

# Risk.

## Impact of having a first-degree relative with affective disorder: a 7-year follow-up study

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### The thesis is based on following papers

- I. Vinberg M, Miskowiak KW, Kessing LV. Risk markers for affective disorder, a seven-years follow-up study of a twin cohort at low and high risk for affective disorder. *J Psychiatr Res.* 2013, 475: 565-71.
- II. Vinberg M, Bech P, Kyvik KO, Kessing LV. Quality of life in unaffected twins discordant for affective disorder. *J Affect Disord.* 2007, 99: 33-8.
- III. Baaré, Vinberg, Knudsen GM, Poulson O, Langkilde A, Jernigan TL, Kessing LV. Hippocampal volume changes in healthy subjects at risk of unipolar depression. *J Psychiatr Res.* 2010, 44: 655-62.
- IV. Vinberg M, Miskowiak KW, Kessing LV. Impairment of executive function and attention predict onset of affective disorder in healthy high-risk twins. *J Clin Psychiatry.* 2013, 8: e747-e757.
- V. Vinberg M, Møllerup E, Andersen PK, Bennike B, Kessing LV. Variations in the serotonin transporter genotype and potential endophenotypes for affective disorder, *Prog Neuropsychopharmacol Biol Psychiatry.* 2010, 34: 86-91.
- VI. Vinberg M, Miskowiak KW, Kessing LV. The Serotonin transporter genotype, salivary cortisol, neuroticism and life events: impact on subsequent psychopathology in healthy twins at high and low risk of affective disorder. *Prog Neuropsychopharmacol Biol Psychiatry.* 2014, 3: 193-8.
- VII. Vinberg M, Trajkovska V, Bennike B, Knudsen GM, Kessing LV. The BDNF Val66Met polymorphism: Relation to familiar risk of affective disorder, BDNF levels and salivary cortisol. *Psychoneuroendocrinology.* 2010, 1: 34: 86-91.
- VIII. Vinberg M, Miskowiak KW, Kessing LV. Brain Derived Neurotrophic factor (BDNF) levels as a possible predictor of psychopathology in healthy twins at high and low risk for affective disorder. *Psychoneuroendocrinology.* 2014, 39: 179-83.

### Terminology and abbreviations

Affective disorders: includes 'single manic episode', bipolar affective disorder, 'depressive episode' and 'recurrent depressive disorder' (ICD-10, DF 30-33)

BDI 21 = Beck Depression Inventory, 21-item version

BDNF = Brain Derived Neurotrophic Factor

Concordance: the probability that a pair of twins will both have a certain characteristic, given that one twin has the characteristic

DPCR = the Danish Psychiatric Central Research Register

DZ = dizygotic

Episode: a period during which syndromal criteria for a disorder are met

G x E = gene x environment interaction

High-risk twins: monozygotic and dizygotic twins with a co-twin with a diagnosis of affective disorder

HR = hazard ratio

HPA = hypothalamus-pituitary-adrenal

LEs = life events

Low-risk twins: monozygotic and dizygotic twins with a psychiatrically healthy co-twin and no first-degree relatives with affective or schizophrenic disorders

MD = major depression

MDQ = Mood Disorder Questionnaire

MRI = magnetic resonance imaging

MZ = monozygotic

QoL = quality of life

SCAN = Schedules for Clinical Assessment in Neuropsychiatry

SD = Standard Deviation

SES = socio-economic status

Syndromal criteria: any of several criterion-based assessment systems.

Unipolar disorder: includes 'single depressive episode' and 'recurrent depressive disorder' (ICD-10, DF 32-33)

WHO = World Health Organisation

5-HTTLPR = the serotonin transporter gene

## Introduction

### *Affective disorders and risk factors*

All mental processes, even the most complex psychological processes, derive from operations of the brain with genes being important determinants of the pattern of interconnections between neurons in the brain and their functions. Genes and specific combinations of genes therefore exert significant control over behaviour (1). Unipolar and bipolar disorders (affective disorders) are destructive not only for those who suffer from them but also for their families. Despite the increasing availability of alternative medical and psychological treatments and modern technology including the tremendous research effort in genomics and brain imaging, the prognosis for severe mental illness, including unipolar and bipolar disorder, remains largely unchanged (2-4). In global terms the main cause of premature mortality in young people is neuropsychiatric disorders (5,6). There is, however, still a lack of understanding of why some people develop psychiatric disorders and others do not. Why are some people better protected against stress and the hardships of living than others? Addressing these questions will pave the way for the development of more successful prevention strategies for affective disorders.

Affective disorder is caused by multiple factors on different levels (physical, psychological, social) and genetic and environmental risk factors seem to interact (7,8). Kraepelin noted that the most potent risk factor for affective disorder is a family history of affective disorder (9) and this has been confirmed in several studies (10,11). Affective disorders run in families and have a significant negative impact on the health and longevity both of those with the disorder and their family (12). Genes predisposing to affective disorders may be transmitted to offspring without expression of the phenotype, and it is probable that affective disorders result from many interacting genes being influenced by environmental factors. In the case of affective disorders the association between genotype and phenotype is complex and involves the interplay of complex genetic mechanisms and non-genetic (environmental) risk factors (13). The specific factors that are transmitted through generations are thus unknown and there is a need for a more integrated approach to studying risk factors for affective disorders. Twin studies are a particularly robust method for studying risk factors. Evidence from twin, family and adoption studies indicates a strong genetic predisposition to affective disorders and a close genetic relationship between unipolar and bipolar disorder (for reviews see 10,14).

Kendler and colleagues were the first to describe a study design that identified twins in four categories of risk by crossing zygosity with family history of affective disorders using a female twin cohort comprising 2,164 individuals, 53,215 person-months of observation, and 492 onsets of depression (15). They found that the best-fitting model for the joint effect of stressful events and genetic liability on onset of major depression suggested genetic control of sensitivity to the depression-inducing effects of stressful life events (LEs). Kendler's study design was used in the research presented in this thesis as it allows one to identify a sample of healthy individuals with a higher genetic liability than samples where 'high-risk' status is based on the status of first-degree relatives. A discordant, healthy, high-risk, monozygotic (MZ) twin has the same genetic make-up as his/her sick co-twin and is thus at very high risk of developing affective

disorder. In contrast, a healthy MZ twin with a healthy co-twin may be at very low risk and may even be protected against developing affective disorder.

The overall aim of the present thesis was to identify risk factors for affective disorders and determine their predictive value using a cohort of twins at high or low risk for affective disorder. Healthy MZ and dizygotic (DZ) twins with and without a co-twin with affective disorder were identified through nationwide registers and assigned to four groups: twins at high risk for development of affective disorder (MZ twin, co-twin affected); twins at moderate risk for development of affective disorder (DZ twin, co-twin affected); twins moderately protected against development of affective disorder (DZ twin, co-twin unaffected); twins at low risk for development of affective disorder (MZ twin, co-twin unaffected).

### *Family studies/ high-risk studies*

The first high-risk family studies were conducted in Germany after the First World War as a follow-up to Kraepelin and colleagues' valuable initial research (16-19). A comprehensive review of family and twin studies was published in Goodwin and Jamison's second edition of the textbook *Manic-Depressive Illness, Bipolar Disorders and Recurrent Depression* (20). Fourteen family studies conducted between 1921 and 1952 were identified, but data from three German studies were not available, leaving 11 studies analysing data on a total of 5515 first-degree relatives of manic-depressive patients; the overall risk of manic-depressive illness in first-degree relatives was estimated to be 12.3 %. After 1960 many family/high-risk studies assessing the independent risk for bipolar disorder and major depression (MD) were conducted. Table 1 shows that 32 studies were conducted between 1960 and 2006, covering 12,245 first-degree relatives of bipolar, bipolar I, bipolar II, MD and control probands (20). These studies found an 11.9 % risk of bipolar illness in first-degree relatives of bipolar probands versus 1.0% in relatives of control probands. First-degree relatives of MD probands had a 20.5 % risk of MD compared with a 5.9 % risk for relatives of control probands. Table 1 also shows that there is considerable overlap in the heritability of MD and bipolar disorder. This is in line with results from a recent study of 679 offspring of 320 parents with affective disorder, which found no difference in the risk of affective disorder in the offspring of parents with bipolar disorder compared with the offspring of parents with MD (21).

Table 1. Family studies conducted after 1960, age-corrected lifetime prevalence in first-degree relatives (%).

Probands	First-degree relatives (n)	BP	BP I	BP II	MD
Bipolar (BP)	9 studies, 3019	11.9	-	-	15.6
Bipolar I	4 studies, 1619	-	5.2	3.8	16.6
Bipolar II	3 studies, 573	-	2.1	6.5	21.6
Major Depression (MD)	11 studies, 5033	1.7	0.8	2.4	20.5
Controls	5 studies, 2001	1.0	0.1	0.9	7.3

Table modified from Goodwin and Jamison, 2007, pp. 416-417.

Twin studies provided further evidence on family risk for affective disorder, with concordance rates for bipolar disorder of 0.36–0.80 in MZ twins and 0.04–0.19 in DZ twins (14,22,23) and 0.23–0.67 and 0.14–0.43 for unipolar disorder for MZ and DZ twins respectively (10,24). There have also been two register studies assessing the risk of affective illness in first-degree rela-

tives. A nationwide Danish population-based sample of all individuals hospitalised or in outpatient clinic contact for the first time with bipolar affective disorder showed that individuals with a first-degree relative with bipolar affective disorder had a 13.63 times greater risk (95 % confidence interval, 11.81-15.71) of developing bipolar affective disorder (25). The other study, a nationwide Swedish study of family risk of depression found a standardised incidence rate of 2.95 among first-degree relatives (siblings) and an incidence rate of 4.57 for twin pairs (26).

