Mineralocorticoid receptor gene variants : implications for stress, blood pressure and personality

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Identification of genetic variation in the Mineralocorticoid Receptor

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Introduction

Genetic variation in the mineralocorticoid receptor (MR) is reported in several studies. Associations with low frequent (<1% in the population) MR mutations have been found to be associated with rare genetic disorders while the more frequent (>1% in the population) single nucleotide polymorphisms (SNPs) have been found to be associated with common variation in healthy individuals, this is described in more detail in chapter 1, paragraph 2.2 of this thesis.
In this study we tried to identify SNPs in the MR, therefore the exonic regions of the MR were screened in fifty individuals from the Leiden population. In addition we performed a computer screening on the genetic variation in the MR to predict possible functionality, we checked if the genetic variation was linked and if haplotypes exist in the MR.

Materials and Methods

Screening exonic regions MR

Fifty anonymous EDTA blood samples were obtained from the general physician laboratory in Leiden, the Netherlands. DNA was isolated using a standard NaCl salting out procedure (Miller et al. 1988). Eighteen primer pairs were developed for PCR reactions covering all exonic regions using Primer3 software (Steve Rozen and Helen J. Skaletsky, 2000; primer sequences on request). All PCR reactions were performed with 50ng DNA, 8.0pM of each primer and AmpliTaq Gold (Applied Biosystems Nieuwekerk a/d IJssel, The Netherlands), according to the manufacturer's protocol. Sequencing of the PCR product was done with BigDye Terminator Sequencing Kit v3.1 (Applied Biosystems Nieuwekerk a/d IJssel, The Netherlands) on an ABI Prism 3100-Avant Genetic Analyzer, according to the manufacturer's instructions, while sequences were analyzed using Chromas Lite (Technelysium Pty Ltd) and Vector NTI (Invitrogen).

Computer screening DNA sequences

The program TFSEARCH (http://www.rwcp.or.jp/papia/ Heinemeyer 1998) was used to identify transcription factor binding sites, UTR-scan was used to identify regulatory regions in the untranslated regions and targetscan (http://www.targetscan.org) was used to identify microRNA target regions.
DNA sequences with and without the SNPs were subjected to the computer programs to reveal a possible effect of the SNP on transcription factor binding sites, regulatory regions and microRNA target regions.

Results

MR SNP identification

The exonic regions of the human MR gene were screened for genetic variation by direct sequencing fifty individuals from the Dutch population. There were no drop outs, all exonic regions were measured in all individuals. Seven SNPs were found in the exonic regions, rs2070951 (Minor allele frequency (MAF) 47%), rs5522 (MAF 11%), rs5523 (MAF 1%), rs5525 (MAF 11%), rs5528 (MAF 1%), rs5534 (MAF 44%) and rs2871 (MAF 22%). No new SNPs were identified, all SNPs were described in the NCBI database (December 2008).

Computer screenings showed that rs2070951 is located in the first Kozak sequence of the gene; this region determines translation of the protein. Other SNPs were not located at predicted transcription binding sites, regulatory regions or microRNA target regions. However rs2871 appeared to be located at a region that is prone to loop formation. This was identified by the primer design software, during development of primers the primer design software indicated possible loop formation of the sequence.

MR genotypes and haplotypes

Linkage Disequilibrium (LD) and haplotype analyses revealed two haplotype blocks. One, containing the three high frequent SNPs in exon 2 rs2070951, rs5522 and rs5525. The other, contains the two high frequent SNPs in exon 9 rs5534 and rs2871 (Fig 1 and 2).

