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## Authors accepted manuscript

**Final version: https://link.springer.com/chapter/10.1007/7854_2024_544#notes**

## Springer Series in Behavioral Neuroscience

New discoveries in the brain sciences of fear and anxiety: from basic to clinical neuroscience

# Genetic vulnerability to social anxiety disorder

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## Abstract

Most anxiety disorders 'run within families': people suffering from an anxiety disorder often have family members who are highly anxious as well. In this chapter, we explore recent work devoted to unravelling the complex interplay between genes and environment in the development of anxiety. We review studies focusing on the genetic vulnerability to develop social anxiety disorder (SAD), as SAD is one of the most prevalent anxiety disorders, with an early onset, a chronic course and associated with significant life-long impairments. More insight in the development of SAD is thus of uttermost importance.

First, we will discuss family studies, twin studies and large-sized population-based registry studies and explain what these studies can reveal about the genetic vulnerability to develop anxiety. Next, we describe the endophenotype approach; in this context, we will summarize results from the Leiden Family Lab study on Social Anxiety Disorder. Subsequently, we review the relationship between the heritable trait 'behavioral inhibition' and the development of SAD, and highlight the relevance of this work for the development and improvement of preventative and therapeutic interventions for socially-anxious youth.

## Introduction

Most anxiety disorders 'run within families': people suffering from an anxiety disorder often have family members who are highly anxious as well. Here, we explore recent work devoted to unravelling the complex interplay between genes and environment in the development of anxiety. We review studies on the genetic vulnerability to develop social anxiety disorder (SAD), as SAD is one of the most prevalent anxiety disorders, with an early onset, a chronic course and associated with significant life-long impairments [1–4]. More insight in the development of SAD is thus of uttermost importance.

First, we will discuss family studies, twin studies and large-sized population-based registry studies and explore what these studies reveal about the genetic vulnerability to anxiety. Next, we describe the endophenotype approach, a method relating the genetic vulnerability to anxiety to neurobiological and psychological characteristics; here we summarize results from the Leiden Family Lab study on Social Anxiety Disorder. Subsequently, we review the relationship between the heritable trait 'behavioral inhibition' and the development of SAD, and highlight the relevance of this work for the development and improvement of preventative and therapeutic interventions for socially-anxious youth.

## Anxiety runs in families

Almost 25 years ago, a meta-analysis investigated available evidence for familial aggregation of panic disorder, generalized anxiety disorder and phobias, including SAD [5]. At that time, twin and family studies on anxiety were scarce and only a limited number of studies met inclusion criteria. Therefore, the results need to be interpreted with caution. However, this meta-analysis provided initial evidence that anxiety disorders have significant familial aggregation. An integrative review of intergenerational family studies on childhood anxiety subsequently confirmed that children whose parents have an anxiety disorder have an increased risk to develop anxiety themselves; moreover, children with anxiety disorders more often have a parent with anxiety [6].

A recent systematic review explored in more detail the association between parental anxiety and emotional development of their offspring [7], revealing that 'the presence of parental anxiety increases the risk of internalizing and externalizing problems in their offspring and is associated with mental health problems in later childhood and adolescence'. Furthermore,

although the association between parental anxiety and negative developmental trajectories in their offspring was present across the whole developmental lifespan, *maternal* anxiety was more influential than *paternal* anxiety during adolescence, pointing at distinct roles for fathers and mothers during these developmental trajectories. Inconsistencies between study methods may explain why studies report different (even sometimes non-significant) associations on the relationship between anxiety in parents and emotional development of their children [7].

A large population-based multi-generational family study on the genetic vulnerability to social anxiety (data on 18,399 individuals with a diagnosis of SAD) confirmed that SAD runs in families and revealed that the risk for SAD in family members was positively related with genetic relatedness: first-degree relatives of individuals with SAD (50% genetic similarity) had a significantly higher risk of having SAD compared to second-degree relatives (25% genetic similarity) and third-degree relatives (12.5 % genetic similarity) [8]. Additionally, this study revealed an increased risk of SAD in partners of people with SAD: these non-biological relatives (defined as 'individuals who have at least one child together with the patient') were four times more likely to have SAD compared to non-biological relatives of controls. Given the early age of onset of SAD, usually during childhood or adolescence (cf. [9, 10] and chapter 'Temperamental risk factors for anxiety' by dr. Perez-Edgar, this volume), the authors assume that these findings reflect 'assortative mating': they consider it most likely that socially-anxious individuals seek partners with similar phenotypes, rather than to attribute the higher SAD prevalence in partners to the observation that partners who cohabit could become more similar in certain respects [8].

