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A study into genes encoding longevity in humans

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CHAPTER 6

Mental Performance in Old Age Dependent on Cortisol and Genetic Variance in the Mineralocorticoid- and Glucocorticoid Receptors

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Abstract

Depression and cognitive decline have been associated with changes in circulating cortisol concentrations. Cortisol exerts its functions through mineralocorticoid (*MR*) and glucocorticoid (*GR*) receptors. However, data on the influence of variations in the *MR* and *GR* genes on depressive symptoms and cognitive functioning in older adults are scarce. Therefore, we explored the impact of *MR*-215G/C, *MR*-I180V, *GR*-ER22/23EK, *GR*-N363S and *GR*-*BclI* polymorphisms on these endpoints in the population-based Leiden 85-plus Study. This prospective study includes 563 participants aged 85 years and older, with a mean follow-up of 4.2 years. In this study, high morning cortisol levels (per 1 SD cortisol) associated with impairments in global cognitive functioning ($p=0.002$) at baseline (age 85). These impairments were mainly attributable to lower attention ($p=0.057$) and slower processing speed ($p=0.014$). Similar effects were also observed during follow-up (age 85-90), where participants with higher cortisol levels (per 1 SD cortisol) had impaired global cognitive functioning ($p=0.003$), as well as impairments in attention ($p=0.034$) and processing speed ($p=0.013$). Changes in depressive symptoms were observed for the *MR*-I180V single nucleotide polymorphism (SNP), where during follow-up the prevalence of depressive symptoms was higher in the 180V-allele carriers ($p=0.049$) compared to non-carriers. Dependent on these polymorphisms, no differences in overall and in specific domains of cognitive functioning were observed. In conclusion, the *MR*-I180V SNP has a specific effect on depressive symptoms, independent from cognitive functioning and other polymorphisms in the *MR* and *GR* genes. In contrast, these genetic variants in the *MR* and *GR* genes do not influence cognitive functioning in old age.

Introduction

In response to a real or imagined threat the organism reacts by eliciting a stress-response in order to cope with the stressor. In humans, cortisol is the primary active stress hormone that mediates counter-responses to stress, aimed to re-establish homeostasis and coordinate behavioral adaptations (de Kloet et al, 1998, 2005; Munck et al, 1984). Cortisol exerts its effects through mineralocorticoid (MR) (NR3C2) and glucocorticoid (GR) (NR3C1) receptors, which are abundantly co-expressed in the neurons of limbic structures (Herman, 2003). MRs, which have ten times higher affinity for cortisol than GRs, determine the threshold or sensitivity of the stress system and thus the onset of the stress response (de Kloet et al, 2005). GRs, on the other hand, represent the slower mode that promotes the termination of the stress response (de Kloet et al, 2005). Balancing these two systems is essential for cell homeostasis, mental performance and health.

Various studies have shown that stress-responsiveness is highly variable among human subjects, and an inadequate stress-response increases vulnerability for disease. Changes in the stress hormone system have been shown to play a role in the development of depression (Belanoff et al, 2001b; Holsboer, 2000, 2001). In addition, changes in the circulating cortisol concentrations have been associated with impairments in various cognitive domains, including attention, perception and memory (Jameison and Dinan, 2001; Lupien et al, 2005; Newcomer et al, 1999). In terms of memory, excess cortisol levels have been shown to impair mainly declarative and not non-declarative memory (Lupien et al, 2005).