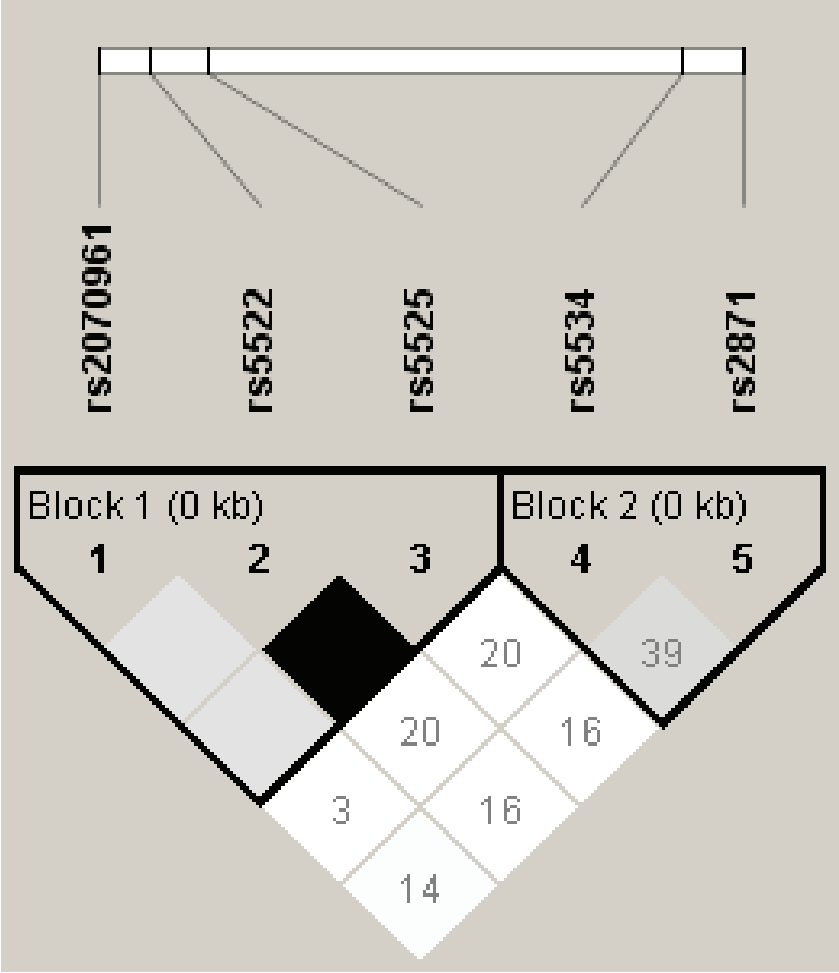

Figure 1. LD between MR SNPs in r^2. Black squares indicate $r^2 = 1$ and shades of grey indicate $r^2 < 1$

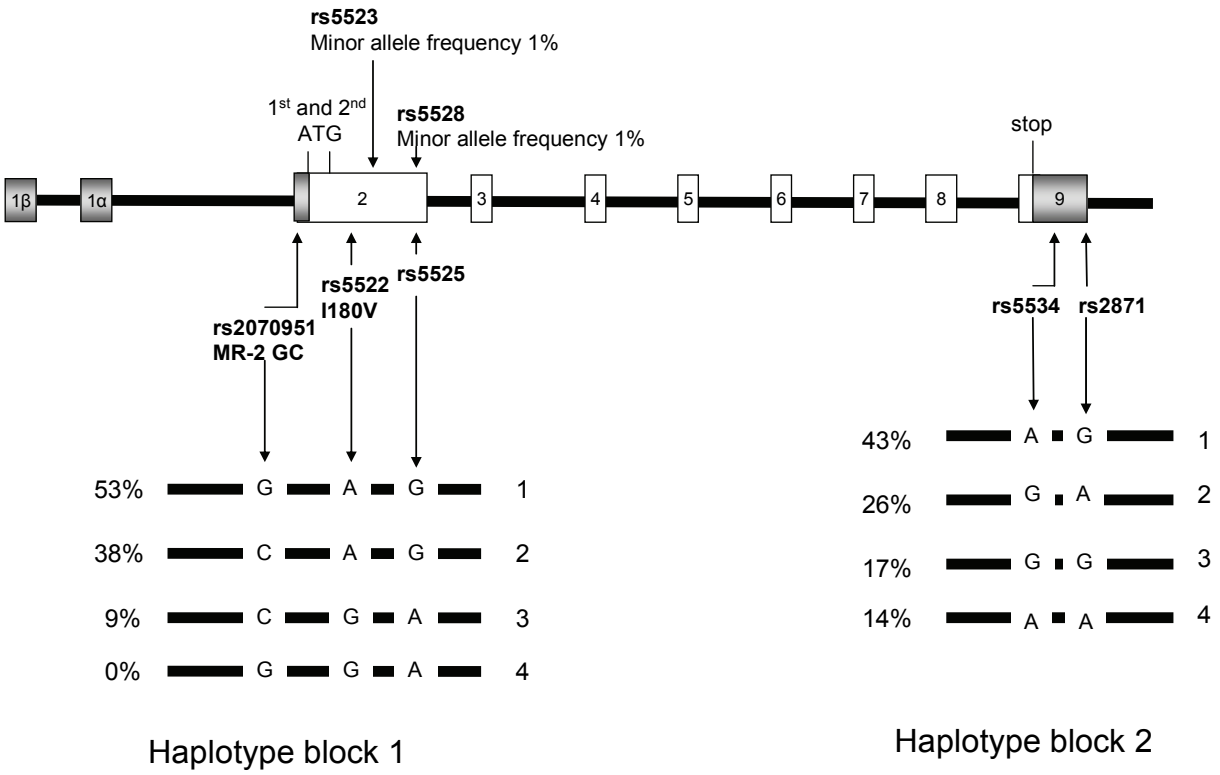

Figure 2. Structure and haplotypes of the hMR gene. Intronic DNA regions are displayed as the black line in the middle; exonic regions are displayed as the boxes on the line. Gray boxes represent the untranslated exons. White boxes indicate translated exons. SNPs and haplotypes are indicated below the DNA

Discussion

Screening the coding region of the MR gene revealed seven single nucleotide polymorphisms (SNPs), single nucleotide changes with a frequency of more than 1% in the population. No new SNPs were identified; these seven SNPs were already described on the NCBI website (http://www.ncbi.nlm.nih.gov/snp). There were no SNPs observed or previously described in the DNA and ligand binding domain. Genetic variation in these regions has probably too severe consequences for physiological processes to occur frequently in a healthy population. This assumption is supported by the finding that if rare genetic mutations occur in these regions, they invariably result in pseudohypoaldosteronism type I (PHA1).

Two haplotype blocks were identified in the MR. One containing the SNPs in exon 2, the other containing SNPs in exon 9. Block 1 contains the previously tested, *in vivo* and *in vitro* functional SNPs MR-2G/C and MRI180V. Functionality of haplotype block 2 or the separate SNPs in haplotype block 2 has not been reported but SNPs in this region might influence mRNA stability, since this has been demonstrated for other genes, including the closely related GR gene.

The frequencies of rs5523 and rs5528, two of the seven SNPs in the coding region, were very low, only 1%, therefore these SNPs can not be used for future association studies in small patient-control cohorts.