An interesting question is: does parental anxiety always lead to anxiety in offspring? Uher and colleagues [11] recently summarized evidence from original family high-risk and registry studies (211 independent studies, >3 million offspring of parents with psychopathology and >20 million offspring of parents without psychopathology) and explored the transdiagnostic risk of developing a mental disorder in offspring of affected parents. This meta-analysis revealed that the relative risk (RR) of developing an anxiety disorder was 2.2 for offspring of parents with anxiety disorders, with a lifetime absolute risk (defined as 'up to age of assessment') of 31%. Risk for developing *any* mental disorder was even higher: the RR to develop a mental disorder appeared to be 3.0 for offspring of parents with anxiety disorders, with a lifetime risk of no less than 55% [11]. Across all mental conditions studied, the risks

for the development of any mental disorder where estimated to be among the highest for offspring of parents with anxiety disorders [11] stressing the need to develop effective preventative interventions for these children at risk.

## Twin studies

Complementing family studies, twin studies increase insights in the extent to which genes contribute to a specific phenotype, providing estimates of heritability, defined as 'the proportion of disease risk that is due to variation in genes in a population' [12]. A meta-analysis by Scaini and colleagues [13] revealed that both genetic (meta-analytic estimate of proportion of total variance: 0.41) and non-shared environmental factors (meta-analytic estimate: 0.54) contribute to individual differences in social anxiety; shared environmental factors appeared less relevant for SAD[1]. Furthermore, the estimates of heritability were higher for original studies measuring social anxiety on a continuous scale (i.e. at the level of symptoms), compared to studies using a categorical approach (i.e. a DSM-based diagnosis of SAD) [13]. This increased sensitivity to detect the genetic contribution to the manifestation of SAD within individuals is in line with the notion that social anxiety is expressed on a continuum of severity, ranging from non-clinical ('discomfort in some situations') to clinical levels (' intense and incapacitating fear in most situations' [14]) and emphasizes the added value of exploring the pathogenesis and pathophysiology of social anxiety on a continuous scale. In addition, a multiple-rater twin study, in which anxiety was rated by adolescent twins, their mothers and their fathers (*n*=1,394), revealed rater-specific genetic effects: analyses based on parent-reported anxiety yielded higher heritability estimates compared to analyses on adolescent self-report data [15]; cf. a recent study exploring why parents' survey reports of adolescent social anxiety fail to predict the anxiety that adolescents self-report [16]. Thus, multiple methodological choices can influence estimates of heritability of anxiety.

[1] The influence of environmental factors on the development of SAD will not be extensively discussed here; for a recent overview on the role of environmental factors in the aetiology of SAD we refer to [161]

## Complex interplay between genes and environment

Most family members share both genes as well as environment. Thus, family studies revealing aggregation of anxiety within families do not necessarily imply a genetic contribution to the risk to develop an anxiety disorder [12]. Likewise, twin studies usually examine phenotypic variation within one generation and are often unable to exclude environmental interaction effects between parents and children [17].

In a systematic review and meta-analysis of genetically informed research, Ahmadzadeh and colleagues focused on gene-environment interactions between parents and offspring [18]. The authors indicate that observational studies reporting on relationships between parent anxiety and internalizing symptoms in their offspring are often biased by unobserved variables, including genetics, hindering distinguishing genetic effects from environmental effects that are also present in the parent-child relationship (like social learning and parental modeling; cf.[19]). [18]Furthermore, they point at passive gene-environment correlations, encompassing the observation that 'the environment shared by parents and their offspring is passively correlated with the genes that they share' [18]. Therefore, Ahmadzadeh et al. [18] meta-analyzed studies which explored the relationship between parent anxiety and offspring internalizing outcomes while accounting for familial genetic resemblance, based on specific quasi-experimental study designs. Examples of such designs are adoption, sibling-comparison, children-of-twins or in vitro fertilization studies. Eight publications (describing data from four independent cohorts) met t inclusion criteria for this meta-analysis [18]. Based on these studies, the authors cautiously conclude that postnatal anxiety in at least one of the parents may be causally related to concurrent internalizing problems in offspring through environmental, nongenetic pathways. Although this observation might seem to contradict the findings of the meta-analysis discussed earlier [13], it is worth noting that these meta-analyses differ in certain respects. Ahmadzadeh et al. [18] included studies with quasi-experimental designs accounting for genetic confounding and explored associations between any anxiety disorder (parents) and broad internalizing problems (offspring), while Scaini et al. [13] focused on SAD and included only studies with a classic twin design. It is possible that, compared to other anxiety disorders, the development of SAD is more influenced by a genetic vulnerability (for example, temperamental traits like behavioral inhibition, which will be discussed in more detail later) than shared parent-child environmental influences. However, as the meta-analyses differed in multiple

methodological aspects, this hypothesis needs to be tested in studies in which the contribution of genetic and environmental factors can be directly compared between several anxiety phenotypes, in both parents and children. Furthermore, the work by Ahmadzadeh and colleagues [18] stresses the need for more research in this field, as their results were based on only four unique datasets. To enable identifying reproducible and reliable causal pathways between parent and offspring anxiety phenotypes, further studies are needed, preferably with a longitudinal design.

