403–432; R. K. Z. Tan and S. C. Harvey, in *Theoretical Biochemistry and Molecular Biophysics*, D. L. Beveridge and R. Lavery, Eds. (Adenine, Guilderland, NY, 1991), vol. 1, pp. 125–137.
23. K. V. Klenin, A. V. Vologodskii, V. V. Anshelevich, A. M. Dykhne, M. D. Frank-Kamenetskii, *J. Mol. Biol.* **217**, 413 (1991).
24. The time-step restriction in typical explicit MD schemes is severely constrained by the most rapid vibrational modes [J. A. McCammon and S. C. Harvey, *Dynamics of Proteins and Nucleic Acids* (Cambridge Univ. Press, Cambridge, 1987)]. This generally limits Δt to the femtosecond range and the trajectory to the picosecond range. Two basic approaches to this problem have emerged. In the first, the fast vibrational modes are effectively frozen by a constrained MD formulation [W. F. van Gunsteren, *Mol. Phys.* **40**, 1015 (1980)]; although this technique increases Δt by a small factor (2 or 5), each time step still requires more work. In the second, the motion is separated into multiple time scales for the slow and fast force components [for example, M. E. Tuckerman, B. J. Berne, A. Rossi, *J. Chem. Phys.* **94**, 1465 (1991); O. Teleman and B. Jönsson, *J. Comput. Chem.* **7**, 58 (1986)]; special systems where such a separation is natural (such as a liquid containing heavy and light particles) are most suitable though this technique is now more generally applied.
25. The resulting discretization of the Langevin equation by the implicit-Euler scheme [G. Dahlquist and Å. Björck, *Numerical Methods* (Prentice-Hall, Englewood Cliffs, NJ, 1974)] produces the following pair of differential equations:

$$M[(\mathbf{v}^{n+1} - \mathbf{v}^n)/\Delta t] = -\mathbf{g}_E(\mathbf{x}^{n+1}) - \gamma M\mathbf{v}^{n+1} + \mathbf{r}^{n+1} \quad (3a)$$

$$(\mathbf{x}^{n+1} - \mathbf{x}^n)/\Delta t = \mathbf{v}^{n+1} \quad (3b)$$

where the random force $\mathbf{r}$ is a stationary Gaussian process with mean and covariance matrix given by

$$\langle \mathbf{r}^n \rangle = 0, \langle \mathbf{r}^n(\mathbf{r}^m)^T \rangle = 2\gamma k_B T M(\delta_{nm}/\Delta t) \quad (3c)$$

The superscripts n represent vector values at time $(n\Delta t)$, $\mathbf{x}$ and $\mathbf{v}$ are the collective position and velocity vectors, respectively; M is the diagonal mass matrix; γ the damping constant; $\mathbf{g}_E$ the gradient vector of the potential energy E, and δ the Kronecker delta function. To calculate $\mathbf{x}^{n+1}$ from $\mathbf{x}^n$ and $\mathbf{v}^n$, we formulate a minimization subproblem (*15, 16*) for the "dynamics function" $\Phi(\mathbf{x})$, where

$$\Phi(\mathbf{x}) = \frac{1}{2}(1 + \gamma\Delta t)(\mathbf{x} - \mathbf{x}_0^n)^T M(\mathbf{x} - \mathbf{x}_0^n) + (\Delta t)^2 E(\mathbf{x}) \quad (4a)$$

$$\mathbf{x}_0^n = \mathbf{x}^n + [\Delta t/(1 + \gamma\Delta t)][\mathbf{v}^n + \Delta t M^{-1}\mathbf{r}^{n+1}] \quad (4b)$$

