GeneQoL Consortium

Negative Psychological Affect/Attributes

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1. Negative Psychological Attributes

It is generally accepted that health-related quality of life is a multidimensional concept incorporating at least three broad domains -- physical, psychological, and social functioning -- that are affected by one's disease and/or treatment. Psychological functioning ranges from severe psychological distress to a positive sense of well-being. The focus of this team's effort is on the negative side of this continuum, including "normal" feelings of depression, anxiety, and distress as a response to a negative life event, e.g. the diagnosis of a disease.

Most biological and genetic research has been conducted in the area of pathology and psychiatry, e.g. clinically diagnosed major depressive and anxiety disorders. We will therefore summarize the major results of this area as we expect a similar biological substrate for non-pathological negative affect as there is evidence to date that negative affect behaves as a continuous trait. One of the goals of the consortium is to determine the usefulness of this approach.

A note of caution is in order. Major depressive and anxiety disorders are distinct from other disorders that can also have substantial depressive symptoms, e.g. bipolar disorder, schizophrenia, schizoaffective disorder, and depressive symptoms resulting from medical conditions (e.g. hypothyroidism). Moreover, there is a large variety of (more or less loosely defined) depressive and anxiety disorders, which are all multi-causal network syndromes, with individually different imbalances of hormones, receptors and neurotransmitters. The following summary can therefore only be sketchy and incomplete. By the time we will move on to data collection and/or analysis, we need to expand the team with experts who can provide a comprehensive picture of the biological pathways and an updated list of potential candidate genes and variants.

2. Potential Biological Pathways

Question 1: Which potential biological pathways have been considered and/or shown to describe a possible genetic disposition for negative psychological affect?

2.1 The Hypothalamo-Pituitary-Adrenal (HPA) System

The HPA-axis is considered to be the ‘final common pathway’ for most of the depressive symptoms (Bao, Meynen, Swaab, 2008). A large part of the environmental and genetic risk factors for depression appear to correlate with increased HPA-axis activity in adults.
The hypothalamus releases corticotropin-releasing hormone (CRH) in response to a stressor. CRH acts on the pituitary gland, triggering the release of adrenocorticotropin (ACTH) into the bloodstream, which subsequently causes the hormonal endproduct of the HPA-axis, corticosteroid release from the adrenal cortex (mainly cortisol in humans). Cortisol normally exerts a negative feedback effect to shut down the stress response after the threat has passed, acting upon the levels of the pituitary and hypothalamus.” (Bao et al., 2008, p.532; see Figure 1).

Figure 2 illustrates schematically the impaired interaction between the decreased activity of vasopressin neurons (AVP) in the superchiasmatic nucleus (SCN; the hypothalamic clock) and the increased activity of corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN). The HPA system is activated in depression and affects mood, via CRH and cortisol. In depression, there is a decreased amount of vasopressin (AVP) mRNA of the SCN. The decreased activity of AVP neurons in the SCN is the basis of the impaired circadian regulation of the HPA system in depression. (Bao et al., p. 541).

The following hypothesis for the pathogenesis of depression was forwarded by Bao et al. (2008). “In depressed patients, stress acting on the HPA system results in a disproportionately high activity of the HPA system because of a deficient cortisol feedback effect due to the presence of glucocorticoid resistance. The glucocorticoid resistance may either be caused by a polymorphism of corticosteroid receptor or by a
developmental disorder. Also AVP neurons in the SCN react to the increased cortisol levels and subsequently fail to inhibit sufficiently the CRH neurons in the PVN of depressed patients. Such an impaired negative feedback mechanism may lead to a further increase in the activity of the HPA system in depression. Both high CRH and cortisol levels contribute to symptoms of depression.” (p. 541).

