Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 8327–8331, September 1992
Genetics

Glucocorticoid-suppressible hyperaldosteronism results from hybrid genes created by unequal crossovers between *CYP11B1* and *CYP11B2*

(dexamethasone-suppressible hyperaldosteronism/hypertension/glucocorticoid remediable aldosteronism/steroid 11-hydroxylase/aldosterone synthase)

LEIGH PASCOE*†, KATHLEEN M. CURNOW*, LILIYA SLUTSKER*, JOHN M. C. CONNELL‡, PHYLLIS W. SPEISER*, MARIA I. NEW*, AND PERRIN C. WHITE*

*Division of Pediatric Endocrinology, Cornell University Medical Center, New York, NY 10021; and ‡MRC Blood Pressure Unit, Western Infirmary, Glasgow G11 6NT, United Kingdom

Communicated by I. S. Edelman, June 5, 1992 (received for review April 15, 1992)

ABSTRACT **Glucocorticoid-suppressible hyperaldosteronism (GSH) is an autosomal dominant form of familial hypertension. The biochemical abnormalities seen in this disorder may be remedied by administration of dexamethasone, implying that aldosterone synthesis is being abnormally regulated by corticotropin. The final three steps of aldosterone synthesis, 11β- and 18-hydroxylation and 18-oxidation, are mediated by a cytochrome P450 in the zona glomerulosa of the adrenal cortex termed CYP11B2. A related isozyme in the zona fasciculata, CYP11B1, is required for cortisol synthesis; this isozyme, which is normally expressed at much higher levels than CYP11B2, only has 11β-hydroxylase activity. These isozymes are encoded by genes on human chromosome 8q22. We have now studied four unrelated patients with GSH. We found that each patient has one chromosome that carries three *CYP11B* genes instead of two. This has presumably been generated by unequal meiotic crossing-over. The extra gene is a hybrid with 5′ regulatory and coding regions corresponding to *CYP11B1* and 3′ coding regions from *CYP11B2*. The breakpoint is in intron 2 in two cases, intron 3 in one, and exon 4 in one. Cells transfected with hybrid cDNAs containing up to the first three exons of *CYP11B1* synthesized aldosterone at levels near that of cells carrying normal *CYP11B2*, but cells transfected with hybrids containing the first five or more exons of *CYP11B1* could not synthesize detectable amounts of aldosterone. These data demonstrate that GSH is caused by expression of a gene that is regulated like *CYP11B1* but that encodes a protein able to synthesize aldosterone.**

Glucocorticoid (or dexamethasone)-suppressible hyperaldosteronism (GSH), also known as glucocorticoid remediable aldosteronism, is a dominantly inherited form of hypertension (1–3). Increased amounts of aldosterone are secreted despite suppressed levels of plasma renin. This hypersecretion is reversed by administration of glucocorticoids such as dexamethasone and exacerbated by administration of corticotropin (ACTH) (4), a characteristic that distinguishes GSH from other forms of hyperaldosteronism such as Conn syndrome. No estimate of the frequency of GSH is available, but 61 patients in 18 families have been reported in the literature (see ref. 5 for an early review).

In the normal adrenal cortex, aldosterone synthesis takes place in the zona glomerulosa and is regulated by angiotensin II and potassium. Synthesis of aldosterone requires successive 11β- and 18-hydroxylations of 11-deoxycorticosterone to corticosterone and 18-hydroxycorticosterone followed by 18-oxidation. Corticosterone is also synthesized in the zona fasciculata under the control of ACTH, but 18-hydroxylase and 18-oxidase activities are normally suppressed in this zone. In contrast, the zona fasciculata, but not the zona glomerulosa, possesses 17α-hydroxylase activity required for synthesis of cortisol, the predominant glucocorticoid in humans. Cortisol is normally secreted at levels 100–1000 times those of aldosterone.

Recent studies have clarified the role of two isozymes, CYP11B1 (P450c11, P450XIB1, steroid 11β-hydroxylase) and CYP11B2 (P450aldo, P450cmo, P450c18, P450XIB2, aldosterone synthase), in steroid biosynthesis. These isozymes, which are 93% identical in amino acid sequence, are encoded by the *CYP11B1* and *CYP11B2* genes (6) located on human chromosome 8q22 (7). CYP11B1 has 11β-hydroxylase activity *in vitro* (8–10), is normally expressed at high levels in the adrenal gland (6, 10), and is regulated by ACTH in cultured cells (10). It is mutated in patients with 11β-hydroxylase deficiency, an inherited disorder in which 11-deoxycortisol is not converted to cortisol (11). Thus, this isozyme is required for cortisol biosynthesis. CYP11B2 has 11β-hydroxylase, 18-hydroxylase, and 18-oxidase activities *in vitro* (8–10), is normally expressed at low levels in the adrenal gland, is regulated by angiotensin II but not by ACTH (10), and is mutated in individuals with an inherited inability to synthesize aldosterone, termed corticosterone methyloxidase II (CMO II) deficiency (12). Thus, this isozyme is normally essential for aldosterone synthesis.

