Cognitive function in old age but not mood is dependent on a ER22/23EK variation in the glucocorticoid receptor gene. The Leiden 85-plus Study.

Cognitive function in old age but not mood is dependent on a ER22/23EK variation in the glucocorticoid receptor gene. The Leiden 85-plus Study.

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Running title: Glucocorticoid receptors and cognitive decline

Number of references: 31

Number of figures: 1

Number of tables: 2

Word count for title: 23

Word count for abstract: 200

Word count for text (excluding references): 2.274

Abstract

Cognitive decline in old age and mood have been associated with changes in cortisol signaling. Cortisol is a stress hormone, which exerts its functions through mineralocorticoid (MR) and glucocorticoid (GR) receptors. At high cortisol levels, GRs get fully activated and therefore it is believed that GRs are resposible for the damaging effects of elevated cortisol. Recently, a ER22/23EK variation in the GR gene was associated with resistance to cortisol. Within the Leiden 85-plus Study we examined the relation between the ER22/23EK variation in the GR gene and cognitive function and mood in old age. In a cross-sectional analysis of 540 participants aged 85 years, ER22/23EK haplotype carriers and non-carriers did not differ in overall cognition and in specific domains of cognitive functioning. However, during mean follow-up of 4.2 years, carriers of the ER22/23EK haplotype had lower attention (2.42 ± 1.19, p=0.043) and slower decline in delayed recall memory function (0.25 ± 0.10, p=0.008). There was no effect of the ER22/23EK variation on depressive symptoms, neither cross-sectionally (0.64 ± 0.52, p=0.22) nor longitudinally (-0.06 ± 0.11, p=0.58). Thus, in elderly subjects aged 85 years and older cognitive function but not mood is dependent on the ER22/23EK variation in the GR gene.

Introduction

In response to a potentially threatening stimulus the organism elicits a hormonal response to restore body homeostasis and to facilitate adaptation. Cortisol is the primarily active glucocorticoid in humans and is secreted by the adrenal gland after the activation of the hypothalamic-pituitary-adrenal (HPA) axis in responses to stress. The physiological functions of cortisol include mobilizing energy resources and restraining potentially concurrent inflammatory and immune reactions [1]. Effective regulation of cortisol secretion depends on the interplay of high affinity mineralocorticoid receptors (MRs) and low affinity glucocorticoid receptors (GRs). MRs stimulate the HPA system and thus cortisol secretion whereas GRs inhibit this effect resulting in GR-mediated feedback inhibition [2]. Thus, depending on cortisol concentration and the number of MRs and GRs present, a biphasic pattern of cortisol secretion results. Disturbances in the pattern of cortisol secretion may negatively affect several aspects of physiology.

Chronically elevated cortisol levels have been shown to affect most adversely the hippocampus, which is implicated in memory functioning [3,4]. Unlike other tissues, the hippocampus expresses both types of receptors, the MRs and GRs [5]. Low doses of cortisol predominantly activate MRs and only few GRs, leading to memory acquisition and retrieval, whereas high doses of cortisol, activating fully both receptors, result in consolidation of learned information [6-10]. Despite these beneficial functions, it appears that cumulative exposure to elevated cortisol during lifetime can lead to cognitive impairments [3]. Changes in cortisol signaling have also been associated with depression [11]. Since only chronically elevated cortisol levels have been associated with these impairments, it is believed that the damaging effects of high cortisol are mainly mediated by GRs. Recently, a ER22/23EK variation in the GR gene (*NR3C1*) was found to be

associated with decreased cortisol suppression in response to dexamethasone administration [12]. As the dexamethasone suppression test measures the sensitivity of the HPA axis to elevated cortisol at the level of pituitary, these results indicate a resistance to cortisol in the negative feedback loop. We hypothesize that the GR-mediated increased cortisol resistance may protect against the damaging effects of high cortisol levels on cognitive decline and depression.

No published study to date has examined the role of GRs in the maintenance of cognitive functions and depressive feelings. In this study we examined the relation between the ER22/23EK variation in the GR gene (*NR3C1*) and cognitive function and mood in old age using a battery of cognitive tests. We performed our analysis in the population-based Leiden 85-plus Study, using cross-sectional and prospective study designs.