This minimization is efficiently performed with the truncated Newton variant for potential energy functions (*11, 14*). After a minimum $\mathbf{x}^{n+1}$ for Φ is calculated, the new velocity vector, $\mathbf{v}^{n+1}$, is calculated directly by Eq. 3b.
26. M. Le Bret, *Biopolymers* **23**, 1835 (1984).
27. D. Joseph, G. A. Petsko, M. Karplus, *Science* **249**, 1425 (1990); H. R. Faber and B. W. Matthews, *Nature* **348**, 263 (1990); B. F. Anderson, H. M. Baker, G. E. Norris, S. V. Rumball, E. N. Baker, *ibid.* **344**, 784 (1990).
28. We thank H. M. Berman, S. Broyde, S. Lifson, M. L. Overton, J. K. Percus, and C. S. Peskin for their inspiration and contributions; C. J. Benham, D. L. Beveridge, N. R. Cozzarelli, S. C. Harvey, J. Hermans, B. M. Pettitt, D. W. Sumners, N. Seeman, I. Tobias, and the reviewers for their valuable comments; and E. Friedman, J. Greenberg, K. Fallon, A. De Falco, and J. Westbrook for their dedicated molecular graphics work. Generous support was provided by the National Science Foundation (CHE-9002146 and Presidential Young Investigator Award ASC-9157582), U.S. Public Health Service (GM 34809), Searle Scholars Program/The Chicago Community Trust, Faculty of Arts and Sciences at New York University, AAUW Educational Foundation, New York State Science and Technology Foundation, and Whitaker Foundation. Computational support was provided by the San Diego Supercomputer Center, the Academic Computing Facility at New York University, and the Rutgers University Center for Computational Chemistry.

17 March 1992; accepted 5 June 1992

A Mutation in the POU-Homeodomain of Pit-1 Responsible for Combined Pituitary Hormone Deficiency

Sally Radovick,* Michelle Nations, Yuefen Du, LaVonne A. Berg, Bruce D. Weintraub, Fredric E. Wondisford†‡

Pit-1 is a pituitary-specific transcription factor responsible for pituitary development and hormone expression in mammals. Mutations in the gene encoding Pit-1 have been found in two dwarf mouse strains displaying hypoplasia of growth hormone, prolactin, and thyroid-stimulating, hormone-secreting cell types in the anterior pituitary. A point mutation in this gene was identified on one allele in a patient with combined pituitary hormone deficiency. Mutant Pit-1 binds DNA normally but acts as a dominant inhibitor of Pit-1 action in the pituitary.

Pit-1–growth hormone factor–1 (Pit-1) is a member of the POU family of transcription factors that regulate mammalian development (*1*). Pit-1 contains two protein domains, termed POU-specific and POU-homeo, which are both necessary for high-affinity DNA binding on the genes encoding the growth hormone (GH) and prolactin (Prl) (*2, 3*). Pit-1 is also important for regulation of the genes encoding Prl and the thyrotropin β subunit (TSH-β) by thyrotropin-releasing hormone (TRH) and cyclic adenosine 3′,5′-monophosphate (*4–7*). Because mutations in the gene encoding Pit-1 (*Pit-1*) have been found in dwarf mice strains (*8*) that lack GH, Prl, and TSH-secreting cell types, we wanted to determine if mutations within this gene resulted in a similar deficiency of pituitary hormones in humans.

The patient (W.T.R.) studied in this report was previously documented to have a deficiency of GH, Prl, and TSH, which was manifest as severe mental retardation and short stature (*9*). Both GH and TSH were undetectable in the plasma before or after provocative stimuli; Prl in the plasma was low (3 μg per liter) and did not respond to stimulation by chlorpromazine or TRH. Baseline gonadotropin amounts, however, were normal [luteinizing hormone (LH), 15 IU per liter, and follicle-stimulating hormone, 7 IU per liter] and responded appropriately to stimulation by gonadotropin-releasing hormone. Serum cortisol levels were normal (8 a.m., 378 nmol per liter; and 4 p.m., 190 nmol per liter); steroid precursors increased appropriately after metyrapone. The patient's mother (E.R.) is of normal stature and has normal pituitary hormone levels. Unfortunately, the remaining family members were inaccessible for study; but, according to historical reports, they all have normal stature, which suggests that this patient may represent a sporadic case of combined pituitary hormone deficiency (CPHD).

The polymerase chain reaction (PCR) was used to amplify genomic DNA fragments from human *Pit-1* (*10*). The POU-specific and POU-homeodomains were amplified separately by means of specific oligonucleotides (*11*). The Pit-1 domains amplified in this study correspond to exons 4 through 6 of the mouse *Pit-1* gene (*8*) and contain most of the POU-specific and the entire POU-homeo domains. As a control in these experiments, genomic DNA from three normal patients was also included. We found no abnormalities in the POU-specific domain after sequencing at least seven independent clones from two separate PCR amplifications from either the patient or normal controls. A C to T mutation of codon 271, however, was found in approximately one-half of independent clones we sequenced from the patient (three of seven cloned fragments); the