Past hypotheses about the etiology of GSH have included partial 17α-hydroxylase deficiency (2) and the abnormal existence of a transitional cell type intermediate between adrenal glomerulosa and fasciculata cells (13, 14). However, it is apparent that GSH can best be explained as a disease of inappropriate expression of a CYP11B2-like activity in the zona fasciculata, where it would be controlled by ACTH (15). This would also explain another characteristic abnormality seen in this disorder, hypersecretion of 18-hydroxycortisol and 18-oxocortisol (16). These 17α-hydroxylated analogs of 18-hydroxycorticosterone and aldosterone cannot be synthesized in the zona glomerulosa because of that zone's lack of 17α-hydroxylase activity.

A priori, several different genetic rearrangements could cause abnormal expression of 18-hydroxylase and 18-oxidase activities. Depending on the location of functionally important amino acid residues, a transfer of coding sequences from *CYP11B2* to *CYP11B1* by a gene conversion-like event could confer these activities on CYP11B1 (10). Alternatively, jux-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GSH, glucocorticoid-suppressible hyperaldosteronism; ACTH, corticotropin; CMO II, corticosterone methyloxidase II.

†To whom reprint requests should be addressed.

taposition of *CYP11B1* regulatory sequences and *CYP11B2* coding sequences should lead to inappropriate expression of 18-hydroxylase and 18-oxidase activities in the zona fasciculata. This could occur in a gene conversion transferring 5′ regulatory sequences from *CYP11B1* to *CYP11B2* or through an unequal crossover between *CYP11B1* and *CYP11B2* during meiosis (17). The latter event would result in gametes carrying one or three *CYP11B* genes (Fig. 1). Depending on the crossover breakpoint and the normal relative positions of *CYP11B1* and *CYP11B2*, one of the gametes should contain a hybrid gene with 5′ regulatory (and some coding) sequences from *CYP11B1* and 3′ coding sequences from *CYP11B2*. If *CYP11B2* normally lies 5′ of *CYP11B1*, this hybrid gene will be on the chromosome carrying the duplication. The product of such a hybrid gene might, depending on the position of the breakpoint, have aldosterone synthetic capacity. A single copy of such a gene would lead to abnormal regulation of aldosterone synthesis and would therefore be expressed and inherited in a dominant fashion, thus explaining fully the characteristics of GSH. This type of chimeric gene has recently been demonstrated by Southern blotting to segregate with GSH in a single kindred (17).

In this study we report on four unrelated patients with GSH, each of whom has a hybrid duplicated *CYP11B* gene. The regions of the breakpoints have been amplified and sequenced. Hybrid cDNAs representing a range of possible breakpoints have been expressed in COS-1 cells. Depending on the position of the breakpoint, certain of these hybrids are indeed able to synthesize aldosterone.

MATERIALS AND METHODS

Subjects. Four unrelated patients with GSH were studied; patients 1 and 2 have been previously reported (2, 14), whereas patients 3 and 4 have not been studied previously. All had positive family histories for GSH consistent with an autosomal dominant mode of inheritance. The specific criteria for diagnosis of GSH were (*i*) mean 24-hr blood pressure >2.5 standard deviations above the mean systolic and/or diastolic levels for age, (*ii*) suppressed upright plasma renin activity, (*iii*) elevations in 24-hr urinary excretion of aldosterone in the basal state, and (*iv*) suppression of these adrenal hormones within 48 hr of administration of dexamethasone, and amelioration of hypertension within 2 weeks of initiation of chronic therapy with dexamethasone.

