**Oxytocin receptor gene variation, behavioural inhibition, and adult separation anxiety –**

**role in complicated grief**

Miriam A. Schiele a,b, Barbara Costa c, Marianna Abelli b, Claudia Martini c,

David S. Baldwin d, e, Katharina Domschke a and Stefano Pini b

**Author affiliations**

a Department of Psychiatry and Psychotherapy, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany

b Department of Clinical and Experimental Medicine, Section of Psychiatry, University of Pisa, Pisa, Italy

c Department of Pharmacy, University of Pisa, Pisa, Italy

d Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, United Kingdom

e University Department of Psychiatry, University of Cape Town, South Africa

**Corresponding author**

Miriam A. Schiele, PhD, Department of Psychiatry and Psychotherapy, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Hauptstrasse 5, 79104 Freiburg, Germany, Phone +49 761 270-66692, Fax +49 761 270-66693, Email: miriam.schiele@uniklinik-freiburg.de

**Abstract**

Objectives: Complicated grief (CG) following bereavement significantly increases the risk for mood and anxiety disorders. The severity of grief reactions may be interactively influenced by temperamental and psychological factors such as behavioural inhibition (BI) and separation anxiety (SA) as well as biological factors. Given its central role in attachment and stress processing, a genetic variant in the oxytocin receptor (*OXTR*) gene was thus investigated in order to elucidate the direction of association as well as its interaction with BI and SA in the moderation of CG severity.

Methods: Ninety-three patients with mood and anxiety disorders were evaluated for CG by means of the Inventory of Complicated Grief (ICG), for BI using the Retrospective Self-Report of Inhibition (RSRI), and for symptoms of SA during adulthood using the Adult Separation Anxiety Scale (ASA-27). All patients were genotyped for *OXTR* rs2254298.

Results: *OXTR* genotype interacted with BI and, on a trend-level, with adult SA, to increase CG. Specifically, higher levels on the RSRI and ASA-27 scales, respectively, were related to higher ICG scores in GG genotype carriers.

Conclusions: The present study for the first time suggests a gene-environment interaction effect of an *OXTR* gene variant with behavioural inhibition and possibly also symptoms of adult separation anxiety in the moderation of vulnerability for complicated grief.

**Keywords**

Temperament, affective disorders, anxiety disorders, gene-environment, bereavement

**Introduction**

Bereavement constitutes one of the most severe stressful life events and is associated with an increased risk for the development of mood and anxiety disorders. Complicated grief (CG) refers to the prolonged (persisting over six months), clinically significant grief reaction following the loss of a loved one that occurs in approximately 10-20% of bereaved individuals (Middleton et al. 1996; Prigerson et al. 1999; Shear and Shair 2005). CG comprises symptoms of separation distress and traumatic distress and has been reported to be highly comorbid with major depression, anxiety disorders, and post-traumatic stress disorder (Melhem et al. 2001; Kersting et al. 2009; Marques et al. 2013; Keyes et al. 2014).

The severity of grief reactions may be subject to the interactions of a range of temperamental and psychological factors, particularly those rooted in childhood, as well as biological factors predisposing the individual to react abnormally to the loss of a beloved person. For instance, temperamental characteristics may influence grief reactions. Behavioural inhibition (BI) is an early temperamental trait characterised by behavioural restraint, fearfulness, and avoidance in response to novel and unfamiliar situations (Kagan et al. 1984), which has been found to be moderately heritable and linked to increased risk for anxiety and affective disorders (Hirshfeld-Becker et al. 2008). Similarly, childhood separation anxiety has been linked to increased CG risk following bereavement later in life, and this association was particularly pronounced if childhood separation anxiety persisted into adulthood (Vanderwerker et al. 2006). Adult separation anxiety (ASA) has also been associated with CG independently of the childhood disorder, and has been reported to be associated with greater symptom severity and impairment of CG (Pini et al. 2012; Gesi et al. 2017). Finally, biological, i.e. genetic predisposition may contribute to the emergence, intensity, and chronicity of grief symptoms (cf. Kersting et al. 2007).

