行政院國家科學委員會補助專題研究計畫 □成果報告 x 期中進度報告

生長激素療效之藥理遺傳學研究

計畫類別：x 個別型計畫　□ 整合型計畫
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計畫參與人員：

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執行單位：中國醫藥大學

中　華　民　國　92　年　9　月　15　日

壹、中文摘要

關鍵字: 生長激素、單核酸多形性、藥物遺傳學

生長是人體健康的一項指標，身高檢查也是兒科門診必要的常規檢查。影響身高的因素很多，環境與基因皆扮演重要的角色。在眾多會影響生長的荷爾蒙中，生長激素是具關鍵的一環。當生長激素缺乏時會導致身材矮小；生長激素缺乏症發生率為萬分之一左右。我們治療117名因生長激素缺乏症的病人，以相同的劑量治療下，反應在療效的生長卻有相當的差異。初步發現這種成長差異與生長激素受體基因上的一個SNP有關聯，與出生體重、父母身高、治療時骨齡以及腦下垂體大小等因素無關。

利用SNP為工具可應用於很多的多因素遺傳疾病，也可有效的鑑別出是否某些基因涉及生長激素的作用而影響到治療的成效，由吾人初步的結果顯示，生長激素受體基因可能與治療的成效有關，因此計劃下一步將分析各種的SNPs在各相關基因的差異與療效的關聯。同時也將分析不同基因形的淋巴球細胞中stat-5a與stat 5-b磷酸化的程度及與生長激素治療的反應，來釐清該基因與生長激素療效間的運作機轉。

貳、英文摘要

Keywords: Growth hormone, single nucleotide polymorphisms, pharmacogenetics

Growth is an important index of physical and mental health. Assessment of growth in stature is an essential part of the pediatric examination. There are many causes of poor childhood growth and short adult height. Both environmental and genetic factors are thought to be involved in the disorders of growth. While multiple hormones influence somatic growth, the major regulator of postnatal growth and metabolic functions is growth hormone (GH) secreted from the anterior pituitary. Its absence is associated with dwarfism, while excessive secretion leads to giantism or acromegaly. The incidence of GH deficiency is estimated to be approach 1:4,000 in the Scottish population and possibly 1:10,000 worldwide, greater than any other disorders of growth (Baxteret al., 1994).

In many endocrine disorders, polymorphisms of relevant human gene have been reported to be associated with polygenic disease. Recently, single nucleotide polymorphisms (SNPs) were used as a tool for mapping the complex disease genes. It could be clinically useful for identifying if the genes involved in the cascade of growth hormone stimulation may influence the efficacy of GH replacement therapy. Our preliminary data indicated one SNP in the GHR gene showed significance in gain of height after growth hormone treatment. In this grant proposal, we plan to analyze more SNPs in the genes that involved in the cascade of growth hormone stimulation. We also proposed to study *in vitro* the difference in the level of phosphorylated Stat-5a and Stat-5b in the established lymphoblastoid cell lines with different genotypes**.**

參、前言

Background and Significance

Growth is an important index of physical and mental health. Assessment of growth in stature is an essential part of the pediatric examination. There are many causes of poor childhood growth and short adult height. Both environmental and genetic factors are thought to be involved in the disorders of growth. While multiple hormones influence somatic growth, the major regulator of postnatal growth and metabolic functions is growth hormone (GH) secreted from the anterior pituitary. Its absence is associated with dwarfism, while excessive secretion leads to giantism or acromegaly. The incidence of GH deficiency is estimated to be approach 1:4,000 in the Scottish population and possibly 1:10,000 worldwide, greater than any other disorders of growth (Baxteret al., 1994).