Amplification and Sequencing of Exons. *CYP11B1* and *CYP11B2* were each amplified in several segments by polymerase chain reactions (PCRs) using oligonucleotides corresponding to intronic sequences where the two genes differed (11, 12). Reactions were performed for 35 cycles at 94°C × 1 min, 60°C or 65°C × 1 min, and 72°C × 2 min with an additional 5 sec each cycle. Fragments containing the breakpoint were amplified from the hybrid gene in each kindred by using a *CYP11B1*-specific 5′ primer and a *CYP11B2*-specific 3′ primer. The specificity of the reactions was confirmed by control reactions on cloned DNA from each gene and by comparison of resulting sequence data to known sequence of *CYP11B1* and *CYP11B2*. Amplified fragments from each gene and the hybrid were purified by agarose gel electrophoresis, recovered on NA45 paper (Schleicher & Schuell, Keene, NH), and sequenced by the dideoxy chain-termination method using DNA denatured in the presence of dimethyl sulfoxide (18). Independent duplicate PCR reactions of the crossover regions were sequenced to reduce the possibility that the breakpoints identified were the result of PCR-mediated artifacts.

Genomic (Southern) Blotting. DNA samples (5 μg) from patients were digested with *Eco*RI, subjected to electrophoresis in a 0.8% agarose gel, and blotted to a nylon membrane (Micron Separations, Westborough, MA). The blot was hybridized overnight at 65°C with a PCR product containing exons 3–6 of *CYP11B1* cDNA that was labeled with ^{32}P by random priming. The hybridization buffer was 0.9 M NaCl/0.09 M sodium citrate/10% dextran sulfate/1% SDS/100 μg of salmon sperm DNA per ml. Final stringent washes were at 65°C in 0.03 M NaCl/0.003 M sodium citrate/1% SDS.

Mutagenesis and Subcloning of Constructs. Each hybrid cDNA was constructed by PCR mutagenesis using *CYP11B1* and *CYP11B2* cDNA (12). Briefly, DNA from the 5′ region of *CYP11B1* cDNA and 3′ region of *CYP11B2* was amplified with *Pyrococcus furiosus* DNA polymerase (Stratagene), so

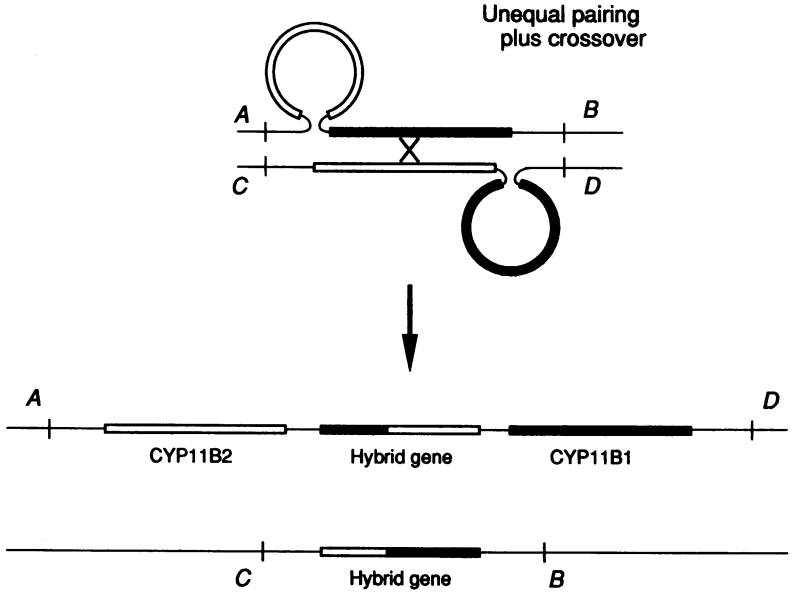

FIG. 1. Unequal crossing-over between *CYP11B1* and *CYP11B2*. *CYP11B2* is on the left and pairs with the 95% similar *CYP11B1*. The direction of transcription is from left to right. A and C and B and D are homologous regions 5′ and 3′, respectively, of the *CYP11B* genes. A crossover between these paired genes during meiosis results in one gamete with duplicated genetic material, containing a hybrid gene with 5′ sequences from *CYP11B1* and 3′ sequences from *CYP11B2*. The presence of a single copy of such an allele is sufficient to explain the features of GSH. The second gamete is deficient, containing only the complementary hybrid gene. The presence of deletions and duplications can be detected by Southern blotting of genomic DNA as a result of the altered restriction map.

that overlapping segments were produced. The 5′ and 3′ amplified segments were then gel purified, combined in a single reaction, denatured, annealed, and extended with *Pfu* polymerase to produce a full-length molecule, which was then amplified with primers from the 5′ and 3′ ends of the coding sequence. Restriction sites were included in these flanking primers to facilitate cloning into the expression vector pCMV4 (19). The complete sequence of each resulting recombinant clone was checked to ensure that no unwanted mutations had been introduced by the PCR. The hybrid constructs each consisted of exons from *CYP11B1* and *CYP11B2* spliced together as depicted in Fig. 2.