Kendler and colleagues also aimed to clarify the sources of parent-offspring transmission of anxiety disorders [20]. Using data from Swedish national samples, the authors determined six types of families in which offspring were raised, and they combined these household data with diagnostic data on anxiety disorders from national medical registries. This approach enabled them to explore three sources of parent-child resemblance: genes plus rearing, genes only, and rearing only [20]. Analyses on this extended dataset of over 2 million offspring revealed that anxiety disorders were transmitted from parents to offspring (intact families $r$=0.16). By comparing data from the six family types, the authors distinguished to which extent genetical influences and rearing effects played a role in the intergenerational transmission of anxiety: genetics explained 70% of the parent-offspring resemblance, while environmental factors (i.e. rearing effects) accounted for the remaining 30%. These data thereby confirm that both genetic and environmental effects play a role in the intergenerational transmission of anxiety disorders [20].

## The search for SAD-genes

Given the evidence summarized above, pointing at genetic influences on the development of anxiety disorders in general, and SAD in particular, the question arises: which particular genes are implicated in this risk? Previous research showed that the exploration of 'SAD-genes' is hindered by multiple factors, including the heterogeneity of the disorder and the fact that the diagnosis is based on clinical assessments and not on biologically-based measurements [21, 22]. Furthermore, accumulating evidence underscores that multiple genetic variants, each with a relatively small effect, interact and increase the risk to develop complex psychiatric disorders, including anxiety, referred to as 'polygenicity'[23–26]. These

genetic variants are in turn influenced by environmental factors [27, 28]. Other articles in this volume will describe in more detail how studies have increased insights in genotypic alterations related to anxiety. Here, we continue with a discussion of an alternative approach to obtain insight in the genetic vulnerability to develop SAD: the endophenotype approach.

## The endophenotype approach[2]

The endophenotype approach has been proposed to facilitate the investigation of genetic factors in psychiatric disorders [29, 30]. Endophenotypes are measurable characteristics on the causal pathway from genotype to phenotype and are thus thought to reflect underlying disease liability [31]. The following criteria are most consistently mentioned to define endophenotypes [22, 31]: 1st an endophenotype is *associated with the disorder of interest*; 2nd an endophenotype is a *stable, state-independent trait*, which is already present in a preclinical state; 3rd an endophenotype is *heritable*; 4th an endophenotype *co-segregates with the disorder within a family*, with nonaffected family members showing altered levels of the endophenotype when compared to the general population. In addition, an endophenotype is ideally more strongly associated with a specific disorder of interest in comparison to other psychiatric conditions [32], but it is also possible that a certain endophenotype affects more than one disorder [33].

Originally, the usefulness of endophenotypes was supposed to lie in discovering the genes predisposing for complex disorders, based on the assumption that endophenotypes have a simpler genetic architecture than the disorders themselves [21, 22]. This idea was challenged by a meta-analytic review [34], which compared the effect sizes of genetic loci contributing to phenotypes (psychiatric disorders) and loci contributing to endophenotypes. Results showed comparable effect sizes, so the assumption that endophenotypes have a simpler genetic architecture than phenotypes was not supported [34]. These findings were empirically confirmed by comparing GWAS-studies investigating the genetic effects related to endophenotypes of schizophrenia (for example, variation in brain structure and measures

[2] This part of the chapter is based on Bas-Hoogendam et al. (2016) [30] and has been updated for the present work; excerpt with permission of Elsevier and Copyright Clearance Center.

of cognitive performance) to studies aimed to identify risk genes for the disorder itself [35]. Again, similar effect sizes were found. So, it is not necessarily true that the genetic architecture of endophenotypes is less complex than that of the disorders themselves.
This does not mean, however, that endophenotypes are of limited value [36]. Their usefulness lies in understanding disease mechanisms: based on the assumption that complex disorders could be divided into simpler and more biologically coherent units, endophenotypes provide insight into the pathways leading to pathology and could help in discerning the origins of mental disorders [35]. Furthermore, endophenotypes support a transdiagnostic perspective on mental disorders, given the fact that endophenotypes could cross traditional diagnostic boundaries [37]. Here, the endophenotype approach fits within the NIMH Research Domain Criteria (RDoC) initiative, a research framework in which not the clinical diagnoses are starting point for investigation, but core features of psychopathology, falling within five research domains [38, 39]. The RDoC initiative explicitly acknowledges that these core features could be present in multiple psychiatric disorders and promotes the integration of data from several levels, from genes to neural systems to behavior [40, 41]. Endophenotypes fit perfectly within this framework, providing a bridge between genetic variations at the one hand and psychiatric disorders at the other.
Endophenotypes could also aid in the development of improved animal models for psychopathology [42]. A recent example is the study by Fox and colleagues, demonstrating that infant inhibited temperament in rhesus monkeys is a heritable trait, which is stable over time and related to anxious behavior in adulthood [43]. A preliminary genome-wide association analysis in these monkeys revealed a promising genetic variant on chromosome 13 (nearby the *CTNNA2* gene) related to inhibited temperament. Interestingly, this *CTNNA2* gene has been previously found in association studies on anxiety in humans, underscoring the value of translational research to explore molecular and genetic underpinnings of anxious behavior based on endophenotypes [43]
Furthermore, endophenotypes can be used to identify individuals at risk, as they are present prior to disease onset. This is of uttermost importance, because early detection of psychopathology and subsequent preventive interventions can improve long-term prognosis, reduce the substantial burden and cost of SAD and lower the risk of developing co-morbid psychopathology [44]. Moreover, endophenotypes could provide clues for

improvement of treatments and guide in the selection of appropriate pharmacological interventions [45].