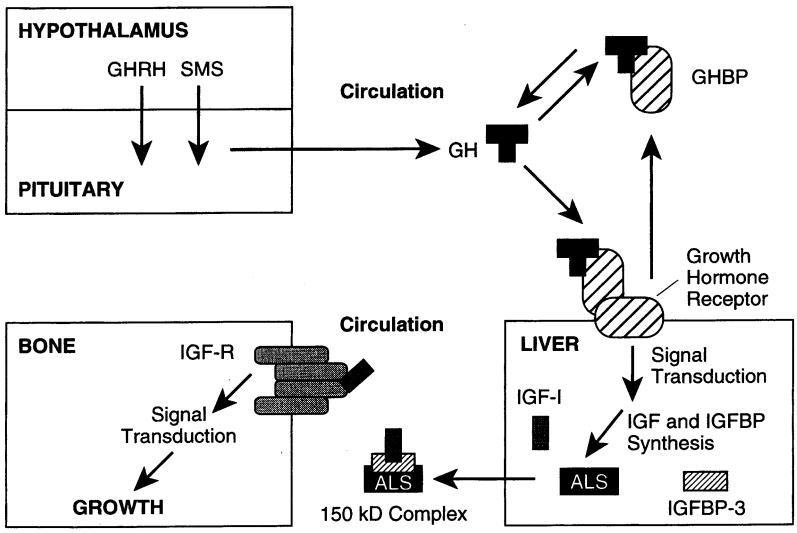

Fig. 1. Diagram showing regulation of growth.

In many endocrine disorders, polymorphisms of relevant human gene have been reported to be associated with polygenic disease. The metabolic effects of GH are to increase bone turnover, lipolysis and protein synthesis by stimulating the uptake of amino acids by muscle and liver cells and turning to fat in order to spare amino acids for protein synthesis (Wuster et al., 1998). GH secretion is mainly under hypothalamic control. The hypothalamic hormones, growth hormone releasing hormone (GHRH) and somatostatin, stimulate and inhibit GH secretion from the anterior pituitary, respectively (Press, 1998). The molecular actions of growth hormone have be elucidated by Ojeda et al. (1996) and shown in **Fig. 1.**. In plasma the majority of GH is bound to a carrier protein termed GH-binding protein (GHBP). GH then binds to its receptor, which is widely expressed in many tissues including liver, heart, kidney, intestine, skeletal muscle, pancreases, brain and testis, and mediates a cascade of cellular events (Rosenbloom et al., 2000). The signal transduction cascade following binding of GH to GHR was shown in **Fig. 2**. Binding of GH to its receptor induces the dimerization of the GH receptor and phosphorylation of JAK2 kinase. These molecules serve in signal transduction by phosphorylating signal transducers and activators of

transcription factors (STAT 5a, 5b) (Silva et al., 1996). STATs then induce the activation of insulin-like growth factors (IGF-I and IGF-II) gene transcription and modulation of other gene expression (Silva et al., 1994; Herrington et al., 2000). IGFs are found in plasma bound to a family of proteins called IGF-binding proteins (IGFBPs). The majority of IGFs are bound to IGFBP-3, together with a third protein known as the acid labile subunit (ALS), form a ternary complex in serum (Leong et al., 1992). The IGF-I is thought to interact with target organs, such as growing cartilage and muscle to induce growth. The IGF-I also has a feedback effect on the pituitary to inhibit GH secretion. Disorders of growth may be a result of a direct abnormality of the axis involving growth hormone (GH), GH binding protein, GH receptor, JAK2, STAT5a, 5b, IGF-1, IGFBP, or ALS.

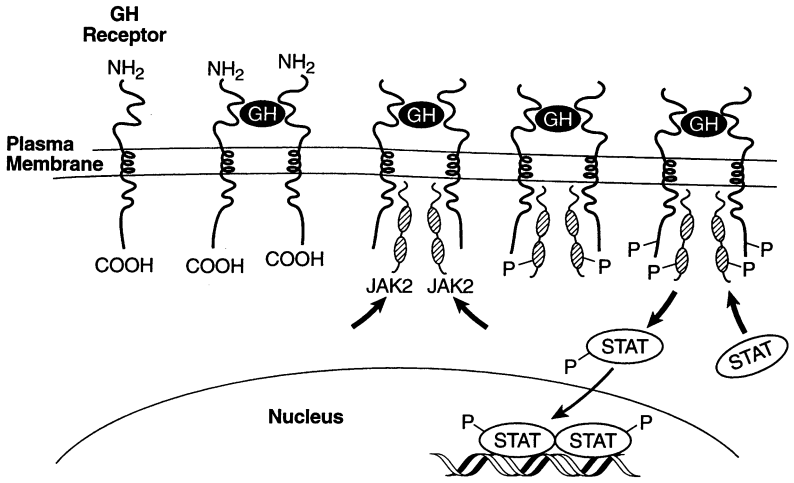

Fig.2. Signal transduction mechanisms associated with binding of growth hormone to its receptors.