Transient Transfection Assays. Plasmid DNA was prepared by the rapid boiling method, treated with Strataclean resin (Stratagene), and ethanol precipitated. DNA was transfected into COS-1 cells using cationic liposomes (Lipofectin, GIBCO) (20). Constructs tested were as follows: pC11-B1, containing the normal sequence for CYP11B1; pC11-B2, containing the normal sequence for CYP11B2; and pC11-H1 to pC11-H7, containing various hybrid cDNAs (Fig. 2). Expression plasmids containing cDNA for human adrenodoxin, pCMV4-HAD, and human adrenodoxin reductase, pCMV4-HAR (12), were also transfected into the cells because previous studies suggested that COS cells do not express sufficient levels of adrenodoxin to permit full activity of mitochondrial cytochrome P450 enzymes (21). Approximately 10^6 cells at 80% confluence in 35-mm culture wells were exposed for 24 hr to 15 μg of Lipofectin, 1 μg of pCMV4-HAD, 1 μg of pCMV4-HAR, and either no additional DNA, 5 μg of pC11-B1 or pC11-B2, or one of the hybrid pC11-H constructs in 1 ml of Opti-MEM reduced serum medium (GIBCO-BRL). Cells were allowed to recover for 24 hr in Dulbecco's modified Eagle's medium with 10% newborn calf serum before addition of 1 nmol of deoxy[^{14}C]-corticosterone. Medium was removed 24 hr later, extracted with 3 ml of methylene chloride, dried under nitrogen gas, resuspended in 20 μl of methylene chloride, and loaded on TLC plates. Plates were developed in 300:20:1 (vol/vol) methylene chloride/methanol/H_2O. Specific products were identified by the use of appropriate labeled and unlabeled steroids as standards. Radioactivity was quantitated with an imaging scanner (System 200, Bioscan, Washington).

In parallel experiments, biosynthesis of corticosterone and aldosterone was quantitated by RIA. Transfected cells were treated identically to those used for TLC assays, except that 1 nmol of nonradioactive deoxycorticosterone was used. Concentrations of corticosterone and aldosterone were determined on duplicate samples as described (22, 23).

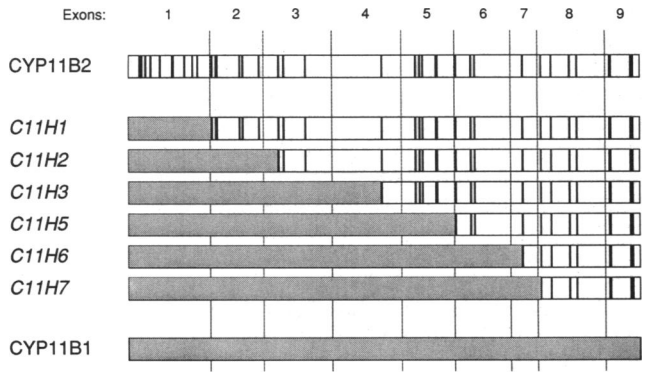

FIG. 2. Diagram of cDNA constructs subcloned into the expression vector pCMV4. The full-length cDNA for CYP11B2 is depicted as an open rectangle with black bars representing positions at which the encoded amino acid sequence differs from that of CYP11B1. Constructs were made so that different 5′ segments of *CYP11B1* (shown as stippled rectangle) are spliced to 3′ sequences from *CYP11B2*. Constructs were made with successively more exons from *CYP11B1* except that no construct was made corresponding to the first four exons of *CYP11B1*, as there was only one amino acid difference in this exon.

RESULTS

Sequencing of *CYP11B1* and *CYP11B2*. Segments of *CYP11B1* and *CYP11B2* were amplified from each affected individual with gene-specific oligonucleotides and sequenced directly. No mutations leading to a change in amino acid sequence were detected in either *CYP11B1* or *CYP11B2*. Additionally, sequences up to 500 base pairs (bp) 5′ of the initiation codon were determined. No polymorphisms related to disease status were identified in these regions.