Given the central role of the oxytocin system in social behaviour, bonding, attachment, anxiety and stress processing (for review, see Gottschalk and Domschke 2017; Kumsta and Heinrichs 2013; Bandelow et al. 2017), the oxytocin receptor (*OXTR*) gene may constitute a key candidate gene in the investigation of the molecular genetic underpinnings of the behavioural and psychological consequences of the loss experiences. In an animal model of depression, depressive-like behaviour following partner loss has been linked to alterations in oxytocin function (Bosch et al. 2016). While to the best of our knowledge, to dateno study has investigated *OXTR* gene variation with regard to CG, variants in the *OXTR* gene have been linked to categorical and dimensional phenotypes of anxiety and depression, either directly or interactively with environmental components including early trauma or attachment styles (e.g., Dannlowski et al. 2016; Notzon et al. 2016; Costa et al. 2017; for review, see Meyer-Lindenberg et al. 2011), which in turn have also been associated with temperamental characteristics and separation anxiety (see for example Muris and Meesters, 2002; Pini et al., 2014; for review, see Baldwin et al., 2016). This association is particularly true for the intronic *OXTR* A/G single nucleotide polymorphism (SNP) rs2254298, which among the approximately 30 known *OXTR* SNPs is one of the most widely studied SNPs so far with regard to its impact on various psychological dimensions, such as affect, temperament, neuroanatomical correlates of social cognition, anxiety and depression (for review see Brüne, 2012; Kumsta and Heinrichs, 2013) as well as its interaction with attachment and childhood trauma (Costa et al. 2009; Thompson et al. 2011), rendering rs2254298 an ideal candidate for the present investigation.

Therefore, the aim of the present study was to explore for the first time the association between *OXTR* rs2254298 with symptoms of complicated grief in patients with mood and anxiety disorders, and whether this relationship was moderated by dimensional risk factors previously linked to traumatic grief and symptoms of mood and anxiety disorders, namely behavioural inhibition and adult separation anxiety. To this end, a gene-environment (GxE) interaction approach (see Figure 1) was applied in order to elucidate the interaction and direction of association of *OXTR* rs2254298 with behavioural inhibition as well as with adult separation anxiety on complicated grief severity.

**Materials and methods**

***Sample characteristics***

In total, 96 patients with DSM-IV axis I mood and/or anxiety disorders as a primary diagnosis and a history of bereavement were recruited at the outpatient clinic at the Department of Psychiatry, University of Pisa, Italy (for details see Table 1). Diagnoses were ascertained by experienced psychiatrists using a structured clinical interview (SCID-I). Exclusion criteria were psychotic symptoms, substance abuse or dependence, or serious medical conditions. All patients were of Caucasian background for at least two preceding generations. The study was approved by the ethical committee of the University of Pisa and conformed to the ethical principles of the Declaration of Helsinki. Written informed consent was obtained in all patients.

***Psychometric assessment***

All participants completed a set of self-report questionnaires selected to address BI, ASA, and CG. BI during childhood was assessed using the Retrospective Self-Report of Inhibition (RSRI; Reznick et al. 1992). The Adult Separation Anxiety Scale (ASA-27; Manicavasagar et al. 2003) was applied to assess symptoms of separation anxiety over the age of 18 years. The Inventory of Complicated Grief (ICG; Prigerson et al. 1995; Italian version: Carmassi et al. 2014) assesses the frequency and severity of grief reactions. Depressive symptom severity was examined using the Hamilton Depression Rating Scale (HAM-D; Hamilton 1960).

***Genotyping***

Patients were genotyped for the *OXTR* rs2254298 variant according to published protocols (Costa et al. 2009). Genotype information was missing for N=3 patients, resulting in a final sample of N=93 for all analyses reported in the following (see Table 1). Hardy-Weinberg criteria as determined by DeFinetti (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) were fulfilled for *OXTR* genotype distribution (GG=69, AG=21, AA=3; p=.396).

***In silico analysis of transcription factor binding***

The sequence containing *OXTR* rs2254298 was analyzed *in silico* to predict whether rs2254298 could affect binding affinity of transcription factors to the surrounding nucleotide sequence using the Transcription Factor Affinity Prediction (TRAP) web tool working on the basis of a biophysical model (<https://omictools.com/transcription-factor-affinity-prediction-tool>; Thomas-Chollier et al. 2011) as described previously (Costa et al. 2017).