As MRs and GRs mediate the cortisol signal, variations in these genes may introduce changes in cortisol signaling dynamics, and thereby lead to changes in mood and cognitive function. In the *MR* gene, the 215G/C and I180V single nucleotide polymorphisms (SNPs) have been shown to change cortisol signaling in vitro (Arai et al, 2003), whereas in the *GR* gene, the ER22/23EK, N363S and *BclI* SNPs have been shown to change HPA-axis reactivity after a dexamethasone suppression test or a psychosocial stressor (Huizenga et al, 1998; van Rossum et al, 2002, 2003). In addition, the ER22/23EK variant has been associated with major depression in two studies (van Rossum et al, 2006; van West et al, 2006). To date, there are no publications that examine the role of these *MR* and *GR* variants in cognitive functioning and as yet no study has assessed the effects of variants in the *MR* gene on mood. Furthermore, a combined effect of the genetic variants in the *MR* and *GR* genes may have a different effect on depressive symptoms and cognitive functioning, owing to the interplay between these genes.

In this study, we examined the influence of cortisol levels and variants in the *MR* and *GR* genes on overall cognitive functioning, attention, processing speed, immediate and delayed memory, and on depressive symptoms in old age. All analyses were performed in the prospective population-based Leiden 85-plus Study. Since in old age cognitive decline and depressive symptoms are more prevalent, the effects of a lifelong exposure to changes in cortisol signaling owing to polymorphisms should be more easily detectable on these endpoints.

Participants and methods

Participants

The Leiden 85-plus Study is a population based prospective study of inhabitants of the city Leiden, The Netherlands. All 85 year old inhabitants were invited to participate. There were no selection criteria related to health or demographic characteristics. The study population consists of 599 participants, all Caucasians and members of the 1912-1914 birth cohorts, who were enrolled in the month of their 85th birthday between 1997 and 1999 (Bootsma-van der Wiel et al, 2002). For the present study DNA was available for 563 participants. The Medical Ethical Committee of the Leiden University Medical Center approved the study and informed consent was obtained from all participants or their guardian.

Cognitive function and depressive symptoms

Global cognitive functioning was assessed in all participants with the Mini-Mental State Examination (MMSE) (Folstein et al, 1975). Participants with a MMSE score 19 points or higher were subjected to additional tests to measure attention (Stroop Test) (Klein et al, 1997), processing speed (Letter Digit Coding Test, LD'T) (Houx et al, 2002), immediate recall memory (Word Learning Test Immediate Recall, WLTI), delayed recall memory (Word Learning Test Delayed Recall, WLTD) (Brand and Jolles, 1985) and depressive symptoms (15-item Geriatric Depression Scale, GDS-15) (De Craen et al, 2003). The tests assessing specific domains of cognitive functioning were not administered in subjects with a MMSE score of 18 points or lower, because diminished reliability and validity in subjects with severe cognitive impairment. All participants were visited annually for re-measurement of cognitive functioning during a mean follow-up period of 4.2 years. Parallel versions of the tests were used. Details of cognitive testing are described elsewhere (Houx et al, 2002).

Cortisol measurement at baseline

All blood samples were drawn in the morning before 11.00 AM. The median time between venipuncture and centrifugation in our laboratory was 50 min (interquartile range: 30–70 min). Non-fasting plasma cortisol levels were determined by a fluorescence polarization immunoassay using the Abbott TDx (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instructions. The within-assay coefficient of variation (CV) was below 5 % at different levels.

Possible confounders

Socio-demographic characteristics, such as sex and level of education were considered as possible confounders. Education was divided into two levels: a lower education level, including individuals without schooling or with only primary school education (less than 6 years of schooling), and those with a higher education level (6 years or more of schooling). Health related

correlates were assessed at baseline (age 85), and included drug use, obtained from the pharmacist's registers, and chronic diseases. Data on the use of oral corticosteroids were available for all participants. Chronic diseases included cardiovascular disease, diabetes mellitus, chronic obstructive pulmonary disease, arthritis, malignancy, dementia and Parkinson's disease. Data were obtained at baseline from a structured questionnaire and based on the diagnoses of the general practitioner.