Subjects and methods

Subjects

The Leiden 85-plus Study is a prospective population based study in which all inhabitants of Leiden, the Netherlands, aged 85 years were invited to take part. There were no selection criteria related to health or demographic characteristics. The study population consists of 599 subjects, all members of the 1912-1914 birth cohort, enrolled in the month of their 85th birthday between 1997 and 1999 [13]. For the present study DNA was available for 563 people. The Medical Ethical Committee of the Leiden University Medical Centre approved the study and informed consent was obtained from all participants and from a gardian in case of severe cognitive impairment.

Cognitive function tests

Overall cognitive function was measured with the Mini-Mental State Examination (MMSE). Individuals who scored equal to or above 19 points also performed tests measuring processing speed (Letter Digit Coding Test)[14], immediate recall memory (Word Learning Test Immediate Recall), delayed recall memory (Word Learning Test Delayed Recall)[15] and attention (Stroop Test, Stroop Interference)[16]. The Stroop Test consists of 3 different parts of which part 1 and 2 reflect the speed of automatic information processing and part 3 reflects attention by the ability of discarding irrelevant information. Stroop Interference is obtained by subtracting the mean scores of Stroop part 1 and 2 from the total Stroop Test score. All participants were visited annually for re-measurement of cognitive functioning during a mean follow-up of 4.2 years. Parallel versions of the tests were used. Details of cognitive testing are described elsewhere [14].

Depressive symptoms

Depressive symptoms were annually assessed with the 15-item Geriatric Depression Scale (GDS-15), a questionnaire especially developed as a screening instrument for depressive symptoms in elderly populations [17]. The GDS-15 was not administered in subjects with a MMSE score of 18 points or lower, because of a lack of reliability and validity in subjects with severe cognitive impairment.

Genotyping

The ER22/23EK variation consists of two single nucleotide polymorphisms (SNPs) in codons 22 (rs6189) and 23 (rs6190), which are in strong linkage disequilibrium. The second polymorphism results in an amino acid change from arginine (R) to lysine (K) [18]. Genotyping was performed using an Assay-by-Design (Applied Biosystems), consisting of PCR primers and TaqMan MGB probes. Amplification reactions were performed at standard conditions except for the following modifications. A qPCR core kit was used (Eurogentec) and half the amount of primers and probes. Real-time PCR was performed on an ABI 7900 HT (Applied Biosystems).

Possible confounders

Socio-demographic characteristics, such as gender 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 primary school education (maximum of 6 years of schooling), and those with a higher education level (equivalent to more than 6 years of schooling). Data on the use of oral corticosteroids were available for all participants.

Statistical analysis

Allele frequencies were calculated and analysed for deviation from Hardy-Weinberg equilibrium using a χ^2-test. The baseline and prospective associations between the genotype, cognitive functioning and depressive feelings were analysed using linear mixed models adjusted for gender and level of education. In the linear mixed model, the term ER22/23EK represents the cross sectional association between ER22/23EK carriership and the scores on the selected cognitive test administered at baseline. The term for time indicates the linear deterioration per year on the cognitive test. The term for the interaction between time and ER22/23EK represents the additional annual deterioration on the selected cognitive test for ER22/23EK carriers. All analyses were performed with SPSS statistical software, version 12.0 (Chicago, Illinois, USA).

Results

Table 1 shows the baseline characteristics of the 85 year-old study subjects. In total 563 participants were genotyped, but the procedure failed in 13 subjects resulting in 550 genotypes. From these, 10 people were excluded from further analysis due to the use of corticosteroids. Therefore, complete data were available for 540 subjects of which 507 (93.9%) had the ER22/23ER haplotype and 33 (6.1%) were ER22/23EK haplotype carriers. No participants with EK22/23EK haplotype were identified (Table 1). The genotype distribution and resulting allelic frequencies of the ER22/23EK variation were in agreement with the distribution predicted by the Hardy-Weinberg equilibrium. There were no statistically significant relations between the ER22/23EK carriership, gender and education.

