Pergamon

J. Steroid Biochem. Molec. Biol. Vol. 64, No. 5-6, pp. 227–230, 1998
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Printed in Great Britain
0960-0760/98 $19.00 + 0.00

PII: S0960-0760(97)00195-7

Common Polymorphisms in Genes Encoding the Human Mineralocorticoid Receptor and the Human Amiloride-sensitive Sodium Channel

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We have examined the human mineralocorticoid receptor gene and the genes encoding the three subunits of the human amiloride-sensitive epithelial sodium channel. Eight new common polymorphisms were identified in these genes which may be useful in genotyping and linkage analysis.
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INTRODUCTION

Sodium balance is crucial for the maintenance of extracellular volume and blood pressure. In the distal part of the nephron Na^+ reabsorption is regulated by aldosterone via the mineralocorticoid receptor (MR) and sodium transport is mediated by the amiloride-sensitive epithelial sodium channel (ENaC) and Na, K-ATPase. Disturbances of sodium homeostasis characterise the inherited diseases pseudoaldosteronism (Liddle's syndrome) and pseudohypoaldosteronism (PHA) [1] where either 'gain of function' mutations in one of the three (α, β, γ) ENaC subunits (Liddle's syndrome) [2–5] or ENaC loss of function mutations (PHA) [6, 7] determine the disease phenotype. ENaC mutations have however, been detected only in some of the families investigated [6, 7] and no pathogenic mutation in the MR gene has been documented to date [8–11], suggesting that further loci may be involved in the pathogenesis of these diseases. To facilitate linkage analysis in families with disturbed sodium homeostasis we have screened the MR gene and the three ENaC subunit-genes for polymorphisms in healthy control subjects.

EXPERIMENTAL

Subjects

Blood samples were from healthy Caucasian males and females and consent was obtained from all participants in accordance with local institutional guidelines.

PCR amplification and direct automatic sequencing

Genomic DNA was isolated by standard procedures. PCR was carried out with primers listed in Table 1 as follows: samples were initially denaturated at 95°C for 5 min and then subjected to 35 rounds of amplification with cycles of 30 s of denaturation at 95°C, 30 s of annealing at 58–62°C (depending on the primers used) and a 150 s elongation phase at 72°C followed by a final incubation at 72°C for 7 min. The MR-intron P1-fragment was obtained by long range (LR)-PCR with the Expand™ Long Template System (Boehringer Mannheim) and an additional 5% DMSO. LR-PCR conditions were an initial incubation for 5 min at 95°C, 10 amplification cycles (20 s at 95°C, 30 s at 61°C, 15 min at 68°C) and 25 cycles as described before, with the elongation step being prolonged for 10 s/cycle. PCR samples were purified by the QIAquick PCR purification system (Qiagen) according to the manufacturer's specifications, with an aliquot of the purified product serving as template in a PRISM™ Ready Reaction Dye Terminator Cycle Sequencing procedure, with AmpliTaq® FS, a Catalyst 800 Mol. Biol. Labstation and a 373 automatic sequencer (Perkin Elmer, Applied Biosystems).

RESULTS

DNA templates for PCR were from healthy Caucasian males and females and, unless otherwise

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Received 17 Jul. 1997; accepted 22 Oct. 1997.

Table 1. Frequencies of intragenic MR and ENaC polymorphisms

Polymorphism[a] (aa change)	Location	Frequency		Primer[b]
		this study	Arai *et al.*	
$CT_{511/512}$ del/ins	MR intron P1	0.58, 0.42		5′-TGCGGTCAGGAGACGAGTTG-3′ (s, A) 5′-ATGACTGATACAAAGTTGCTGCG-3′ (as, A) 5′-GTGGCGGGCGAGTGATC-3′ (s, S)
$T_{1118} \rightarrow C$	MR intron P1	0.83, 0.17		5′-CCGCCTCGGGCTTTGGCG-3′ (s, S)[c]
$G_{3514} \rightarrow C$	MR intron A	0.50, 0.50		5′-CTTTATCATTGCGGACGCTC-3′ (s, A) 5′-CAGTAGCAGAGTAAGGATG-3′ (as, A, S)
$C_{8336} \rightarrow G$	MR exon 2	0.61, 0.39		5′-TAGCTAGAAATCGTTGCTC-3′ (s, A) 5′-TTCCATATCTAGACCTTCAG-3′ (as, A, S)
$A_{8915} \rightarrow G(I_{180} \rightarrow V)$	MR exon 2	0.83, 0.17	0.89, 0.11	5′-TCCGTGAATGGTGGCGTC-3′ (s, A, S) 5′-TGCAAACCGAAGATGTCATG-3′ (as, A)
$C_{9099} \rightarrow T$ ($A_{241} \rightarrow V$)	MR exon 2	1.00, –	0.62, 0.38	5′-TTCCATTTATGGATGGCTCG-3′ (s, A, S) 5′-ATAGCAGAGGAAGGGATGC-3′ (as, A)
$C_{9874} \rightarrow T$ (D_{499} silent)	MR exon 2	0.85, 0.15		5′-GATTTAAAAACTTGCCTCTTG-3′ (s, A, S) 5′-ATTAAAGACTAGGTCTGGTG-3′ (as, A)
$G_{1266} \rightarrow T$ or nt del	αENaC intron d, Alu	nd		5′-CCTGCACTGCTGGATCTG-3′ (s, A) 5′-GTTGCAGACAGCCGAGATC-3′ (as, A, S)
$G \rightarrow A$ ($A_{663} \rightarrow T$)	αENaC exon 13	0.54, 0.46		5′-GGTAGCCTCCACCCTGGC-3′ (s, A, S) 5′-GCCTTGGTGTGAGAAACCTC-3′ (as, A)
$C \rightarrow G$ (L_{649} silent)	γENaC exon 13	0.70, 0.30		5′-GCAGAAAGCCAAGGAGTGG-3′ (s, A, S) 5′-TGGTCAGAGTATCTGAAAAGC-3′ (as, A)