Genotyping

The GR-ER22/23EK polymorphism consists of two single nucleotide polymorphisms (SNPs) in codons 22 (rs6189) and 23 (rs6190). The MR-I180V (rs5522), GR-ER22/23EK, GR-N363S (rs6195) and GR-BcII (van Rossum et al, 2003) variants were genotyped using an Assay-by-Design (Applied Biosystems, Foster City, CA), consisting of PCR primers and TaqMan MGB probes, on an ABI 7900 HT with real-time PCR (Applied Biosystems). MR-215G/C (rs2070951) was genotyped using the MassArray platform according to the protocols of the manufacturer (Sequenom, San Diego, CA).

Statistical analysis

Allele and genotype frequencies were calculated and analyzed for deviation from Hardy-Weinberg equilibrium using the χ^2 -test. Cortisol levels were converted into z-scores ((individual level – mean level)/SD) in order to normalize the data and provide comparable estimates per 1-SD increase in cortisol level. The cross-sectional (age 85) and longitudinal (age 85-90) associations between baseline cortisol levels, cognitive functioning and depressive symptoms were analyzed using a linear regression and a linear mixed model, respectively. Differences in cortisol levels dependent on polymorphisms were assessed with a univariate general linear model. Associations between genotypes, cognitive functioning and depressive symptoms were analyzed using the linear mixed model, estimating the overall mean difference in cognitive functioning or depressive symptoms during follow-up. In multivariate analyses, the other polymorphisms were added to the model as independent covariates. The analyses of cognitive functioning were adjusted for sex and level of education, and the analyses of depressive symptoms were adjusted for sex and presence of chronic diseases. All these analyses were also performed with additional adjustment for cortisol levels or chronic diseases. The analyses were performed with SPSS statistical software, version 12.0 (Chicago, IL, USA).

Results

Baseline characteristics of the participants are shown in Table 1. From the 563 participants, 12 were excluded from the analyses owing to the use of corticosteroids. Global cognitive functioning was assessed in all participants using MMSE. Some 460 (83 %) scored 19 points or more on MMSE, and these subjects performed additional tests, measuring attention, processing speed, immediate and delayed recall memory, and depressive symptoms. All 551 subjects were genotyped for the MR-215G/C, MR-I180V, GR-ER22/23EK, GR-N363S and GR-BcII poly-

Table 1. Baseline characteristics of study participants

Number	551
Age ¹	85 (-)
Female (%)	365 (66 %)
Low level of education (%)	356 (65 %)
Chronic diseases	391/461
Cortisol ($\mu\text{mol/l}$) ¹	0.49 (0.15)
Mini-Mental State Examination (points) ¹	23.9 (6.27)
Mini-Mental State Examination \geq 19 points (%)	460 (83 %)
Specific domains of cognition ¹	
Stroop Test (seconds)	82.4 (34.3)
Letter Digit Coding Test (digits)	17.1 (7.12)
Word Learning Test Immediate Recall (pictures)	23.9 (6.00)
Word Learning Test Delayed Recall (pictures)	8.65 (2.76)
15-item Geriatric Depression Scale (points) ¹	2.32 (2.44)
Polymorphisms (minor allele frequency)	
MR - 215G/C	0.47
MR - I180V	0.14
GR - ER22/23EK	0.03
GR - N363S	0.05
GR - BclI C/G	0.34

¹ Data presented as means with standard deviations (SD)

morphisms. The overall genotype distributions and resulting allelic frequencies of the variants were in agreement with the distribution predicted by the Hardy-Weinberg equilibrium, and in case of the *GR* gene similar to the frequencies observed in other studies. This is the first study reporting allele frequencies for the SNPs in the *MR* gene. Because of the low allele frequencies of the SNPs (Table 1), the heterozygous and homozygous minor allele carriers of all the polymorphisms were combined. However, for the *MR*-215G/C and *GR*-*BclI* variants, the analyses were also repeated using all genotypes, as these polymorphisms have higher allele frequencies.

