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COMMENT

Cyclin-Dependent Kinase Inhibitor Gene Polymorphisms in Pituitary Gigantism

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Gigantism and acromegaly are mostly caused by growth hormone (GH)–secreting pituitary adenomas *(1)*. Gigantism occurs from a distinct group of GH-secreting adenomas arising early in life, and usually derived from more primitive pituitary stem cells or mammosomatotroph cells. Genetic abnormalities may be more prevalent in these sporadic childhood and adolescent tumors than those arising in adults. Cyclin-dependent kinase inhibitors (CKI) play an important role in preventing tumorigenesis *(2)*. Mice deficient in CKI p18 or p27 develop intermediate lobe pituitary tumors, p21 deletion facilitates such tumor growth in Rb-deficient mice, and p18 expression is low in GH-secreting pituitary adenomas *(3–6)*. p21 and p27 gene mutations have not been detected in pituitary tumors *(7,8)*. We found no p27 gene mutations in DNA extracted from eight GH-secreting pituitary adenomas *(7)*. In this study, we tested for germline mutations in CKI p21, p18, and p27 in three patients harboring GH-secreting tumors with gigantism.

Informed consent was obtained after Institutional Review Board approval. Clinical information is summarized in Table 1. Whole blood DNA was extracted from each patient by standard procedure. To amplify all coding exons of p21, p18, and p27, the following primers were used. 5'-AGG GCC TTC CTT GTA TCT CTG CTG-3' and 5'-AAA GTC CTT CCG TGC ACA TGT CCG-3' for p21 exon 2; 5'-ACA TCT GTG AAG CAT GGT GGG ACA-3' and 5'-ACT AAG GCA GAA GAT GTA GAG CGG-3' for p21 exon 3; 5'-AGC CTG GTT AGG AGC AAA GGA AAG-3' and 5'-CTA AGA CCA AAT CTG GAC CCC ACT-3' for p18 exon 2; 5'-GCA CTT GAA GGA TTC TAC CAT TTC-3' and 5'-AAG TCA GGA GAG CTA CTC AGT TAA-3' for p18 exon 3; 5'-AGT CGC TGG GCT TCC GAG AGG GGT-3' and 5'-CTA TGG TTG GGA AAG GGT CAT TAC-3' for p27 exon 1; and 5'-TCA CTA GCA ACT CCT AGG TAT GTG-3' and 5'-TCC TTT AGT GAT CAA CCC ACC GAG-3' for p27 exon 2. PuReTag Ready-to-Go PCR beads were used. PCR conditions: 95°C for denaturing, 62°C for annealing, 72°C for polymerization. PCR products were purified and sequenced in both directions using the primers for PCR.

In all three patients, coding sequences for p21, p18, and p27 did not contain point mutations or frame shifts. A known polymorphism in p21 codon 31 (AG<u>C</u> to AG<u>A</u>, Ser to Arg) *(9)* was dectected. Patient 3 was heterozygous for Arg/Ser, and the other two patients appear to be homozygous for Ser/Ser. A known polymorphism in p27 codon 109 (G<u>T</u>C to G<u>G</u>C, Val to Gly) *(7)* was found in patient 2, while the other two patients both had Val/Val alleles at this site.

As the GH-secreting pituitary tumor causing gigantism may be more influenced by genetic factors, we were prompted to sequence three CKI genes in white blood cells of these patients to identify potential germline mutations. Undetectable germline mutations in p21, p18, and p27 gene coding regions in these three patients with gigantism demonstrate that these mutations are not commonly encountered in this rare group of patients. Our approach cannot, however, reliably identify gene deletions. We found two known polymorphisms: $31^{Ser \rightarrow Arg}$ in p21 and $109^{Val \rightarrow Gly}$ in p27. The significance of these polymorphisms is not clear. p21 codon 31 polymorphism has been associated with various tumors, but the phenotype is not consistently associated with tumorigenesis *(9)*.

The most prevalent p21 polymorphism in the Caucasian population is Arg/Arg (80%) while Arg/Ser and Ser/Ser are rare (<10%) but notably two of the three gigantism patients, all Caucasian, have Ser/Ser polymorphism and one has Arg/Ser polymorphism. We demonstrated previously that p27 $109^{Val \rightarrow Gly}$ polymorphism does not appear to be involved in pituitary tumorigenesis, as the prevalence of this polymorphism is similar to that expected in the general population *(7)*. Interestingly, the rare Val/Gly polymorphism was detected in patient 2, who was diagnosed at the age of 8 yr with gigantism.

In summary, we sequenced coding exons of the p21, p18, and p27 genes from white blood cells derived from three patients with gigantism and found no mutations. Although disruption of these genes leads to mouse pituitary tumori-

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Table 1
Clinical Characteristics of Three Patients with Gigantism

	Patient 1	Patient 2	Patient 3
Sex	Female	Female	Male
Age at diagnosis (yr)	15	8	18
Height at diagnosis (cm)	175.3	148.8	213.4
IGF-1[a] (ng/mL)	845 (242–660)	1188 (88–474)	1031 (182–780)
GH (ng/mL)	79	N/D	20
Prolactin (ng/mL)	8700	88	9.1
Pituitary MRI	Invasive macroadenoma	Macroadenoma	Macroadenoma
Treatment	Surgical + medical	Medical	Medical

[a]Normal range of IGF-1 is shown in parentheses.

genesis, they may not play an important role in pathogenesis of rare growth hormone-secreting pituitary tumors occurring in patients with gigantism.

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