **Growth hormone** deficiency may occur during infancy or later in childhood. **GHD** occurs three or four times more often among boys than girls.

**Growth hormone**, also known as somatropin, acts directly and indirectly on the growth of bones, tissues and organs. The elderly naturally produce less growth hormones than young adults. **Growth hormone** is usually secreted in response to sleep, exercise, and hypoglycemia and promotes growth and metabolic functions.

A deficiency of **growth hormone** may occur alone or it may be associated with other hormone deficiencies. Frequently, **sex hormones**, thyroid stimulating hormone (TSH), and the hormone which stimulates the adrenal gland (ACTH), are affected.

**What causes growth hormone deficiency?**

Growth hormones are produced by the pituitary gland, which is attached to the hypothalamus (a part of the brain that affects the pituitary gland) located at the base of the brain.

When the pituitary gland or the hypothalamus are malformed or damaged, growth hormone deficiency may result. Damage to the pituitary gland or hypothalamus may occur as a result of abnormal formation of these organs before a child is born (congenital, or present at birth), or as a result of damage that occurred during or after birth (acquired).

Researchers have found that growth hormone deficiency may also be part of a genetic syndrome. However, in some cases, the cause of growth hormone deficiency is unknown (idiopathic).
The Pit-1 Transcription Factor

If a particular transcription factor is synthesized in only one type of cell, it can activate only the target genes in that cell type, and the proteins encoded will be synthesized only in those cells. For example, the transcription factor Pit-1 is synthesized only in the pituitary gland and regulates pituitary-specific expression of the genes encoding proteins such as growth hormone and prolactin (Figure 1). PIT1 is a pituitary-specific transcription factor responsible for pituitary development and hormone expression in mammals and is a member of the POU family of transcription factors that regulate mammalian development. The POU family is so named because the first 3 members identified were PIT1 and OCT1 of mammals, and Unc-86 of C. elegans. PIT1 contains 2 protein domains, termed POU-specific and POU-homeo, which are both necessary for high affinity DNA binding on genes encoding growth hormone. PIT1 is also important for regulation of the genes encoding prolactin and thyroid-stimulating hormone beta subunit by thyrotropin-releasing hormone and cyclic AMP.

Mutations of specific transcription factors can produce developmental abnormalities. One of the best characterized of such mutations involves the Pit-1 factor, which plays a critical part in regulating gene expression in the pituitary. Mutations in the gene encoding Pit-1 have been identified in patients with combined pituitary hormone deficiency, in which there is no production of growth hormone, prolactin, and thyrotropin, resulting in mental retardation and growth deficiency.

The mutant Pit-1 in patients with this disease can still bind to its DNA-binding site in target genes, but unlike the normal transcription factor, it does not activate transcription after binding (Figure 1). The mutant protein not only fails to stimulate gene expression after binding but can also inhibit gene activation by preventing the normal protein from binding to DNA (Figure 1). These molecular findings explain the dominant nature of the disease, which can be caused by a single mutant gene even when a wild-type gene encoding functional Pit-1 is present.
The Pit-1 transcription factor plays a critical role in cell differentiation during organogenesis of the anterior pituitary in mammals and is a transcriptional activator for pituitary gene transcription. Increased expression of Pit-1 has been reported in human tumorigenic breast cells. Here, we found that Pit-1 overexpression or knockdown in human breast cancer cell lines induced profound phenotypic changes in the expression of proteins involved in cell proliferation, apoptosis, and invasion.

Several studies have suggested that Pit-1 is directly involved in pituitary cell proliferation, apoptosis, and possibly in the pathogenesis of pituitary adenomas. However, other studies have not found any relation between Pit-1 expression and pituitary tumorigenesis. Given that the original studies describing Pit-1 expression and function were restricted to the pituitary gland and pituitary cell lines, the function of Pit-1 in extrapituitary tissues had not been well evaluated previously. In the mammary gland, Pit-1 also regulates GH and PRL, increases cell proliferation, and has higher expression in breast tumors than in normal mammary gland, suggesting that Pit-1 could be involved in mammary tumorigenesis. In present studies, demonstrate that Pit-1 was also involved in extrapituitary regulation of cell death.
Figure 7-3. Transcription factors and gene regulatory proteins. (A) The three-dimensional structure of the Pit-1 homeodomain protein is shown. The 60 amino acid homeodomain of Pit-1 protein is coded for by the PIT1 gene containing a 180 base pair sequence called the homeobox sequence. Pit-1 binding at the transcription-initiation (TI) complex is required for transcription of the genes for growth hormone (GH), thyroid-stimulating hormone (TSH), and prolactin (PRL). A mutation in the gene for Pit-1 will result in the combined deficiency of GH, TSH, and PRL, causing pituitary dwarfism. (B) The three-dimensional structure of a specific zinc finger protein (i.e., the glucocorticoid receptor that acts as a gene regulatory protein). The glucocorticoid receptor has a DNA-binding region and a hormone-binding region. In the presence of glucocorticoid hormone, the glucocorticoid receptor will bind to a gene regulatory sequence known as the glucocorticoid response element (GRE), which loops in order to interact with the TI complex and allows the start of gene transcription. (C) The three-dimensional structure of a leucine zipper protein (Jun) forming a leucine zipper homodimer (Jun–Jun). L = leucine. (D) The three-dimensional structure of a helix-loop-helix (HLH) protein forming an HLH homodimer.
Cystic fibrosis (CF) is a common disease which affects the entire body, causing progressive disability and often early death. The name cystic fibrosis refers to the characteristic scarring (fibrosis) and cyst formation within the pancreas.