There are two main hypotheses concerning the HPA-axis hyperdrive and depression: (1) The CRH hypothesis, according to which CRH not only causes the neuroendocrine-hyperdrive, but is also responsible for depressive signs and symptoms via CRH innervation of brain areas. (2) The glucocorticoid hypothesis, which states that increased cortisol plasma levels are causal to the development for depressive symptoms (Bao et al., 2008; p. 545).

2.2. Subsystems

2.2.1 Dopamine
Dopamine is an important neurotransmitter for stable moods, such as depression. Some forms of depression are believed to be a result of impaired dopamine system. There are mainly three pathways: (a) the nigrostriatal pathway, which is primarily responsible for motor planning, movement, and cognition; (b) the mesolimbic pathway, which is important for reward, motivation, and the experience of pleasure, and (c) the mesocortical pathway, which is essential for concentration and working memory (Dunlop & Nemeroff, 2007).

2.2.2 Serotonin
Serotonin levels play an important role in anxiety (Maron & Shlik, 2006). Serotonin is a mono-amine neurotransmitter (also known as 5-hydroxytryptamine/5-HT) that is mainly synthesized by serotonergic neurons of the raphe nuclei. Serotonin disruption in the projection areas of the raphe nuclei is thought to be of importance in anxiety disorders (Lesch, Zeng, Reif & Gutknecht, 2003). More specifically, it is suggested that a decreased level of serotonin in the brain is related to panic disorders (Maron & Shlik, 2006) and mood disorders, although this relation is most probably reflecting a vulnerability to suffer from depressive disorders (Ruhe, Mason & Schene, 2007). In patients with panic disorder, anxiety symptoms can be attenuated by increasing serotonin levels through drug administration with selective serotonine uptake inhibitors (SSRIs) (Maron & Shlik, 2006). Depressed patients also respond to SSRIs (e.g. Belmaker & Agam, 2008).

2.2.3 Sex hormone level fluctuations
Based on the findings that men show higher HPA-axis activity than women; that 2) the lifetime prevalence of major depression is twice as high in women as in men; and 3) the prevalence of mood disorders increases during the reproductive years, especially during times of change in gonadal hormone levels it has been speculated that the changes of sex hormone levels may play a more important role in the vulnerability to mood disorders than the basal absolute levels (Bao et al., 2008, p. 539). However, studies regarding this
theory have been controversial. While a few studies showed that testosterone administration may be effective in mood disorders, also estrogen substitution in postmenopausal women with depressive symptoms seemed to be effective in some studies but not in others.

2.2.4 Circadian system
The SCN that induces circadian and circannual variations in neuronal activity is supposed also to be related to circadian and circannual fluctuations in mood and to sleeping disturbances in depression. A polymorphism in the clock gene NPAS2 appeared to be associated with seasonal affective disorder, indicating that the SCN may also play a causal role in this type of depression (Bao et al., 2008, p. 540).

3. Polymorphisms
Question 2: Which genes and genetic variants have been considered and/or shown to have a potential association with negative psychological affect?

3.1 Genes significantly associated to depression in meta-analysis
The following five genes were significantly associated to major depression disorder in meta-analysis for polymorphisms that had been investigated in at least three studies (López-León et al., 2008):

- APOE (apolipoprotein E)
- GNB3 (guanine nucleotide-binding protein)
- MTHFR (methylenetetrahydrofolate reductase)
- SLC6A3 (dopamine transporter)
- SLC6A4

While these genes were significantly associated to depression in meta-analysis, this does not mean that there are no other potentially important genes. Insignificant results may stem from small sample size, low frequencies of the risk variant and heterogeneity between studies. Moreover, most studies have investigated only one single polymorphism per gene, whereas other, unstudied variants in the same gene may be associated to depression. Hence, the candidate genes listed in the following paragraphs need to be taken seriously.