Global cognitive functioning, attention, processing speed, memory and depressive symptoms were assessed at baseline and re-examined annually during a mean follow-up period of 4.2 years. During follow-up, there was a significant increase in depressive symptoms and a decline in cognitive functioning in all participants (all $p < 0.001$). The influence of plasma cortisol levels on depressive symptoms and cognitive functioning was assessed at baseline (age 85) and also during follow-up (age 85-90). The cross-sectional analysis at age 85 revealed that higher cortisol levels (per 1 SD cortisol) were associated with impairments in overall cognitive performance. This was observed in the whole study population ($p = 0.008$), as well as in the restricted sample (MMSE \geq 19 points) ($p = 0.002$) (Table 2). These differences were attributable to lower attention ($p = 0.057$) and slower processing speed ($p = 0.014$) (Table 2). Similar effects were also observed during follow-up (age 85-90), where participants with higher cortisol levels (per 1 SD cortisol) had impaired global cognitive functioning ($p = 0.004$), attention ($p = 0.034$) and processing speed ($p = 0.013$) (Table 2). The overall difference in cognitive functioning and depressive symptoms

Table 2. Cortisol, cognitive functioning and depressive symptoms at baseline (age 85), and during the annual follow-up (age 85-90)

	Age 85		Age 85-90	
	Estimate (SE)	p-value	Estimate (SE)	p-value
All participants (n=551)				
Mini-Mental State Examination (points)	-0.67 (0.25)	0.008	-0.78 (0.27)	0.004
Restricted sample (n=460) ¹				
Mini-Mental State Examination (points)	-0.40 (0.13)	0.002	-0.37 (0.12)	0.003
Specific domains of cognitive function				
Stroop Test (seconds)	3.15 (1.65)	0.057	2.86 (1.34)	0.034
Letter Digit Coding Test (digits)	-0.79 (0.32)	0.014	-0.74 (0.30)	0.013
Word learning Test Immediate Recall (pictures)	-0.39 (0.29)	0.187	-0.38 (0.28)	0.186
Word learning Test Delayed Recall (pictures)	-0.21 (0.14)	0.129	-0.19 (0.13)	0.160
15-item Geriatric Depression Scale (points)	0.18 (0.12)	0.146	0.10 (0.13)	0.424

¹ Restricted to participants who scored on MMSE \geq 19 points

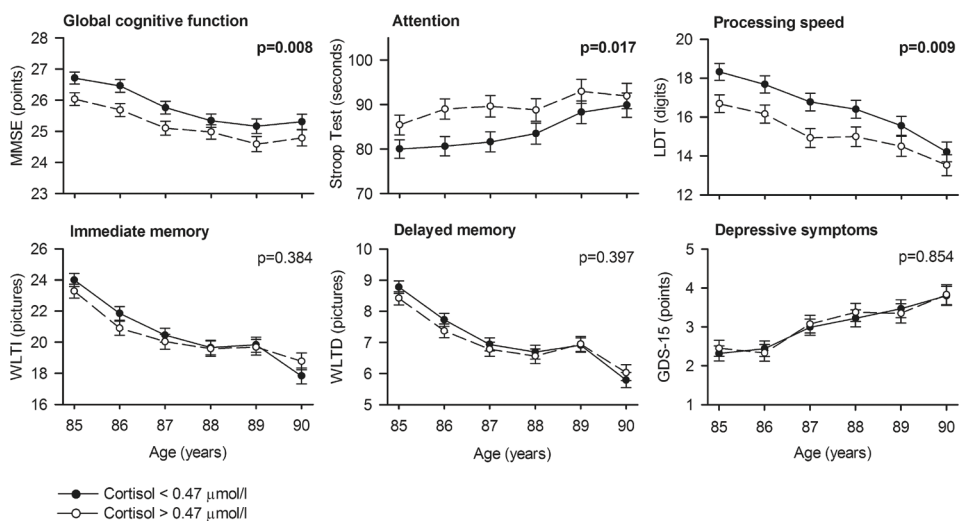


Figure 1. Cognitive functioning and depressive symptoms during the mean 4.2 year follow-up period in participants with low (<0.47 $\mu\text{mol/l}$) and high (>0.47 $\mu\text{mol/l}$) cortisol levels. The p-values represent the overall mean difference in cognitive functioning and mood during the follow-up between the two groups