GH-deficient children require recombinant GH treatment as a subcutaneous injection at a dose ranging from 0.18 to 0.3 mg/kg/wk. The increase in growth rate is most marked during the first year of therapy. The therapeutic effect can be improved by treated earlier, greater dosage and more frequently injected with recombinant GH (Vander Ley et al., 1998). We had invested 117 GH-deficient children. We found that the increase of growth rate differs after these GH-deficient children treated with recombinant GH during the first year treatment. Recently, single nucleotide polymorphisms (SNPs) were used as a tool for mapping the complex disease genes. It could be clinically useful for identifying if the genes involved in the cascade of growth hormone stimulation may influence the efficacy of GH replacement therapy. We will compare allelic frequencies in control group with recurrent GH-deficient children by screening the GH receptor, JAK2, STAT5a, 5b, IGF-1, IGFBP, or ALS polymorphisms listed on Table 1 to identify useful genetic markers for this efficacy research. To elucidate if those polymorphisms could be a marker of prognosis for GH therapy in GH-deficient children. Because height is determined by various genetic factors as

well as other nutritional and environmental factors, the relevance will be investigated in the difference of growth response after one-year-GH treatment, the genetic polymorphisms and clinical variants such as body weight, height of parents, and hypothalamic condition using statistical analysis. This method will be beneficial in improving growth velocity and adult height prediction.

肆、研究目的

The main specific aims of this proposed research include: (1) to evaluate the efficacy of recombinant growth hormone treatment in short stature patients with growth hormone deficiency through single nucleotide polymorphism "fingerprinting" approach, (2) to identify new targets for efficative growth hormone treatment. Our future objectives will be: (1) establishment of personal SNP file for short stature patients for more effective treatment, (2) development of new drug for short stature patients.

伍、結果與討論

The original specific aims proposed for this study were: (1) to evaluate the efficacy of recombinant growth hormone treatment in short stature patients with growth hormone deficiency through single nucleotide polymorphism "fingerprinting" approach, (2) to identify new targets for efficative growth hormone treatment. To accomplish the proposed specific aims, four phases of work have been undertaken:

(1). Recruitment and evaluation of patients with growth hormone deficiency and genomic DNA extration.

After careful clinical evaluation, a total of 117 patients were recruited. Clinical records were collected before treatment, including birth weight, height of parents, maximal peak GH value and the size and shape of pituitary gland detected by MRI. A total of 10 ml whole blood was collected from patients and their parents. Genomic DNA was extracted.

(2). Genotyping on SNP markers selected through candidate genes involved in growth regulation and statistical analysis of assessed SNP genotypes.

In the initial stage, 4 SNPs in GHR genes and one SNP each in JAK2, STAT5a, IGF1, IGFBP3, ALS were selected in genotyping. All selected SNPs are in coding region. Of those selected SNPs, rs6182 showed association with hormone treatment efficacy. SNP rs6182 has a nucleotide change of G to T which would lead to a nonsynonymous amino acid change of cysteine to phenylalanine at codon 440. Statistical analysis indicated that T/T genotype has larger height gain than T/G and G/G genotype.

(3). Establishment of Epstein-barr (EB) virus transformed lymphoblastoid cell lines for each genotype.

To evaluate the mechanism that underlie the efficacy of growth hormone treatment, EB virus transformed cell lines of different genotypes were established.

(4). *in vitro* study of growth hormone regulation using the established EB virus transformed cell lines.

Cells were grown and stimulated with a pulse of human growth hormone (hGH) (500ng/ml), cells treated with hGH in 1, 5, 10, 15, 20, 30, 60 and 120 min. Whole cell extracts (20 μg of protein/lane) were resolved on SDS-polyacrylamide gels. Proteins are then electrotransferred onto polyvinyllidene diflouride (PVDF) membrane using a semi-dry transfer unit. Membranes were then probed with primary antibody for STAT-5a and STAT-5b. Enhanced chemiluminescences were then used to identify the phosphorylated proteins. The initial result indicated that cell lines with T/T genotype has higher level of phophorylated STAT5a/5b detected than cells with other genotypes (T/G and G/G).