***Statistical analysis***

Questionnaire raw data were checked for missing data. Missing values were imputed by the individual total score divided by the number of completed items. A cut-off of >20% of missing data was defined as an exclusion criterion. No patient had to be excluded from the reported analyses applying this criterion. For group comparisons, *OXTR* genotype was grouped into GG vs. AG/AA carriers (cf. Costa et al. 2009). Genotype group differences regarding continuous variables were assessed using independent samples t-tests. Differences regarding categorical variables were analysed by Chi square (χ2) tests. Pearson’s correlations were used to test for associations between dimensional variables and possibly confounding gene-environment correlations (rGE). Hierarchical multiple regression was used to determine the effects of *OXTR* genotype, BI, and ASA, on ICG scores, as well as the respective two-way interactions effects (*OXTR* x BI, *OXTR* x ASA). Genotype groups were coded as GG=.5 and AG/AA=-.5, and RSRI mean and ASA-27 sum scores were centred (mean=0) to avoid statistical inference errors (Kraemer and Blasey, 2004). Regression analyses were run in two steps, with the first step containing all main effects (grouped *OXTR* genotype, centred RSRI or ASA-27 scores, respectively), and the second step including the respective interaction term (*OXTR* x BI, *OXTR* x ASA). A p-value≤.05 was considered statistically significant. Given the explorative nature of the study, no correction for multiple testing was applied (cf. Bender and Lange, 2001). All statistical tests were computed using SPSS version 20 (SPSS Inc., Chicago, Illinois, USA).

**Results**

***Descriptive statistics***

Regarding loss characteristics, 35.5% (N=33) of patients reported the loss of a grandparent, 31.2% (N=29) loss of a parent, 10.8% (N=10) loss of a sibling, 9.7% loss of a friend, 8.6% (N=8) loss of another relative, 3.3% (N=3) loss of another significant person, and 1.1% (N=1) loss of a child. On average, the loss event occurred 9.73 (±10.35) years prior to the assessment. Descriptive statistics of the whole sample as well as stratified by grouped *OXTR* genotype are given in Table 1. No sex differences were observed regarding the dependent (ICG score, p=.259) or independent variables (RSRI score: p=.239; ASA-27 score: p=.558), and no associations with age emerged (all ps≥.296). *OXTR* groups did not differ with respect to depressive symptom severity as assessed by the HAM-D (p=.285) or its anxiety/somatisation factor (p=.593) (Cleary and Guy 1977), and HAM-D scores were not related to ICG total score (p=.104). No significant correlations (rGE) were observed between *OXTR* and RSRI or ASA-27, respectively (ps≥.352).

- Table 1 -

***Interaction analyses of OXTR genotype with BI and ASA on ICG scores***

The relationships between grouped *OXTR* genotype, RSRI and ASA-27, respectively, and ICG are depicted in Figure 2.

- Figure 2 -

With respect to behavioural inhibition, in the first and second steps of the regression analysis, no significant main effects of *OXTR* genotype or RSRI score on the outcome variable were observed. The second step, however, yielded a significant interaction effect of *OXTR* x BI, which accounted for a significant increase in explained variance, indicating differential dynamics in RSRI scores depending on genotype. In a similar vein, regarding adult separation anxiety, no main effects of *OXTR* genotype or ASA-27 score were observed in either the first or the second step of the regression analysis, however, a trend for a significant two-way interaction of *OXTR* x ASA emerged in step two, accounting for a marginally significant increase in total variance (see Table 2 for detailed results) and pointing to increases or decreases in ASA scores in a genotype-dependent (GG vs. AG/AA) fashion as depicted in Figure 1..

- Table 2 -

***Predictive transcription factor binding analysis of OXTR rs2254298***

In order to explore the potential functionality of the intronic *OXTR* rs2254298 SNP, an *in silico* analysis was performed to evaluate potential transcription factor binding affinities at the nucleotide sequence containing the G or A allele, respectively, using the online tool Transcription Factor Affinity Prediction (TRAP) (see methods; Thomas-Chollier et al. 2011).

Among the top 10 predictions, a significant difference was observed in binding affinity for the transcription factor GATA4. Presence of the G allele was related to altered GATA4 sequence motifs, leading to loss of GATA4 binding affinity (for details, see Table 3).