## Neurobiological endophenotypes of SAD

The Leiden Family Lab study on Social Anxiety disorder (LFLSAD was especially designed to investigate endophenotypes of SAD, using a two-generation design [46]. Data-acquisition for this study took place between 2013 and 2015 and electroencephalography (EEG) and magnetic resonance imaging (MRI)-data revealed multiple promising candidate endophenotypes of SAD. Here, we describe the design of the LFLSAD and discuss several neurobiological endophenotypes based on MRI.

To the best of our knowledge, the LFLSAD is the first and only multiplex (i.e., multiple cases of the disorder within one family), multigenerational family study designed to unravel neurobiological SAD-endophenotypes, by exploring two endophenotype criteria. First, the family design enabled investigating whether a presumed endophenotype *co-segregated with the disorder* within the families. Furthermore, the *heritability* of candidate endophenotypes was estimated, using a joint mixed model taking the ascertainment process and familiar relationships into account [47].

As described in a dedicated design paper[46], the multiplex, multigenerational design maximized statistical power to detect genetic and environmental influences on SAD-related characteristics. Families were considered eligible for inclusion when they contained at least one adult with a primary diagnosis of SAD (‘proband’), of whom at least one child showed SAD symptoms at a clinical or subclinical level (‘proband's SA-child’, age 8-21 years). In addition to the proband and its SA-child, the proband's spouse, other children (age ≥8 years) and the proband's sibling(s) and their spouse(s) with their child(ren) (age ≥8 years) were invited to participate. Families with at least eight family members were included to enable reliable estimations of the relation between endophenotype and SAD. This multiplex design enriched the sample for a heritable basis of SAD and facilitated the detection of genetic factors. Furthermore, a sample consisting of large families, composed of several related nuclear families (parents with their children), is likely to share more heritable factors than a same-sized sample of unrelated nuclear families, hence more statistical power to distinguish shared environmental effects from genetic effects [48].

Based on an extensive literature review [30], we identified several neurobiological measurements as SAD-endophenotypes, namely 1st the function and functional connectivity of the amygdala, 2nd the function of the medial prefrontal cortex (PFC), 3rd whole-brain functional connectivity and 4th structural-anatomical brain changes. These candidate endophenotypes were explored in a one-hour MRI session (neuroimaging data from 8 families, total *n*=110, age range 9.2–61.5 years). Results are summarized below, partly based on a discussion previously published in the thesis 'Extremely Shy & Genetically Close'[49].

**Functional brain characteristics as SAD-endophenotypes**

Previous functional (f)MRI studies indicated an *association* between SAD and hyperactivation of subcortical, frontal, parietal and occipital brain areas, evoked by paradigms addressing specific SAD-related fears [[50, 51]. In the LFLSAD, we employed two functional paradigms: a neutral faces paradigm and a social norm processing task. Each paradigm targeted different neurocognitive components of SAD. We used these paradigms to examine evidence for brain activation as candidate endophenotype of SAD, by exploring the *co-segregation within families* and *heritability* criteria.

***Amygdala and hippocampus activation as candidate SAD-endophenotypes***

The first fMRI paradigm concerned the processing of faces with a neutral expression, being strong social stimuli with an ambiguous meaning. The Neutral Faces Paradigm (NFP) was created to explore brain activation related to two different aspects of processing such faces. In the habituation phase (HP), we tested whether impaired habituation to neutral faces (i.e. the adaptive decline in brain activation to a stimulus which is presented multiple times without meaningful consequences [52, 53]) could be considered a candidate SAD-endophenotype. This hypothesis was based on previous research considering individuals with inhibited temperament, which is an important risk factor for SAD, and in participants with high levels of social fearfulness [54, 55]; both studies reported failed habituation. We found that the neural habituation response in the right hippocampus and amygdala was impaired in family-members with high levels of social anxiety, providing support for the endophenotype criterion of *co-segregation within the families*. Subsequent *heritability*