[a] nt positions in the MR gene are according to our sequencing results deposited in the GenBank®/EMBL Data Bank (acc. No. X97925, with nt position 1 given to the first base of exon 1β) and aa numbering is according to Ref. [12]. Characterization of the human αENaC gene (acc. Nos. Z92978–ZS92981) led to the identification of the polymorphic Alu sequence located in intron d (acc. No. Z92980) (Ludwig *et al.*, manuscript in preparation).

[b] Abbreviations used: s, sense; as, antisense; A, amplification primer; S sequencing primer.

[c] PCR product obtained by primers given for the CT-deletion/insertion polymorphism. γENaC primers were designed according to Ref. [13].

stated, 100 chromosomes were investigated in case of each polymorphism.

Sequence analysis of the human MR gene identified five new silent polymorphisms (Table 1). There were three intronic polymorphisms; in addition, a C/G could be observed at position −2 from the translation start site (nt 1 of exon 2) and the triplet encoding Asp_{499} was found to be either GAC or GAT. One of the two previously reported [10, 11] polymorphisms A-to-G (Ile_{180}→Val) showed a similar heterozygote frequency in our study. We did not, however, find the C-to-T (Ala_{241}→Val) polymorphism reported by this group. Furthermore, none of 100 chromosomes bore the silent G/A (Glu_{838}) variant observed twice in the homozygous state [11].

Screening the ENaC genes for polymorphisms (Table 1) revealed αENaC amino acid 663 to be either threonine (ACC) or alanine (GCC) with a heterozygote frequency of 0.49 (58 alleles tested). One Alu element identified in αENaC intron d (acc. No. Z92980) showed a polymorphism (G/T, or deletion of this nucleotide) in its polyA tract. Furthermore, a silent polymorphism could be identified in the γENaC gene where the triplet encoding $leucine_{669}$ was found to be either CTC or CTG, with an observed heterozygote frequency of 0.42 (40 alleles tested).

DISCUSSION

Multiple genes have been shown to cause impaired maintenance of extracellular volume and blood pressure. For this reason genotyping of affected kindreds and investigation of a probable co-segregation of the disease with a given marker should simplify mutation analysis.

Screening of DNA samples of Caucasian origin enabled us to establish the presence of five previously unreported MR gene polymorphisms (Table 1). Interestingly, the C/G polymorphism of the first nucleotide of exon 2 seems not to alter splicing at this 3′ (acceptor) splice site, in accordance with the data of Padgett *et al.* [14]. In contrast with the invariant A (−2) and G (−1) in the 3′ splice site consensus sequence, a G at position +1 was observed in only 55% of the vertebrate genes investigated. Therefore, this splice site position does not appear critical for the accuracy of cleavage and rejoining. In addition, initiation of translation seems not to be affected by this sequence variation. A C-residue is found at position −2 in all three different rat MR-mRNA species [15], however, only the purine at position −3 and the G at +4 were shown to be critical with respect to the Kozak consensus (GCCGCC*A*/GCCAUG*G*, nucleotides −3 and +4 are given in italics) for the translational start site [16].

Since both the human MR gene polymorphisms described so far [10] show low heterozygote frequencies and only one common ENaC variant has been found (βENaCGT-1) [3], the polymorphisms described here should be of considerable use for the investigation of disorders of sodium absorption in the distal nephron. Furthermore, they might also be of use for a more precise mapping of genes located around their chromosomal regions.