- Table 3 -

**Discussion**

The present study for the first time explored the interactive role of *OXTR* rs2254298 with dimensional measures of behavioural inhibition (BI) and adult separation anxiety (ASA), in the moderation of symptoms of complicated grief (CG) in patients with mood and anxiety disorders. While no main effects of *OXTR* genotype, BI, or ASA on CG could be discerned, *OXTR* genotype interacted with BI and, on a trend-level, with ASA, to increase complicated grief symptoms. Specifically, higher levels of both BI and ASA were related to elevated CG scores in patients homozygous for the G allele. Interestingly, as depicted in Figure 2, this pattern appears to be reversed when BI, and to an extent ASA, is low, which accords with the “differential susceptibility hypothesis” by Belsky et al. (2009), proposing that genes are responsive to the environment as a whole and can therefore either increase or decrease vulnerability – in this case to CG – depending on the quality of non-genetic influences, which in the present study were operationalized as differing levels of BI and ASA. Indeed, *OXTR* rs2254298 has previously been suggested to confer differential susceptibility to psychopathology (Brüne 2012). Though preliminary, the present results add further support to this notion by suggesting that while *OXTR* GG genotype carriers are at greater risk for developing CG following the death of a loved one in the context of a risk factor constellation comprising high levels of inhibited temperament or separation anxiety, they are the least prone to develop clinically relevant grief symptoms when scoring low on these intermediate phenotypes of anxiety and depression. On a molecular level, the mechanisms by which environmental factors or person characteristics may serve to activate or counteract a genetically determined biological susceptibility may be facilitated via dynamic alterations of epigenetic mechanisms such as DNA methylation in response to negative or positive environmental influences, respectively (cf. Domschke et al. 2012; Domschke et al. 2013; for review see Schiele and Domschke 2017), and, crucially, DNA methylation risk patterns have been shown to be reversible by successful psychotherapy (Ziegler et al. 2016).

From a clinical perspective, the present findings may be relevant for the identification of at-risk individuals and, consequently, the targeted application of early preventive interventions, as well as for the improvement of therapeutic options by taking into account a patient’s individual risk factor constellation towards the development of a personally tailored treatment approach. Especially early preventive strategies, ideally set during childhood, aimed towards building and strengthening resources are needed in order to increase resilient functioning especially when faced with later-life adversity in at-risk populations. For instance, early prevention programs such as the “Cool Little Kids” program centred on early risk factors like BI have been shown to be effective in reducing anxiety disorder risk (Rapee et al. 2005; Rapee et al. 2010) and may therefore constitute promising tools in the prevention of bereavement-associated mental health problems. For the treatment of complicated grief, effective psychotherapeutic and pharmacological (e.g. Shear et al. 2005; Hensley et al. 2009; Glickman et al. 2016; Shear et al. 2016; Bryant et al. 2017) interventions are available. However, since the presence of ASA symptoms has been shown to affect CG symptom severity and impairment either directly (Pini et al. 2012; Gesi et al. 2017) or in interaction with genetic variation (as shown in the present study), consideration of biological susceptibility factors and comorbid symptoms may help improve treatment options and so reduce the problem of treatment resistance.

On a molecular level, the investigated single nucleotide polymorphism (SNP) is located inside a region involved in the epigenetic transcriptional regulation of the *OXTR* gene (Mizumoto et al. 1997). As is the case for most *OXTR* loci, the functionality of this SNP on the level of gene expression regulation is still unknown. However, results from a hypothesis-generating *in silico* approach using a web tool designed to explore the theoretical transcription factor binding affinity of a given sequence and its variation revealed that the G allele might abolish the binding affinity of the transcription factor GATA4, mainly known as a transcription factor involved in mammalian cardiac development (Lentjes et al. 2016). Additionally, GATA4 is expressed in the embryonic and adult central nervous system (CNS) and participates in the regulation of CNS function (Lawson et al. 1996; Agnihotri et al. 2009). Whether differential GATA4 binding could account for patterns of altered *OXTR* gene expression in specific neuron populations, and the possible way whereby *OXTR* rs2254298 might differentially influence oxytocinergic function is, however, still highly speculative and remains to be elucidated in future experimental studies, particularly given the intronic location of OXTR rs2254298.