analyses revealed that the neural habituation response within the right hippocampus was at least moderately heritable, in line with work in monkeys [56]. These findings indicate that altered neural habituation in the hippocampus is a putative SAD-endophenotype [57].
The second phase of the NFP concerned the social-evaluative conditioning of the faces [58]. By consistently pairing three neutral faces with social-evaluative sentences with a positive ('He says you are smart'), negative ('He says you are stupid') or neutral ('He says you are in Leiden') content, participants learned the social-evaluative value of each face. Previous work indicated amygdala engagement during this learning process [59], but the relation between amygdala activation related to this social-evaluative conditioning paradigm (SCP) and social anxiety has not been investigated, let alone within families genetically enriched for SAD. Data from the LFLSAD indicated bilateral amygdala hyperactivation to faces conditioned with a social-evaluative meaning; levels of this amygdala activation *co-segregated* with social anxiety and displayed at least moderate *heritability*. Interestingly, this amygdala hyperreactivity was present for all conditions of the SCP, indicating that being directly addressed ('He says you are…') strongly activates the amygdala in socially-anxious family-members, independent from the content of the evaluation (i.e. positive, negative or neutral). These results provide evidence for amygdala activation in response to faces with a learned social-evaluative meaning as a neurobiological candidate endophenotype of SAD [58].

***Medial PFC activation as candidate SAD-endophenotype***

The second fMRI paradigm employed in the LFLSAD, the Social Norm Processing Task (SNPT), taps into the fear of socially-anxious individuals that they will *unintentionally* break a social norm in the presence of others. In this task, three different types of stories on social situations are presented: stories on situations in which *no social norm* (SN) was violated, stories describing *unintentional SN violations* and stories outlining *intentional SN violations*. These conditions enable investigating the behavioral and neurobiological correlates of processing intentional and unintentional social norm violations [60]. Building upon previous versions of the SNPT [60, 61], we created a revised version of the paradigm (SNPT-R) which facilitates using the paradigm in participants of different ages; furthermore, methodological improvements were made and we ensured that the SNPT-R is now publicly available [62]. A validation study in healthy individuals demonstrated that participants rated the stories

differently, depending on the intention underlying the social norm violation: *intentional* social norm violations were considered as more inappropriate and more embarrassing when compared to *unintentional* social norm violations. fMRI data revealed both overlapping as well as differential brain activation patterns for reading intentional and unintentional social norm violations [63]. Next, we demonstrated a positive relationship between social anxiety and the ratings, with the most pronounced effect for the embarrassment ratings of the *unintentional* social norm violations. While individuals with low-to-intermediate social anxiety levels rated the unintentional social norm transgressions as less embarrassing when compared to the intentional social norm transgressions (i.e., these individuals make a distinction between breaking conventional rules, by *intention*, and committing a blunder, *unintentionally*, when they rate the stories on embarrassment), individuals with high social anxiety levels consider unintentional social norm violations as equally embarrassing as intentional social norm transgressions [64].

Consequently, we explored within the LFLSAD whether the neurobiological and behavioral correlates of processing unintentional social norm transgressions could serve as endophenotypes of SAD. Brain responses to unintentional social norm violations were positively *related with levels of social anxiety* within the LFLSAD-families, in the medial PFC and in a cluster encompassing the medial temporal gyrus, superior temporal gyrus and superior temporal sulcus; these brain activation levels were at least moderately *heritable* [65]. Our hypothesis with respect to the ratings of embarrassment was partly supported: data revealed a positive correlation between social anxiety and embarrassment, but this effect was not specific for the unintentional condition and heritability estimates of these ratings were low or even absent. Thereby, this study provided evidence for hyperactivation in the medial PFC and temporal brain regions as putative SAD-endophenotypes [65].

**Brain connectivity as SAD-endophenotype**

Brain regions do not function in isolation but are part of large-scale networks [66], and changes in brain connectivity have been associated with psychopathology in studies on individuals with SAD and children at risk for developing social anxiety [67–72]. Within the

LFLSAD sample, both intrinsic functional connectivity and structural brain connectivity were examined.

Functional brain networks were derived from fMRI data acquired during resting-state, and analyses were performed within the default mode network, dorsal attention network, executive control network, frontoparietal network, limbic network and salience network. Alterations in intrinsic functional connectivity in the dorsal attention network and the frontoparietal network met both endophenotype criteria (*co-segregation* and *heritability*) [73], making them promising SAD-endophenotypes.

Structural white matter (WM) tracts were investigated using diffusion tensor imaging scans. We explored WM-microstructure in the uncinate fasciculus, superior longitudinal fasciculus and inferior longitudinal fasciculus, building on prior work [74–76]. Increased fractional anisotropy in the bilateral superior longitudinal fasciculus co-segregated with social anxiety within families enriched for SAD, and, in line with previous work, all WM characteristics were estimated to be at least moderately heritable [77]. Thus, altered WM-microstructure could be a candidate SAD-endophenotype [78], but more research is needed to determine which WM-alterations are reliably and reproducible related to SAD (cf. this recent meta-analysis [79]).