Chimeric gene sequences were sought by performing PCR amplifications of patient and control DNA, using 5′ primers specific for *CYP11B1* and 3′ primers specific for *CYP11B2* (Fig. 3). Fragments were amplified from all four patients but not from unaffected individuals, and the actual crossover points were determined by direct sequencing. The two genes are identical in sequence in many stretches of DNA throughout their length, so it was only possible to delimit the breakpoints to regions between two points where the sequences differed. Two unrelated patients had crossovers in the same region of intron 2, a third crossed over in intron 3, and the fourth patient had a crossover in exon 4. The presence of a chromosome carrying a duplicated *CYP11B* gene was demonstrated on Southern blots of three of the four patients (Fig. 4). In a sample from one patient, the duplication was confirmed as a 40-kb increase in size of a 280-kb *Bss*HII or 250-kb *Sac* II fragment as resolved by field inversion gel electrophoresis (data not shown).

Transfection Results. As previously observed (10, 12), cells transfected with pC11-B2 were able to convert deoxycorticosterone to corticosterone, 18-hydroxycorticosterone, and aldosterone, whereas cells transfected with pC11-B1 were able to synthesize corticosterone, a barely detectable amount of 18-hydroxycorticosterone, and no detectable aldosterone (Fig. 5, Table 1).

Cells transfected with the hybrid cDNA constructs, pC11-H1, pC11-H2, and pC11-H3, all converted deoxycorticoste-

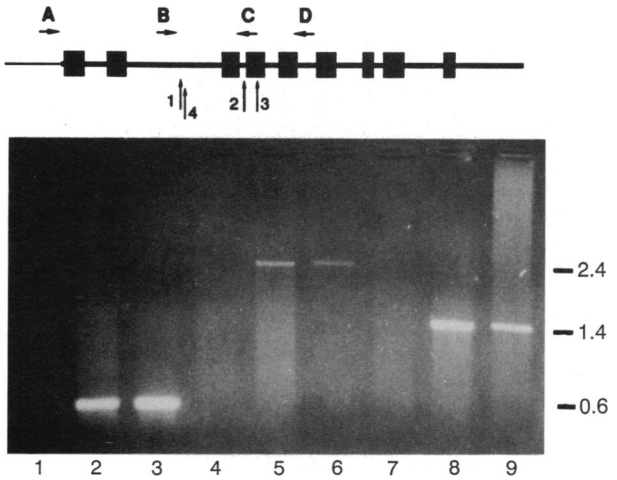

FIG. 3. Amplification of breakpoint regions from GSH patients. Genomic DNA from patients and an unaffected control individual was amplified by PCR using a *CYP11B1*-specific 5′ primer and a *CYP11B2*-specific 3′ primer. Positions of oligonucleotides used (not to scale) and positions of breakpoints for patients, determined by sequencing of PCR products, are shown on the diagram above. PCR products were resolved on a 1% agarose gel and visualized by ethidium bromide staining. Lanes: 1–3, oligonucleotides B and C with DNA from a control and patients 1 and 4, respectively; 4–6, oligonucleotides A and C with the same DNA samples; 7–9, oligonucleotides B and D with DNA from a control and patients 2 and 3.

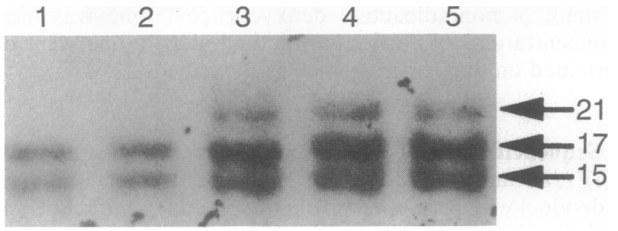

FIG. 4. Southern blots of genomic DNA from GSH patients. *Eco*RI-digested DNA was hybridized with a ^{32}P-labeled PCR product spanning exons 3–6 of *CYP11B2* cDNA. Lanes: 1 and 2, DNA from unaffected individuals; 3–5, DNA from patients 1, 2, and 3, respectively. The 15-kilobase (kb) and 17-kb fragments correspond to *CYP11B1* DNA and *CYP11B2* DNA, respectively, whereas the 21-kb fragment indicates the presence of a single copy of a hybrid gene containing the 5′ end of *CYP11B1* and the 3′ end of *CYP11B2*.

rone to corticosterone, 18-hydroxycorticosterone, and aldosterone, although a slight decrease in the ratio of aldosterone to corticosterone synthesis was consistently observed relative to cells transfected with pC11-B2. In contrast, cells transfected with pC11-H5, pC11-H6, and pC11-H7, plasmids containing more *CYP11B1* sequences, had activities similar to cells transfected with pC11-B1 in that they all made corticosterone but only minimal amounts of 18-hydroxycorticosterone and no detectable aldosterone. Similar results were obtained in assays using TLC and RIAs. The success of the transfection procedure was confirmed by detection of full-length message on a Northern blot (data not shown) as well as the fact that all constructs had 11β-hydroxylase activity. Cells transfected with pCMV4-HAR and pCMV4-HAD but not pC11 DNA gave no detectable activity by RIA or on autoradiography of TLC plates.