High-risk studies offer a way of analysing relatively rare outcomes and can provide insight into inherited vulnerability, potential risk factors and intermediate causal pathways and facilitate identification of prodromal stages without the confounding effects of the changes associated with the burden of the illness (27). High-risk studies thus offer an opportunity to study risk factors before these are influenced by the effect of repeated episodes of mood disorder and are a powerful tool for identifying prognostic risk factors and early clinical manifestations of affective disorders (28).

The heritability estimates indicate a strong genetic influence on affective disorders but the genetic factors presumably operate by making individuals more vulnerable to environmental risks (gene-environment interaction; G x E) (29). Environmental factors may also help to explain why risk factors have different effects in different situations (30); however G x E interactions have rarely been investigated in high-risk studies. At the time we planned and started our initial high-risk study (2001–2002) there was a lack of recent prospective high-risk studies based on more advanced techniques such as genomics, proteomics and the newer neuroimaging techniques. Our aim was to provide new insight into risk and prospective factors for affective disorders by analysing a high risk sample identified using an innovative approach based on the unique Danish registers and making use of what were, at that time, advanced techniques to investigate G x E interactions.

#### *Summary of previous cross-sectional findings from the present study cohort*

Some of the cross-sectional results have previously been presented in a PhD thesis entitled *A comparison of psychopathology, socio-economic status, cognitive function, personality traits and salivary cortisol in twins with and without a co-twin history of affective disorder* (31). In summary, twins at familial risk for affective disorders had a higher level of subclinical depressive and anxiety symptoms than twins without familial risk. They presented with more minor psychiatric diagnoses (i.e. phobia, alcohol abuse and stress reactions) (32). Their psychomotor speed and ability to solve complex cognitive tasks and memory capacity were slightly impaired (33) compared with twins at low familial risk. Familial risk for affective disorder was further associated with higher neuroticism scores but the association interacted with other risk factors: female gender, minor psychopathology and recent LEs (34). Finally, twins at familial risk exhibited higher evening salivary cortisol levels but not higher morning cortisol levels than twins without familial risk for affective disorder (35). Although it was impossible to establish whether these variables predict subsequent affective disorder, some of the differences may reflect subsyndromal affective states which are in the process of becoming syndromal. A follow-up study of the participants offered the opportunity to determine whether the variables identified in the cross-sectional study predicted onset of affective disorder.

#### *Hypotheses and aims*

The overall aim was to identify risk and protective factors for onset of affective disorders among a cohort of DZ and MZ twins discordant for affective disorders (high-risk) or with no predisposition to affective disorder (low-risk) in both cross-sectional and prospective studies. As described in section 2.3, some of the results have been reported before (31). This thesis presents additional cross-sectional data from the baseline assessment and the results of the prospective follow-up study. The following potential risk factors for affective disorder were investigated cross-sectionally: quality of life (QoL), brain structure, G x E interactions between the serotonin transporter gene (5-HTTLPR) polymorphism and LEs, and interactions between Val66Met polymorphism, familial risk, and levels of peripheral brain-derived neurotrophic factor (BDNF) and cortisol. The following risk factors were investigated prospectively as potential predictors of illness: familial history of affective disorder, subclinical depressive and anxiety symptoms, trait neuroticism, QoL, LEs and cognition. We also investigated two biomarkers - salivary cortisol levels and whole-blood BDNF levels - and two-way interactions involving cortisol, LEs and the 5-HTTLPR polymorphism and the two-way interaction between the Val66Met polymorphism and family risk.

Risk factors were organised into three domains:

A. The impact of familial risk, subclinical symptoms, trait neuroticism, LEs (longitudinally) and QoL (cross-sectionally) (hypotheses 1 and 2; papers I, II).

B. Risk factors associated with brain function: structural brain changes (cross-sectionally) and cognitive function (longitudinally) (hypotheses 3 and 4; papers III, IV).

C. Biomarkers, genomics, G x E interactions and two-way interactions between risk factors: salivary cortisol and whole-blood BDNF levels as predictors and the possible influence of two candidate polymorphisms, SERT (cross-sectionally) and BDNFVal66Met (longitudinally), in interaction with other risk factors (hypotheses 5–8, papers V–VIII).

To summarise, the following a priori hypotheses were investigated and the findings reported in the correspondingly numbered paper (I–VIII):

1. Onset of affective disorder is predicted by familial risk for affective disorder, subclinical psychopathology, neuroticism, LEs, sex and age at baseline. A prospective study.

Familial risk for affective disorder is associated with a lower quality of life. A cross-sectional study.

2. Familial risk for affective disorder is associated with structural brain changes e.g. hippocampal volume. A cross-sectional study.

3. Cognitive function in a healthy, never-depressed cohort of twins at familial risk for affective disorder predicts onset of affective disorder. A prospective study.

4. Familial risk for affective disorder is associated with variations in the 5-HTTLPR and interacts with LE in relation to depressive symptoms, neuroticism, and awakening and evening salivary cortisol levels, respectively. A cross-sectional study.

5-6. Salivary cortisol levels at baseline predict onset of psychiatric disorder either alone or in interaction with familial risk for affective disorder.

The two-way interaction between high neuroticism and LEs and the two-way interaction between the short allele of 5-HTTLPR and LEs predict onset of affective disorder. A prospective study.

7-8. Familial risk for affective disorder is associated with variations in the BDNF Val66Met polymorphism. Variations in the Val66Met polymorphism interact with the familial risk for affective disorder.

tive disorder in relation to whole-blood BDNF levels, and awakening and evening salivary cortisol levels, respectively. A cross-sectional study.

Whole-blood BDNF predicts subsequent onset of affective disorder in a cohort of twins at high versus low familial risk for affective disorder and to investigate. The two-way interactions between the Val66Met polymorphism and BDNF levels and between familial risk and the Val66Met polymorphism predict illness onset. A prospective study.

## Methods

### The registers

The Danish Civil Registration System assigns a unique personal identification number to all residents in Denmark. All other Danish registers use the same unique identifier and thus residents in Denmark can be tracked in all public registers. The Danish Psychiatric Central Research Register (DPCR) is nationwide and contains data on all psychiatric admissions and, since 1995, outpatient hospital contacts in Denmark for the country's 5.3 million inhabitants (36, 37). From April 1969 to December 1993, diseases were classified according to the International Classification of Diseases, 8th revision (ICD-8) (38) and from January 1994 according to the International Classification of Diseases, 10th revision (ICD-10) (39). Denmark decided in 1978 not to introduce the 9th revision (ICD-9) for reasons of economy and continuity, as it involved only small changes from ICD-8 (40).

The Danish Twin Registry was initiated in 1953 and contains information on 75,000 twin pairs born between 1870 and 2003 (41). The completeness varies with the birth cohort and is approximately 70% for the period before the establishment of the Civil Registration System and close to 100% thereafter (41,42). The Twin Registry contains information about the zygosity of same-sex twins based on mailed questionnaires. The questionnaire method used in the Danish Twin Register has been found to result in error rates of less than 5% when compared against serological and DNA tests (43,44).

### Record linkage

A cohort of 'high-risk' twins was identified using the linkage between the Danish Twin Register, the DPCR and the Danish Civil Register. We identified 204 same-sex twin pairs, aged between 22 and 70 years old, in which one twin had been treated as a psychiatric inpatient for an affective episode (index twin) and the co-twin had not been treated for affective disorder (healthy, high-risk co-twin). Approximately one third of the twins were MZ twins, which is in line with the normal distribution of twin zygosity. Probands or index twins were defined as twins who were discharged between 1968 and 2005 from their first psychiatric hospital admission with a diagnosis of depression or recurrent depression (ICD-8 codes: 296.09, 296.29; ICD-10 codes: F32-33.9) or manic or mixed episode or bipolar affective disorder (ICD-8 codes: 296.19, 296.39; ICD-10 codes: F30-31.6, F38.00). The control twins (low-risk) were defined as twins from a twin pair in which the co-twin (control index twin) had no known personal history of hospital contact due to affective disorder or another psychiatric disorder, and were matched to the high-risk twins with respect to age, sex and zygosity.

### Participants

#### Inclusion and exclusion criteria for high-risk twins

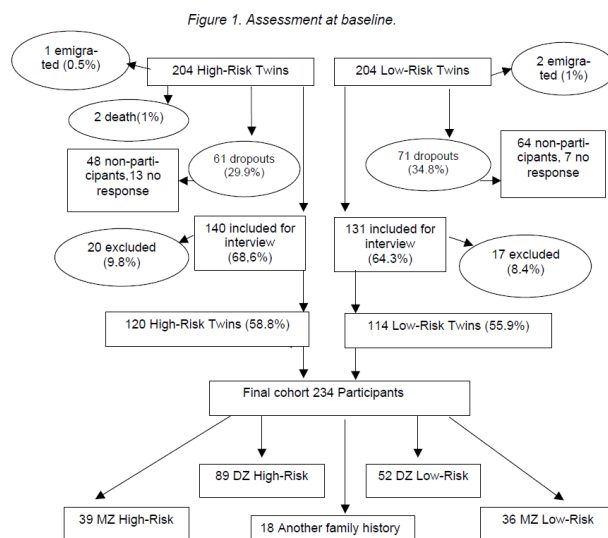
Inclusion criteria: having a MZ or a DZ co-twin with a diagnosis of affective disorder (co-twin history of affective disorder) according to information from the DPCR. Exclusion criteria: a personal history of severe to moderate depression, earlier medical treatment for an affective episode, severe organic brain disease or schizophrenia.

