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A *de novo* missense mutation of the β subunit of the epithelial sodium channel causes hypertension and Liddle syndrome, identifying a proline-rich segment critical for regulation of channel activity

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ABSTRACT **Liddle syndrome is a mendelian form of hypertension characterized by constitutively elevated renal Na reabsorption that can result from activating mutations in the β or γ subunit of the epithelial Na channel. All reported mutations have deleted the last 45–76 normal amino acids from the cytoplasmic C terminus of one of these channel subunits. While these findings implicate these terminal segments in the normal negative regulation of channel activity, they do not identify the amino acid residues that are critical targets for these mutations. Potential targets include the short highly conserved Pro-rich segments present in the C terminus of β and γ subunits; these segments are similar to SH3-binding domains that mediate protein–protein interaction. We now report a kindred with Liddle syndrome in which affected patients have a mutation in codon 616 of the β subunit resulting in substitution of a Leu for one of these highly conserved Pro residues. The functional significance of this mutation is demonstrated both by the finding that this is a *de novo* mutation appearing concordantly with the appearance of Liddle syndrome in the kindred and also by the marked activation of amiloride-sensitive Na channel activity seen in *Xenopus* oocytes expressing channels containing this mutant subunit (8.8-fold increase compared with control oocytes expressing normal channel subunits; $P = 0.003$). These findings demonstrate a *de novo* missense mutation causing Liddle syndrome and identify a critical channel residue important for the normal regulation of Na reabsorption in humans.**

Hypertension is a common disorder imparting an increased risk of death from stroke, myocardial infarction, and end-stage renal disease. In a substantial subset of the hypertensive population, blood pressure is sensitive to the effects of dietary salt; the precise mechanism(s) underlying this salt-sensitivity remains unknown in the vast majority of affected subjects. Liddle syndrome represents an extreme form of such salt-sensitive hypertension. This disorder is inherited as an autosomal dominant trait and is characterized by hypertension associated with low plasma renin activity, low aldosterone level, and hypokalemia (1). The hypertension and hypokalemia are corrected by low salt diet in conjunction with therapy with triamterene or amiloride but are not affected by mineralocorticoid antagonists; renal transplantation also corrects the defect (2). Elucidation of the pathogenesis of this trait may provide insights into the mechanisms of other forms of salt-sensitive low-renin hypertension.

We have demonstrated (3, 4) that Liddle syndrome is a disease of the amiloride-sensitive epithelial Na channel (ENaC). This channel is composed of three subunits—α (αENaC), β (βENaC), and γ (γENaC)—of similar structure: each subunit is composed of intracytoplasmic N and C termini, two transmembrane-spanning domains, and a large extracellular loop (5–7). Patients with Liddle syndrome have mutations in βENaC (gene symbol *SCNN1B*) or γENaC (gene symbol *SCNN1C*) that cosegregate with the syndrome in extended kindreds (3, 4). To date, all of these mutations have introduced premature termination codons or frameshift mutations into the segments encoding the cytoplasmic C termini of one of these subunits, deleting the last 45–76 normal amino acids from the encoded subunits (3, 4). Expression of truncated βENaC in conjunction with normal αENaC and γENaC subunits in *Xenopus* oocytes results in markedly increased amiloride-sensitive Na transport compared with oocytes expressing three normal subunits (8); expression of similarly truncated γENaC subunits also results in increased Na transport, and the coexpression of the two mutant subunits results in an additive increase in activity beyond that seen for channels bearing a single truncated subunit (8).

These findings demonstrate the importance of the cytoplasmic C termini of βENaC and γENaC in the normal negative regulation of channel activity and indicate that Liddle syndrome results from constitutively increased Na reabsorption via this channel in the distal nephron. These findings fail, however, to pinpoint critical amino acid residues in these segments in which mutation can result in activated channel activity. Potential targets for such mutations include the highly conserved Pro-rich segments present in the C termini of both the β and γ subunits; these segments have features similar to SH3 binding domains, segments that can mediate protein–protein interaction (9, 10).

We now report a kindred with Liddle syndrome due to a missense mutation in βENaC that results in substitution of a Leu residue for one of the highly conserved Pro residues in the Pro-rich segment of the C terminus of this protein (βENaC[L616]). This is a *de novo* mutation, appearing concordantly with Liddle syndrome in the kindred. The functional significance of this mutation is further indicated by its expression in *Xenopus* oocytes, which demonstrates that this mutation results in markedly activated amiloride-sensitive Na channel activity. These findings provide insight into the specific amino acid residues of these subunits that are essential for the normal regulation of channel activity and have implications for

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Abbreviations: SSCP, single-strand conformational polymorphism; cRNA, complementary RNA; ENaC, epithelial Na channel; K242, kindred 242.