S. Radovick and Y. Du, Division of Endocrinology, Department of Pediatrics, Rainbow Babies and Childrens Hospital and Case Western Reserve University Medical School, Cleveland, OH 44106.
M. Nations, L. A. Berg, F. E. Wondisford, Division of Endocrinology and Hypertension, Department of Medicine, University Hospitals of Cleveland and Case Western Reserve University Medical School, Cleveland, OH 44106.
B. D. Weintraub, Molecular and Cellular Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

*Present address: Division of Endocrinology, Department of Medicine, The Children's Hospital, 300 Longwood Avenue, Boston, MA 02115.
†Present address: Division of Endocrinology, Department of Medicine, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215.
‡To whom correspondence should be addressed.

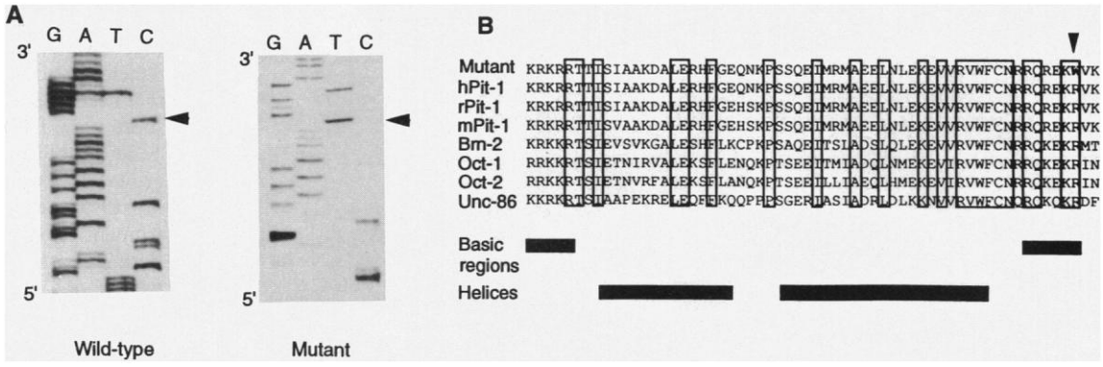

Fig. 1. Human *Pit-1* and a point mutation in the POU-homeodomain in a patient with CPHD. (**A**) Representative autoradiograph of both alleles from the patient W.T.R. Arrowheads denote the C to T mutation in codon 271 (GGG to TGG). (**B**) Alignment of POU proteins in their homeodomain (*8*). The species of Pit-1 is indicated by an h (human), r (rat), or m (mouse). At any position, amino acid residues that are identical are boxed. The residue mutated in this patient is marked by an arrowhead. Regions rich in basic amino acids are indicated by the black boxes. Regions that could form α helices according to a consensus prediction from both the Chou-Fasman (*29*) and Robson-Garnier methods (*30*) are also shown by black boxes (MacVector Sequence Analysis Software, IBI, New Haven, Connecticut). Introduction of a Trp at codon 271 did not alter these predictions. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

other four clones had a normal sequence, which indicates the presence of only one mutant allele at this site (Fig. 1A). This mutation was confirmed by allele-specific amplification of the POU-homeodomain (*12*). DNA from the patient's mother did not contain this mutation. This mutation alters the predicted amino acid from Arg to Trp at codon 271 and thus reduces the positive charge in a basic amino acid region of Pit-1 (Fig. 1B). An Arg residue is strictly conserved at this location among *Pit-1* gene products from several species and other related POU proteins (Fig. 1B). Moreover, either Arg or Lys is found at this position in all other homeobox proteins we analyzed (*13*). On the basis of the protein conformation model of Ingraham and co-workers (*2*), codon 271 is 3′ to the α-helical domains thought to be important for DNA binding in the Pit-1 homeodomain (Fig. 1B).

To test whether this mutation would alter Pit-1 DNA binding, we used an avidin-biotin complex DNA binding assay with wild-type or mutant ^{35}S-labeled Pit-1 from an in vitro transcription-translation reaction (Fig. 2A). Both proteins are of the correct apparent molecular size of 33 and 30 kD (depending on the methionine used for translation initiation). As shown in Fig. 2B, both wild-type and mutant Pit-1 proteins displayed high-affinity binding to a Pit-1 DNA binding site from the rat gene encoding GH (−89 to −60 bp) (*14*). The dissociation constant of DNA binding was approximately 6 nM for either protein and may have been somewhat greater for the wild-type protein. This result was predicted because the amino acid change in the mutant protein lies outside the α-helical domains important for DNA binding. Binding of either wild-type or mutant Pit-1 protein to the unrelated adenovirus 5 (Ad5) DNA fragment was negligible (Fig. 2B).