Table 3. Cortisol levels ($\mu\text{mol/l}$) dependent on the MR and GR polymorphisms

	Non-carriers		Carriers		p-value
	n	mean (SE)	n	mean (SE)	
MR - 215G/C	135	0.52 (0.01)	376	0.48 (0.01)	0.008
MR - I180V	394	0.49 (0.01)	145	0.48 (0.01)	0.423
GR - ER22/23EK	504	0.49 (0.01)	33	0.49 (0.03)	0.895
GR - N363S	494	0.49 (0.01)	50	0.50 (0.02)	0.491
GR - BclI C/G	234	0.47 (0.01)	288	0.50 (0.01)	0.084

during follow-up dependent on cortisol levels (two groups based on median cortisol level $0.47 \mu\text{mol/l}$) is shown in Figure 1.

The influence of MR and GR gene polymorphisms on plasma cortisol levels is shown in Table 3. Plasma cortisol levels were associated with the 215G/C variant in the MR gene, where the C-allele carriers had lower cortisol levels than non-carriers ($p=0.008$) (Table 3). In addition, for the GR-BclI C/G SNP, a trend for an increase in cortisol levels in G-allele carriers was observed ($p=0.084$) (Table 3).

Cognitive functioning and the prevalence of depressive symptoms were also analyzed dependent on the MR and GR gene polymorphisms. Differences in depressive symptoms were observed for the MR-I180V SNP, where carriers of the 180V-allele had more depressive symptoms than non-carriers ($p=0.049$) (Table 4, Figure 2). This difference increased after adjusting for cortisol levels (0.54 points, SE (0.27), and $p=0.043$). On the other hand, the analyses with cognitive functioning dependent on the polymorphisms revealed no differences, either in overall (Table 4) or in specific domains of cognitive functioning (data not shown). The results remained unaltered after the analyses with a multivariate model, or adjustment for depressive symptoms and cortisol concentrations (data not shown). The analyses using all the MR-215G/C and GR-BclI genotypes did not provide any extra information.

All the cognitive functioning analyses were repeated with additional adjustment for chronic diseases, as participants with higher cortisol levels had a higher prevalence of chronic diseases at baseline. This adjustment only marginally changed the results (data not shown).

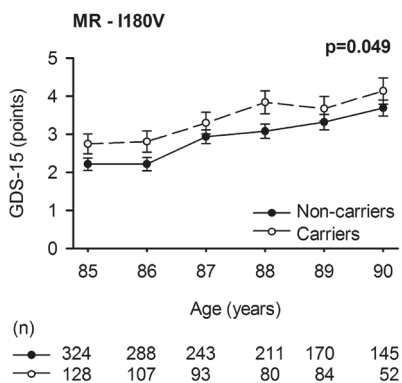


Figure 2. Prevalence of depressive symptoms in non-carriers and carriers of the MR-I180V polymorphism during the mean 4.2-year follow-up period. P-value represents the overall mean difference in the 15-item Geriatric Depression Scale (GDS-15) scores during follow-up between the two groups. (n) represents the number of participant

Table 4. Cognitive functioning and depressive symptoms during the mean 4.2 year follow-up period dependent on the *MR* and *GR* polymorphisms