#### Inclusion and exclusion criteria for low-risk twins

Inclusion criteria: no history of affective disorder in the MZ or DZ co-twin and no history of affective disorder or other severe psychiatric illness among other first-degree relatives. Exclusion criteria: as for high-risk twins, plus inpatient or outpatient psychiatric treatment. The DPCR records for control twins and their co-twins did not include any diagnoses of mania or depression; however, it was possible that they or their co-twin had received another diagnosis e.g. schizophrenia. If it was revealed during the recruitment interview that a first-degree relative to a low-risk twin had a history of severe psychiatric illness, the low-risk twin was reassigned to the group of twins with another family history of psychiatric illness involving a non-twin first-degree relative. This group with "another family history" (n = 18; MZ twins = 6, DZ twins = 12; men = 6, women = 12; mean age 36.1 years) was made up of twins with a first-degree family history of affective disorder or schizophrenia not involving the co-twin (see Figure 1, bottom box in the middle, page 13). The group of twins with another family history of severe psychiatric disorder was followed in the same way as the rest of the cohort.

#### The cohort at baseline (Figure 1)

As can be seen from Figure 1, 204 high-risk and 204 low-risk twins were invited to participate in the study. A total of 271 twins agreed to participate; 37 twins were subsequently excluded (mainly because of a prior or current affective episode), leaving 234 participants. One hundred and thirty-one twins did not wish to participate in the study, but 112 of these agreed to participate in a short telephone interview or fill in a questionnaire sent by post. We were unable to get responses from 20 twins. The 234 participants were divided into five groups according to their risk of affective disorder. These 234 participants have been assessed every 6 months since the baseline data were collected.



### Cross-sectional comparison of participants and non-participants

Four hundred and eight twins, 204 high-risk and 204 low-risk twins, were invited to participate in the study. One hundred and thirty-one refused, 20 could not be contacted and 23 had had an affective episode and were therefore excluded (Figure 1). There were no significant differences between participants and non-participants in terms of zygosity, urban status, education level, and civil status. The only group difference was that non-participants were slightly older than the participants (participants: 43.9 years, SD 13.3; non-participants: 47.2 years, SD 13.0,  $p = 0.03$ ) (31).

Table 2. Characteristics of participants and non-participants

	Participants N = 234	Non-Participants* N = 112	p
Women (%)	64.5	66.7	0.7
Age (mean (SD))	43.9 (13.3)	47.2 (13)	0.03
Zygosity (% MZ)	32.4	33.3	0.4
Years of education (mean (SD))	12.8 (3.2)	12.4 (3.2)	0.4

\* Excludes the 20 non-participants from whom no response was obtained

### Baseline assessment

During the cross-sectional recruitment period (May 2003 to September 2005) 408 twins were invited to participate in the study by letter, with a return envelope for the response. Persons who did not respond within two weeks were contacted by telephone. If contact was not established, another letter including a short questionnaire was posted. Those who did not wish to participate were asked to participate in a short telephone interview or fill in a short questionnaire (N =112, as seen from Table 2). They were asked about family history of affective disorder, education, work status and somatic and psychiatric health. The interviewer was initially blind to the risk status of the participants and did not know whether the co-twins of the participants had been admitted to a psychiatric hospital or treated in an outpatient setting. All participants were offered compensation to cover loss of normal earnings and travel expenses.

Participants assessed face-to-face using semi-structured interviews. Diagnoses were obtained using Schedules for Clinical Assessment in Neuropsychiatry (SCAN) version 2.1 (45). All persons with a lifetime (current or past) diagnosis of affective disorder, schizoaffective disorder or schizophrenia according to the SCAN interview were excluded from the study. At the end of the interview, participants were asked about the lifetime psychiatric history of first-degree relatives (their biological parents, co-twin, siblings and offspring) based on the Brief Screening for Family Psychiatric History questionnaire described by Weissmann and colleagues (46). Low-risk twins who had a family history of affective disorder or schizophrenia involving a first-degree relative other than their co-twin were followed as an intermediate high-risk group. The 21-item Beck Depression Inventory, BDI 21, was used to obtain self-reports of psychopathology; the recommended cut-off score of 11 was applied (47) and anxiety symptoms using the 14-item Anxiety Subscale (48). Manic symptoms were assessed with the Mood Disorder Questionnaire (MDQ) (49) and further depressive symptoms were assessed using the Major Depression Inventory (MDI) (50,51). Quality of life was assessed with the World Health Organisation Quality of Life Assessment

(WHOQoL) (52), the Danish version was used. The World Health Organisation Quality of Life brief version (WHOQoL-BREF is a 26-item questionnaire developed from the original 100-item questionnaire the WHOQOL-100. The WHOQoL-BREF covers four domains: physical health (energy and fatigue; pain and discomfort; sleep and rest), psychological health (bodily image and appearance; negative feelings; positive feelings; self-esteem; thinking; learning; memory; concentration), social relationships (personal relationships; social support; sexual activity), environment (financial resources; freedom; physical safety and security; health and social care: accessibility and quality; home environment; opportunities for acquiring new information and skills; opportunities for, and participation in recreation/leisure; physical environment: pollution, noise, traffic and climate; transport). The global score provides a measure of overall quality of life (overall QoL). Items on the WHOQoL-BREF are scored from 1 to 5 on a response scale; higher scores indicate better quality of life. A pilot study found that the Danish WHOQoL-BREF had adequate internal consistency and psychometric validity (53).

Participants were asked about LEs in the year prior to the interview (recent LEs) and earlier LEs, using a Danish version (translated with the authors' permission) of the questionnaire used by Kendler and colleagues (24). Participants were asked about personal events and network events (events that occurred primarily to, or in interaction with, a member of the participant's social network). We collected data on 9 types of personal event: assault; serious marital problems; divorce or separation; job loss; loss of a confidant; serious illness; major financial problem; being robbed; serious legal problems. We also collected data on 22 types of network event: death or severe illness of the participant's spouse, child, parent, co-twin, other sibling, other relative or other individual close to the participant; serious trouble getting along with a parent, child, co-twin, sibling, in-laws, other relative, neighbour or close friend.

Personality traits were assessed using the Danish version of the Eysenck Personality Questionnaire (EPQ) (54). The EPQ comprises 101 items intended to measure the broadly defined traits neuroticism, extroversion and psychoticism. The Danish version of the EPQ has shown alpha coefficient values of 0.87 for neuroticism and 0.84 for extraversion (55).

The Cognitive assessment; Global cognitive function was assessed using the Cambridge Cognitive Examination (CAMCOG) (56). CAMCOG is a detailed neuropsychological instrument incorporating a brief neuropsychological battery which is especially sensitive to mild cognitive dysfunction; its ability to distinguish between demented, depressed and normal individuals has been demonstrated (57). The test is capable of measuring the more general and diffuse cognitive symptoms. The CAMCOG subscales are orientation, memory (recent and remote) and learning, language (comprehension and expression including verbal fluency), attention and praxis. The maximum total score on the CAMCOG is 105 (58). The items measuring general knowledge (e.g. 'When did World War II start?') were not standardised for younger persons, so six items (items 166-171) were omitted resulting in a maximum total CAMCOG score of 99.

Three specific cognitive tests were used (Trail Making A, Trail Making B and the Stroop test). The Trail Making Test (59) is a test of executive function, including selective and sustained attention. In Trail Making A the participant has to connect printed circles

numbered 1 to 25 on one worksheet in consecutive numerical order. In the second part, Trail Making B, the participant has to connect the numbers 1 to 13 and letters A to K (e.g. 1-A, A-2, 2-B, B-3) on a new worksheet. In this study the difference (Trail A minus Trail B) was used as a measure of selective and sustained attention. All participants were given the same instructions and urged to work as fast as possible. Errors were corrected and the trails were timed, a high score indicated poor performance.

The Stroop test is a test of frontal executive function and attention (60,61). The Stroop test requires the respondent to process information from his or her environment and to react selectively to this information (61). The test consists of three pages. Each page contains 100 items, presented in 5 columns of 20 items. The first page (Word, W) comprises colour names, which have to be read as quickly as possible. The second page (Colour, C) comprises colour patches (XXXX printed in red, blue or green); the respondent is required to name the colours. The third page (Colour-Word, CW) consists of colour names printed in incongruent colours (e.g. the word 'red' printed in green or blue ink); the respondent is required to name colour of the ink, and try to avoid attending to the word itself. Participants were all given the same instructions and asked to read aloud from each page for 60 seconds. The number of words read was recorded as the score. We analysed raw Colour-Word scores and Stroop scores (defined as the interference score  $(W * C / (W + C)) = CW$  predicted and Stroop score =  $CW - CW$  predicted), which is not dependent on the participant's reading or colour-naming speed (61). In cases where a person can inhibit the word-naming response, the Colour-Word score will be higher than predicted, yielding a positive value for the interference score and vice versa.

Finally, all participants were asked to provide routine blood samples and collect four salivary cortisol samples.