We next tested whether this mutant Pit-1 protein would activate pituitary gene expression. Luciferase reporter constructs that contained the genes encoding GH, Prl, or TSH-β promoters were cotransfected with either a wild-type or a mutant Pit-1 expression vector (300 ng) into a Pit-1–deficient cell line derived from a human choriocarcinoma (JEG-3 cells). In these experiments (Fig. 3A), the wild-type Pit-1 expression vector activated expression from the GH and Prl promoters (approximately threefold) as reported (*3*, *15*). Expression from the TSH-β promoter, however, was not activated by the wild-type construct in JEG-3 cells. This is a result of a fundamental difference in the Pit-1 DNA binding elements present in these genes. TSH-β elements, unlike those present in the genes encoding GH and Prl, have a lower affinity for Pit-1 and appear to be less important for cell-specific expression of this gene in the anterior pituitary (*16*). However, Pit-1 is clearly important for development and hormonal regulation of the thyrotroph in the anterior pituitary (6–8). To prevent promoter competition in transfection assays, we used a relatively small amount of Pit-1 expression vectors (300 ng), which could explain the lower degree of transactivation we observed.

When a mutant Pit-1 expression vector

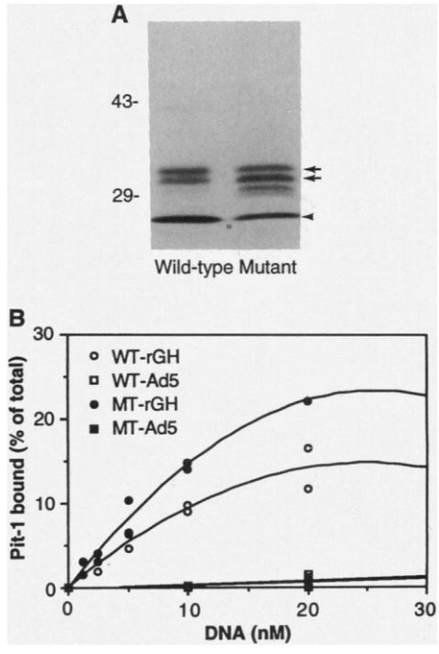

Fig. 2. Binding of radioactively labeled wild-type and mutant Pit-1 to a high-affinity Pit-1 DNA binding site. (**A**) Radioactively labeled proteins used in the DNA binding assay. A coupled in vitro transcription-translation reaction (Promega, Madison, Wisconsin) was used to synthesize ^{35}S-labeled wild-type and mutant Pit-1 proteins. Wild-type and mutant *Pit-1* cDNA were cloned into pGEM3Z at Xba I–Bam HI sites. Mutant *Pit-1* cDNA was generated from wild-type cDNA by PCR and a specific mutant 3′ oligonucleotide (G to A mutation in codon 271 on the antisense strand) and confirmed with DNA sequencing. In vitro transcription-translation reactions were programmed with pGEM3Z alone, pGEM3Z containing a wild-type *Pit-1* cDNA insert, or pGEM3Z containing a mutant *Pit-1* cDNA insert. Labeled bands corresponding to the two major species of 33 and 30 kD (arrows) were detected. No such bands were detected in a control reaction with pGEM3Z. Free [^{35}S]methionine is indicated by an arrowhead. (**B**) Avidin-biotin complex DNA binding (ABCD) assay. DNA fragments that contained either a high-affinity Pit-1 DNA binding site [−89 to −60 bp of the rat gene encoding GH (rGH) (*14*)] or unrelated DNA sequences from the long terminal repeat of Ad5 were synthesized as complementary oligonucleotides and biotinylated as described (*7*). The ABCD assay was performed in duplicate at each DNA concentration as described (*7*) with various concentrations of either rGH or Ad5 and either wild-type (WT)– or mutant (MT)-labeled Pit-1. DNA affinity constants for binding to wild-type and mutant Pit-1 proteins were determined by a Hill plot of the data (*31*).