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the mechanism by which these subunits regulate channel activity.

METHODS

Characterization of Kindred 242 (K242). Subjects III-1 and III-2 were evaluated at the Children's Medical Center at Stony Brook. Clinical data of other family members were obtained from their physicians. Venous blood samples were collected from family members, and total genomic DNA was prepared by standard methods (3). The research protocol was approved by the Human Investigation Committee of the Yale University School of Medicine.

Single-Strand Conformation Polymorphism and DNA Sequence Analysis. The last coding exon of the genes encoding βENaC and γENaC were screened for molecular variants by single-strand conformational polymorphism (SSCP) (11) as described (3, 4). In brief, specific primers were used to direct PCR with genomic DNA as template; five primer pairs, each amplifying 190- to 275-bp segments of the C terminus of βENaC or γENaC, were employed. Products were labeled by inclusion of [α-^{32}P]dCTP in the PCR. PCR products were denatured and fractionated at room temperature or 4°C by electrophoresis under nondenaturing conditions on polyacrylamide gels or MDE gels (AT Biochem, Malvern, PA). After autoradiography of gels, conformers were identified, excised from the gel, and eluted. The products were then reamplified by PCR and subjected to direct DNA sequence analysis using an ABI 373 automated DNA sequencer and a standard protocol (3). DNA sequences were confirmed by sequencing both DNA strands.

Genotyping and Parental Testing. Genotyping was performed by PCR, amplifying specific segments from genomic DNA with specific primers for each marker locus; loci employed for parental testing included *D3S1764, D4S1627, D5S819, D7S820, CPY19, D14S48, D16S420*, and *D16S398* (12). PCR with specific primers was performed under conditions optimized for each individual primer pair. Genotypes were analyzed by electrophoresis on standard DNA sequencing gels and subsequent autoradiography. All genotypes were scored independently by two investigators. If one of a child's reported parents is in fact biologically unrelated, for a given marker, the likelihood that the marker allele from the true (missing) parent that is seen in the child will match one of the two alleles of the unrelated (nonbiological) parent is $[1 - (1 - p)^2]$, where p is the average allele frequency of alleles of the marker; the likelihood that n markers will all show such a match is $[1 - (1 - p)^2]^n$. For a marker of heterozygosity h, the average allele frequency $p = 1 - h$ (13).

Construction of βENaC[L616]. The Pro → Leu mutation at codon 616 was introduced into the rat βENaC cDNA by two-step PCR mutagenesis. The first PCR step used a 5′ mutagenic primer and a normal 3′ primer, using cloned rat βENaC cDNA (6) as a template. The product of this PCR was used as the 3′ primer in conjunction with another 5′ primer to again direct PCR with the βENaC cDNA as template. The product contained the desired mutation flanked by single *Bam*HI and *Spe* I restriction endonuclease cleavage sites. In parallel, corresponding segment of rat βENaC cDNA in vector PSD5 (8) was removed by digestion with *Bam*HI and *Spe* I. The mutant PCR product was then ligated into the βENaC cDNA and transformed into *Escherichia coli* DH5α. The DNA sequence of resulting clones confirmed the introduction of the single expected mutation.

Expression Studies of Normal and Mutant ENaC in *Xenopus* Oocytes. Complementary RNAs (cRNA) of α, β, and γ subunits were synthesized *in vitro*, and equal saturating concentrations of each subunit cRNA (5–8 ng of cRNA encoding each subunit per oocyte) were injected into stage V and VI oocytes as described (8). Twenty-four hours after injection, whole-oocyte currents were measured with a two-electrode voltage-clamp technique in 115 mM NaCl/5 mM KCl/1.8 mM $CaCl_2$/10 mM Hepes·NaOH, pH 7.2. The expressed ENaC activity was assessed by measurement of the amiloride-sensitive Na current, defined as the difference between the Na current recorded at a membrane potential of 100 mV in the absence and presence in the medium of 5 μM amiloride. The currents produced by injection of saturating amounts of cRNA of mutant or wild-type constructs into oocytes obtained from the same animal on the same day were compared in a series of five to seven paired experiments, with 8–10 oocytes studied with each construct in each experiment. The statistical significance of results was analyzed by use of the paired t test.

