European Review for Medical and Pharmacological Sciences 2016; 20: 4078-4081

Effect of single nucleotide polymorphisms on CDK4 and Zn supplementation in children with growth hormone deficiency

Y. LIU, Z.-X. ZHANG

Department of Pediatrics, China-Japan Friendship Hospital, Beijing, China

Abstract. – OBJECTIVE: Precision medicine is a personalized disease prevention and treatment program combining modern genetic technology, molecular imaging techniques, and biological information with patients' living environment and clinical data, for accurate classification and diagnosis of diseases.

CASE REPORT: Our study presents the case of a 7-year-old female patient with clinical manifestations of growth hormone (GH) deficiency. After treatment with recombinant human GH for 2 years, the patient showed a reduced growth rate. Then single nucleotide polymorphisms according to GHD was analyzed, and the 6009 site within cyclin-dependent kinase 4 gene showed a weak response to IGF-1 which was a downstream signal molecules of GH.

CONCLUSIONS: Based on these results, both Zn and GH were supplied to the patients, and the growth rate increased significantly. Precision medicine needs more studies on patients to make accurate treatment.

Key Words:

Precision medicine, Cyclin-dependent kinase 4, Single nucleotide polymorphisms, Serum Zn.

Introduction

Precision medicine focus on the customization of healthcare, with decisions and practices tailored to an individual patient based on their intrinsic biology in addition to clinical "signs and symptoms", including optimal therapeutics and accurate medication[1,2]. The optimal therapeutics can be prescribed according to specific individual etiologies and genetic factors, including haploidy, single-nucleotide polymorphism (SNP), and gene expression[3]. Accurate medications in the pathology field contain accurate diagnosis of disease and molecular genetic testing[4]. For the patients with growth hormone deficiency (GHD), most of them were supplied recombinant human growth hormone (rhGH) clinically. Precision medicine was also important based on the different physical condition.

There are numerous genes and SNP sites related to GHD have been tagged and investigated[5]. The genomic deletion of the exon 3 in the GH receptor (GHR) has been linked to the growth responses to high-dose rhGH therapy for the treatment of short children[6]. Some SNPs related to lipid metabolism contribute to individual differences in baseline serum lipid profiles[7]. Stevens found that six SNPs in five different genes of children with GHD are significantly associated with 1-month IGF-1 SDS. In children with GHD, the AA genotype of 6500 sites (SNP rs2270777) within *CDK4* was associated with high IGF-1 response, and GG type means weak IGF-1 response[8]. In a female patient, the GG genotype of 6009 sites (SNP rs2069502) in *CDK4* exhibits a weak response to IGF-1 while the AA showed a high response[8].

The growth rate of patients with GHD is not only influenced by the polymorphism of gene markers, Zn deficiency has also been reported to be associated with growth impairment[9]. Studies[10,11] suggested that Zn can mediate growth stimulation by altering the levels of circulating IGF-1. During the growth period, Zn deficiency results in growth failure and lack of gonadal development[8]. Zn supplementation exerts evident effects on inducing the growth rate of both healthy and short children with Zn deficiency[12,13].

We report here a 7-year-old female patient with GHD. After treatment with rhGH for two years, the growth rate was lower year by year, but the dosage of rhGH on the second year is higher than the first year. The SNPs of this patient was analyzed and the genotype of *CDK4* showed less response to IGF-I which is a downstream signal molecules of GH. Because serum Zn is associated with IGF-I, both Zn and rhGH were supplied

Corresponding Author: Zhixin Zhang, MD; e-mail: zhangzhixin032@163.com

and the growth rate increased significantly. This case was a good example in patients with GHD on precision medicine.

Case Presentation

A 7-year-old female child was admitted to our department on an emergency basis because of short stature 112.8 cm, which is below the third percentile for her gestational age. She was born by cesarean delivery with 50 cm and a birth weight of 3.9 kg. She had normal eating and sleeping, with normal spine and extremital symmetry. Her father was 168 cm and her mother was 155 cm, with a target height of 161 cm. No abnormal findings were revealed through analysis of complete blood test, liver and renal function test, hyperthyroidism, and sex hormone index. Moreover, normal results were obtained from type-B ultrasound check of the uterus and ovary, abdominal ultrasound, double adrenal ultrasound, and pituitary magnetic resonance imaging.