#### *Follow-up. Definition of psychiatric illness and outcome assessment*

Our outcome was "onset" defined as development or occurrence of a mood disorder or other psychiatric disorder during the follow-up period. Onset was assessed with a SCAN interview at follow up. A multiplicity of methods was used to identify all individuals with a potential outcome. After baseline assessment, the participants were followed longitudinally at 6-monthly intervals. To obtain information on the development of an affective episode, participants received a letter containing the Beck Depression Inventory 21 item (BDI 21) (47) and the Mood Disorder Questionnaire (MDQ) (49), every 6 months as well as a questionnaire assessing potential LEs annually (a modum Kendler et al. 1993). The follow-up assessment was conducted from 01 January 2010 to 30 April 2012. At follow-up, all participants underwent a telephone interview. A SCAN interview was performed if participants according to the telephone interview had had 1) any contact to a psychologist or psychiatrist, 2) had been on sickness leave because of personal difficulties 3) were prescribed any psychopharmacological medicine, or additionally if 4) their answers in the questionnaires (BDI 21, MDQ) raised the suspicion of onset of psychiatric disorder or 5) they received a first psychiatric diagnosis in the DPCR during follow-up (this was possible as the personal identification numbers of all participants were linked to the DPCR).

#### 3.2.7 Ethics

The Danish Ministry of Health, the Danish Scientific Ethics Committee ((KF)-12-122/99 and (KF)-01-001/02) and the Data

Protection Agency approved the study protocol. The study was conducted in accordance with the most recent version of the Declaration of Helsinki. All participants gave written, informed consent.

#### *Statistical analyses*

Multiple group comparisons between onset participants and the healthy participants were performed using one-way analysis of variance (ANOVA) in the case of continuous variables or chi-square tests in the case of categorical variables, with onset as the dependent variable. The Kaplan-Meier method for estimation with censored observations was used to calculate the probability of remaining healthy (i.e. without onset of a psychiatric disorder) and the Log Rank test was used to estimate possible differences in onset rates between high-risk and low-risk twins. Participants were censored at the time of death or withdrawal from the study. Hazard ratios (HR) were estimated in separate models using Cox proportional hazards regression to determine significant predictors of time to onset of a psychiatric disorder. All survival analyses were adjusted for the effects of age and sex. In analysis of two-way interactions, HR was estimated in a multivariate Cox model using a forward conditional procedure to determine significant predictors of time to onset of a psychiatric disorder, including main effects for all variables and two-way interactions. The level of significance was set at  $p < 0.05$  (two-tailed). The Statistical Package for the Social Sciences was used to create a database and to undertake the statistical analyses (SPSS, version 15 for Windows).

#### **Results**

This section presents results relating to:

- A. The impact of familial risk, subclinical symptoms, neuroticism, LEs and QoL (Sections 4.1 and 4.2).
- B. Structural brain changes cross-sectionally and the impact of cognitive function longitudinally (Sections 4.3 and 4.4.)
- C. Biomarkers, genomics and G x E interactions (Sections 4.5 and 4.6).

#### *Family history, subclinical symptoms, life events and the trait neuroticism as predictors of onset of psychiatric illness (Paper I).*

The aim of this study was to investigate whether onset of affective disorder was predicted by family predisposition to affective disorder, subclinical psychopathology, neuroticism, LEs, sex or age. Having a family history of affective disorders increased the risk of onset of psychiatric illness by more than one third (Figure 2). Subclinical symptoms at baseline measured with clinical scales and self-rating scales were also found to predict onset of affective disorder. Female sex and younger age were associated with development of affective disorder. Onset of psychiatric illness was also predicted by neuroticism, lifetime experience of severe LEs and LEs during the follow-up period. In further analyses we showed that the probability of developing a psychiatric disorder increases with the number of even minimal subclinical symptoms measured with the 17-item Hamilton Depression Rating Scale (HDRS 17) or with the self-report scales BDI 21 and the 14-item Anxiety scale (62).



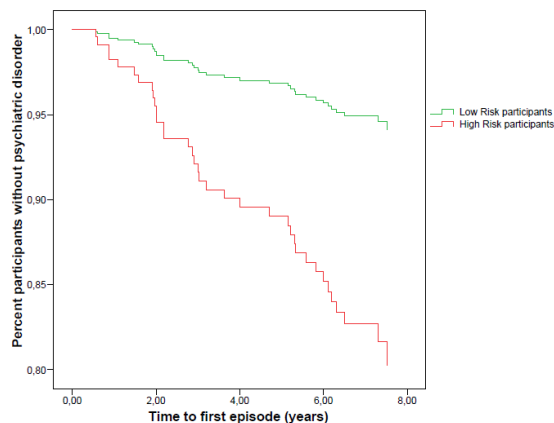


Figure 2. Risk of onset of a psychiatric disorder according to risk status (high or low: with or without a family history of unipolar or bipolar disorders).

### Cross-sectional analysis of quality of life (Paper II)

The disability and hardship associated with affective disorder is shared by the family members of patients and may have a detrimental affect on their QoL (63,64). The aim of this study was to investigate the hypothesis that familial risk for affective disorder was associated with a lower QoL in healthy individuals. Paper II concluded that being at familial risk for affective disorder seems to have a negative influence on the QoL of the healthy co-twin even when the healthy twin did not share a home with the affected co-twin. We therefore had to consider the possibility that this was due to other factors known to affect QoL e.g. coping style, personality and, in particular physical health, as the high-risk twins had significant lower scores on the domain physical health in the cross-sectional study than the low-risk twins and the normal control persons (Paper II). Because the analysis was cross-sectional it was not possible to distinguish between subsyndromal state and trait scores (65), so it was of interest to estimate the predictive value of the above-described results.

For the predictive impact of the cross-sectional findings from paper II, the following analyses were conducted (not published elsewhere): in univariate analysis, the four baseline domains 1) physical health 2) psychological health 3) social relationships 4) environment and the overall WHOQoL-BREF score (52), were significantly impaired for the 36 onset participants compared with the healthy participants. In further analyses, a multivariable Cox regression model with illness onset as the dependent variable and risk status, gender, age, Hamilton score and overall QoL as covariates found that the following variables were associated with onset: Hamilton score at baseline (HR = 1.22, CI: 95 % 1.03-1.45,  $p = 0.02$ ); risk status (HR = 1.29, 95 % CI: 1.01-1.66,  $p = 0.05$ ) and female sex (HR = 2.58, 95 % CI: 1.06-6.28,  $p = 0.04$ ), but overall QoL was not (HR = 0.99, 95 % CI: 0.97-1.02,  $p = 0.72$ ). The same pattern was observed for all subscales of the WHOQoL-BREF (results not presented). Overall, WHOQoL-BREF scores have limited predictive value; the observed baseline differences did not predict onset after controlling for variance in subclinical depressive symptoms. Hamilton score seems to be an independent, more robust predictor of onset than the various WHOQoL-BREF scores. In conclusion, QoL did not predict onset although cross-sectional analysis demonstrated that high-risk twins had lower QoL. This association may be a state phenomenon derived from

and explained by the higher level of subclinical depressive symptoms in the high-risk group. Together these analyses suggest that there seems to be a bidirectional association between depressive symptoms and QoL.

### Cross-sectional analysis of structural brain abnormalities (Paper III)

In the cross-sectional part of the study we obtained magnetic resonance imaging (MRI) brain scans for a proportion of the participants. Following comments by several reviewers we decided to exclude twins with a bipolar proband or other first-degree relatives with severe psychiatric disorders from the analysis, leaving a sample comprising 59 healthy, high-risk twins (co-twin with unipolar depression) and 53 healthy, low-risk twins (no first-degree family history of major psychiatric disorder). As it is unclear whether the structural brain abnormalities associated with unipolar depression are present in healthy persons at risk of developing the disorder we investigated whether a genetic predisposition to unipolar depression was associated with structural brain abnormalities. We hypothesised that high-risk twins would have lower hippocampal volume than low-risk participants. The results confirmed our hypothesis: high-risk twins had smaller hippocampal volumes than low-risk twins ( $p < 0.04$ ) although there were no group differences in global brain tissue volumes or regional tissue volumes based on exploratory, voxel-wise, whole-cerebrum analyses. In conclusion, lower hippocampal volume may indicate a predisposition to depression (66) and ongoing analyses will reveal whether lower hippocampal volume predict unipolar depression (67).

### Cognition as a predictor of onset of illness (Paper IV)

Affective disorders seem to affect cognitive function in a proportion of patients (68). At present it is unclear whether the cognitive deficits are attributable to core mood symptoms or constitute a separate cluster of symptoms, with independent effects on prognosis and functional status (69). It is therefore possible that cognitive impairment is present before the onset of depression rather than developing as part of the disease process; this would be consistent with our cross-sectional findings (33). No high-risk studies have investigated cognitive function as a predictor of illness onset, which raises the intriguing question of whether cognitive impairments reflect neurodevelopmental changes as well as neurodegenerative processes occurring as a result of the illness. The aim of Paper IV was, therefore, to investigate whether baseline cognitive function predicted later occurrence of depression. The findings presented in Paper IV suggest that in healthy individuals lower baseline cognitive performance predicts subsequent psychiatric illness. This association was significant even after controlling for variance in baseline subclinical depressive symptoms and well-established risk factors, namely a family history of affective disorder, youth and sex (70). This suggests that the cognitive impairment is present before the onset of affective disorder and therefore partly reflects neurodevelopmental processes. The existence of an ongoing neurodevelopmental process in healthy individuals at risk of affective disorder is further supported by our demonstration of lower hippocampal volume in healthy high-risk twins from this cohort, suggesting that a reduction in hippocampal volume may be part of the diathesis (66) (Paper III).