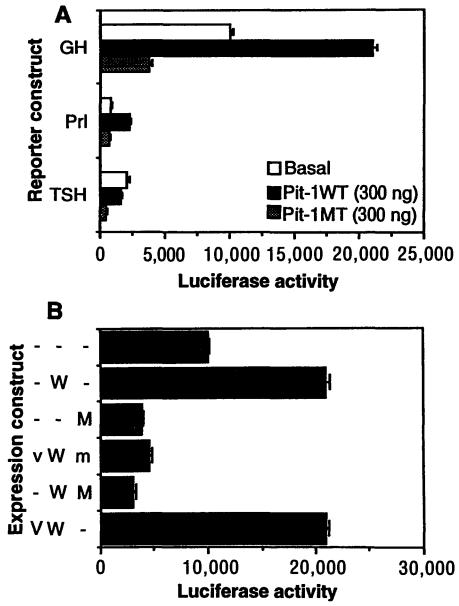

Fig. 3. Transfection of wild-type and mutant Pit-1 expression vectors in JEG-3 cells. (**A**) 1.7 kb of the rat GH promoter (GH) (*32*), 1.0 kb of the bovine prolactin promoter (Prl) (*33*), or 1.2 kb of the human TSH-β promoter (TSH) (*34*) was inserted upstream of the luciferase reporter gene in pSVOALΔ5′ (*34*). We constructed the wild-type (WT) and mutant (MT) Pit-1 expression vectors by inserting cDNAs from pGEM3Z into the pCMV expression vector at the Xba I–Bam HI sites. All constructs were confirmed with DNA sequencing and restriction enzyme analysis. Three micrograms of a reporter construct, 300 ng of a pCMVPit-1 expression construct, and 600 ng of pTKGH were transfected per plate into JEG-3 cells with a calcium-phosphate precipitation technique. Luciferase activity was corrected for human GH secretion in the medium as described [relative light units/GH in medium (ng/ml)] (*34*). Data were derived from three independent experiments and are presented as the mean ± SE. (**B**) The GH reporter construct (3 μg) and pTKGH (600 ng) was transfected into JEG-3 cells with the indicated combination of expression constructs. Uppercase (300 ng) or lowercase (150 ng) letters indicate the expression vector and amount transfected. V, v = empty vector; M, m = mutant Pit-1 expression vector; W = wild-type Pit-1 expression vector. Data were derived from three independent experiments and are presented as mean ± SEM. Human GH secretion was not significantly different between transfection experiments or experimental conditions.

was cotransfected, however, no transactivation was observed. In fact, basal activity from each promoter was inhibited 20 to 80% by the mutant construct (Fig. 3A). This effect, however, was not a result of a general squelching of transcription because expression of a cotransfected GH reporter construct that contained the thymidine kinase promoter (pTKGH) was not inhibited by either the wild-type or mutant Pit-1 constructs (basal, 0.8 ± 0.1; wild-type, 1.0 ± 0.1; and mutant, 1.4 ± 0.2 ng per milliliter of human GH in media from three independent experiments). Because mutant Pit-1 binds normally to a high-affinity Pit-1 DNA binding element from the rat gene encoding GH, these data indicate that Arg^{271} must be critical in Pit-1 transactivation. Moreover, these data indicate that the mutant protein acts as an inhibitor and not as an activator of transcription. Although mutant POU proteins have been described that do not bind DNA and inhibit transcriptional activation by forming inactive hetero- and homodimers (*8*, *17*), this mutant POU protein binds DNA and also inhibits transcriptional activation.