RESULTS

Liddle Syndrome K242. Liddle syndrome K242 was ascertained through an 11-year-old African American female, first reported to have elevated blood pressure at 18 months. At the age of 6 years, she was found to have systolic and diastolic blood pressures >3 SDs above the mean for her age and sex (14) and hypokalemia (Table 1; the index case number is subject III-1, representing generation III, subject number 1). Physical exam revealed no signs of virilization, and blood pressures in the lower extremities were greater than in the upper extremities. Other electrolytes, hematocrit, blood urea nitrogen, creatinine, and urinalysis were within normal limits. Further evaluation revealed suppressed plasma renin activity (0.1 ng per ml per hr, supine; 0.3 ng per ml per hr, upright) and suppressed plasma aldosterone concentration (3.7 ng/dl, supine; <2.5 ng/dl, upright). Measurements of urinary cortisol/cortisone metabolite ratio, 24-hr urinary 17-ketosteroids, 24-hr urinary free cortisol, and urinary catecholamines were all within normal limits. Renal ultrasound was normal, and a renal scan revealed equal perfusion of both kidneys with a normal estimated glomerular filtration rate (105 ml/min).

The patient was treated with a variety of antihypertensive medications with inadequate control of blood pressure, leading to hospital admission at age 8 years and 5 months. Blood pressures ranged from 152 to 161 mmHg (systolic) and from 93 to 108 mmHg (diastolic) (1 mmHg = 133 pa) in the hospital (mean, 156/100 mmHg). Serum potassium was 2.9 mM. The patient's blood pressure and hypokalemia did not improve in response to the mineralocorticoid antagonist spironolactone or to dexamethasone but ultimately improved in response to treatment with triamterene in conjunction with a low sodium diet, with a mean blood pressure of 115/63 mmHg.

The age of onset and severity of this subject's hypertension in conjunction with her hypokalemia prompted evaluation of other members of her family. Her father had died from trauma;

Table 1. Clinical features of subjects of Liddle syndrome K242

	I-1	I-2	II-1	II-2	II-3*	II-4	II-5	II-6	III-1*	III-2*
Age, years	76	71	43	46	41	34	36	40	11	13
Age at Dx of HTN, years	—	42	41	—	15	—	—	—	1.5	1.5
BP, mmHg	146/72	170/90	142/100	130/80	180/140	120/80	112/78	112/84	140/95	130/90
K^+, mM	5.0	4.2	4.2	ND	2.8	3.7	ND	4.6	2.9	3.3

Dx, diagnosis; HTN, hypertension; BP, blood pressure; K^+, serum potassium, lowest off therapy (normal, 3.5–5.0 mM); ND, not done.
*Subjects diagnosed with Liddle syndrome.

he had no history of hypertension. The index subject's older brother (subject III-2) also had blood pressure >3 SDs above the mean for age and sex and spontaneous hypokalemia (Table 1); he also had suppressed plasma renin activity (<0.2 ng per dl per hr with concurrent 24-hr urinary Na of 34 meq) and suppressed plasma aldersterone concentration (3.6 ng/dl). Renal function was normal. He was treated with a low salt diet and triamterene, with a resulting mean ambulatory blood pressure of 122/70 mmHg. The index subject's mother, subject II-3, was diagnosed as hypertensive at age 15; severe hypertension and hypokalemia were documented (Table 1). Rapid sequence intravenous pyelorogram, computed tomography of the adrenal glands and kidneys, and renal arteriogram were normal. Her clinical course was complicated by a stroke at age 21, which left her with mild residual right-sided weakness.

The findings of hypertension in childhood with hypokalemia, suppressed plasma renin activity and plasma aldersterone concentration, and the absence of clinical response to exogenous glucocorticoids, in conjunction with the vertical transmission of early hypertension and hypokalemia are diagnostic of Liddle syndrome and exclude the diagnosis of both glucocorticoid-remediable aldosteronism and the syndrome of apparent mineralocorticoid excess, the two other mendelian forms of hypertension in the differential diagnosis (15). The clinical features of this family are indistinguishable by any criterion from Liddle syndrome found in kindreds harboring mutations in βENaC or γENaC (3, 4).