*Cross-sectional and prospective analysis of associations between affective disorder and the 5-HTTLPR polymorphisms, familial risk, salivary cortisol levels and subclinical psychopathology (Papers V and VI)*

A functional deletion/insertion polymorphism in the promoter region of the gene for the serotonin transporter gene (5-HTTLPR) creates a short (s) allele and a long (l) allele, which alters the promoter activity. The long variant has approximately threefold higher transcriptional activity than the short variant (71). Activation of the hypothalamus-pituitary-adrenal (HPA) axis is part of the primary response to stress. The serotonergic system has a complex influence on the HPA axis (72) and can either stimulate or inhibit it (73). In the cross-sectional part of the study we investigated whether the distribution of the alleles on 5-HTTLPR was associated with a genetic predisposition to bipolar or unipolar disorder and whether variations in 5-HTTLPR interact with LEs in relation to depressive symptoms, neuroticism and awakening and evening salivary cortisol levels. Analysis of 81 l/l individuals and 125 l/s and s/s individuals provided no evidence of an association between the allele distribution and a genetic predisposition to affective disorders. Presence of the short allele of 5-HTTLPR and LEs were associated with a higher neuroticism score, but not with depressive symptoms or salivary cortisol levels (74) (Paper V).

In the follow-up study we investigated the predictive value of baseline cortisol levels and the two-way interactions between familial risk for affective disorder and LEs, high neuroticism and LEs, and 5-HTTLPR and LEs. Baseline salivary cortisol levels did not predict occurrence of psychiatric illness, however lifetime history of severe LEs and the interaction between morning cortisol and lifetime history of LEs (low morning cortisol was associated with more LEs) predicted later onset. The cross-sectional finding that neuroticism scores were predicted by the two-way interaction between LEs and the short allele of the 5-HTTLPR gene (34) is consistent with this follow-up study finding that neuroticism and the LEs x 5-HTTLPR interaction predicted occurrence of psychiatric illness. The finding that carriers of the short allele with a lifetime history of a high number of LEs were at increased risk of illness onset is consistent with previous research and with our hypothesis. Familial risk of affective disorder predicted illness, as shown in Paper I (62) and we unexpectedly found that an established risk factor, being at familial risk for affective disorder, was amplified in individuals carrying the short allele of 5-HTTLPR (75) (Paper VI).

*Cross-sectional and prospective analysis of associations between affective disorder and BDNF Val66Met polymorphisms, familial risk, whole-blood BDNF levels (Papers VII and VIII).*

BDNF is a member of the nerve growth factor family and is involved in promoting neuronal differentiation, synaptic connectivity, neuronal repair and survival (76). It has been shown that serum BDNF levels are reduced during depressive and in manic episodes, but return to normal in the euthymic phase (77,78). Several brain-imaging studies have demonstrated the functional influence of the Val66Met polymorphism on the expression of BDNF (79,80) and Val66Met has been associated with stress-related dysfunction e.g. depression (81). Furthermore, stress is known to affect the HPA axis and increased secretion of cortisol has been observed in a proportion of patients with affective disorder (82,83). There is thus compelling evidence that both neurotrophic and HPA systems are involved in the pathophysiology of affective disorder (84,85). While the relationships described

above suggest an association between the Val66Met polymorphism, BDNF levels and the HPA axis, associations between this polymorphism, peripheral BDNF levels and cortisol have not been studied in a single sample. The aim of the cross-sectional analysis was to investigate whether the BDNF Val66Met polymorphism was associated with a familial predisposition to bipolar and unipolar disorder and to investigate whether variations in Val66Met polymorphism interacted with a familial predisposition to affective disorder in relation to whole-blood BDNF levels, and awakening and evening salivary cortisol levels. We found no differences between healthy high-risk and low-risk twins with respect to the distribution of the alleles of the BDNF Val66Met polymorphism, indicating that there was no association between specific alleles and familial risk of affective disorders (86). We found that high-risk twins carrying the met allele presented with higher whole-blood BDNF and higher evening salivary cortisol. There were no associations involving awakening cortisol levels (86) (Paper VII).

BDNF is a potential biomarker of affective disorder. There is, however, a lack of longitudinal studies evaluating its value as a predictor of psychopathology, so in the follow-up study we assessed the predictive value of baseline whole-blood BDNF levels and the two-way interactions between BDNF levels and the Val66Met polymorphism and between familial risk and the Val66Met polymorphism. Baseline BDNF levels did not predict onset of illness. The Val66Met polymorphism was not an independent predictor of illness and nor was it a predictor in two-way interactions with the other risk variables analysed (87) (Paper VIII).

## Discussion

### Main results

These results suggest that familial risk, impaired stress tolerance and cognitive dysfunction may be important predictors of later occurrence of illness and that it is possible to identify a cluster of prodromal symptoms: subclinical symptoms of anxiety and depression, higher neuroticism and cognitive problems. The cognitive problems may be related to the cross-sectional finding that high-risk twins have lower hippocampal volumes than low-risk twins. Furthermore, two genetic polymorphisms, 5-HTTLPR and BDNF Val66Met, were not directly associated with familial risk for affective disorder and were not prospective predictors of illness. Similarly, salivary cortisol levels and whole-blood BDNF levels were not predictive of subsequent illness. However, the more complex two-way interactions between 5-HTTLPR and LEs suggest that if high-risk individuals are exposed to more stressors this increases their overall risk of developing an affective disorder and may accelerate onset. In contrast, low-risk individuals seem to experience fewer LEs and may also be more resilient to them.

### Comparison with other studies

Our finding of increased risk for affective disorders in the high-risk twins is in line with the overall conclusions from family studies conducted over the last 90 years (20). Our results are also consistent with the impressive research carried out by Weissmann's group, which followed the offspring of depressed parents through three generations, and concluded that they constituted a high-risk group for early psychiatric problems and that their high-risk status persisted during adulthood (88,89). Four prospective studies of high-risk subjects, including bipolar probands and their offspring, have been following children into adulthood. Together these studies have followed 523 offspring for up to 34 years; all four studies have reported high rates of psychopathology in their



high-risk subjects although onset of bipolar I disorder was still relatively rare among offspring with a parent with bipolar disorder (90-93). The results are also consistent with a meta-analysis (94) showing that offspring of a parent with bipolar disorder have a 2.7 times higher risk of developing a mental disorder and 4 times higher risk of developing a mood disorder than the offspring of healthy parents.

The results from paper I are also in line with the results of a large family study (95) which investigated subthreshold depressive symptoms and self-reported symptom inventories in individuals who did not meet the criteria for major depressive disorder. The study found familial aggregation of psychopathology according to self-reported symptoms and concluded that subthreshold depressive symptoms might be a forerunner of major depressive disorder. In the cross-sectional paper on personality traits we showed that high-risk twins had higher neuroticism scores (34); however when included in a more complex multiple regression model neuroticism was also correlated with female sex, minor psychopathology and recent LEs. In Paper I we reported that higher baseline neuroticism scores were also predictive of later illness, in line with previous high-risk studies showing that neuroticism seems to be a precursor of major depression (for a review see 96). However the other risk factors, namely family risk, female gender, youth, LEs and subclinical symptoms, were all predictors of subsequent illness, which underscores the existence of strong correlations among risk factors. Our finding that LEs are a risk factor for later development of psychiatric illness corroborated the findings of Kendler and colleagues (15). Their study also used a population-based registry and had the strength that the twins were ascertained independent of treatment facilities because all twins in the register were contacted. Both members of twin-pairs were enrolled, but a limitation was that only female twin pairs were included.

The cross-sectional finding that high-risk twins had lower hippocampal volume than low-risk twins is consistent with another study which compared middle-aged (mean age = 37.25 years, SD = 14.24) first-degree relatives of patients with major depression to healthy controls (mean age = 35.65 years, SD = 11.73) and found that high-risk individuals with a history of emotional abuse had significantly smaller left and right hippocampal heads than the individuals without (97). Furthermore, a recent two-year follow-up study of a cohort of initially unaffected young adults at high familial risk for affective disorder found that reduced cortical thickness in the right parahippocampal and fusiform gyri were familial trait markers for vulnerability to mood disorders (98).

In Paper IV, we concluded that cognitive impairment in affective disorders may reflect both ongoing neurodevelopmental and neurodegenerative processes. The results of the cross-sectional analysis of our cohort supported the hypothesis that cognitive abnormalities are present before the onset of unipolar or bipolar disorder (33). In the prospective study, we found that baseline cognitive deficits, particularly in executive function and attention, predicted subsequent affective illness. It is a limitation of the study that we did not repeat the neuropsychiatric battery at the follow-up assessment and this result is based solely on the baseline cognitive assessment. However, because cognitive function was assessed at the start of the study we can be confident that the results were not influenced by factors such as medication for affective disorder, LEs or physical illness during the follow-up period. It is, however, not possible to determine whether partici-

pants who experienced onset of affective disorder during the follow-up period also exhibited a worsening of the cognitive impairment.

The HPA system may mediate the ability of the central nervous system to respond to stressors early in life. Early exposure to stress may induce a long-lasting increase in responsiveness to stress. In other words, it appears that early adversity results in a more reactive HPA system and thus is a candidate biological mechanism for the process whereby severe LEs increase an individual's risk of developing psychopathology. This proposal is consistent with our finding that individuals with lower baseline cortisol levels and a lifetime history of a large number of LEs were more likely to develop illness. Lower baseline morning cortisol levels may thus represent a compensatory adaptation to a hyper-reactive HPA system. It is possible that psychiatric illness occurs when such compensatory mechanisms break down. This is in line with recent results from a two-year follow-up study of healthy individuals, indicating that low salivary cortisol may be a risk factor for depression (99). In addition a 5.5-year prospective study of patients with recurrent depression showed that lower cortisol levels predicted recurrence and that childhood trauma was associated with lower cortisol levels (100).