DISCUSSION

Rearrangements of *CYP11B* Genes. In the mouse the homologs of *CYP11B1* and *CYP11B2* (i.e., the genes respectively encoding 11β-hydroxylase and "aldosterone synthase" isozymes) are oriented so that both genes are transcribed in the same direction with the homolog of *CYP11B2* 5′ of the *CYP11B1*-like gene; the 5′ ends of the two genes are about 13 kb apart (24). Although genomic clones linking the human *CYP11B1* and *CYP11B2* genes have not yet been isolated, several lines of evidence suggest that the human genes are arranged similarly to those of the mouse. The fact that the *CYP11B1/2* hybrid gene in five unrelated GSH kindreds [this work and the kindred studied by Lifton *et al.* (17)] is on a chromosome carrying a duplication rather than a deletion is most easily explained if *CYP11B2* is normally 5′ of *CYP11B1*. In Iranian Jewish patients with CMO II deficiency, a mutation in exon 7 of *CYP11B2* is in complete linkage disequilibrium with an *Msp* I polymorphism in *CYP11B1*, whereas a mutation in exon 3 of *CYP11B2* does not segregate completely with the *Msp* I polymorphism (12, 25). This again suggests that the 3′ end of *CYP11B2* is closer to *CYP11B1*. Finally, preliminary experiments using field inversion gel electrophoresis indicate that a 40-kb segment is duplicated in a GSH patient, suggesting that this represents the normal distance between *CYP11B2* and *CYP11B1*.

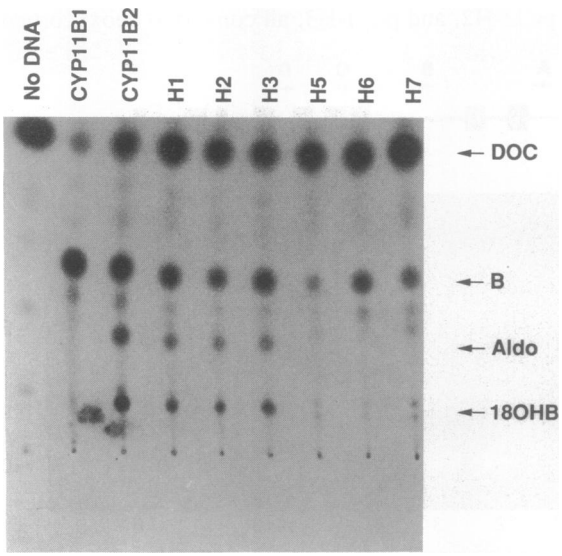

FIG. 5. Enzymatic activity of cells transfected with normal and hybrid cDNA constructs. COS-1 cells were transfected with 1 μg each of pCMV4-HAD and pCMV4-HAR and 5 μg of pC11-B1 (CYP11B1), pC11-B2 (CYP11B2), or pC11-H1 to pC11-H7, or no additional DNA (No DNA). The transfected cells were incubated with 11-deoxy[^{14}C]corticosterone, and the resulting metabolites were identified by TLC chromatography and autoradiography. Positions of steroids are marked on the autoradiogram as DOC (11-deoxycorticosterone), B (corticosterone), 18OHB (18-hydroxycorticosterone), and Aldo (aldosterone).

Table 1. Enzymatic activities of COS cells transfected with pC11 constructs

Construct	% conversion			Aldo:B	
	B	18-OHB	Aldo	TLC	RIA
B1	74	0	0	0	0
B2	30	22	11	0.36	0.40
H1	21	7	5	0.24	0.31
H2	22	8	6	0.24	0.31
H3	28	9	6	0.21	0.23
H5	8	0	0	0	0
H6	12	0	0	0	0
H7	13	0	0	0	0

Constructs are described in the legend to Fig. 2. Percent conversion of deoxycorticosterone to corticosterone (B), 18-hydroxycorticosterone (18-OHB), and aldosterone (Aldo) is shown for each construct. These figures are derived from the experiment shown in Fig. 5. The ratio of aldosterone to corticosterone (Aldo:B) produced by cells transfected with each construct is shown for this experiment (TLC) and for RIA of an independent experiment.