A Missense Mutation in βENaC in K242. We screened the last coding exon of both βENaC and γENaC of these three members of K242 for molecular variants by SSCP (11). This analysis identified a variant in one segment of βENaC in all three subjects (Fig. 1*A*); no other variants in βENaC or γENaC were detected. This variant was not seen in 1000 alleles from control subjects of Caucasian, African American, or Asian descent, indicating that this variant does not represent a common polymorphism. Direct sequence analysis of this variant demonstrates the presence of a single base substitution in this segment of βENaC, a C → T transition that changes the encoded Pro at codon 616 to Leu (Fig. 2). This variant, βENaC[L616], is in a 13-aa Pro-rich segment of the C terminus of βENaC (Fig. 2*B*) that is completely conserved from *Xenopus laevis* to rats to humans (3, 6, 16); moreover, while the C termini of βENaC and γENaC are generally highly divergent,

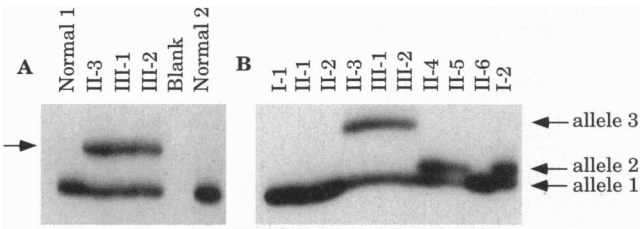

FIG. 1. Variant in βENaC in K242. (*A*) SSCP of the C terminus of βENaC in affected subjects of K242. A segment encoding a portion of the C terminus of βENaC was amplified from the three clinically affected individuals with Liddle syndrome in K242 and fractionated by electrophoresis; primers used were βENaC 1800 (5′-CTGGTGGAGGCCCACACCAAC-3′) and βENaC 2000 (5′-TGGCTGCTCAGTGAGTTTCAG-3′). Autoradiography reveals the presence of a variant in affected members of K242 (indicated by arrow). Members of K242 are numbered as in Table 1 and Fig. 3; the lane labeled "Blank" contains the product of a PCR to which no genomic DNA was added. (*B*) SSCP of the extended family of K242. Genomic DNA of members of the extended kindred of K242 (for pedigree structure, see Fig. 3) was used as template to direct PCR as in *A*, and the products were analyzed as above. Alleles are numbered at the right, with the allele corresponding to the common allele in normal subjects designated allele 1. The variant allele 3 is seen in all three subjects with Liddle syndrome and is absent in all other members of the kindred, including subjects I-1 and I-2, the parents of affected subject II-3.

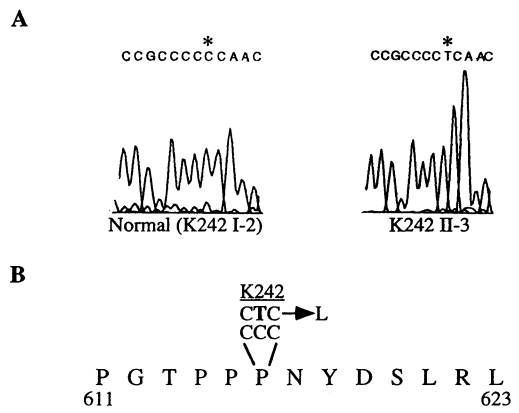

FIG. 2. Missense mutation in codon 616 of βENaC in K242. (*A*) Sequence of wild-type and mutant alleles from K242. The variant identified in subject II-3 in Fig. 1 was subjected to DNA sequence analysis; the resulting sequence from codons 614 to 617 is shown and was compared to the normal sequence (3), in this case obtained from allele 1 of subject I-2 shown in Fig. 1*B*. The site of the single-base difference between these two alleles is indicated by an asterisk. (*B*) Effect of the SSCP variant on encoded βENaC. The amino acid sequence of the completely conserved 13-aa Pro-rich segment of the C terminus of βENaC is shown; the point mutation in subject II-3 shown in *A* changes codon 616 from Pro to Leu.

10 of these 13 aa, including Pro-616, are conserved in these two subunits (6). This segment has features similar to those of SH3-binding domains that can mediate protein–protein interaction (9, 10).