The present negative findings concerning the two biomarkers BDNF and cortisol (Papers VII and VIII) suggest that peripheral BDNF and cortisol levels cannot be used as a marker of vulnerability to affective disorder. Given the limitations of this study - namely the small sample, a limited number of onset cases and that only one measurement of whole-blood BDNF and four measurements of cortisol levels were taken, at baseline - there is a need for further longitudinal research to evaluate the predictive value of a broad range of biomarkers in high-risk cohorts and across mood states.

#### *Strengths and limitations*

In the following sections methodological factors which have a bearing on the interpretation of results are discussed: study design, recall bias, selection bias, drop-out rates, statistical techniques, use of registers, age, sex, socio-economic status (SES), comorbidity, baseline assessment tools, substance abuse, medical treatment and representativeness.

#### *Study design*

It is impossible to draw firm conclusions about causality on the basis of cross-sectional data; see, for example, Paper II. Cross-sectional data revealed that high-risk twins had lower QoL than low-risk twins, but lower QoL proved not to be predictive of subsequent illness; it seems rather that it is attributable to the higher levels of anxiety and depressive symptoms found among the high-risk twins. The prospective design is thus a more robust design as it offers the opportunity to observe the longitudinal effects of variables and determine their predictive value.

#### *Recall bias*

It is possible that low-risk twins recall previous depressive or difficult periods in their life at a lower rate than high-risk twins, who may be personally affected by their co-twin's disease. We cannot, therefore, exclude the possibility that a recall bias may partly account for the higher rate of LEs among the high-risk twins in both the cross-sectional and prospective studies. Furthermore, the experience of having a co-twin with affective disorder may influence an individual's perception of his or her own subclinical

symptoms and act as chronic stressor, e.g. because the individual worries about his or her co-twin or experiences more LEs as a result of the co-twin's illness; in the long term this may increase the risk of the healthy twin developing an affective episode (34). It should be noted, however, that affected co-twins had their first admission in adulthood, after the age of 18 years, and that only a few of the investigated high-risk twins had been sharing a home with their co-twin (shared environment) at the time of admission.

#### *Selection bias and dropout rates*

Using registers instead of asking probands for permission to contact their relatives reduced the risk of selection bias. In this study, the group of twins ( $N = 20$ ) with whom we were unable to establish contact (no response, Figure 1), was characterised by a relatively high proportion of men (45 %) and disproportionate number of high-risk twins (65 %) (31). It is likely that psychopathology was more prevalent in this group and in the group of non-participants and the group who refused to be contacted by the Danish Twin Registry than in the group of participants (31). A participation rate around 65 % (Figure 1) at baseline was satisfactory. There was a tendency towards a higher participation rate in the high-risk groups; this was anticipated and is assumed to be due to motivation to help one's co-twin. The extensive assessment and the fact that some participants had to travel more than 300 km to participate may have selected for a group of participants with more energy than the group that was unable to participate.

Seven participants died during the follow-up period (five high-risk participants and two low-risk participants; four died from cancer, one from a heart attack and two from unknown causes; there were no deaths by suicide), one (low-risk) participant emigrated and three (one high-risk, two low-risk) were impossible to trace. The remaining 223 eligible participants were contacted by letter, then phone call, and invited to participate in the follow-up interview; 218 participants (98%) agreed to do so. In longitudinal research one must consider the risk that fewer responses will be obtained from participants with more severe psychopathology; however the high participation rate for the follow-up part of the study suggests that this bias was minimal in our study. One must also consider the possibility that participation in the research induced a heightened awareness of psychiatric symptoms, although the risk is equal for high and low-risk twins. At baseline, the participants were asked questions about psychiatric well-being and subsequently they received questionnaires dealing with this twice a year.

#### *Statistical analyses*

Follow-up studies often comprise incomplete sets of observations (censored data), as some patients die, emigrate or drop out. Using survival analyses in follow-up studies is mandatory to take account of the length of time for which data on these censored individuals is available. Statistical power in studies of predictors of outcome is influenced by sample size, the presumed impact of the risk factor, the presumed prevalence of the outcome and the duration of the observation period. The Cox proportional hazard model was used to analyse time to event (onset of psychiatric illness) (101). The hazard function is the rate at which an event occurs with a specified time interval, given that the event has not occurred before that interval. The Cox model is commonly used for analysis when the effects of covariates are of interest, as it allows covariates to affect the hazard of an event and to vary with time. As the outcome in this prospective study was uniform 'on-

set of psychiatric illness' the Cox model was used; however other models could have been used e.g. multi-state models which allow individuals to change state over time (e.g. from anxiety disorder to bipolar disorder).

The limited number of participants is a limitation of this naturalistic register linkage study. The initial cohort consisted of only 234 participants and in the follow-up part of the study 36 participants (15 %) developed psychiatric illness. This means that the statistical power may not have been sufficient to detect the effects of the weaker risk factors (e.g. the impact of two-way interactions involving genetic polymorphisms). We cannot exclude the possibility that the negative findings might reflect a type 2 error. Paper VII reports the results of a post hoc statistical power calculation using the open source statistical power calculation tool [http://www.openepi.com/v37/Menu/OE\\_Menu.htm](http://www.openepi.com/v37/Menu/OE_Menu.htm), with the predictive value of BDNF levels as an example. This power calculation showed that given a difference in whole-blood BDNF levels of 3.0 ng/l between those who went on to develop psychiatric illness and those who remained healthy, our sample size of  $n = 234$  with mean (SD) whole-blood BDNF levels of 18.6 (6.1) ng/l, yielded a power of 0.77 for a group difference at a significance level of  $p < 0.05$  (two-tailed). If group difference in whole-blood BDNF levels was only 2.5 ng/l, the power would be reduced to 0.62 (87): a difference of 0.5 ng/l does matter. At present setting thresholds for specific biomarkers would be a somewhat arbitrary process and such thresholds would be in many ways preliminary. As others have pointed out larger sample sizes will be needed to detect complex gene-environment interactions (102,103).

#### *Using registers*

The sample was population-based and using registers meant that we did not have to have the proband's permission to contact the high-risk twin; this is the normal procedure in other high-risk studies (104) and can result in selection bias. The high- and low-risk groups were chosen using the same criteria, which reduced selection bias in this study. By using register linkage and by including twins discordant for unipolar disorder it was possible to identify a considerably large sample over a limited time period.

Using registers has some disadvantages when it comes to the diagnosis of probands of: diagnoses are clinical rather than research diagnoses, before 1995 only hospitalised probands were included in the DPCR and only a few studies have looked at the validity of affective diagnoses in the DPCR (105). These studies showed that the ICD-8 diagnoses of affective disorders in the DPCR were correct in 94 % of cases when ICD-10 diagnoses made on the basis of case notes using OPCRIT were used as the reference (106). Similarly, the ICD-10 diagnoses of affective disorders were correct in 86.4 % of cases, with a SCAN interview as the reference (107).

Addressing the diagnostic crosstalk between ICD-8 and ICD-10 is difficult, since the two systems are not identical. ICD-10 differs fundamentally from ICD-8 and ICD-9 as it is based on phenomenological descriptions, whereas the two earlier systems were based on etiological principles (40). This means that aetiologically derived terms in ICD-8, such as 'neurotic depression' (classified under 'neurosis'; code 300.xx) and 'endogenous depression' (classified under 'manic depressive psychosis'; code 296.xx) and psychogenic depression (classified under 'other psychosis'; code 298.xx) are not used in ICD-10. Instead these conditions are grouped together as 'affective disorders' as they share depressive

symptomatology. A register-based comparison of ICD-8 and ICD-10 diagnoses of affective disorders was carried out in Denmark in connection with the introduction of ICD-10 in 1994. The study assessed the overlap between the affective diagnosis given in 1993 using ICD-8 and that given in 1994 using ICD-10; the results indicated that the differences between ICD-8 and ICD-10 were minor with respect to major affective disorders and that the ICD-10 concepts appeared broader and more comprehensive than those of ICD-8 (108).

It is unknown whether the diagnosis of the proband has changed as the study used register information about the diagnosis given at the first discharge. There have been two studies using the Danish registers to investigate diagnostic stability for affective disorders. The first study identified 4116 patients who had received a diagnosis of mania/bipolar disorder at least once; 2315 of these patients (56.2 %) received the diagnosis at the first contact, whilst the remaining patients (43.8 %) got the diagnosis at a later contact. Approximately 30% of patients with an initial diagnosis of mania/bipolar disorder received a different diagnosis during follow-up (109). There were 39,741 patients diagnosed with depressive disorder at least once; 81 % were diagnosed at the first contact. In approximately 56 % of these patients the initial diagnosis of depressive disorder changed during follow-up, most often to a diagnosis of schizophrenic spectrum (16 %), and to a lesser extent to personality disorders (9 %), neurotic, stress-related and somatoform disorders (8 %), and bipolar disorder (8 %) (110). Lastly, it should be noted that most individuals with affective disorder do not seek or receive treatment and it is likely that hospitalised patients had more severe forms of the disorders (111).

The choice of comparison group can substantially affect the results in family studies (112). Using registers meant that the high- and -risk groups were chosen using the same criteria, which may have reduced selection bias. It was not necessary to ask the proband for permission to contact his or her relatives. This method also means that recall bias in relation to the diagnosis of the probands is avoided because data are collected routinely and independently of researchers (32). Selection bias related to SES is minimised as the registers are nationwide, treatment in Denmark is free of charge and only a few private psychiatric outpatient and inpatient facilities exist. In addition, psychiatric care is well developed and the Danish population is socially and ethnically homogeneous. Furthermore, the inclusion of twins offers an opportunity to study high-risk individuals who are at a higher risk than is usual for high-risk studies, which usually use presence of the disease in any first-degree relative as the criterion for high-risk status. Lastly, it is possible to sample larger cohorts using registers. Using the registers as an easy way to obtain a sample of at-risk individuals does, however, have disadvantages: we could not determine the sample size in advance and estimates of the risk of developing mental disorders were pragmatic i.e. based solely on information from previous family and twin studies. As the Danish population is rather small we invited all identified high-risk individuals below the age of 70 years to participate and the present study sample ended up having a higher mean age than we had anticipated.

