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Serotonin transporter genotype, salivary cortisol, neuroticism and life events: Impact on subsequent psychopathology in healthy twins at high and low risk for affective disorder

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ABSTRACT

Objective: To investigate if cortisol alone or in interaction with other risk factors (**familial risk**, **the serotonin transporter genotype**, **neuroticism and life events** (**LEs**)) predicts onset of psychiatric disorder in healthy individuals at heritable risk.
Matrial and methods: In a high-risk study, 234 healthy monozygotic and dizygotic twins with or without a co-twin history of affective disorder (high and low risk twins) were baseline assessed. Participants were followed up for seven years and then reassessed with a personal interview revealing whether they had developed psychiatric illness.
Results: 36 participants (15.4%) developed psychiatric disorder. Using Cox proportional hazards ratio (HR) estimates neither morning nor evening salivary cortisol at baseline did predict illness onset. In multivariate Cox models, the two-way interaction between morning cortisol and LEs lifetime before baseline was significantly associated with onset. Further, the HR of onset was higher concerning individuals carrying the short allele of the 5-HTTPLR and having experienced more LEs lifetime. **Familial risk for affective disorder predicted illness and the risk of onset was further increased in individuals at familial risk carrying the short allele of the 5-HTTPLR**.
Conclusions: Cortisol levels alone do not increase the risk of onset of psychiatric illness but the interaction of a lower cortisol level and the experience of more LEs do. The 5-HTTLPR genotype seems to interact and contribute to increased stress vulnerability in combination with other stress indicators of illness thereby adding to the risk of subsequent psychopathology.

1. Introduction

Genetic factors play an important role in an individual's response to stress and trauma (Feder et al., 2009) and heritability is a potent risk factor for affective disorders (Merikangas and Low, 2004; Mortensen et al., 2003). However, the mechanisms of the intergenerational transmission are still unclear (Southwick and Charney, 2012). Affective disorders are also closely related to altered stress response but the relation between individual stress response and potential development of affective disorder is not unidirectional (Brietzke et al., 2012). This was emphasized in the landmark study by Caspi and colleagues (Caspi et al., 2003) who concluded that the interaction between stressful life events (LEs) and a genetic variation in the serotonin transporter gene plays a key role in the vulnerability to depression. Serotonin has an important role in the corticolimbic circuits where the serotonin transporter is involved in the main reuptake mechanism. Similarly the hypothalamus–pituitary–adrenal (HPA) axis may be a central mediator in the pathophysiology of affective disorder (Holsboer and Ising, 2010; Spijker and van Rossum, 2012). There is lack of studies concerning the prospective value of HPA axis dysregulation and it is unknown whether an altered HPA axis physiology is part of an individual's vulnerability to psychopathology (Rosmalen et al., 2005). Despite thorough research, targeting the HPA axis and gene × environment interactions, the knowledge regarding the complex relation between stress and development of affective disorder is still limited.

In the cross sectional part of the present study we found that individuals at high familial risk for affective disorders exhibited elevated evening cortisol levels (Vinberg et al., 2008). We showed no association between familial risk and 5-HTTLPR polymorphism but the presence of

Abbreviations: LEs, Life events; HR, Hazard Ratio; HPA, Hypothalamus–pituitary–adrenal; 5-HTTPLR, serotonin-transporter-linked polymorphic region; MZ, monozygotic; DZ, Dizygotic; ICD-8, International Classification of Diseases "8th" revision; ICD-10, International Classification of Diseases "10th" revision; SCAN, Schedules for Clinical Assessment in Neuropsychiatry; EPO, Eysenck Personality Questionnaire; BDI, Beck depression Inventory; MDQ, Mood Disorder Questionnaire; SD, Standard Deviation; CI, Confidence Interval.

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the short allele of the 5-HTTLPR and experience of LEs were associated with a higher neuroticism score, but not with morning or evening salivary cortisol levels (Vinberg et al., 2010).

1.1. Aims of the study

The aims of the present study were to determine whether cortisol, at baseline, predicts onset of psychiatric disorder either alone or in interaction with familial risk for affective disorder or LEs in a cohort of twins at high versus low familial risk for affective disorder. Further to investigate whether the cross sectional finding of a two-way interaction between high neuroticism and Les, and whether the two-way interaction between the short allele of 5-HTTPLR and LEs predicts onset of affective disorder.

2. Materials and methods

2.1. Design

The present study sample is part of an on-going high-risk study elucidating risk factors of affective disorder. Healthy monozygotic (MZ) and dizygotic (DZ) twins with and without at co-twin history of affective disorder were identified through nationwide registers. Two risk groups were identified: the high-risk group: twins at risk of development of affective disorder (DZ or MZ twin, index co-twin affected), the low-risk group (control group): twins at low risk for development of affective disorder (DZ or MZ twin, index co-twin not affected) (Christensen et al., 2007).