Global cognitive functioning, attention, processing speed, memory and depressive feelings were assessed at baseline and re-examined annually during a mean follow-up period of 4.2 years. At baseline, there were no statistically significant differences in overall cognition and in specific domains of cognitive functioning between carriers of the ER22/23EK haplotype and non-carriers (Table 2).

During follow-up, all participants had highly significant decline in all cognitive domains ($p<0.001$) and an increase of depressive symptoms ($p<0.001$). The additional annual change due to genotype was significantly different between the ER22/23EK haplotype carriers and non-carriers only in respect to attention and delayed recall memory. The carriers of the ER22/23EK haplotype had lower attention compared to non-carriers (2.42 ± 1.19, $p=0.043$) as measured with the Stroop Test. A similar result was obtained by

Stroop Interference, which reflects attention independent from cognitive speed (2.36 ± 1.18, 0.045) (Table 2). The result remained unaltered after adjustment for depressive feelings. The performance of delayed recall memory was significantly better in carriers of the ER22/23EK haplotype compared to non-carriers (0.25 ± 0.10, p=0.008) (Table 2, Figure 1). However as seen in Figure 1, both groups show a gradual annual decline in delayed recall memory test on the period 85-90, which is interrupted at age 89. We believe that this better performance of all participants in delayed recall memory test at age 89, is due to the version of the test, which was easier than those applied in other years.

In this study carriers of the ER22/23EK haplotype did not suffer more from depressive symptoms than non-carriers, neither cross-sectionally (0.64 ± 0.52, p=0.22) nor longitudinally (-0.06 ± 0.11, p=0.58).

Discussion

The aim of our study was to examine the relation between a ER22/23EK variation in the glucocorticoid receptor (GR) gene (*NR3C1*) and cognitive function and mood in old age. The results of our propsective study show that the variation is associated with lower attention and has a specific beneficial effect on delayed recall memory during ageing. There was no relation between the ER22/23EK variation and depressive symptoms.

Several studies have examined cortisol levels in relation to ageing and cognitive functioning and shown that elevated cortisol levels correlate with impairments in overall cognition [19-21]. In this study, we examined a genetic variation (ER22/23EK) in the GR gene (*NR3C1*) which has been shown to cause a mild cortisol resistance [12]. At baseline, we did not find any significant differences in overall cognition and in specific domains of cognitive functioning between carriers of the ER22/23EK haplotype and non-carriers. However at baseline the interindividual variance was very big, as seen from the standard errors and therefore we tend to believe that the model was unable to distinguish the subtle differences. On the other hand, the prospective analysis is more sensitive since it measures the intraindividual variance during time. In this study, when exploring the longitudinal effect of the variant on specific domains of cognitive functioning we found that the carriers of the ER22/23EK haplotype had lower attention and significantly slower decline of delayed recall memory during the follow-up. Since it is known, that depression can influence attention, we repeated the analyses with the adjustment for depressive feelings. After this adjustment, the deficits in attention seen for the ER22/23EK haplotype carriers remained unaltered, indicating that the haplotype affects attention independently from depression. We had expected that the ER22/23EK variation in the GR gene would protect the brain from the damaging effects of cortisol, which would result in better

attention within the ER22/23EK haplotype carriers, unless there were other mechanisms involved in this process. Possibly the latter is the case. It has been shown that states of endogenous hypercortisolemia lead to increased levels of adrenocorticotropic hormone (ACTH), which has been shown to de-focus attention and produce an inability to filter out the processing of irrelevant stimuli. Thus, in these cases the impairments in attention have been related to levels of ACTH rather than to levels of cortisol [22,23]. Nevertheless, we cannot exclude that also other mechanisms influence attention.

A lately emerged hypothesis suggests that increased cortisol resistance is a key mechanism in the pathogenesis of depression [11,24]. As the ER22/23EK carriers were found to be slightly more resistant to cortisol, we expected them to have more depressive feelings. Although we observed a slight tendency towards higher levels of depression at baseline, this effect was not consistently observed over the years. In line with our findings, a recent study found no relation between cortisol levels and depression [25].