3.2 Other candidate genes

3.2.1 The Hypothalamo-Pituitary-Adrenal (HPA) System
The following list of genes is provided by Prof. D. Swaab, neurobiologist (personal communication):

Cortisol is a major stress hormone that acts on many organs and brain areas through two types of receptors:

- Mineralocorticoid receptor (MR) / AR / CRHR1
- Glucocorticoid receptor (GR) (NR3C1) / ER1,2 / CRHR
Further candidate genes are:

- VMAT2
- Arginine vasopressin (AVP), AVPR1b/1a
- Oxytocin (OXTR)
- DA receptors
- Opiate receptors
- SHT receptors (5HT2A/1A); 5HT transporter
- GABA receptors / Glutamate
- CR (cannabis)
- BDNF / Trx B
- Melatonin receptors
- Clock genes (NPAS2, ARNTL, PER3)
- Urocortin receptors
- NPY receptors
- Orexin receptors
- Adrenergic receptors (α2 A)
- Thyroid hormones, receptors and transporters

3.2.2 Dopamine
The polymorphisms in the (mesolimbic) dopamine pathway thought to be most important in depression, are those that alter function of dopamine transporter (DAT), Catechol-O-methyltransferase (COMT), and the D4-receptor (DRD4) (Sullivan et al., 2000).

1. The dopamine transporter (DAT) is associated with depression, but also with the reward system. Many polymorphisms of this gene exist, and probably not all the polymorphisms of the DAT gene are known, consequently the whole DAT gene is a candidate.

2. Catechol-O-methyltransferase (COMT) gene, because high COMT activity will result in less dopamine and less COMT activity will result in more dopamine. Whereas the Val158 (perhaps lower QOL) and Met158 (perhaps higher QOL) alleles are particular interesting (Lohoff et al., 2008), the entire COMT gene will be a candidate. Silent polymorphisms can also affect mRNA translation/stability (Science, 2006).

3. D4-receptor (DRD4). The most investigated polymorphism in DRD4 is the 48-bp repeats in the third exon which gives rise to nine alleles varying from two to ten repeats (Reif & Lesch, 2003). Whereas the different amounts of repeats in the third exon will be the candidate polymorphism, the entire DRD4 gene will be a good candidate (see also López-León et al., 2005).

3.2.3 Serotonin
To date, no gene polymorphism is unquestionably related to anxiety disorders. However, three genes have been targeted in several association studies, as they exert a decreasing influence on serotonin levels: 5-HTT, COMT, and monoamide oxidase (MAO-A).

1. The serotonin transporter 5-HTT. It should be noted that mixed results have been found regarding the association of 5-HTT with anxiety disorders, with both positive (Melke et al., 2001; Greenberg et al., 2000) and negative (Hamilton et al., 1999) findings.
Relationships between 5-HTTLPR and SSRI-response were also examined (e.g., Belmaker et al., 2008).

2. Catechol-O-methyltransferase (COMT). A locus for panic disorders is situated within or in the immediate proximity of the COMT gene on chromosome 22 (Hamilton et al., 2002). On the other hand, a lack of association between panic disorders in Japanese patients and the COMT polymorphism has also been described (Ohara et al., 1998).

3. A functional polymorphism in the promoter region of the MAO-A gene has been described. Sabol and colleagues (1998) revealed 4 variations in the upstream sequence of the MAO-A gene: alleles containing 3 repeats of a 30bp sequence; 3.5 repeats; 4 repeats; and 5 repeats, respectively. Moreover, several research groups found that female patients suffering from panic disorder showed a significantly higher frequency of the longer alleles (specifically the alleles containing 3.5 and 5 repeats), and therefore the more active MAO-A enzyme compared to control groups not suffering from panic disorder (Deckert et al., 1999; Maron et al., 2005).

Thus, of all three targets, the results confirming the importance of the MAO-A promoter were the most promising and consistent (Deckert et al., 1999; Samochowiec et al., 2004; Maron et al., 2005). One might hypothesize that longer alleles of the MAO-A promoter might be responsible for a more effective enzyme activity of MAO-A, which in turn could possibly lower the levels of serotonin, which in itself is associated with panic disorder. The question arises whether longer alleles of the MAO-A promoter are predictive for impaired quality of life (Audureau et al., 2008).