**Structural brain characteristics as SAD-endophenotypes**

Previous work provided evidence for associations between SAD and gray matter brain characteristics, although results are often inconsistent between studies, probably due to small sample sizes and heterogenous methodology [80]. Building on these studies, data from the LFLSAD allowed for exploring evidence for the *co-segregation* of social anxiety with gray matter characteristics within families. Analyses were restricted to regions of interest (exploratory results on all areas are available online [81]), revealing a positive association between pallidum volume and social anxiety, supporting the *co-segregation* endophenotype criterion. Furthermore, pallidum volume was moderately *heritable*, making pallidum volume a promising candidate SAD-endophenotype. Moreover, several cortical gray matter characteristics, extracted from frontal, parietal and temporal regions, *co-segregated* with social anxiety within the families and had moderate to high *heritability*. It should be noted

that these association results did not survive correction for the number of statistical tests, but these findings provide preliminary evidence that gray matter characteristics of various brain regions are candidate SAD-endophenotypes [82, 83].

**Insights into the genetic vulnerability to SAD from the LFLSAD**

When considering these results from the LFLSAD together with other recent neuroimaging work on SAD, several interesting patterns emerge which provide novel insights into the genetic vulnerability to SAD.

First of all, results illustrate that brain alterations underlying the genetic vulnerability to SAD are diffusely spread over the brain, as they involve subcortical, prefrontal, parietal and temporal regions (Figure 1). These regions, whose function and/or structure qualifies as candidate SAD-endophenotypes, are to a great extent in line with the regions summarized in a neurobiological model of SAD proposed by Brühl and colleagues a decade ago [50]. This model, based on a qualitative review and meta-analysis of 76 neuroimaging studies on adults with SAD, described SAD-related changes in brain function in subcortical, frontal, parietal and occipital areas, as well as alterations in the connections between these regions. Brühl et al. [50] extended an older neurobiological model outlined by Etkin and Wager, describing functional alterations in the so-called 'fear circuit' (amygdala, parahippocampal gyrus, pallidum, insula, inferior frontal gyrus) as well as in the fusiform gyrus and superior temporal gyrus [84]. The results from the LFLSAD in turn further extend the Brühl-model [50], because the LFLSAD provided insights in SAD-related brain characteristics which are not just *biomarkers* (i.e. associated with the disorder, but not necessarily located on the causal pathway from genotype to phenotype), but qualify as candidate *endophenotypes* and are as such most likely part of the neural circuitry that translates genetic effects into disorder phenotypes [85]. This distinction is important, as it implies that these brain alterations likely reflect the genetic vulnerability to *develop* SAD and are not the *result* of the (often chronic) course of the disorder, nor could they be attributed to the effects of psychological treatment, pharmacological medication, or comorbid psychopathology [32, 37]. As such, the LFLSAD findings indicate that SAD is a multi-encompassing brain disorder already at the level of the endophenotype.

[INSERT Figure 1 around here}

Second, the LFLSAD-results stress the importance of considering the dorsal striatum, including the pallidum and putamen, in research on SAD. These subcortical brain areas have increasingly received attention in anxiety research [86–88], but were not yet part of the neurobiological model by Brühl [50]. In two separate studies, being a mega-analysis on a large international dataset of individuals with SAD and healthy participants [89] and the endophenotype LFLSAD-study [82], we found associations between social anxiety and gray matter volume of this region; furthermore, these alterations co-segregated with social anxiety within families and were moderately heritable. These findings were supported by other studies with relevance for SAD. Research on healthy participants demonstrated a positive correlation between the concept 'intolerance of uncertainty' and striatal volume [90], while a study on healthy women demonstrated that socially-anxious tendencies were associated with an enlarged striatum [91]A recent mega-analysis within the ENIGMA (*E*nhancing *N*euro*I*maging *G*enetics by *M*eta-*A*nalysis)-Anxiety working group [92] on data from 37 international samples revealed alterations in putamen and pallidum volumes in adults with SAD [93]. Furthermore, a meta-analysis of fMRI studies on pathological and induced anxiety revealed increased activation of, among others, the putamen [94]. Interestingly, a transdiagnostic study on the common underlying structural brain alterations across multiple psychiatric disorders reported strong evidence for putamen enlargement as a transdiagnostic marker of the familial vulnerability to psychopathology [95]. The findings from the LFLSAD concur with this result, not only with respect to the involvement of the dorsal striatum in psychopathology but also in light of the genetic susceptibility to develop psychopathology. However, these observations also question the specificity of striatal enlargement as an endophenotype for SAD, suggesting that the putamen is a transdiagnostic marker of the vulnerability to develop psychopathology. Nevertheless, specificity is not a prerequisite for an endophenotype [33]: endophenotypes could predispose for multiple anxiety and mood disorders. In line with this reasoning, we propose that striatal enlargement reflects the shared genetic background of anxiety disorders, depression and related phenotypes [96]; cf. work of the Brainstorm consortium showing a high degree of genetic correlation among psychiatric disorders [97]. The idea of striatal alterations as a

transdiagnostic feature is reinforced by a review by Lago and colleagues, highlighting the important role of the striatum in multiple behavioral processes that are very relevant in psychopathology [98]. Furthermore, a large (>30,000 MRI scans) genome-wide association study revealed several genetic variants influencing variation in putamen volume; these genetic variants were thought to affect developmental pathways such as apoptosis, axon guidance and vesicle transport, and could aid in determining mechanisms of neuropsychiatric disorders [99]. Taken together, the role of the dorsal striatum in anxiety, both with respect to its structure as well as its function, deserves attention in future research on the genetic vulnerability to psychopathology, and SAD in particular.