	Non-carriers mean (SE)	Carriers mean (SE)	p-value
Mini-Mental State Examination (points) ¹			
MR - 215G/C	25.2 (0.25)	25.6 (0.15)	0.179
MR - I180V	25.5 (0.15)	25.5 (0.23)	0.926
GR - ER22/23EK	25.5 (0.14)	25.8 (0.48)	0.458
GR - N363S	25.5 (0.13)	25.3 (0.43)	0.776
GR - BcII C/G	25.5 (0.19)	25.4 (0.17)	0.809
15-item Geriatric Depression Scale (points) ¹			
MR - 215G/C	3.12 (0.24)	2.98 (0.15)	0.594
MR - I180V	2.90 (0.15)	3.42 (0.23)	0.049
GR - ER22/23EK	3.02 (0.13)	3.53 (0.48)	0.297
GR - N363S	3.04 (0.13)	2.55 (0.41)	0.248
GR - BcII C/G	3.26 (0.19)	2.89 (0.17)	0.133

¹ Restricted to participants who scored on MMSE \geq 19 points

Discussion

In this study, we assessed the impact of cortisol levels and of variations in the *MR* and *GR* genes on cognitive function and depressive symptoms. The results revealed associations between high cortisol levels and cognitive impairment, but not with depressive symptoms. In contrast, the prevalence of depressive symptoms was dependent on a variation in the *MR* gene, where carriers of the 180V-allele had more depressive symptoms compared to the non-carriers. This association remained unchanged after taking into account the influence of the other polymorphisms in the *MR* and *GR* genes. Variations either in the *MR* gene or in the *GR* gene were not associated with cognitive function.

Similar to observations in other studies, we found an association between high cortisol levels and cognitive impairments. These differences were observed at baseline (age 85) and also during follow-up period (age 85-90). Impairments in global cognitive functioning, owing to high cortisol, were observed in the whole study population, and also in the restricted sample (MMSE \geq 19 points). In the restricted sample, the estimates were without exception smaller however, they had increased significance. This implies that by excluding severely cognitively impaired participants, we lose some power but increase specificity, as these participants may have impaired cognition caused by other factors than high cortisol. From the specific domains of cognitive functioning, attention and speed of information processing were most affected, whereas for memory and mood only a modest influence was observed. Intriguingly, in literature high cortisol levels have been mainly associated with memory impairments and depression (Holsboer, 2000, 2001; Lupien et al, 2005). Most of these studies have been conducted with exogenous administration of glucocorticoids or with depressed patients, with higher cortisol concentrations than observed in this study. Therefore, it might be that cortisol levels higher than the average values in older

adults are necessary to affect memory function and depressive symptoms. However, the lack of associations could also be related to the single cortisol measurement, or to the tests used in this study. It has been shown, that not the acute basal cortisol levels, but rather the change in cortisol levels over time predicts cognitive impairments in aging (Belanoff et al, 2001a). In addition, it has been shown that in some cases, cortisol effects on cognitive performance are very difficult to detect with the use of standardized, less sensitive cognitive tests. Thus, it is plausible that the memory tests used in this study are not sensitive enough to detect cognitive impairments related to cortisol.

MRs have been implicated in the control of HPA-axis activity, as they determine the threshold of stress system's activation (de Kloet et al, 2005). To date, there is no information on how polymorphisms in the *MR* gene influence these functions. In this study, we found that plasma cortisol levels were lower in C-allele carriers of the 215G/C SNP in the *MR* gene. As the 215G/C SNP precedes the start codon of the *MR* gene (Arai et al, 2003), it could interfere with the translational start site and thus, lead to a less efficient translation and change in MR-A and MR-B balance (Pascual-Le Tallec et al, 2004). However, the influence of the different *MR* isoforms on cortisol levels is not known. From the other *MR* polymorphisms, we found that the I180V amino acid change in the *MR* gene was associated with higher prevalence of depressive symptoms. It has been reasoned that this SNP changes MR's transactivational properties (Arai et al, 2003), which might change stress responsiveness and lead to increased vulnerability to develop depressive symptoms. On the other hand, carriers of the MR-I180V polymorphism did not display any deficits in overall or in specific domains of cognitive functioning, which suggests that the polymorphism specifically influences depressive symptoms and not cognitive functioning. The mechanisms behind cortisol mediated effects on cognitive functioning and depressive symptoms are most probably different, and modulated by different neuronal pathways and/or contextual conditions (Lupien et al, 2005).