3.3 Considered and rejected biological pathways/polymorphisms

Given the methodological challenges to genetic research, it is perhaps premature to exclude genes that were found to be insignificantly associated to depression. However, the following genes were qualified in the literature.

Until the seventies, deficits in serotonin (HT5) and norepinephrine (NE) were thought to play the predominant role in the pathophysiology of depression. However, antidepressant drugs made to replenish the lack of these neurotransmitters, like serotonin reuptake inhibitors (SSRIs) showed not to be effective in large groups of patients suffering from depression (Dunlop & Nemeroff, 2007). The current view is that antidepressants may improve mood via an indirect mechanism of action, in which increased serotonergic (and/or norepinephrinergic) neurotransmission improve the regulation of emotions (Ruhe, 2008).

A genome-wide association study and replication efforts for major depressive disorder examining a possible role for the presynaptic protein piccolo (PCLO) did not yield "proof beyond a reasonable doubt" level of evidence for an association between this gene and major depression (Sullivan et al., 2008). However, authors find it premature to exclude PCLO as a possible relevant gene for some forms of major depression, and hypothesize that an association may be detected only in population-based cases.
4. Potential Datasets
Question 3: What datasets are available to explore the association of genes and negative psychological affect?

4.1 Existing datasets

4.1.1 General population
- Australian studies: Appendix 1 provides an overview of the number of genotyped individuals (related versus unrelated/males versus females) that were phenotyped according to emotional well-being (one item); depression; any anxiety; obsessive compulsive disorder; social phobia; panic or agoraphobia; psychosis, extroversion, and neuroticism (EPQ); Tridimensional Personality Questionnaire; and the General Health Questionnaire.
- Framingham Sleep Heart Health Study: 2772 subjects; SF-36 items (4 negatively worded items of mental health scale).
Request on behalf of the Consortium submitted.
- Framingham Share: Offspring of above study: 3000 subjects; SF-36 items; GWA available.

4.1.2 Disease populations
- GAIN Major Depressive Disorder Study: Netherlands Twin Register (NTR) and Netherlands Study of Depression and Anxiety (NESDA) biobanks: 1821 MDD cases and 1822 controls; range of questionnaires; GWAs available. Co-Principal Investigator: Dorret Boomsma

4.2 Future datasets

4.2.1 General population
- LIFEGENE: Swedish general population (N=500,000); SF-36 items (4 negatively worded items of mental health scale) + CES-D; collection of blood. Co-Principal Investigator: Nancy Pedersen

4.2.2 Disease populations
- Breast and prostate cancer: Linneus project "Individualized prediction and prevention of breast and prostate cancer"; Swedish study; Co-Principal investigator: Per Hall
- Congenital heart disease: Concor dataset: Dutch database of 1,400 patients with congenital heart disease; SF-36 + disease specific quality of life + HADS; blood samples available; Co-Principal investigator: Mirjam Sprangers
5. Future Studies

Question 4: How would you design a new prospective study to explore the association of genes and negative psychological affect?

5.1 Operationalization of negative psychological affect

Candidate standardized, self-report questionnaires that are used widely include:

A) anxiety/depression subdomains or individual items of generic quality-of-life questionnaires: e.g.,
- SF-36 Health Survey (SF-36) and its derivatives (e.g., SF-12)
- Euroqol EQ-5D (EQ-5D)
- Nottingham Health Profile (NHP)
- McMaster Health Index Questionnaire (MHIQ)
- Psychological General Wellbeing Index (PGWI)
- Affect Balance Scale (ABS)

B) Anxiety and depression questionnaires, e.g.:
- Hospital Anxiety Depression Scale (HADS)
- Center for Epidemiologic Studies-Depression scale (CES-D)
- State Trait Anxiety Inventory (STAI)
- Inventory of Depressive Symptomatology (IDS-SR)
- Beck Depression Inventory (BDI)
- General Health Questionnaire (GHQ)

5.2 Genes and Genotyping

We have to make a decision which approach we will follow.