#### *Substance abuse and medical treatment*

All participants were asked about substance use; the baseline SCAN interview indicated that 12 of the 234 participants had an abuse disorder. Two of these 12 participants gave information

about previous cocaine abuse, one had a history of cannabis abuse; three had a history of alcohol abuse and six participants (five high-risk, one low-risk) at baseline reported ongoing periodic alcohol abuse, which could have influenced their cognitive scores and cortisol levels. Fifty-six participants (24 %) were taking daily medication (oral contraceptives excluded) but there was no significant baseline difference in daily medicine intake between the risk groups (31). Only a few participants had been prescribed anxiolytics or sleeping pills (used only occasionally) that might slow cognitive performance. It is strength of the study that none of the participants had ever been treated with psychoactive drugs when the study started; this is a common problem in the studies of cognition in patients with affective disorder because of the possible cognitive side effects of the medical treatment.

#### *Age and sex*

A limitation of the study is that there were significant age differences between the high- and low-risk groups at inclusion. This was not intentional, as the high- and low-risk groups were matched for age, but we had difficulty recruiting older control twins. It is possible that this was because they were less motivated to participate than twins with an affected co-twin. Analyses in the eight papers controlled for age effects and it was found that youth was a significant predictor of subsequent illness (Paper I).

Onset of most mental disorders, including mood disorders (113), occurs mainly during the first four decades of life. The heritability of unipolar disorder may also depend on the age of onset; it has been reported in a twin study that early-onset unipolar disorder (onset before 30 years) is more heritable than late-onset unipolar disorder (47 % vs. 10 %) (114). It has also been established that delay between onset and initiation of effective treatment is typically 5–10 years (115). The twins included in this study had a mean age of 43.9 years (SD = 13.3) at baseline; we therefore have to consider the possibility that the participating high-risk twins had passed the risk period. In this study we also found that youth was associated with subsequent illness (Paper I). Nevertheless, as can be seen from Figure 2, the risk of onset of psychiatric illness was increased by more than one third in the high-risk cohort and seemed to increase steadily throughout the observation period in individuals at familial risk. This suggests that although the older high-risk twins included in this study may have had some resilience. Nevertheless, their familial risk seems to contribute to a lifelong vulnerability (after follow-up was completed a 70-year-old high-risk female MZ participant informed us that she had been admitted to psychiatric hospital (August 2012) with severe depression which was treated with electro-convulsive treatment).

There were no sex differences between the high- and low-risk twins who participated in the study and the sex distribution was one third men and two third women, reflecting the known sex difference in unipolar disorder (116,117). In the follow-up part of the study female sex was found to be a significant predictor of subsequent illness (Paper I).

#### *Socio-economic status*

In the cross-sectional part of the study the high-risk twins had lower SES (lower educational level and a lower status occupation) than the low-risk twins (32). In the follow-up part of the study post hoc analyses using a Cox regression model (including risk status, sex, age and BDI 21 scores) showed onset of psychiatric illness was not predicted by SES (HR = 0.99, 95 % CI: 0.99-1.01, p =

0.48) or educational level (HR = 0.87, 95 % CI: 0.72-1.06,  $p = 0.17$ ) (results not presented elsewhere). Although there was a cross-sectional association between lower SES and familial risk, the longitudinal follow-up showed that SES was not predictive of illness onset. This may be because SES is a multi-dimensional construct, so analysis of a larger sample over a longer follow-up period would be needed to reject the hypothesis that SES is a predictor of subsequent illness. We also cannot exclude the possibility that the high-risk twins lower SES in comparison with the low-risk twins observed in the high-risk twins cross-sectional part of the study was a result of parental SES because we assessed only the participants' SES; not their parents' SES. As Denmark has a well-developed social security system and a relatively homogeneous social culture the consequences of low SES, e.g. early retirement, may not be as strongly associated with social stigmatisation as elsewhere. In contrast to our findings, analysis of a nationally representative sample of 5692 US adults found that childhood financial hardship predicted onset of all classes of mental disorders at every life stage, with odds ratios of 1.7-2.3 (118).

### *Comorbidity*

At baseline minor psychiatric diagnoses (e.g. previous alcohol abuse, phobia, stress-depression/anxiety reactions) were more prevalent in the high-risk twins, a result which is consistent with a review of 23 twin studies and 12 family studies of comorbidity of anxiety and depression (119). This review concluded that comorbidity involving different anxiety disorders and between anxiety disorders and depression was explained by a shared genetic vulnerability to both disorders. It is therefore debatable whether participants with a current or historical diagnosis of minor psychiatric illness should be rigorously excluded. We decided to include both high- and low-risk participants with current or historical minor psychiatric diagnoses (defined as non-organic, non-schizophreniform and non-affective disorders) due to the risk of type 2 error if they were excluded, and to avoid a bias towards a sample of resilient, healthy individuals. In the cross-sectional studies there were associations between current or historical minor psychiatric diagnosis and neuroticism and subclinical anxiety scores, but not cognitive performance, BDI 21 score, Hamilton scores or cortisol levels (32-35). However, in the follow-up analyses comorbidity did not predict subsequent illness (62).

### *Baseline assessment tools*

Our primary criteria for selecting assessment tools were validity and established effectiveness. We used the SCAN interview as it is a well-established diagnostic, semi-structured interview protocol. The questionnaires (described in details in section 3.2.5) were selected according to the same criteria, with the proviso that they had to be available in Danish. We had to translate some questionnaires into Danish, e.g. Kendler LEs and the Coping Inventory For Stressful Situations (120,121); our translations were not validated using a Danish sample. Our cognitive assessment battery was selected to include well-known, validated tests e.g. Trail Making A and B, the Stroop test, and CAMCOG, which has been used previously by our research group (122). It was also important that the cognitive tests should be practical, easy to administer and not too time-consuming, as there was a time constraint on the duration of the whole assessment (7-8 hours including MR-scan); this meant that we used a rather narrow cognitive battery. Including a broader range of tests, in particular computerised tests, might have proved more informative.

LEs were assessed retrospectively at baseline; we asked separately about lifetime history of LEs and experience of LEs during the year before the baseline interview. During the follow-up part of the study participants were asked to report LEs once a year, using a self-report checklist. The checklist is used to report occurrence of LEs regardless of their personal significance, duration and objective and subjective impact. A better method of capturing LE data would be to use semi-structured interviews because they are more valid and reliable; however, they are also more time consuming than checklists and would have been difficult to use during follow-up (123,124).

### *Representativeness*

Using twins, especially MZ twins, as high-risk participants gives the researcher a unique opportunity to study individuals at higher familial risk. Not all conditions that run in families are necessarily genetic, however; conditions can also be influenced by environmental factors such as social status, education level, nutrition, favourite TV programmes and religion (103). It is therefore difficult to determine whether a given risk factor is solely environmental or genetic (103). If the findings from a twin study are to be generalised, the twins must be representative of the general population. A Danish study of bipolar disorder found equal rates of bipolar disorder in twins and singletons (125). No study has investigated the relative prevalence of unipolar depression in singletons and twins, but a study based on the Danish Twin Registry showed that twins were slightly protected against suicide (126). Another study reported similar levels and variability of common psychiatric symptoms in twin and non-twin populations (127).

The sample for the studies reported in this thesis had a wide age distribution (22-70 years) but was restricted to twins born in Denmark who had agreed to be contacted through the Danish Twin Registry. It is unclear whether the results can be extrapolated to other ethnic or geographic populations. Finally, the substantial variation in the length of the period between the baseline assessment and the onset of the co-twin's affective disorder contributed to the heterogeneity of the cohort.

The number of cases of illness onset was not as high as one might have expected: 15% for the whole cohort over a period of seven years. Low incidence of onset was anticipated in the case of the low-risk cohort (five low-risk twins succumbed to illness: 6 %). In the high-risk group 31 participants (24 %) experienced onset of psychiatric illness during the study; the figure for the broader high-risk group, i.e. including the group of 18 high-risk twins with another family history involving a non-twin first-degree relative was 21 %. We have further information that at least three high-risk twins experienced onset after the end of the follow-up period, including them would increase the percentage to 26% or 23% including the 18 high-risk twins with another family history. In brief, almost one out of four middle-aged high-risk twins developed psychiatric illness during the seven-year study period; it is also worth noting that the risk seems to persist over time, as illustrated in Figure 2. It will therefore be of interest to reinvestigate the cohort again e.g. after 14 years.

### **Overall conclusions**

First-degree relatives of patients with affective disorders have a two to three times higher risk of developing affective disorder and this was confirmed in this study. The cross-sectional findings that twins at familial risk for affective disorder had higher rates of

subclinical anxiety and depressive symptoms, higher neuroticism scores and had experienced more LEs were reflected the follow-up study which found that all these variables predicted subsequent illness. The observed baseline cognitive impairment in high-risk twins also predicted subsequent illness. These findings support the notion that subclinical symptoms and increased stress sensitivity reflect latent psychopathology. Furthermore, it is possible that the genetic variables involved in familial risk act both directly and indirectly, for example genetic predisposition may affect exposure to risky environments and sensitivity to environmental stressors (128,129).