2.2. The registers

The Danish Civil Registration System assigns a unique personal identification number to all Danish residents and thus Danish residents can be tracked in all the public registers through record linkage. The Danish Psychiatric Central Research Register is nationwide, with registration of all psychiatric admissions and since 1995 outpatient hospital contacts in Denmark for the country's 5.3 million inhabitants (Mors et al., 2011; Munk-Jorgensen and Mortensen, 1997). From April 1969 to December 1993, diseases were classified according to the International Classification of Diseases,"8th" revision (ICD-8) (WHO, 1974) and from January 1994 according to the International Classification of Diseases, "10th" revision (ICD-10) (WHO, 1993). The Danish Twin Registry contains information on 75,000 twin pairs born from 1870 to 2003 (Harvald et al., 2004).

2.3. The linkage

Through record linkage between the Danish Twin Register, the Danish Psychiatric Research Register and the Danish Civil Register, a cohort of "high-risk" and "low-risk" twins was identified. This linkage identified same gender twin pairs in which one twin had been treated in a psychiatric hospital setting for an affective episode (the index twin) and the co twin had not been treated for affective disorder (the healthy high-risk co-twin). Probands/index twins (the affected index co-twins, not included in the present study) were identified as twins who on their first admission, in the period between 1968 and 2005, were discharged from a psychiatric hospital with a diagnosis of depression or recurrent depression (ICD-8-codes: 296.09, 296.29, ICD-10-codes: F32–33.9) or a first diagnosis of manic or mixed episode or bipolar affective disorder (ICD-8-codes: 296.19, 296.39; ICD-10-codes: F30-31.6, F38.00). The low-risk twins were identified as twins from psychiatric healthy twin pairs where the co-twin had no known personal history of hospital contact due to affective/psychiatric disorder, and were matched to high-risk twins in terms of age, gender and zygosity.

2.4. Ethics

The Danish Ministry of Health, the Danish Scientific Ethic Committee ((KF)-12-122/99 and (KF)-01-001/02) and the Data Inspection Agency approved the study. The study was conducted in accordance with the latest version of the Declaration of Helsinki. All procedures were carried out with the adequate understanding and written informed consent from the participants.

2.5. Baseline sample

During the baseline recruitment period May 2003 to September 2005, a total of 204 high risk and 204 low risk twins, were invited to participate in the study, 271 twins agreed to participate of whom 37 were excluded. All participants were of Caucasian origin. The remaining 234 participants were divided into groups according to risk of affective disorder. The cohort is described in detail elsewhere (Christensen et al., 2007)).

2.6. Assessment at baseline

Participants were rated in a face-to-face interview using semi-structured interviews: diagnoses were obtained using Schedules for Clinical Assessment in Neuropsychiatry (SCAN) version 2.1 (WHO, 1999). All persons with a lifetime (current or past) diagnosis of affective disorder, schizoaffective disorder or schizophrenia according to SCAN interview were excluded from the study.

2.6.1. Personality measure

Personality dimensions were assessed using the Eysenck Personality Questionnaire (EPQ), Danish version. The EPQ comprises 101 items intended to measure a broad dimension of neuroticism, extroversion and psychotism (Eysenck, 1975). The Danish version of the EPQ has shown coefficient alpha values of 0.87 for neuroticism (Mortensen et al., 1996).

2.6.2. Life events

Participants were at baseline asked about: 1) severe LEs in their lifetime before (prior LEs) using a Danish version of the questionnaires developed by Kendler and colleagues (Kendler et al., 1995). The participant completed ten questions concerning severe LEs lifetime before including: sexual assault or rape, other physical assault, life-threatening accident, life-threatening illness, unexpected death of a loved one, abortion, broken engagement/marital separation, miscarriage or stillbirth, prolonged life-threatening illness of a loved one, and major property loss.

2.6.3. Genotyping

Whole blood samples were collected in tubes containing EDTA and immediately frozen. Genomic DNA was extracted from peripheral blood lymphocytes using FlexiGene DNA Kit (Qiagen, Maryland, USA) according to the manufacturer's instruction. The 5-HHTLPR polymorphic at the SCL6A4 gene promoter (Klauck et al., 1997) was amplified by PCR using the forward primer (5′-GGCGTTGCCGCTCTGAATGC-3′) and reverse primer (5-′GAGGGACTGAG-CTGGACAACCAC-3′) in a solution containing genomic DNA and Taq polymerase (Tempase Ampliqon, Copenhagen, Denmark). The PCR products were analysed in an agarose gel 3% (NuSieve GTG Agarose, Cambrex, Inc.Rockland, ME USA) stained with ethidium bromide. Blood for genetic analyses was not collected from participants who were telephone interviewed or from participants who did not wish to participate in the genetic part of the assessment, or from those who had needle phobia. This left 207 Caucasian participants for genetic analyses (Table 1).

2.6.4. Cortisol measure

Participants were instructed to collect saliva 15 min after awakening before eating, drinking, smoking or brushing teeth and to collect an