To determine if the mutant construct would interfere with transactivation by the wild-type Pit-1 construct, we cotransfected different ratios of wild-type and mutant constructs with a GH-luciferase reporter construct (Fig. 3B). The total amount of expression vector DNA was kept constant with empty cytomegalovirus vector DNA. The mutant construct inhibited transactivation by the wild-type construct. At a twofold excess or equal ratio of wild-type to mutant construct DNA, expression was inhibited 80 and 85%, respectively, compared to the level of expression observed with the wild-type construct alone (Fig. 3B). This was not a nonspecific effect of the expression vector alone, because an equal amount of empty vector DNA did not alter the degree of activation observed with the wild-type construct (Fig. 3B). Because Pit-1 forms dimers on DNA through POU-specific domain interactions (*2*), three possible mechanisms could account for the dominant negative effect, but no mechanism is mutually exclusive of the other. First, mutant Pit-1 may form homodimers that compete for DNA binding and inhibit the action of wild-type Pit-1 homodimers. Second, mutant Pit-1 may form heterodimers with wild-type Pit-1 on DNA and inhibit its action. Third, mutant Pit-1 may interact with, and inhibit the function of, a limiting transcription factor when bound to DNA. Regardless of the mechanism, however, these data indicate that mutant Pit-1 expression from one allele is clearly sufficient to block normal Pit-1 action in the pituitary. In an analogous system, thyroid hormone receptor mutations at one allele result in a dominant negative effect on thyroid hormone action in patients with thyroid hormone resistance syndromes (*18–20*). Although this syndrome is usually familial (autosomal dominant), sporadic cases are frequently identified (*21*).

The etiologies of pituitary deficiency in humans are numerous and include trauma, infection, tumor, autoimmune disorders, vascular disease, developmental abnormalities, and genetic disorders. A genetic basis for monotrophic pituitary hormone deficiency has been demonstrated for GH (*22*), TSH (*23–25*), and LH (*26*). This study indicates that CPHD can be a result of a mutation of the *Pit-1* gene, which encodes a transcription factor necessary for development and expression of multiple pituitary cell types (*8*). Disorders of GH, Prl, and TSH deficiency, however, are heterogenous, exemplified by two families with complete deficiency of GH and Prl and only partial deficiency of TSH (*27*). Because an alteration in Pit-1 function depends on the location and type of Pit-1 mutation and because Pit-1 appears to have different actions in the somatotroph, lactotroph, and thyrotroph, hormone deficiency states caused by mutations of *Pit-1* are likely to be variable in humans. Further study will be required, however, to determine how common these gene mutations are in patients with CPHD.

Note added in proof: After this manuscript was submitted, researchers reported a family with CPHD that was a result of a *Pit-1* gene mutation (*28*).

REFERENCES AND NOTES

1. M. G. Rosenfeld, *Genes Dev.* **5**, 897 (1991).
2. H. A. Ingraham *et al.*, *Cell* **61**, 1021 (1990).
3. L. E. Theill, J-L. Castrillo, D. Wu, M. Karin, *Nature* **342**, 945 (1989).
4. R. N. Day and R. A. Maurer, *Mol. Endocrinol.* **3**, 3 (1989).
5. G-Z. Yan and C. Bancroft, *ibid.* **5**, 1488 (1991).
6. H. J. Steinfelder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3130 (1991).
7. H. J. Steinfelder *et al.*, *J. Clin. Invest.* **89**, 409 (1992).
8. S. Li *et al.*, *Nature* **347**, 528 (1990).
9. A. D. Rogol and C. R. Kahn, *J. Pediatr.* **88**, 953 (1976).
10. A. M. Lew and H. P. Elsholtz, *Nucleic Acids Res.* **19**, 6329 (1991).
11. Oligonucleotide primers used for PCR amplification were chosen on the basis of the published human *Pit-1* cDNA sequence (*10*) and genomic structure in the mouse (*8*) and are as follows: 4, GGTGAATTCGATACACCCAGACAAATGTTG; 5, GGTGGATCCCTCCTACTTGCTCAGCTTCCTT; 12, GTTGGAATTCTGCTAAAGATGCTCTGGAGAG; and 13, GTTGGGATCCGAAGAGAAAGGAATGAAACGG. Primers 4 and 5 and 12 and 13 were used to amplify most of the fourth and fifth exons and the sixth exons, respectively. PCR products of the correct size were digested with Eco RI and Bam HI and cloned into these sites in pGEM4Z. Both DNA strands were sequenced from individual clones, and mutations were confirmed on both strands.
12. PCR amplification of the POU-homeodomain from the mutant *Pit-1* allele was performed with two specific primers: 8, GGTGAATTCCATTGCTGCTAAAGATGCTCT; and W.T.R., ATTCAGACTTGTTTTCACCCA. The 3′ primer (W.T.R.) contains the mutation at its 3′ terminus (underlined). DNA from W.T.R. but not from his mother, E.R., or from normal subjects yielded a 174-bp PCR product, which indicates the presence of the mutant allele in W.T.R.
13. S. Radovick *et al.*, data not shown.
14. C. Nelson, V. R. Albert, H. P. Elsholtz, L. I.-W. Lu, M. G. Rosenfeld, *Science* **239**, 1400 (1988).