The present findings should be interpreted in light of some limitations. Given that the present study for the first time explored a complex interaction of *OXTR* gene variation with behavioural inhibition and adult separation anxiety in the moderation of complicated grief, the promising pilot data presented here are to be perceived as primarily hypothesis-generating. Along these lines, the exploratory nature of the present study requires confirmatory testing in future studies, and the relatively small sample size warrants replication in larger, sufficiently powered samples. Similarly, given the diagnostic heterogeneity of the present sample, investigation of the reported interaction effect in more narrowly defined patient groups may constitute a promising avenue in disentangling the involvement of *OXTR* variation, BI, and ASA in grief symptoms across specific diagnostic groups. Since no control group was investigated in the present study, the message of the present results is limited to the proposition of *OXTR*, BI and ASA impacting CG as a possible intermediate phenotype of mood and anxiety disorders (cf. Kersting et al. 2007). Therefore, future, ideally longitudinal and case-controlled studies would extend knowledge of the interactive impact of genetic susceptibility and bereavement on the development of pathological grief and related clinical phenotypes. Additionally, although all assessment instruments used in this study exhibit good validity and reliability, data were assessed retrospectively using self-report questionnaires and so may be subject to recall bias. Given the small sample size, it was not feasible to address the possible three-way interaction of *OXTR* genotype, BI, and ASA. Therefore, future research in sufficiently powered samples might want to address these factors simultaneously. Additional studies may address the effect of further moderating factors including attachment characteristics (Costa et al. 2009; Notzon et al. 2016), stressful life events or protective factors associated with adaptive coping (cf. Schiele et al. 2016) in the moderation of grief reactions. In a similar vein, specific loss characteristics such as cause and time of death or relationship to the deceased person may be differentially related to the severity of grief reactions. Future research might want to take into account such potentially confounding factors and also address possible differences regarding the genetic and psychological impact on grief symptoms with respect to loss characteristics. While the *OXTR* gene may play a promising, although understudied, role in the moderation of grief reactions, future studies may also want to examine other candidate genes such as the monoamine oxidase A (*MAOA*) gene, which has previously been linked to vulnerability to CG (Kersting et al. 2007). Finally, future research on moderators of CG in affective and anxiety disorders may want to take into account *OXTR* DNA methylation (cf. Ziegler et al. 2015), an epigenetic mechanism known to crucially modify gene function (see Schuebel et al. 2016).

In summary, the present study for the first time provides preliminary evidence for a GxE effect of a variant in the *OXTR* gene with the temperamental trait behavioural inhibition and symptoms of adult separation anxiety in the moderation of complicated grief symptoms in mood and anxiety disorders. This finding encourages further research in larger, case-controlled samples and may inform therapeutic options in the prevention and treatment of complicated grief after bereavement based on biological and trait/psychological markers.

**Acknowledgements**

This work was supported in part by Fondazione Cassa di Risparmio di La Spezia and the CRC-TRR58 (projects C02 and Z02 to KD) funded by the German Research Foundation (DFG). DSB, KD and SP are members of the Anxiety Disorders Research Network (ADRN), European College of Neuropsychopharmacology (ECNP).

**Statement of interest**

None to declare.

**Table 1.** Sample characteristics for whole sample and stratified by *OXTR* rs2254298 genotype

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Whole sample** | ***OXTR* rs2254298** |  |  |
|  **(N=93)** | **GG****(N=69)** | **AG/AA****(N=24)** | **t/** χ2 | **p** |
| Age (mean±SD) | 40.01 ±12.74 | 40.61±12.96 | 38.29±12.22 | -.791 | .431 |
| Sex (female, %) | 68 (73.1%) | f=52, m=17 | f=16, m=8 | .685 | .408 |
| RSRI (mean±SD) | 2.49±.60 | 2.47±.55 | 2.54±.72 | -.478 | .634 |
| ASA-27 (mean±SD) | 31.54±15.17 | 32.41±15.56 | 29.04±13.98 | .935 | .352 |
| ICG (mean±SD)HAM-D (mean±SD) | 10.04±11.679.66±6.23 | 10.88±11.469.25±6.07 | 7.63±12.1710.83±6.68 | 1.181-.290 | .241.772 |
| Diagnostic composition |  |  |  |  |
| *DSM-IV mood disorders* |  |  |  |  |
| MDD | N=43 (44.8%) | 29 (42.0%) | 13 (54.2%) | 2.39 | .880 |
| BD-I | N=9 (9.4%) | 8 (11.9%) | 1 (4.2%) |
| BD-II | N=29 (30.2%) | 23 (33.3%) | 6 (25.0%) |
| *DSM-IV anxiety disorders* |  |  |
| OCD | N=23 (24.0%) | 18 (26.1%) | 5 (20.8%) |
| SP | N=9 (9.4%) | 6 (8.7%) | 2 (8.3%) |
| SpP | N=24 (25.0%) | 17 (24.6%) | 7 (29.2%) |
| PD | N=54 (56.3) | 41 (66.1%) | 13 (59.1%) |