βENaC[L616] Is a *de Novo* Mutation. To assess the functional significance of βENaC[L616], we investigated the grandparents and five maternal aunts and uncles of the index subject (Table 1 and Fig. 3). None of these individuals had a history of hypertension in childhood or hypokalemia. The maternal grandmother had been diagnosed with mild hypertension at age 42; all potassium levels were >4.0 mM, including a potassium of 4.2 mM while on a thiazide diuretic. One sibling of subject II-3 had been diagnosed with mild hypertension at age 41, having had normal blood pressure at younger ages and normal potassium levels on repeated measurement. On the basis of the absence of early onset of hypertension and the absence of hypokalemia, none of these patients were felt to have findings suggestive of or diagnostic of Liddle syndrome.

We screened these additional members of K242 for the presence of βENaC[L616]. By SSCP analysis, neither of the parents of affected subject II-3 nor any of her five siblings displayed this variant (Fig. 1*B*). This finding could be explained if this mutation was *de novo* in subject II-3; alternatively, this result could be seen if one or both reported parents were not a true biological parent of subject II-3.

To test for parentage in this kindred, we determined the genotypes of eight highly informative genetic markers from seven chromosomes in K242; the reported heterozygosity of these markers ranged from 80% to 83% (12); 13 of the 16 genotypes of these eight markers in subjects I-1 and I-2 were heterozygous, consistent with such high heterozygosity. For each marker, subject II-3 and all of her siblings shared 1 allele with each of the reported parents (data not shown), strongly supporting subjects I-1 and I-2 as the biological parents of all subjects in generation II (the probability of achieving this result if one of the reported parents of subject II-3 were biologically unrelated equals 0.0003 by assuming heterozygosity of each marker of 0.8).

If the Leu-616 mutation arose *de novo* in patient II-3, the βENaC allele on which it occurred should not harbor this mutation in the parent from whom it was inherited. We can identify the appropriate parental allele by analysis of haplotypes spanning the βENaC locus, taking advantage of our prior

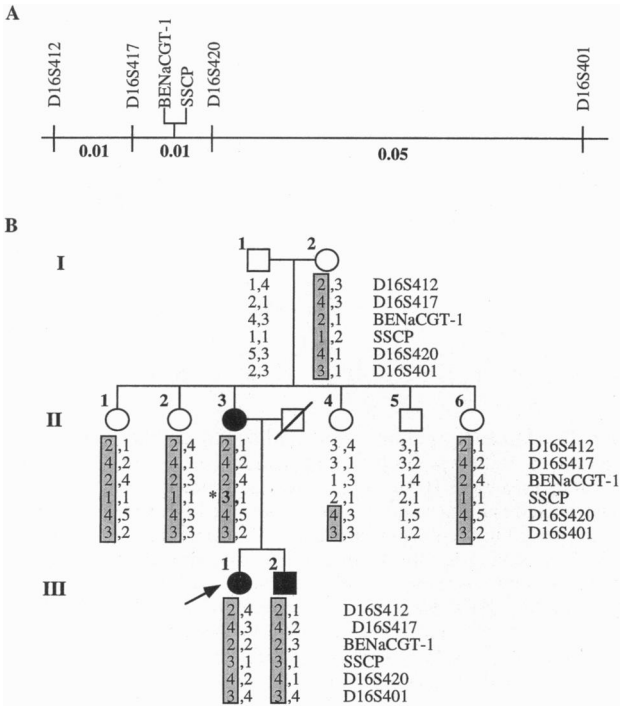

FIG. 3. βENaC[L616] is a *de novo* mutation. (*A*) Genetic map spanning the locus encoding βENaC. A segment of the genetic map of human chromosome 16p (12) spanning the βENaC locus is shown. The order of loci and the recombination fraction between adjacent loci is indicated. The marker BENaCGT-1 and the SSCP variants identified in the C terminus of βENaC are both intragenic. (*B*) Genotypes and haplotypes spanning βENaC in K242. The family relationships of members of K242 are shown. Individuals classified as affected with Liddle syndrome are shown as solid symbols; individuals classified as unaffected are shown as open symbols. The index subject is indicated by the arrow and the individuals are numbered as in Table 1 and Fig. 1. Below each symbol, genotypes of loci spanning the βENaC locus are shown. Unambiguous haplotypes can be determined, demonstrating that the mutant allele encoding βENaC[L616] arose *de novo* in subject II-3 on a haplotype inherited from subject I-2. Segments derived from this haplotype are shaded and the mutation is indicated by an asterisk.

localization of βENaC on the genetic map of human chromosome 16 (3). We determined genotypes in K242 of five highly informative markers closely linked to βENaC in K242 (Fig. 3). Unambiguous haplotypes were defined, providing strong additional support that subjects I-1 and I-2 are the biological parents of all subjects in generation II. The SSCP variant bearing Leu-616 in all three Liddle subjects (allele 3 from Fig. 1) is seen to be linked to a haplotype inherited from subject I-2 (Fig. 3). This variant SSCP allele, however, is not present on this haplotype in subject I-2 nor in subject I-2's three other offspring who have inherited this haplotype (Fig. 3).