Summarising the investigated risk factors, a dysfunctional stress response is one possible final common pathway for affective disorders and would also apply to the closely related symptom complex of anxiety disorders. The dysfunctional stress response seems to have a genetic origin and be partly related to the functioning of the serotonin system, a lower serotonin traffic due to the short allele meaning that individuals carrying the s allele may begin from a higher baseline level of stress reactivity (81). Another possible pathway involves cognitive dysregulation, which could create a secondary vulnerability by reducing the individual's capacity to cope with stress. Neurocognitive function may represent an independent indicator of genetic risk for psychiatric disorders in general and it is possible that this risk contributes separately to cognitive functioning independently of diagnosis. The results of our study add to our understanding of the multiple levels of risk factors, including their interactions and potentially cumulative influence on vulnerability to full syndromal affective disorder.

There has been a renewal of interest in familial transmission of psychiatric illness, but most studies examine single disorders e.g. schizophrenia, bipolar disorder, anxiety disorder, without calculating comorbidity (130). It also seems that the earliest expressions of psychopathology are a non-specific mixture of affective dysregulation, aberrant resistance, motivational changes and anxiety states (131). Being at familial risk for affective disorders may lower the threshold for subclinical psychopathology due to pooling of multiple illness related genes and innate hyper-reactivity to stress. However familial risk may not necessarily apply to a single disorder; it may instead represent a vulnerability to a spectrum of psychiatric disorders (132). As psychiatric disorders have overlapping aetiologies and co-aggregate in families, research should move from using traditional, descriptive diagnoses to clinical entities or categories that are more closely related to the underlying workings of the brain as outlined in (133).

Overall, having a first-degree relative with affective disorder matters when it comes to risk of developing affective disorder. This thesis illustrates that family and high-risk studies are highly informative, and allow us to observe the pathological processes occurring prior to the onset of illness. Using nationwide registers to sample high-risk individuals is a practical method of avoiding selection bias and it is possible to sample larger cohorts using this method. Finally, as the treatment methods used so far in psychiatry mainly target end-state disorders the perspectives are identification of high-risk individuals and mapping their individual risk profiles aiming to develop treatments targeting earlier stages of the disorders.

## **Clinical and research implications**

### *Possible clinical implications*

Affective disorders can be seen as the end state of a long process. In 1993, Fava and Kellner introduced the concept of staging to psychiatric classification and developed staging models for several psychiatric disorders including bipolar and unipolar disorders (134). The staging model is based on longitudinal rather than cross-sectional observations and characterises disorders according to severity, development and features and potentially allows the clinician to select different treatments for earlier stages (135). An important objective in psychiatry is to shift from the traditional focus on treating end-stage illness to intervening in the earlier stages, as already happens in other medical areas (e.g. heart diseases) (4). Although the mean age of this cohort was higher than high-risk studies of the offspring of parent with affective disorder (children and adolescents) we found a similar pattern of results, with affective instability and anxiety emerging as precursors of subsequent illness. Nevertheless, one should be careful in extrapolating from children and adolescents to an adult cohort. Since the impact of family risk seems to persist into middle-age, and youth contributed significantly to the risk of illness onset in the follow-up period the staging model may be clinically relevant also when assessing older at risk individuals. Finally, more insight into how molecular genetic risk factors influence cellular processes and behaviour is needed. Integrating our current knowledge of clusters of candidate biomarkers will also be essential (136).

These findings may add weight to the clinical arguments for earlier and more thorough psychiatric assessment including neuropsychological testing in individuals with a family history of affective disorder in order to achieve earlier diagnosis and easier admission to psychiatric treatment. For example, cognitive impairment has a substantial impact on functional outcomes, e.g. affecting patients' ability to work and seems to have an adverse influence on the course of illness (137-139). Our results indicate that it would be of benefit to develop preventive treatments targeted at individuals at familial risk who also exhibit a discrete cognitive impairment of the form outlined in a recent review (140) or signs relevant to the depression and anxiety spectrum. A further clinically important finding is that such individuals seem to be at increased risk of developing prolonged and pathological reactions when exposed to severe adverse events and this risk seems to persist over time. Clinical staging could be used to ensure that high-risk individuals have easy access to psychiatric assessment and hence to interventions to improve coping strategies or reduce LE exposure as well as to prompt psychological and, if necessary, medical treatment. Such an approach would help to avoid psychopathology going untreated for lengthy periods and might halt the progression of disorders as well as potentially being more effective. However, these post hoc hypotheses need to be tested in a randomised clinical research design to determine whether the suggested approach to monitoring, diagnosis and treatment would be of social, clinical and economic benefit.

### *Research implications*

This study has enabled us to delineated risk factors for affective disorder with the aim of advancing understanding of the pathophysiological mechanisms involved, and in the longer term facilitating earlier diagnosis and better treatment. Family studies using national registers to obtain a larger cohort are recommended in order to replicate and extend the findings presented in this thesis. Research into developing a broad psychosocial interven-

tion directed at selected high-risk cohorts including adults at increased risk for psychiatric spectrum disorders would be a natural extension of this study. Such an intervention could be tested in a randomised controlled trial to determine whether a structured, psychosocial intervention (e.g. a psychoeducation programme) prevents onset of illness and improves functional outcome. It is recommended that such an intervention should integrate the accessible, observable factors such as cognition, dispositional variables, subclinical symptoms, family history, stress measures and potential risk gene polymorphism, for example the 5-HTTLPR gene polymorphism. When this study began there was a lack of newer high-risk studies integrating the technical advances of the last twenty years in genetics and imaging. Our hypotheses were therefore developed to refer to a broad range of risk factors from different domains and, in particular gene x environment interactions. Now, a decade later, new and promising research results on metabolic and immune markers, oxidative stress and emotional processing have emerged and it is regrettable we did not assess these parameters at baseline. It is recommended that future high-risk studies include prospective assessments of a broad selection of relevant clusters of candidate biomarkers. Finally, there is a lack of twin studies in which participants are seen clinically and comprehensively assessed. Future researchers are therefore encouraged to use the Scandinavian registers to sample a large cohort of twins and to consider the both twins in a pair regardless of concordance. Our group is currently conducting a multidisciplinary study of 200 MZ twins (100 pairs) involving a comprehensive clinical assessment encompassing diagnosis, mood symptoms, cognitive tests, blood samples, neuroimaging and questionnaires.

Overall, identification of individual risk factors, including individual biomarkers, will be a key factor in future diagnostic procedures and strategies for prevention of affective disorders. Studying individuals at high risk of developing affective disorder offer a unique opportunity to evaluate how well potential risk factors discriminate between healthy and affected individuals. This thesis end by quoting Kandel's words from 15 years ago, urging the development of a new intellectual framework for psychiatry, as they remain pertinent today: "The analysis of interactions between social and biological determinants of behaviour can best be studied by also having the full understanding of the biological components of behaviour" (1).

### Summary

Risk impact of having a first-degree relative with affective disorder: a 7-year follow-up study (risk and protective factors in affective disorders).

This study investigated a high-risk sample in order to elucidate risk factors for affective disorder. Healthy monozygotic (MZ) and dizygotic (DZ) twins with and without a co-twin with a history of affective disorder were identified through nationwide registers. Two risk groups were identified: the high-risk group comprised twins at risk of developing affective disorder (DZ or MZ twin; index co-twin affected); the low risk group (control group) comprised twins at low risk of developing affective disorder (DZ or MZ twin; index co-twin not affected). At baseline 234 participants were divided into groups according to their risk for affective disorder; they were followed up at 6-month intervals with posted questionnaires assessing depression. After a mean follow-up period of seven years, the participants were invited to participate in an individual interview. A total of 36 participants (31 high-risk

twins and 5 low-risk twins) developed a psychiatric disorder during the seven-year follow-up period: 24 developed mood disorder (67 %), 7 anxiety disorder (19 %) and 5 (14 %) substance abuse, schizophrenia or personality disorder.

The results showed that familial risk, impaired stress tolerance and discrete cognitive dysfunction seem to be core predictors of affective illness. It is possible to identify a cluster of prodromal symptoms encompassing subclinical anxiety and depressive symptoms, higher neuroticism and cognitive problems. The cognitive problems may further be related to the cross-sectional finding that high-risk twins had lower hippocampal volumes. Further, two genetic polymorphisms: the 5-HTTLPR and the brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms were not directly associated with familial risk for affective disorder and did not predict illness onset. Similarly, salivary cortisol levels and whole-blood BDNF levels did not predict subsequent illness. The more complex two-way interactions between 5-HTTLPR and LEs suggested that high-risk individuals and individuals carrying the short s allele are exposed to more stressors and that this seems to contribute to an overall enhanced risk and thus accelerate the onset of illness. Low-risk individuals seem to experience fewer LEs and may exhibit resilience to their adverse psychological effects.

Overall, having a first-degree relative with affective disorder matters. This thesis demonstrates that high-risk studies are informative, allowing observation and investigation of the pathological processes that occur prior to the onset of illness. There is a lack of prospective intervention studies assessing psychopathology in well-defined, high-risk samples and it is obvious that future research must transcend diagnostic boundaries in order to have an impact on prevention. Furthermore, there is a need to move beyond the notion of 'magic bullets', instead developing an integrated paradigm encompassing clusters of biomarkers related to behavioural measures of developmental psychopathology. Finally, as most psychiatric treatment developed to date target end-state disorders, the identification of high-risk individuals and mapping of individual risk profiles should be a priority in order to facilitate early treatment and prevention.

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