While case-control studies provide insights in the *association with the disorder*, and LFLSAD-data revealed initial evidence for *co-segregation within families* and *heritability,* these insights into the genetic susceptibility to SAD need to be complemented by further work focusing on the endophenotype criterion of *state-stability* [22, 31, 100]. As the LFLSAD was a cross-sectional study [46], data from this project did not allow for investigating this criterion. To examine the stability of the candidate endophenotypes over time, longitudinal neuroimaging family-studies with a substantial sample size, involving adults with SAD as well as their offspring at risk, are important [101]. To the best of our knowledge, no such studies have been undertaken, probably due to the high costs involved and the labor-intensity of such work.

## Inhibited temperament as a marker of the genetic vulnerability to SAD

An alternative way to investigate stable neurobiological characteristics associated with the heritable risk to develop SAD, is to focus on *inhibited temperament.* Temperament involves the 'innate individual differences in behavioral and emotional tendencies that appear in infancy and are relatively stable across context and time' [102]. One of the most commonly used constructs distinguishes individuals based on their tendency to *approach* or *avoid* novelty. The propensity to withdraw from new persons, situations or objects is referred to as 'behavioral inhibition' or 'inhibited temperament' (IT) [102–104]. IT is a heritable trait, constant across time and also measurable in animals, offering opportunities for translational research [105–107]. Variations in IT are present at a continuous scale within the population, and around 20% of young children present with high IT [108]. Essentially, having an IT at a young age has often long-term consequences: a study with a prospective longitudinal design

over three decades revealed that infants with IT (determined at 14 months of age) became introverted adults, with poorer outcomes when it comes to social relationships; furthermore, infant IT was a major risk factor for internalizing psychopathology [109]. Especially stably elevated childhood IT is associated with an increased risk of developing social anxiety [110–112]. A meta-analysis quantified that almost half of all children with elevated IT will develop SAD, compared with only 12% of non-inhibited children [113], and social anxiety shares a substantial proportion of genetic and environmental variance with IT, with tentative evidence for causality [114]. Taken together, these findings indicate that IT is a strong innate risk factor for the later development of SAD, although it should be noted that not all children starting with similar levels of IT become anxious adults ('multifinality') [115, 116].

It has been proposed that brain characteristics provide the foundation for IT and the associated anxiety vulnerability [105]. However, our knowledge with respect to this 'neural risk signature' is still limited, although several neuroimaging studies have examined neurobiological characteristics associated with IT [115]. As also summarized in [117], some studies used a *cross-sectional* approach, examining children and early adolescents with high IT [118] or including young adults who displayed inhibited behavior as a child (determined retrospectively) and at present [119, 120]. Other studies have *longitudinal* designs, in which infant temperament was established during early childhood, while neuroimaging was subsequently performed at various ages [121–127]. These studies suggest that IT is associated with structure and function of brain networks involved in processing fear, reward and emotion regulation; key regions are the anterior cingulate cortex, insula, amygdala, PFC and striatum (cf. [102, 105]). However, such studies into the neurobiological characteristics associated with the temperamental vulnerability to develop SAD are scarce and the majority of them are based on a single longitudinal sample; furthermore, analyses are often restricted to a limited number of regions, and findings lack replication [105]. Moreover, sample sizes of current studies are relatively small (between 22-135 participants, including subjects with various levels of IT).

In a recently pre-registered ENIGMA-Anxiety mega-analysis [117], combining structural MRI data and data on temperament assessments from almost 5,000 young participants (age 6–25 y), we aim to unravel which structural brain characteristics are associated with the temperamental risk for SAD. As described in more detail in the pre-registration [117], we

expect that structural alterations in multiple brain regions, in particular gray matter volumes of several subcortical structures (amygdala, hippocampus, striatum including caudate and putamen), and characteristics of frontal and temporal cortical areas are related to inhibited temperament during childhood.

## Moving forward: neurobiological endophenotypes as targets for interventions[3]

As argued above, unraveling neurobiological endophenotypes of SAD is important to gain insights in the genetic vulnerability to develop anxiety. Another avenue for future research concerns the use of candidate SAD-endophenotypes as targets for therapeutic or even preventive interventions. Nowadays, there are several cutting-edge techniques which enable altering brain function of specific regions or brain networks. Here, we highlight the possibilities of real time fMRI-based neurofeedback and non-invasive brain stimulation, and take amygdala hyperreactivity [58] as an example of a promising treatment target.