Knowledge that the βENaC[L616] mutation in subject II-3 arose on the haplotype bearing SSCP allele 1 in subject I-2 permitted us to sequence this allele from this subject; this sequence reveals only the normal or wild-type sequence including the normal codon encoding Pro-616 (Fig. 2*A*). SSCP variant allele 2 from subject I-2 was also sequenced and also bears Pro-616; this conformer does contain a different variant, with a Thr → Met replacement at codon 592. This latter variant is common in African Americans (R.S. and R.P.L., unpublished data) and is present on the haplotype that is not transmitted to subject II-3. These findings demonstrate that βENaC[L616] arose as a *de novo* mutation in subject II-3; the simultaneous appearance of Liddle syndrome and βENaC[L616] in patient II-3 in K242, coupled with transmission of both the clinical syndrome and the mutation to her offspring provides genetic evidence that βENaC[L616] is the functional mutation causing Liddle syndrome in this kindred.

Expression of Mutant βENaC Increases ENaC Activity in *Xenopus* Oocytes. We can further assess the functional significance of βENaC[L616] by expression of subunits bearing this mutation in *Xenopus* oocytes. If βENaC[L616] causes Liddle syndrome, we anticipate that oocytes expressing channels containing this mutant subunit will display similarly increased amiloride-sensitive Na conductance.

We introduced the Leu-616 mutation into the corresponding conserved position of the rat βENaC cDNA, expressed this mutant or the wild-type βENaC in *Xenopus* oocytes in conjunction with the wild-type α and γ subunits, and measured the resulting whole-cell amiloride-sensitive Na current by two-electrode voltage clamp. In addition, oocytes expressing channels containing truncated βENaC or both truncated βENaC and truncated γENaC were studied as positive controls for activated channel activity (8). Oocytes expressing βENaC[L616] displayed a highly significant 8.8-fold increase in amiloride-sensitive Na current compared with oocytes expressing wild-type channels ($P = 0.003$; Fig. 4). This finding demonstrates that this single amino acid substitution results in a marked increase in Na channel activity and demonstrates at a biochemical level that this mutation can account for channel activation and Liddle syndrome in this family.

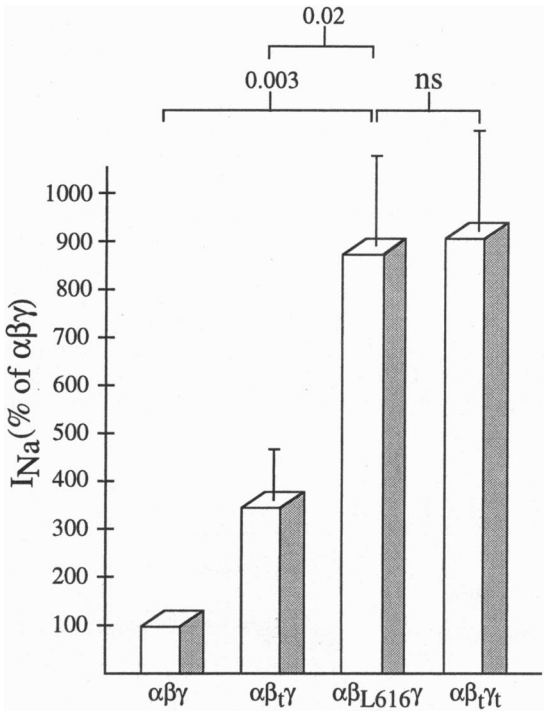

FIG. 4. Expression of channels containing βENaC[L616] in *Xenopus* oocytes. cRNAs encoding normal or mutant α, β, and γ subunits of the rat ENaC were coinjected into *Xenopus* oocytes, and the resulting amiloride-sensitive Na current was measured. Wild-type α, β, and γ cRNAs were coinjected as normal controls (represented as αβγ); expression of channels containing βENaC truncated at position 564 ($\alpha\beta_T\gamma$) or channels containing both the truncated β subunit and a γ subunit truncated at position 571 ($\alpha\beta_T\gamma_T$) served as positive controls for activated Na channels (8). The current in oocytes expressing the mutant βENaC[L616] (denoted as αβL616γ) was measured and compared to the current seen in paired oocytes expressing control channels. The results are expressed as the mean and SEM of the percentage of wild-type current. The *P* values for paired *t* tests comparing activity of αβL616γ and control channels are shown. The mean amiloride-sensitive Na current for oocytes expressing wild-type channels was 3.63 ± 0.68 μA. ns, Not significant; I_{Na}, Na current.