Available GWAS data with the proper phenotypic information can be used to identify risk factors but we should keep in mind that this is not likely to give strong modifiers. In addition mild modifying loci might be missed but a network-based approach, in which the analysis is based on pathways, might help to reduce the number of false negatives.

Sequencing of whole genomes is a better approach but this is technically and financially not yet feasible. Sequencing a selection of candidate genes is a viable option and can identify both common and rare variants. In that case, we need to ensure that we will start from an updated list of potential candidate genes and variants. To that purpose, we need to invite other experts to our team. For example, Prof. Dick Swaab, professor of neurobiology and Prof. Aart Schene, professor of psychiatry, both at the University of Amsterdam, have the required expertise, and expressed willingness to collaborate. Other experts also need to be sought.

For Copy Number Variant (CNV) analysis one can analyze existing GWAS data, but as indicated in appendix 2, many CNVs will be missed. However, in case an association is found with a quality-of-life dimension/phenotype one can perform a detailed analysis of
these regions. This can be either direct copy number analysis or a sequence-based approach.

5.3 Statistical analysis
A range of different approaches will be adopted. First, for each different gene polymorphism, subjects will be categorized accordingly. For example, they may be dichotomized by presence or absence of the particular polymorphism (e.g. Val158 of COMT gene present/absent), or grouped according to the number of possible alleles (e.g. in case of D4DR gene). Analysis of Variance (ANOVA) will then be conducted on the quality-of-life scores.

Second, patients can be grouped according to their scores on the quality-of-life outcome (e.g. in quartiles). On the basis of a Genome Wide Analysis set, associations with SNPs can be explored.

Clearly, we need pooling of a wide range of studies that have used the same questionnaire (e.g. SF-36, HADS) to allow the performance of ten-thousands of t-tests/ANOVAs.
Acknowledgments

We are greatly indebted to Dick Swaab, professor of neurobiology at the University of Amsterdam, for his insightful comments and advice. Information about the HPA-axis is based on his review (Bao et al., 2008) and presentation “The Neurobiology of Depression”. For the information on the dopamine and serotonin systems, we have made gratefully use of two Masters theses that were prepared under supervision of Frank Baas: (1) Harg van der J, Hoogland L, Maaden van der T, Vissers B. The Genetic Influence on Quality of Life. Amsterdam, 2008; and (2) Audureau N, Kouwenhoven W, Naninck E. Polymorphisms in the MAO-A promoter gene as predictive parameters for impaired quality of life. Amsterdam, 2008. We are also indebted to insightful comments from Dr. Eric Ruhé, Department of Psychiatry, University of Amsterdam.
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Appendix 1: Overview of Australian studies (prepared by Miriam Mosing)

Explanation of abbreviations:
OCD stands for Obsessive Compulsive Disorder. As diagnoses of Social Phobia and Panic or Agoraphobia, diagnoses of OCD are based on the DSM-III-R and the DSM-IV.

Furthermore, TPQ stands for Tridimensional Personality Questionnaire, a questionnaire developed by Cloninger. The original Cloninger long form TPQ instrument had 110 items (version 4 - revised 26-10-87). The AL1/AL2/AR1/AR2 TPQ contain 54 items. An example of an item would be: "I usually am confident that everything will go well, even in situations that worry most people."

Within the TPQ there are 12 subscales. The subscales fall into 3 categories, Novelty Seeking (NS), Harm Avoidance (HA) and Reward Dependence (RW), each of which contains 4 subscales.

The NS subscales are: NS1 - Exploratory excitability vs stoic rigidity; NS2 - Impulsiveness vs reflection; NS3 - Extravagance vs reserve; NS4 - Disorderliness vs regimentation.