15. H. J. Mangalam *et al.*, *Genes Dev.* **3**, 946 (1989).
16. H. J. Steinfelder, S. Radovick, F. E. Wondisford, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
17. M. N. Treacy, X. He, M. G. Rosenfeld, *Nature* **350**, 577 (1991).
18. S. J. Usala *et al.*, *Mol. Endocrinol.* **2**, 2217 (1988).
19. S. J. Usala *et al.*, *J. Clin. Invest.* **85**, 93 (1990).
20. V. K. K. Chatterjee *et al.*, *ibid.* **87**, 1977 (1991).
21. R. Parrilla *et al.*, *ibid.* **88**, 2123 (1991).
22. J. A. Phillips III, in *The Metabolic Basis of Inherited Disease*, C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Vale, Eds. (McGraw-Hill, New York, 1989), pp. 1965–1983.
23. Y. Hayashizaki, Y. Hiraoka, Y. Endo, K. Miyai, K. Matsubara, *EMBO J.* **8**, 2291 (1989).
24. C. Dacou-Voutetakis, D. M. Feltquate, M. Drakopoulou, I. A. Kourides, N. C. Dracopoli, *Am. J. Hum. Genet.* **46**, 988 (1990).
25. F. E. Wondisford *et al.*, in preparation.
26. J. Weiss *et al.*, *N. Engl. J. Med.* **326**, 179 (1992).
27. J. M. Wit *et al.*, *Horm. Res.* **32**, 170 (1989).
28. K.-I. Tatsumi *et al.*, *Nature Genetics* **1**, 56 (1992).
29. P. Y. Chou and G. D. Fasman, *Biochemistry* **13**, 211 (1974).
30. J. Garnier, D. J. Osguthorpe, B. Robson, *J. Mol. Biol.* **120**, 97 (1978).
31. F. W. Dahlquist, *Methods Enzymol.* **49**, 270 (1978).
32. S. A. Lira, E. B. Crenshaw III, C. K. Glass, L. W. Swanson, M. G. Rosenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4755 (1988).
33. S. A. Camper, Y. A. S. Yao, F. M. Rottman, *J. Biol. Chem.* **260**, 12246 (1985).
34. D. L. Bodenner, M. A. Mroczynski, B. D. Weintraub, S. Radovick, F. E. Wondisford, *ibid.* **266**, 21666 (1991).
35. This work was supported by grants from NIH, American Cancer Society, the Marguerite M. Wilson Foundation, and the Departments of Medicine and Pediatrics at University Hospitals of Cleveland. We thank P. Gorden, C. R. Kahn, A. D. Rogol, and J. Robbins for their evaluation of this patient, R. P. Owens for clinical assistance, and F. M. Rottman for the bovine Prl promoter plasmid.

15 April 1992; accepted 17 June 1992

Mutation of the POU-Specific Domain of Pit-1 and Hypopituitarism Without Pituitary Hypoplasia

R. W. Pfäffle,* G. E. DiMattia, J. S. Parks,† M. R. Brown, J. M. Wit, M. Jansen, H. Van der Nat, J. L. Van den Brande, M. G. Rosenfeld, H. A. Ingraham‡

A point mutation in the POU-specific portion of the human gene that encodes the tissue-specific POU-domain transcription factor, Pit-1, results in hypopituitarism, with deficiencies of growth hormone, prolactin, and thyroid-stimulating hormone. In two unrelated Dutch families, a mutation in Pit-1 that altered an alanine in the first putative α helix of the POU-specific domain to proline was observed. This mutation generated a protein capable of binding to DNA response elements but unable to effectively activate its known target genes, growth hormone and prolactin. The phenotype of the affected individuals suggests that the mutant Pit-1 protein is competent to initiate other programs of gene activation required for normal proliferation of somatotrope, lactotrope, and thyrotrope cell types. Thus, a mutation in the POU-specific domain of Pit-1 has a selective effect on a subset of *Pit-1* target genes.