Surprisingly, the amiloride-sensitive Na current resulting from this single amino acid substitution was 2.5-fold greater than that seen in oocytes expressing βENaC[564stop], which deletes the last 75 aa of the C terminus of βENaC (Fig. 4; $P < 0.02$). We have shown (8) that coexpression of truncated β and truncated γ subunits (γENaC[571stop]) increases Na channel activity in an additive fashion when compared with the effects of either truncation alone. The magnitude of the current in channels containing βENaC[L616] is indistinguishable from that seen with this double mutant (Fig. 4), suggesting that βENaC[L616] in some fashion mimics the double β-γ deletion. If this is correct, we would expect that coexpression of a truncated γ subunit would not further augment channel activity. Coexpression of βENaC[L616] with a truncated γ subunit (γENaC[571stop]) and wild-type αENaC resulted in channel activity not significantly different from that seen with βENaC[L616] and wild-type α and γ subunits (8.8 ± 1.39 times increase compared with wild-type for βENaC[L616], 6.6 ± 1.24 times increase compared with wild-type for the βENaC[L616], γENaC[571stop] double mutant).

DISCUSSION

We have identified a *de novo* missense mutation causing Liddle syndrome, a salt-sensitive form of human hypertension. From a clinical standpoint, the finding of a *de novo* mutation among the small number of Liddle syndrome mutations described thus far reveals the potential unreliability of using the absence of a family history of Liddle syndrome to exclude the diagnosis in an apparently sporadic subject with low-renin hypertension associated with hypokalemia and suppressed aldosterone levels. This underscores the difficulty in establishing or excluding this diagnosis on clinical grounds.

All previously reported Liddle mutations have resulted in deletion of at least 45 normal amino acids from the C terminus of either βENaC or γENaC (3, 4); βENaC[L616] demonstrates that this trait can be produced by missense mutation and identifies one critical amino acid required for the normal negative regulation of channel activity. This mutation occurs in a completely conserved Pro-rich segment with some features of an SH3 binding domain (9, 10), raising the possibility that a normal function of this segment might be to bind to another protein; in this case, the βENaC[L616] mutation might cause Liddle syndrome by disrupting or altering this interaction. Another Pro-rich segment of the C terminus αENaC has been shown to bind to an SH3 domain of spectrin, supporting this possibility (17). This observation leads to the prediction that a protein specifically binding to this segment is essential for normal regulation of channel activity and that Pro-616 is required for this binding. Further work will be required to test this hypothesis and to define other residues in this segment that affect channel activity. In addition, it will be of considerable interest to determine whether similar missense mutations in the γ subunit have comparable effects on Na transport.

We have shown (8) that expression of channels containing either truncated β or γ subunits results in activation of channel activity and channels containing both truncated subunits show additive effects; these findings have been replicated in the present study (Fig. 4). The findings that βENaC[L616] has a larger effect on Na channel activity than deletion of the entire cytoplasmic C terminus and that addition of a truncated γ subunit does not further augment channel activity suggest that channels containing βENaC[L616] may behave functionally like channels containing both truncated β and γ subunits. These findings would be consistent with a model in which the mutant βENaC[L616] subunit, in addition to abolishing the negative regulatory effects of βENaC, also eliminates the remaining negative regulatory effect of γENaC, suggesting a functional interaction between the C termini of βENaC and γENaC. Further work will be required to elucidate the molecular mechanism(s) by which these cytoplasmic C termini regulate channel activity.

The study of patients with Liddle syndrome has demonstrated that mutations resulting in an intrinsic increase in renal sodium reabsorption can result in a distinct form of salt-sensitive hypertension and demonstrate a molecular mechanism of hypertension that is entirely intrinsic to the kidney. These findings raise the possibility that other inherited variants in these channel subunits or other regulators of channel activity could contribute to the development of low-renin salt-sensitive hypertension and motivate further examination of this pathway in patients with essential hypertension.

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