Acknowledgements—This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Bi 306/3-2).

REFERENCES

1. Lifton R. P., Molecular genetics of human blood pressure variation. *Science* **272** (1996) 676–680.
2. Schild L., Canessa C. M., Shimkets R. A., Warnock D. O., Lifton R. P. and Rossier B. C., A mutation in the epithelial sodium channel causing Liddle's disease increases channel activity in the *Xenopus laevis* oocyte expression system. *Proc. Natl. Acad. Sci. USA* **92** (1995) 5699–5703.
3. Shimkets R. A., Warnock D. G., Bositis C. M., Nelson-Williams C., Hansson J. H., Schambelan M., Gill J. R. Jr., Ulick S., Milora R. V., Findling J. W., Canessa C. M., Rossier B. C. and Lifton P., Liddle's syndrome: Heritable human hypertension caused by mutations in the β subunit of the epithelial sodium channel. *Cell* **79** (1994) 407–414.
4. Hansson J. H., Nelson-Williams C., Suzuki H., Schild L., Shimkets R., Lu Y., Canessa C., Iwasaki T., Rossier B. and Lifton R. P., Hypertension caused by a truncated epithelial sodium channel γ subunit: Genetic heterogeneity of Liddle syndrome. *Nature Genet.* **11** (1995) 76–82.
5. Hansson J. H., Schild L., Lu Y., Wilson T. A., Gautschi I., Shimkets R., Nelson- Williams C., Rossier B. C. and Lifton R. P., A de novo missense mutation of the subunit of the epithelial sodium channel causes hypertension and Liddle syndrome, identifying a proline-rich segment critical for regulation of channel activity. *Proc. Natl. Acad. Sci. USA* **92** (1995) 11495–11499.
6. Chang S. S., Gründer S., Hanukoglu A., Rösler A., Mathew P. M., Hanukoglu I., Schild L., Lu Y., Shimkets R. A., Nelson-Williams C., Rossier B. C. and Lifton R. P., Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nature Genet.* **12** (1996) 248–253.
7. Strautnieks S. S., Thompson R. J., Gardiner R. M. and Chung E., A novel splice mutation in the γ subunit of the epithelial sodium channel gene in three pseudohypoaldosteronism type 1 families. *Nature Genet.* **13** (1996) 248–250.
8. Komesaroff P. A., Verity K. and Fuller P. J., Pseudohypoaldosteronism: Molecular characterization of the mineralocorticoid receptor. *J. Clin. Endocrinol. Metab.* **79** (1994) 27–31.
9. Zennaro M. C., Borensztein P., Jeunemaitre X., Armanini D. and Soubrier F., No alteration in the primary structure of the mineralocorticoid receptor in a family with pseudohypoaldosteronism. *J. Clin. Endocrinol. Metab.* **79** (1994) 32–38.
10. Arai K., Tsigos C., Suzuki Y., Irony I., Karl M., Listwak S. and Chrousos G. P., Physiological and molecular aspects of mineralocorticoid receptor action in pseudohypoaldosteronism: A responsiveness test and therapy. *J. Clin. Endocrinol. Metab.* **79** (1994) 1019–1023.
11. Arai K., Tsigos C., Suzuki Y., Listwak S., Zachman K., Zangeneh F., Rapaport R., Chanoine J. P. and Chrousos G. P., No apparent mineralocorticoid receptor defect in a series of sporadic cases of pseudohypoaldosteronism. *J. Clin. Endocrinol. Metab.* **80** (1995) 814–817.
12. Arriza J. L., Weinberger C., Cerelli G., Glaser T. M., Handelin B. L., Housman D. E. and Evans R. M., Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. *Science* **237** (1987) 268–275.

13. Voilley N., Bassilana F., Mignon C., Merscher S., Mattéi M. G., Carle G. F., Lazdunski M. and Barbry P., Cloning, chromosomal localization, and physical linkage of the β and γ subunits (SCNN 1B and SCNN 1G) of the human epithelial amiloride-sensitive sodium channel. *Genomics* **28** (1995) 560–565.
14. Padgett R. A., Grabowski P. J., Konarska M. M., Seiler S. and Sharp P. A., Splicing of messenger RNA precursors. *Ann. Rev. Biochem.* **55** (1986) 1119–1150.
15. Kwak S. P., Patel P. D., Thompson R. C., Akil H. and Watson S. J., 5′-Heterogeneity of the mineralocorticoid receptor messenger ribonucleic acid: Differential expression and regulation of splice variants within the rat hippocampus. *Endocrinology* **133** (1993) 2344–2350.
16. Kozak M., An analysis of vertebrate mRNA sequences: Intimations of translational control. *J. Cell Biol.* **115** (1991) 887–903.