The reciprocal product of the unequal crossover causing each GSH allele is a deleted chromosome carrying only a single hybrid gene containing a 5′ region derived from *CYP11B2* and a 3′ region derived from *CYP11B1*. Such a gene should be expressed at low levels and only in the zona glomerulosa. The corresponding hybrid protein should have 11β-hydroxylase activity but, depending on the breakpoint, not 18-hydroxylase or 18-oxidase activity. Such a gene should function essentially as a null allele. Carriers could be expected to suffer from 11β-hydroxylase deficiency (due to low levels of expression of the hybrid gene) or CMO II deficiency (due to lack of 18-oxidase activity of the encoded enzyme) when they inherit a second defective allele with a mutation in *CYP11B1* or *CYP11B2*, respectively. Though chromosomes with such deletions are presumably segregating in populations, none has been detected to date in 30 independent 11β-hydroxylase deficiency alleles (unpublished observations).

The unequal crossovers involving the *CYP11B* genes are reminiscent of those seen in globin genes, causing Hb-Lepore (26), and in another disorder of adrenal steroid biosynthesis, congenital adrenal hyperplasia due to 21-hydroxylase deficiency. About 20% of mutant alleles in the latter disease are net deletions of the active *CYP21* gene due to unequal crossing-over between *CYP21* and the 30-kb distant *CYP21P* pseudogene (reviewed in ref. 27). Deletions or duplications of *CYP21P* are seen in about 10% of all normal chromosomes. It is not clear why unequal crossovers involving the *CYP21*

genes are apparently so much more common than similar rearrangements of the *CYP11B* genes, given that the distance between the members of each pair of genes is similar. The *CYP21* genes are located within the *HLA* major histocompatibility complex on chromosome 6p21.3, and frequent rearrangements involving genes for transplantation antigens are characteristic of this chromosomal region. The mechanism(s) promoting such rearrangements is not known.

Function of the Hybrid Gene Products. All constructs used in this study confer 11β-hydroxylase activity on COS cells. Replacement of the first three exons of *CYP11B2* by *CYP11B1* sequences leaves the 18-hydroxylase and 18-oxidase activities of CYP11B2 intact and we infer that the CYP11B2-specific residues essential for these activities occur after residue 247. There are only 19 differences in the amino acid sequences of CYP11B1 and CYP11B2 that are 3′ of this residue; further studies of mutant cDNAs may be able to identify particular residues that are necessary for aldosterone synthetic activity.

The activities observed in the transient transfection assays suggest limits on the positions of breakpoints that will lead to GSH. Breakpoints through exon 4 (codon 247) result in a hybrid protein that could be expected to lead to full GSH. Conversely, breakpoints after codon 302 result in proteins with 18-hydroxylase and 18-oxidase activities that are undetectable in *in vitro* assays, although a low level of activity (a few percent of the activity of CYP11B2) cannot be ruled out. Carriers of such hybrid genes may be expected to be asymptomatic or perhaps suffer a relatively mild version of the disease.

An important aspect of GSH is that affected individuals have elevated blood pressure due to hypersecretion of aldosterone and possibly other mineralocorticoids such as 18-oxocortisol. Although classic GSH is a rare cause of hypertension, it is possible that substantially larger numbers of patients with hypertension have milder forms of this disorder due either to crossovers located after exon 5 or to gene conversions. Such a situation would be analogous to that seen in 21-hydroxylase deficiency, where mild forms of the disease are about 100 times more frequent than the classic disorder. Elucidating the molecular basis of GSH may thus have important ramifications for the larger problem of essential hypertension.

We thank Mr. C. Crawford for assistance with preparation of figures. This work was supported by Grants DK42169 from the National Institutes of Health and 6-609 from the March of Dimes as well as a grant from the Horace Goldsmith Foundation. K.M.C. is supported by a fellowship from the Klosk Foundation, and P.C.W. is a Scholar of the Irma T. Hirschl Trust.