The most adverse effects of cortisol have been seen in hippocampus dependent cognitive tasks. The hippocampus is an important receptor site for cortisol and a key in the GR mediated negative feedback control of cortisol secretion. The hippocampus is also described as the "cognitive arm" of the limbic system because it plays a pivotal role in memory formation [26]. Unlike other tissues, the hippocampus expresses both MR and GR receptors, which work in a coordinated manner, dependent on cortisol level and receptor numbers. It has been shown that high cortisol levels impair memory, whereas intermediate doses enhance memory [9,27-31]. In cases of high cortisol levels, GRs get fully activated (besides MRs) and therefore it is believed that the GR activation contributes to the damaging effects of elevated cortisol. We reason that the ER22/23EK variation in the

GR gene, resulting in a slight resistance to cortisol mimics the situation with the intermediate cortisol doses. In this situation all MRs are fully activated and only few GRs, creating appropriate conditions for memory formation and diminishing the chance for GR-mediated damage. Since the ER22/23EK variation results in a mild cortisol resistance, the protective effect would be moderate. However the accumulation of the moderate effects during lifetime could lead to a substantial beneficial effect later in life. On the other hand, cortisol signaling is a complex system and most probably other factors also influence the effects of cortisol, such as its interaction with tissue specific proteins and enzymes.

The strength of this study is the large community-based sample of the oldest old with high prevalence of cognitive decline and the annual repeated assessments of various cognitive domains. The limitation of this study is the lack of 24-hour cortisol measurements, which unables us to establish a relationship between the ER22/23EK variation, cortisol concentration and cognitive functioning. Nevertheless, the present study adds strong evidence for a relation between cortisol receptor mechanisms and cognitive function. This calls for more in-depth analysis of cortisol-cognition relationships in dedicated studies.

In conclusion, this prospective study of elderly subjects aged 85 years and older shows that, the cognitive function in old age but not mood is dependent on the ER22/23EK variation in the GR gene.

Acknowledgements

We thank Prof E. Ronald de Kloet and Roel H. de Rijk for fruitful discussions. This work was supported by an IOP grant (Innovative Orientated Research) from the Dutch Ministry of Economic Affairs (grant number IGE010114) and by a Marie Curie Fellowship of the European Community programme EUROGENDIS "The Genetic Basis of Disease" under contract number QLGA-GH-00-60005-59.

Table 1. **Baseline characteristics of the Leiden 85-plus Study**

Number	540
ER22/23ER	507 (93.9%)
ER22/23EK	33 (6.1%)
EK22/23EK	-
Minor allele frequency	0.03
Age	85
Female (%)	359 (67%)
Low education (%)	348 (65%)
MMSE ≥ 19 (%)	452 (84%)

MMSE = Mini-Mental State Examination

Table 2. **Decline in various domains of cognitive function and depressive symptoms dependent on the ER22/23EK haplotype**

	Baseline difference		**Additional annual change**	
	Estimate (SE)	p-value	Estimate (SE)	p-value
Global cognitive function (points)	0.32 ± 1.14	0.78	0.23 ± 0.14	0.11
Attention (seconds, Stroop Test)	2.83 ± 5.65	0.62	2.42 ± 1.19	**0.043**
Attention (seconds, Stroop Interference)	4.41 ± 5.16	0.39	2.36 ± 1.18	**0.045**
Processing speed (digits)	0.29 ± 1.20	0.81	0.12 ± 0.19	0.55
Immediate memory (words)	-0.46 ± 1.19	0.70	0.32 ± 0.20	0.10
Delayed memory (words)	0.05 ± 0.56	0.92	0.25 ± 0.10	**0.008**
Depressive feelings (points)	0.64 ± 0.52	0.22	-0.06 ± 0.11	0.58

All estimates were calculated using linear mixed models, adjusted for gender and level of education. Attention, processing speed, immediate recall memory, delayed recall memory and depressive feelings were not administered in participants with MMSE-scores below 19 points. All p-values < 0.05 are indicated in bold.

SE = standard error

Figure legends

Figure 1. The annual decline in delayed memory is indicated from age 85 to 90. At each year the number of subjects analysed is indicated for the two haplotypes. The p-value indicates the difference between the two lines.

Figure 1. **Decline in delayed recall memory dependent on the ER22/23EK variation**