The symptoms of cystic fibrosis are:-

- Difficulty breathing is the most serious symptom and results from frequent lung infections.
- sinus infections.
- diarrhea
- poor growth and poor weight gain despite a normal food intake.
- accumulation of thick, sticky mucus, frequent chest infections and coughing or shortness of breath.
- Males can be infertile due to congenital absence of the vas deferens.
- Symptoms often appear in infancy and childhood, such as bowel obstruction.

Causes:-

- CF is caused by a mutation in the gene cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation, ΔF508, is a deletion (Δ) of three nucleotides that results in a loss of the amino acid phenylalanine (F) at the 508th (508) position on the protein.
- Although most people have two working copies (alleles) of the CFTR gene, only one is needed to prevent cystic fibrosis. CF develops when neither allele can produce a functional CFTR protein. Thus, CF is considered an autosomal recessive disease.
- The CFTR gene, found at the q31.2 locus of chromosome 7, is 230,000 base pairs long, and creates a protein that is 1,480 amino acids long.
- Structurally, CFTR is a type of gene known as an **ABC gene**. The product of this gene (the CFTR) is a **halide anion** channel important in creating **sweat**, **digestive** juices and **mucus**. This protein possesses two **ATP-hydrolyzing domains** which allows the protein to use **energy** in the form of **ATP**.

- It also contains two domains comprising 6 **alpha helices** apiece, which allow the protein to cross the cell membrane. A regulatory **binding site** on the protein allows activation by **phosphorylation**, mainly by **cAMP-dependent protein kinase**. The **carboxyl terminal** of the protein is anchored to the **cytoskeleton** by a **PDZ** domain interaction.

**Pathophysiology**

- There are several mechanisms by which mutations cause problems with the CFTR protein. ΔF508, for instance, creates a protein that does not **fold** normally and is degraded by the cell.

- Several different mutations result in proteins that are too short because **production** is ended prematurely.

- Less common mutations produce proteins that do not use energy normally, do not allow **chloride**, **iodide** and **thiocyanate** to cross the membrane appropriately, or are degraded at a faster rate than normal.

- The protein created by this gene is anchored to the **outer membrane** of **cells** in the **sweat glands**, lungs, pancreas, and other affected **organs**. The protein spans this membrane and acts as a **channel** connecting the inner part of the cell (**cytoplasm**) to the **surrounding fluid**. This channel is primarily responsible for controlling the movement of halogens from inside to outside of the cell; however, in the sweat ducts it facilitates the movement of chloride from the sweat into the cytoplasm. When the CFTR protein does not work, chloride and thiocyanate are trapped inside the cells in the airway and outside in the skin. Then **hypothiocyanite**, OSCN, cannot be produced by immune defense system. Because chloride is **negatively charged**, this creates a difference in the electrical potential inside and outside the cell causing **cations** to cross into the cell. Sodium is the most common cation in the extracellular space and the combination of
sodium and chloride creates the salt, which is lost in high amounts in the sweat of individuals with CF.

- How this malfunction of cells in cystic fibrosis causes the clinical manifestations is not well understood.

- One theory suggests that the lack of halogen and pseudohalogen (mainly, chloride, iodide and thiocyanate) exodus through the CFTR protein leads to the accumulation of more viscous, nutrient-rich mucus in the lungs that allows bacteria to hide from the body's immune system.

- Another theory proposes that the CFTR protein failure leads to a paradoxical increase in sodium and chloride uptake, which, by leading to increased water reabsorption, creates dehydrated and thick mucus.

- Yet another theory focuses on abnormal chloride movement out of the cell, which also leads to dehydration of mucus, pancreatic secretions, biliary secretions, etc.