1. Sutherland, D. J. A., Ruse, J. L. & Laidlaw, J. C. (1966) *Can. Med. Assoc. J.* **95**, 1109–1119.
2. New, M. I. & Peterson, R. E. (1967) *J. Clin. Endocrinol. Metab.* **27**, 300–305.
3. New, M. I., Oberfield, S. E., Levine, L. S., Dupont, B., Pollack, M. S., Gill, J. R. & Bartter, F. C. (1980) *Lancet* **i**, 550–551.
4. Oberfield, S. E., Levine, L. S., Stoner, E., Chow, D., Rauh, W., Greig, F., Lee, S. M., Lightner, E., Witte, M. & New, M. I. (1981) *J. Clin. Endocrinol. Metab.* **53**, 158–164.
5. New, M. I. & Borelli, P., eds. (1986) Dexamethasone-Suppressible Hyperaldosteronism, Serono Symp. 10, Ares-Serono Symposia, Rome.
6. Mornet, E., Dupont, J., Vitek, A. & White, P. C. (1989) *J. Biol. Chem.* **264**, 20961–20967.
7. Chua, S. C., Szabo, P., Vitek, A., Grzeschik, K. H., John, M. & White, P. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7193–7197.
8. Kawamoto, T., Mitsuuchi, Y., Ohnishi, T., Ichikawa, Y., Yokoyama, Y., Sumimoto, H., Toda, K., Miyahara, K., Kuribayashi, I., Nakao, K., Hosoda, K., Yamamoto, Y., Imura, H. & Shizuta, Y. (1990) *Biochem. Biophys. Res. Commun.* **173**, 309–316.
9. Ogishima, T., Shibata, H., Shimada, H., Mitani, F., Suzuki, H., Saruta, T. & Ishimura, Y. (1991) *J. Biol. Chem.* **266**, 10731–10734.
10. Curnow, K. M., Tusie-Luna, M. T., Pascoe, L., Natarajan, R., Gu, J. L., Nadler, J. L. & White, P. C. (1991) *Mol. Endocrinol.* **5**, 1513–1522.
11. White, P. C., Dupont, J., New, M. I., Leiberman, E., Hochberg, Z. & Rosler, A. (1991) *J. Clin. Invest.* **87**, 1664–1667.
12. Pascoe, L., Curnow, K. M., Slutsker, L., Rosler, A. & White, P. C. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4996–5000.
13. Gomez-Sanchez, C. E. (1984) *Endocrin. Res.* **10**, 609–615.
14. Connell, J. M. C., Kenyon, C. J., Corrie, J. E. T., Fraser, R., Watt, R. & Lever, A. F. (1986) *Hypertension* **8**, 669–676.
15. White, P. C. (1991) *Endocrinol. Res.* **17**, 85–108.
16. Ulick, S., Chan, C. K., Gill, J. R., Gutkin, M., Letcher, L., Mantero, F. & New, M. I. (1990) *J. Clin. Endocrinol. Metab.* **71**, 1151–1157.
17. Lifton, R. P., Dluhy, R. G., Powers, M., Rich, G. M., Cook, S., Ulick, S. & Lalouel, J. M. (1992) *Nature (London)* **355**, 262–265.
18. Winship, P. R. (1989) *Nucleic Acids Res.* **17**, 1266.
19. Andersson, S., Davis, D. L., Dahlback, H., Jornvall, H. & Russell, D. W. (1989) *J. Biol. Chem.* **264**, 8222–8229.
20. Fuerst, T. R., Niles, E. G., Studier, R. W. & Moss, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8122–8126.
21. Zuber, M. X., Mason, J. I., Simpson, E. R. & Waterman, M. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 699–703.
22. Rauh, W., Levine, L. S., Gottesdiener, K. & New, M. I. (1978) *Klin. Wochenschr.* **56**, 161–167.
23. Chakmakjian, Z. H., Pryor, W. W. & Abraham, G. E. (1974) *Anal. Lett.* **7**, 97–108.
24. Domalik, L. J., Chaplin, D. D., Kirkman, M. S., Wu, R. C., Liu, W., Howard, T. A., Seldin, M. F. & Parker, K. L. (1991) *Mol. Endocrinol.* **5**, 1853–1861.
25. Globerman, H., Rosler, A., Theodor, R., New, M. I. & White, P. C. (1988) *N. Engl. J. Med.* **319**, 1193–1197.
26. Flavell, R. A., Kooter, J. M., De Boer, E., Little, P. F. R. & Williamson, R. (1978) *Cell* **15**, 25–41.
27. White, P. C. & New, M. I. (1992) *J. Clin. Endocrinol. Metab.* **74**, 6–11.