The HA subscales are: HA1 - Anticipatory worry & pessimism vs uninhibited optimism; HA2 - Fear of uncertainty; HA3 - Shyness with strangers; HA4 - Fatigability & asthenia.

The RD subscales are: RD1 - Sentimentality; RD2 - Persistence; RD3 - Attachment; RD4 - Dependence.

Each item asks for a true/false answer and the items are phrased in such a way that for some items a true answer adds to the subscale score (+) and for others a false adds to the subscale score (-).
Appendix 2: Background on Genetic Approaches (prepared by Frank Baas)

Understanding the genetic basis for human disease or phenotype is a major challenge for medical genetics. Thus far the genetic basis of many monogenic disorders has been discovered; however, most of these disorders are rare. Our understanding of the genetics of common disease, i.e. diseases with a high prevalence in the population, is incomplete. These traits are considered to be complex in the sense that multiple acquired and genetic factors interact in varying combinations. Therefore there is no simple model for the genetics of these diseases. For example, common disease can be considered as the result of mainly a combination of multiple common sequence variants each with a small effect (common disease- common variant hypothesis; CD-CV) or of only a few rare sequence variants, each with a greater impact on the phenotype than the common variants. With the rapid development of both DNA-array and DNA-sequencing technology it is becoming possible to test these disease models by analyzing large populations for sequence variants. Genome wide association studies (GWAS) use DNA-array technology to identify common haplotypes amongst affected individuals, whereas the population based resequencing approach uses novel sequence technologies to identify sequence variants in a defined set of candidate genes already known to be implicated in the disease. As a result, there is experimental support for both the models mentioned above. Large GWASes have identified risk factors for several complex diseases and traits like macula degeneration, breast cancer, prostate cancer, diabetes, obesity, height, coronary heart disease and inflammatory bowel disease (examples are: Li et al, Nat. Genet 38, 1049-1054 (2006), McPearson et al., Science 316:1488-1491 (2007); Rioux et al. Nat Genet 39:596-604 (2007); Haiman et al. Nat genet 39: 631-637 (2007); WTCC, Nature 447; 661-678, 2007). As postulated, the common variants that were identified indeed have only small effects on the susceptibility for the disease. Odds ratios usually are between 1.2 and 1.5. One might argue that in most GWASes common SNPs in linkage disequilibrium with the ‘real’ mutation were discovered. Maybe, the causative sequence variant has a lower frequency and larger effect on the phenotype, but this is still hypothetical.

The question remains what the contribution of rare variants is. There is evidence that not all complex disorders are caused by high frequency variants. It is expected that most rare missense alleles are deleterious in the human population and thus subject to negative selection (Kryukov et al Am J Hum Genet 69: 124-137; 2007). Therefore, even mildly deleterious mutations cannot exist as common polymorphisms in the human population as a whole. Resequencing studies showed that systematic sequencing of genes can identify both common and rare variants associated with disease (e.g. Sandilands et al. Nat Genet, 39: 650-654 (2007). A recent study in search for genetic determinants for blood pressure showed that rare sequence variants in genes involved in renal salt handling also contribute to blood pressure (Li et al, Nature Genetics; 40: 592-599 (2008). Apparently, rare sequence variants with a relatively strong impact also play a role in a common trait.

In addition to simple sequence variations, changes in copy number of a small part of the genome occur in man. These changes are called copynumber variants (CNVs). CNVs have recently been implicated is several diseases including psychiatric disorders like
schizophrenia (Vrijenhoek et al. Am J Hum Genet. 83:504-10 (2008), Stefansson et al. Nature; 455:232-6 (2008)). CNV analysis is still in its infancy, microarray based approaches like CGH or some specialized SNP arrays can detect CNVs but the sensitivity of these technologies is limited. Small duplications or deletions can easily be missed. Therefore there is not a single technology that can be applied for all approaches/models.