Legend to Table 1

OXTR: oxytocin receptor; RSRI: Retrospective Self-Report of Inhibition; ASA-27: Adult Separation Anxiety Questionnaire; ICG: Inventory of Complicated Grief (in the whole sample, seven patients fulfilled criteria for categorical CG when applying a cut-off of ICG score >29 and loss > 6 months); MDD: major depressive disorder; BD-I: bipolar disorder I; BD-II: bipolar disorder II; OCD: obsessive-compulsive disorder; SP: social phobia; SpP: specific phobia; PD: panic disorder

**Table 2.** Results of hierarchical multiple regression analyses on complicated grief symptoms (Inventory of Complicated Grief total score)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Predictor** | **R2** | **∆R2** | **∆F** | **B** | **SE B** | **β** | **t** | **p** |
| ***Model 1 – OXTR GG vs. AG/AA x behavioural inhibition*** |
| *Step 1* | .016 | .016 | .744 |  |  |  |  | .478 |
| *OXTR* |  |  |  | 3.304 | 2.777 | .125 | 1.190 | .237 |
| RSRI |  |  |  | .370 | 1.135 | .034 | .326 | .745 |
| *Step 2* | .066 | .050 | 4.784 |  |  |  |  | **.031\*** |
| *OXTR* |  |  |  | 3.271 | 2.720 | .123 | 1.202 | .232 |
| RSRI |  |  |  | -.294 | 1.152 | -.027 | -.255 | .799 |
| *OXTR* x RSRI |  |  |  | 5.041 | 2.305 | .232 | 2.187 | **.031\*** |
|  |  |  |  |  |  |  |  |  |
| ***Model 2 – OXTR GG vs. AG/AA x adult separation anxiety*** |
| *Step 1* | .038 | .038 | 1.784 |  |  |  |  | .174 |
| *OXTR* |  |  |  | 2.864 | 2.756 | .108 | 1.039 | .301 |
| ASA-27 |  |  |  | 1.715 | 1.168 | .152 | 1.468 | .146 |
| *Step 2* | .075 | .037 | 6.529 |  |  |  |  | **.064+** |
| *OXTR* |  |  |  | 3.125 | 2.721 | .118 | 1.148 | .254 |
| ASA-27 |  |  |  | .209 | 1.404 | .019 | .149 | .882 |
| *OXTR* x ASA-27 |  |  |  | 5.274 | 2.807 | .233 | 1.879 | **.064+** |

Legend to Table 2

OXTR: oxytocin receptor; RSRI: Retrospective Self-Report of Inhibition; ASA-27: Adult Separation Anxiety Questionnaire; \* significant at p≤.05; + trendwise significant at p≤.10.

**Table 3.** Top 10 predicted transcription factors with differential affinities to the two alternative sequences of *OXTR* rs2254298 using TRAP

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Rank order | Difference log(p) for the two sequence | P valueG allele | P valueA allele | Matrix\_ID | Matrix name |
| 1 | -1.909 | 0.280 | 0.0034 | M00632 | V$GATA4\_Q3 |
| 2 | 0.537 | 0.073 | 0.2521 | M00087 | V$IK2\_01 |
| 3 | 0.293 | 0.326 | 0.6397 | M01281 | V$NFAT1\_Q6 |
| 4 | 0.236  | 0.422  | 0.2449 | M01012 | V$HNF3\_Q6\_01 |
| 5 | 0.178 | 0.769 | 0.5103  | M00724 | V$HNF3ALPHA\_Q6 |
| 6 | 0.169 | 0.336 | 0.4959  | M00496 | V$STAT1\_03 |
| 7 | -0.148 | 0.250  | 0.1780 | M00317 | V$LDSPOLYA\_B |
| 8 | 0.144 | 0.460 | 0.6415 | M00139 | V$AHR\_01 |
| 9 | -0.141 | 0.667 | 0.4828 | M00791 | V$HNF3\_Q6 |
| 10 | 0.137 | 0.448 | 0.6144 | M00750 | V$HMGIY\_Q6 |

Legend to Table 3

Values are sorted from highest to lowest “absolute difference log(p) for two sequences”. Table shows results obtained selecting as a matrix and a background model “Transfac\_2010.1 vertebrates” and “human promoter”, respectively.

**Figure 1.**

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**Figure 2.**



Figure captions

**Figure 1.** Schematic overview of the two proposed interaction models of *OXTR* rs2254298 with a) behavioural inhibition and with b) adult separation anxiety on symptoms of complicated grief.

**Figure 2.** Interaction of *OXTR* variation with behavioural inhibition and adult separation anxiety, respectively, on complicated grief

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