### Real-time fMRI-based neurofeedback

In the last decade, real-time fMRI-based (rtfMRI) neurofeedback has increasingly attracted attention in the neuroimaging field. RtfMRI neurofeedback uses the latest developments of data processing and pattern analysis, making it possible to provide immediate information about brain activation or brain connectivity to participants, who, in turn, can learn to control the level of these indices [128, 129]. There are several important questions that need to be resolved, for example with respect to the sustainability, transferability, and feasibility of the technique [130–132], and the impact of psychological factors like attention, motivation and mood influence on the outcome of rtfMRI neurofeedback experiments [133]. However, multiple studies reported promising findings with respect to the possibility of rtfMRI neurofeedback to alter amygdala activation or amygdala connectivity. Two studies indicated that participants were able to downregulate activation of the amygdala during viewing faces with a negative expression (proof-of-principle study and replication: [134] and [135]).

[3] This section is partly based on the discussion of the thesis 'Extremely Shy & Genetically Close' [49] and updated for the present chapter.

Furthermore decreased amygdala activation was found during cognitive reappraisal in participants who received rtfMRI neurofeedback based on lateral PFC activation [136], and two studies demonstrated that participants could regulate functional connectivity between regions of the PFC and the amygdala [137, 138]. Furthermore, studies on small clinical samples, involving individuals with borderline personality disorder [139] post-traumatic stress disorder [140–142] and depression [143], yielded promising results with respect to their ability to regulate amygdala activation and connectivity. Lastly, work in adolescent girls described modulation of functional connectivity between the PFC and amygdala [144, 145].

To the best of our knowledge, rtfMRI neurofeedback has not been applied in participants with (sub)clinical social anxiety. Based on the findings summarized above, we speculate that rtfMRI neurofeedback could be a useful tool to downregulate the hyperresponsiveness of the amygdala, offering the possibility to alter the expression of this SAD-endophenotype [58]. Furthermore, the effect of this downregulation on behavioral outcomes and levels of social anxiety is worthy of investigation. Confirmative results open the way for the use of rtfMRI neurofeedback in preventive and therapeutic interventions [146]

**Brain stimulation**

Another method to non-invasively influence brain activation is brain stimulation, either by repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS) [147–149]. Research on non-invasive brain stimulation in the context of anxiety disorders, and SAD in particular, is still scarce [150–152]. Two case-studies from the same research group demonstrated reductions in the level of social anxiety following rTMS over the right ventromedial PFC [153, 154], while a double-blind within-subject protocol in individuals with SAD revealed that tDCS over the left dorsolateral PFC reduced the attentional bias to threat, but not levels of social anxiety [155]. Unfortunately, these proof-of-principle studies did not include fMRI measurements to assess the effect of stimulation on amygdala activation.

Recently, a groundbreaking study combined rTMS with fMRI, in a considerable sample of 117 participants, exploring the neural circuit underlying emotion regulation [156]. Active rTMS (versus sham) over the right ventrolateral PFC was associated with reduced amygdala

activation during reappraisal of pictures depicting social exclusion; furthermore, active rTMS was related to lower negative feelings during reappraisal. Although these findings concern healthy participants, they highlight the potential of rTMS to effectively influence emotion regulation at the neurobiological and behavioral level. Furthermore, these findings align with the recommendations from a systematic review, in which the authors propose a treatment model in which stimulation of the dorsolateral PFC, via the corticolimbic pathway, results in down-regulation of the amygdala [157]. Although this model is speculative, a randomized tDCS study in women with high trait anxiety yielded promising results, by revealing that active tDCS (versus sham stimulation) of the dorsolateral PFC significantly reduced bilateral amygdala reactivity to threat [158]. Another study indicated that rTMS over the right dorsolateral PFC attenuated the amygdala response to visual stimuli with a strong negatively-loaded emotional content [159]. Based on these encouraging results, we plead for double-blind placebo-controlled studies in healthy participants, individuals with SAD and young people with the genetic vulnerability to develop SAD, in order to characterize the neurobiological effects of non-invasive brain stimulation on amygdala reactivity, to determine the long-term outcomes of stimulation, and to explore the potential of these stimulation methods in the treatment and prevention of SAD (cf. the recommendations by [160] regarding the application of non-invasive brain stimulation in the treatment of generalized anxiety disorder).

To conclude, the work reviewed in this chapter highlights the impact of genetics on the development of SAD. Recent work on endophenotypes and inhibited temperament has further informed our understanding of this genetic vulnerability and state-of-the art neuroimaging and neuromodulation techniques open the way to develop novel interventions to target this genetic vulnerability. More research, preferably in large samples is needed to further elucidate reliable and robust targets for these interventions.

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**Figure caption**

**Fig. 1**

Summary of candidate SAD-endophenotypes based on findings from the Leiden Family Lab study on Social Anxiety Disorder (LFLSAD).

A. Subcortical brain regions.

B. Cortical brain regions.

(Adapted from [49]).