The pituitary transcription factor Pit-1 is part of the large POU-domain gene family defined by a highly conserved, bipartite DNA binding domain that consists of the POU-specific domain (POU-S) and POU-homeodomain (POU-HD) regions (*1*). Biochemical and ontogeny studies have shown that Pit-1 is the critical, cell-specific transcription factor for activating expression of the prolactin (*Prl*) and growth hormone (*GH*) genes in the anterior pituitary gland (*2*, *3*). Additionally, Pit-1 acts to regulate expression of its own encoding gene (*4*). Detailed analyses have suggested that both the POU-S and POU-HD contact DNA, with the POU-S domain required for high-affinity, site-specific binding and DNA-dependent cooperative binding events observed on many response elements (*5*). Deficiencies of GH, Prl, and thyroid-stimulating hormone (TSH) in the Snell dwarf mouse are caused by a point mutation [$Trp^{261} \rightarrow Cys$ (W261C)] in the POU-HD that eliminates binding of the defective protein to the *GH* and *Prl* genes (*6*). Homozygosity for the Snell mutation results in severe hypoplasia of the anterior pituitary gland, with the absence of somatotropes, lactotropes, and thyrotropes (*7*).

There have been several reports of multiple pituitary hormone deficiency involving GH, Prl, and TSH in humans (*8*, *9*). We examined the possibility of *Pit-1* gene (*Pit-1*) mutation in two unrelated Dutch families, each with of two affected and three unaffected siblings (*8*). Studies were conducted with informed consent. The affected children in Family I had severe growth impairment with heights 10.2 standard deviations (SD) below the mean for age before beginning human GH (hGH) treatment. The older affected child in Family II had a height of −8.2 SD at age 3.5, but the younger child's height was only 1.0 SD below the mean before hGH treatment was started at 9 weeks of age. All four failed to increase serum GH and Prl levels above detection limits of 0.5 μg/liter and 50 mU/liter in response to GH-releasing hormone and thyrotrophin-releasing hormone (normal > 10 μg/liter and >500 mU/liter). The affected children in Family I had normal serum T4 amounts before GH treatment, but these amounts decreased to 36 and 45 nmol/liter (normal is 70 to 170 nmol/liter) after 1 year of hGH treatment. The affected children in Family II had more severe, symptomatic central hypothyroidism that was recognized shortly after birth.

The normal human *Pit-1* cDNA sequence was determined to provide a basis for comparison with the sequences in the affected children (*10*). A 2.31-kb isolate was found to contain the entire coding region and all but ten nucleotides of the 5′ untranslated region, as judged by the transcriptional start site of the rat *Pit-1* gene. The 873-bp open reading frame showed 85% DNA homology and greater than 95% amino acid sequence homology with bovine, rat, and mouse sequences. Our results are in agreement with those of Lew and Elsholtz (*11*). There were no differences between the bovine Pit-1 protein in the POU-S domain and a single, conservative Met-for-Leu substitution within the POU-HD. The first 40 amino acids were the most divergent region among the four species, exhibiting nonconservative differences.

In Northern (RNA) blot analysis, the human *Pit-1* cDNA hybridized to a single band of approximately 2.4 kb in human pituitary polyadenylated RNA. No Pit-1 message was observed in RNA from human decidua or placenta. This suggests that the *Pit-1* DNA response elements in *Prl* and chorionic somatomammotropin genes do not contribute to their regulation in these tissues (*12*).

Genomic cloning was done to examine regulatory elements and define exon-intron boundaries (*13*). The *Pit-1* autoregulatory binding sites described for the rat and mouse in the 5′ flanking and 5′ untranslated re-

R. W. Pfäffle, J. S. Parks, M. R. Brown, Division of Pediatric Endocrinology, Department of Pediatrics, Emory University School of Medicine, 2040 Ridgewood Drive, Atlanta, GA 30322.
G. E. DiMattia, M. G. Rosenfeld, H. A. Ingraham, Howard Hughes Medical Institute, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093.
J. M. Wit, M. Jansen, J. L. Van den Brande, Department of Pediatrics, University of Utrecht, Utrecht, The Netherlands.
H. Van der Nat, Laboratory for Virology, National Institutes of Public Health and Environmental Protection, Bilthoven, The Netherlands.

*Present address: Department of Pediatrics, RWTH Aachen School of Medicine, 5100 Aachen, Germany.
†To whom correspondence should be addressed.
‡Present address: Department of OB/GYN and Reproductive Sciences, University of California, San Francisco, CA 94143.