- These theories all support the observation that the majority of the damage in CF is due to blockage of the narrow passages of affected organs with thickened secretions. These blockages lead to remodeling and infection in the lung, damage by accumulated digestive enzymes in the pancreas, blockage of the intestines by thick faeces, etc.
Summary:

1. Cystic fibrosis is the most common serious single-gene inherited disease in the Western world. Many organs are affected. Sticky mucus builds up in the reproductive tract and in the lungs. The pancreas is always affected and usually fails completely.
2. Electrical measurements show that the basic problem is in chloride transport.
3. CF is a classic recessive disorder. The gene is on chromosome 7.
4. A combination of hard work and novel techniques helped isolate a small part of the CFTR gene. This sequence helped to isolate the normal CFTR cDNA from a sweat gland clone library.
5. From the cDNA it was possible to identify the gene and infer its amino acid sequence in both normal and mutated forms. The gene codes for a chloride channel protein called CFTR. Hundreds of different CFTR mutations have now been found.
6. For the time being gene therapy only holds out hope of alleviating the lung problems, leaving other less accessible organs untreated. Tests have been devised for prenatal diagnosis and to detect carriers of CF.

**nuclear lamina**

The nuclear envelope is composed of the nuclear lamina, the nuclear pore complexes, and the nuclear membranes. The nuclear lamina is a proteinaceous layer apposed to the inner nuclear membrane. It is composed of a family of polypeptides, the lamins, highly conserved in evolution.

Mutations in lamin A and lamin C —nuclear intermediate filament proteins that are expressed in nearly all somatic cells —cause tissue-specific diseases that affect striated muscle, adipose tissue and peripheral nerve or skeletal development.

Mutations in the nuclear envelope proteins emerin and lamin A cause a number of diseases including premature aging syndromes, muscular dystrophy, and cardiomyopathy. Emerin and lamin A are implicated in regulating muscle- and heart-specific gene expression and nuclear architecture. For example, lamin A regulates the expression and localization of gap junction and intercalated disc components. Additionally, emerin and lamin A are also required to maintain nuclear envelope integrity. Demonstrating the importance of maintaining nuclear integrity in heart disease, atrioventricular node cells lacking lamin A exhibit increased nuclear deformation and apoptosis.

Mutations in *LMNA*, the gene encoding A type lamins, cause numerous human diseases, including the segmental premature aging disease Hutchinson–Gilford progeria syndrome (HGPS). HGPS is an accelerated aging disease that is lethal, on average, by the age of 13 due to heart disease in 90% of the cases. The hypothesis for the cause of HGPS was sporadic autosomal dominant inheritance.
The figure to the left is the summary map used to identify the candidate region on chromosome 1 where the gene mutation responsible for the HGPS phenotype is present. The arrows indicate microsatellite markers and the horizontal bars show the BAC probes used for FISH. The region considered was a total of 4.82 Mb in length and represented approximately 80 genes.

**Fig. 2** Point mutations in exon 11 of *LMNA* gene
a) Sequences from one healthy individual and two patients with HGPS.  
b) Schematic of hypothesis for the activation of a cryptic splice site.  
c) RT-PCR products indicating an abnormal splice product of 489 bp. Evidence for activation of cryptic splice site.  
d) Western blot using antibody against lamin A/C. Lanes 1, 5, 8, 9 are from patients carrying the amino acid missense mutation GGC>GGT. Lane 4 is from a patient with amino acid missense mutation GGC>AGC. Lanes 2, 3 are from healthy parents with affected offspring. Lane 6 is from a father with an affected child and lane 7 is from a mother of an affected child. Lane 10 is a protein sample from HeLa cells.
Schematic representation of the LMNA gene encoding lamin A and lamin C proteins, showing position of disease-causing mutations.

Disease-causing mutations: * DCM  △ LGMD-1B  ○ FPLD  □ AD-EDMD  ● CMT2

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Figure 12.3. (A) Pedigree of Duchenne type muscular dystrophy, an example of X-linked recessive inheritance. Males may be homozygous for the DMD gene (X) because the Y chromosome has no corresponding DMD gene. Heterozygous recessive (X) males are affected by Duchenne type muscular dystrophy, but heterozygous (X) females are not. (B) Location of the DMD gene on chromosome X (Xp21). The DMD gene consists of exons that are separated by numerous introns. The DMD gene codes for a 4000-amino acid protein called dystrophin. Dystrophin anchors actin filaments to the extracellular matrix through a transmembrane protein that consists of α-dystroglycan and β-dystroglycan. RNA = ribonucleic acid.