The growth rate of this patient was only 4 cm per year. Based on clonidine and arginine, the growth hormone stimulation experiment showed the peak GH was only 4.11 ng/mL, which meant the patient was serious GHD. Then the rhGH was supplied to this patient on the dosage of 0.12 in the first year and 0.15 in the second year. In the first year, the growth rate was 9.8 cm/y, but in the second year, the growth rate was only 7.2 cm/y (Table I). These results suggested that the response to rhGH was weak in this patient.

It has been reported that the GH-IGF-1 was an important factor in growth regulation The IGF-I was a downstream signal molecular of GH, and the serum Zn was associated with IGF-1. Therefore, the serum Zn level of the patient was detected with the concentration of 147.3 mol/L Zn, which is lower than normal concentration. The SNPs related to GHD were also analyzed and the 6009 site in *CDK4* was GG (Table II), which means weak response to GH. Then both Zn (1 mg/kg/d) and more rhGH (0.25 U/kg/d) were supplied to the patient. After 3 months, the growth rate increased significantly to 8.5 cm/y (Table I).

Discussion

Precision medicine aims to minimize iatrogenic damage, reduce treatment costs, and maximize the effectiveness of treatments[14]. Precision medicine is not only confined to treatment at the molecular level (e.g., genomic markers) but is also combined with other technologies, such as traditional medicine, including PET-CT, pathological examination[15]. As we know, gene polymorphisms can alter gene function.

In the rhGH therapy, the polymorphisms of genes could affect the signaling pathways that participate in growth responses to GH[16]. The 6009 and 6500 sites of *CDK4* were important SNPs, which means different response to GH. The GG in 6009 site means weak response to GH, while the AA means a normal response[8]. The AA in 6500 site means normal response, while the GG means weak response. In this patient, the 6009 site is GG and the 6500 site is AA. Therefore, the response to GH maybe weak compared with a normal child. A high dose of rhGH is needed in the treatment.

The GH/IGF-1 pathway plays essential roles in growth in childhood. As a key marker of GH activity, IGF-I has been shown to be useful in monitoring and adjusting GH dose during treatment of GHD[17]. In this case, when rhGH was used the IGF-I level reduced from 196 ng/mL to 160 ng/mL, while IGF-I level increased to 242

Table I. Baseline characteristics of the patient.

Characteristic	Pre-treatment	Post-treatment without Zn supplementation	Post-treatment without Zn supplementation	Post-treatment with Zn supplementation
Age (years)	7	8	8.11	9.2
ΔH (cm/yr)	4	9.8	7.2	8.5
Weigh (kg)	20.5	23.5	28.5	29
Bone age (year)	7	8	9	9
rhGH dosage (U/kg/d)	–	0.12	0.15	0.25
IGF-1 (ng/mL)	196	160	174	242
Serum Zn (μmol/L)	–	–	147.3	154.7

Table II. Polymorphism of *CDK4* of the patient.

Gene name	Mutation site	Genotype
CDK4	6009	GG
CDK4	6500	AA

ng/mL when both rhGH and Zn were used. Although the IGF-I level was in the normal range, IGF-I cannot be used as an accurate marker of GH in this case. Maybe, the IGF-I can be regulated by GH and other factors together, and Zn is important in this process.

Growth retardation related to Zn deficiency may have resulted from low serum IGF-1 concentrations and inhibition of the anabolic actions of IGF-1[12]. Zn deficiency reduces IGF-1 gene expression, which can be reversed by Zn supplementation but not by GH administration. Zn deficiency can also lead to pubertal arrest possibly through its effect on hypothalamic function, which can be reversed by Zn supplementation[12]. In this case, the concentration of serum Zn was lower than normal, suggested the concentration of IGF-I maybe low to show a normal effect on growth. Therefore, Zn should be supplied to the patient in this case.

Conclusions

In this paper, we demonstrated that the polymorphism located at the 6009 site of *CDK4* is associated with GH response in patient with GHD during rhGH treatment. The patients with GG genotype in *CDK4* means a low response of GH and should be supplied by a high dose of rhGH. We also found that the serum Zn concentration was related to serum IGF-I concentration and its function in growth. Therefore, we assumed that the different genotype of patients and serum Zn concentration should be supplied with different treatment with Zn or rhGH dosage.

Acknowledgements

This study was supported by the "National Science and Technology Infrastructure Program of China" (NO 81270496).

Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Gourraud PA, Henry RG, Cree BA, Crane JC, Lizee A, Olson MP, Santaniello AV, Datta E, Zhu AH, Bevan CJ, Gelfand JM, Graves JS, Goodin DS, Green AJ, von Büdingen HC, Waubant E, Zamvil SS, Crabtree-Hartman E, Nelson S, Baranzini SE, Hauser SL. Precision medicine in chronic disease management: the multiple sclerosis BioScreen. Ann Neurol 2014; 76: 633-642.

2) Rubin EH, Allen JD, Nowak JA, Bates SE. Developing precision medicine in a global world. Clin Cancer Res 2014; 20: 1419-1427.

3) Deluche E, Onesti E, Andre F. Precision medicine for metastatic breast cancer. Am Soc Clin Oncol Educ Book 2015; e2-7.

4) Tsimberidou AM, Eggermont AM, Schilsky RL. Precision cancer medicine: the future is now, only better. Am Soc Clin Oncol Educ Book 2014; 61-69.

5) Goode EL, Fridley BL, Vierkant RA, Cunningham JM, Phelan CM, Anderson S, Rider DN, White KL, Pankratz VS, Song H, Hogdall E, Kjaer SK, Whittemore AS, DiCioccio R, Ramus SJ, Gayther SA, Schildkraut JM, Pharaoh PP, Sellers TA. Candidate gene analysis using imputed genotypes: cell cycle single-nucleotide polymorphisms and ovarian cancer risk. Cancer Epidemiol Biomarkers Prev 2009; 18: 935-944.

6) Ranke MB, Lindberg A; KIGS International Board. Prediction models for short children born small for gestational age (SGA) covering the total growth phase. Analyses based on data from KIGS (Pfizer International Growth Database). BMC Med Inform Decis Mak 2011; 11: 38.

7) Glad CA, Barbosa EJ, Filipsson Nyström H, Carlsson LM, Nilsson S, Nilsson AG, Svensson PA, Johannsson G. SNPs within the GH-signaling pathway are associated with the early IGF1 response to GH replacement therapy in GHD adults. Eur J Endocrinol 2014; 170: 101-107.

8) Stevens A, Clayton P, Tatò L, Yoo HW, Rodriguez-Arnao MD, Skorodok J, Ambler GR, Zignani M, Zieschang J, Della Corte G, Destenaves B, Champigneulle A, Raelson J, Chatelain P. Pharma-

cogenomics of insulin-like growth factor-I generation during GH treatment in children with GH deficiency or Turner syndrome. Pharmacogenomics J 2014; 14: 54-62.

9) Tuerk MJ, Fazel N. Zinc deficiency. Curr Opin Gastroenterol 2009; 25: 136-143.

10) Oner G, Bhaumick B, Bala RM. Effect of zinc deficiency on serum somatomedin levels and skeletal growth in young rats. Endocrinology 1984; 114: 1860-1863.

11) Clegg MS, Keen CL, Donovan SM. Zinc deficiency-induced anorexia influences the distribution of serum insulin-like growth factor-binding proteins in the rat. Metabolism 1995; 44: 1495-501.

12) Karaca Z, Tanriverdi F, Kurtoglu S, Tokalioglu S, Unluhizarci K, Kelestimur F. Pubertal arrest due to Zn deficiency: the effect of zinc supplementation. Hormones (Athens) 2007; 6: 71-74.

13) Hamza RT, Hamed AI, Sallam MT. Effect of zinc supplementation on growth hormone-insulin growth factor axis in short Egyptian children with zinc deficiency. Ital J Pediatr 2012; 38: 21.

14) de Ridder MA, Stijnen T, Hokken-Koelega AC. Prediction model for adult height of small for gestational age children at the start of growth hormone treatment. J Clin Endocrinol Metab 2008; 93: 477-483.

15) Guo XQ, ??LIST ALL THE AUTHORS??. Cancer precision Medicine. Science(KEXUE) 2015; 67: 28-31.

16) Clayton P, Chatelain P, Tatò L, Yoo HW, Ambler GR, Belgorosky A, Quinteiro S, Deal C, Stevens A, Raelson J, Croteau P, Destenaves B, Olivier C. A pharmacogenomic approach to the treatment of children with GH deficiency or Turner syndrome. Eur J Endocrinol 2013; 169: 277-289.

17) Pawlikowska-Haddal A, Cohen P, Cook DM. How useful are serum IGF-I measurements for managing GH replacement therapy in adults and children? Pituitary 2012; 15: 126-134.