Previously, it has been shown that the *GR* gene polymorphisms alter HPA-axis responsiveness and thereby cortisol sensitivity. The ER22/23EK variation has been associated with resistance to cortisol (van Rossum et al, 2002), whereas the N363S and *BclI* polymorphisms were shown to increase the sensitivity to cortisol (Huizenga et al, 1998; van Rossum et al, 2002, 2003). We reasoned, that the increased resistance to cortisol attained by the ER22/23EK variant would protect against the damaging effects of high cortisol levels on cognitive decline and depression, whereas increased cortisol sensitivity would have opposite effects. In this study, we found no influences of the analyzed polymorphisms on plasma cortisol levels, or on cognitive functioning. For depressive symptoms, only a trend for an influence by the ER22/23EK variant was observed. In contrast, the 22/23EK-allele carriers tended to have more depressive symptoms than non-carriers, without any deficits in cognitive functioning. The lack of a significant difference in depressive symptoms between the ER22/23EK carriers and non-carriers in our study may result from the low number of participants carrying the variant allele. Despite that, the trend observed in our study, is in accordance with two recent studies, showing an enrichment of the 22/23EK-allele in patients with major depression (van Rossum et al, 2006; van West et al, 2006). It could be that in our study the lack of a distinct influence of the *GR* gene SNPs on cognitive functioning and mood is a result of the non-stressful testing conditions. GRs are activated in response to stress and they promote the termination of the stress response and normalization of homeostasis (de Kloet et al, 2005). This suggests that the effects of the polymorphisms are only seen in response to stress or challenge, and that testing in basal conditions does not reveal any

differences in cognitive functioning and depressive symptoms.

The response to stress is modulated by the interplay between MRs and GRs, and a combined effect of genetic variants in these genes may have a different effect on depressive symptoms and cognitive functioning. In this study, we attempted to take into account the influence of genetic variability in both the *MR* and *GR* genes. However, the multivariate analysis did not add any extra information. The cortisol signaling cascade is complex and involves numerous chaperones, accessory proteins, co-regulators and interacting transcription factors that permit differentiation between the MR- and GR-mediated actions (De Bosscher et al, 2003; Pascual-Le Tallec and Lombes, 2005). For instance, recently it was found that increase in recurrence of depressive episodes is associated with a variant in a gene (*FKBP5*) that regulates GR activity (Binder et al, 2004). Therefore, not only genes directly involved in stress-induced signaling pathways, but genes regulating their activity also appear to be involved in the development of these disorders, and their actions should be analyzed in more detail.

Strengths of the present study include the population-based sample of the oldest old with high incidence of depression and cognitive decline, and the annual repeated assessment of depressive symptoms and various cognitive domains. A major limitation of the study is the one time cortisol measurement. Cortisol is secreted diurnally, and the single measurement of cortisol over an entire day of the circadian cortisol secretion, considerably weakens the value of this measure. Cortisol levels measured only once could be confounded by acute effects, and not reflect the basal concentration. It has been shown, that cortisol is relatively stable from day to day but it is not indisputably stable across the years. Therefore, the results on the predictability of baseline cortisol levels on cognitive decline over the years should be interpreted with care. Another limitation of the study is the lack of data on HPA-axis reactivity, or on other parameters that reflect the stress system's activity.

In conclusion, this is the first study looking into the influence of polymorphisms in the *MR* and *GR* genes on mood and cognitive functioning in older adults. We found an association between high baseline cortisol levels and impairments in cognitive functioning during follow-up. However, no differences in cognitive functioning were observed dependent on the polymorphisms in the *MR* and *GR* genes. On the other hand, the I180V variant in the *MR* gene influenced the prevalence of depressive symptoms, independently from cognitive functioning.

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