General Introduction

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behavior-genetic studies of twins, siblings and other pairs of relatives are very useful to identify the heritable components of complex traits such as those related to well-being (WB). From these studies, we know that individual differences in well-being are accounted for by both genetic as well as environmental factors, although the range in estimates is large and varies between $0\%$\(^1\) and $64\%$\(^2\). From a recent systematic review study, we know that the average heritability for WB tends to hover around $40\%$\(^3\). These heritability studies, based on both classical and extended twin designs, are valuable, as they allow us to gauge the relative influence of our genetic make-up on WB. Nevertheless, it is important to note that classical twin designs do not give insight into the specific genomic regions that may be involved in explaining the heritability of well-being. One of the most exciting directions for research in behavioral genetics is the combination of quantitative behavioral genetics and molecular genetics in an attempt to identify the specific genes underlying the substantial heritability estimates of complex behaviors. Although the GWAS Era is in full bloom in 2018, many obstacles has to be conquered to get to this point. Therefore, the present chapter can be seen as an overview of molecular genetic studies trying to associate genetic variants to WB up until 2014, the beginning of my PhD trajectory.

**Linkage analysis**

Traditionally, the search for genes involved in both normal behavior and behavioral disorders began with linkage analysis, aimed at localizing genetic variants that regulate a trait of interest. It is well known that during recombination, deoxyribonucleic acid (DNA) is not copied straightforwardly from parents to offspring, but shuffled to create a “unique” DNA template. Linkage studies rely on the assumption that genes located closer to each other are more likely to be transmitted together (linked) than genes that are located separately on a chromosome. Therefore, within linkage analysis, the distance between a DNA marker (i.e., a landmark of known location in the genome) and a locus involved in a particular phenotype is analyzed in small numbers of large multigenerational pedigrees. For linkage analysis to succeed, markers that flank the trait of interest must co-segregate along familial lineages in extended pedigrees. This method has been very successful in mapping rare genetic diseases of large effect, such as DNA linkage for Huntington’s Disease on chromosome $4^4$. 
Linkage analysis: complex traits

Although linkage analysis of large pedigrees has been very effective for locating genes for rare single-gene disorders, it is less powerful when many genes with small effect sizes are involved in the trait of interest. To overcome this problem, the “classical” linkage analysis has to be extended in a way to gain more power to detect genes with smaller effect sizes. With the development of the quantitative trait loci (QTL) linkage design, these problems could be (partly) addressed. Rather than studying large pedigrees in a few families, this method studies many families with a small number of relatives, usually siblings. QTL linkage examines allele sharing between two individuals of a sibling pair, also known as identity-by-descent (IBD). Full siblings receive each an allele from the father as well as an allele from the mother. The total IBD value of a sibling pair can therefore range between 0 and 2. QTL linkage is based on the assumption that linkage is supported if sibling pairs with two alleles IBD are significantly more alike on the (complex)-trait of interest than sibling pairs that share only 1 or 0 alleles IBD, respectively. The strength of linkage is denoted as a logarithm of odds (LOD) score, which compares the likelihood of whether two loci are indeed linked to the likelihood that the observed linkage is present purely by chance. The first success for QTL linkage studies came with the identification and replication of linkage for reading disability on chromosome 6 (6p21)⁵, but QTL linkage has also been successful for complex traits. For instance, Cloninger et al.⁶ found a significant linkage between the personality trait Harm Avoidance, a measure of anxiety proneness, and a locus on chromosome 8 which explained 38% of the trait variance.

Linkage analysis and well-being

To date, only one genome-wide linkage study has been performed to disentangle sources of individual differences in happiness⁷. Happiness was assessed with the four-item Subjective Happiness Scale⁸, and a total of 1,157 offspring from 441 families were genotyped with an average of 371 micro-satellite markers per individual. Using QTL linkage, authors found suggestive linkage (LOD score of 2.73, \(p = .10\)) at the end of the long arm of chromosome 19 (q13.43) and at the short arm of chromosome 1 (LOD score of 2.37, \(p = .21\)). This result indicates that happiness might be positively associated with particular genes located in these genomic regions. However, after closer inspection, none of the genes (e.g. DUXA, AURKC, USP29, and several zinc finger protein genes) appeared to play a plausible role in happiness. For instance, DUXA genes are thought to be involved in early embryonic development, whereas USP29 is associated with Angelman Syndrome. In addition, zinc finger proteins are one of the most abundant proteins in the genome with extreme diverse functioning, including...
DNA recognition, RNA packaging, and transcriptional activation\textsuperscript{9}.

**Association studies**

**Candidate genes**

Over the past few years, linkage analysis has lost its predominance in favor of *allelic association* analysis. In contrast to linkage studies, which are very systematic but often suffer from a lack of statistical power, association studies are more powerful but were initially less systematic. Association studies are more powerful because they do not rely on recombination within families as in linkage analysis, but simply compare allelic frequencies for groups such as low-scoring versus high-scoring individuals on a quantitative trait\textsuperscript{10}. However, the strength of allelic association is also its biggest weakness, as allelic association can only be detected if a DNA marker is itself the functional genetic variant (i.e. *direct association*) or is located closely to the functional variant (i.e., indirect association or *linkage disequilibrium*). As a consequence, hundreds of thousands, or even millions of DNA markers must be *genotyped* in order to capture genetic variation across the genome. Therefore, for a long time, allelic association has been used primarily for fine-mapping of linkage regions and testing of potential candidate genes rather than scanning the genome in a systematic way. There are several strategies for the selection of candidate genes. For instance, the selection of genes based on their hypothesized involvement in physiological systems thought to influence the trait of interest or selection of chromosomal regions in animals that are known to influence the trait of interest as a starting point. To date, there are only two candidate gene studies that investigated subjective well-being (SWB), one involving the serotonin transporter gene (5-HTTLPR) and the other the Monoamine oxidase A (MAOA) gene which encodes the MAO-A enzyme.

**Candidate genes: serotonin and life satisfaction**

The neurotransmitter serotonin is believed to play an important role in human mental states and is therefore one of the most intensively studied neurotransmitters in the central nervous system (CNS). When serotonin is released in the synaptic cleft, serotonin transporters that are placed in the cell wall will recycle most of it. The 5-HTT gene that encodes for this transporter contains a VNTR in the promotor region (5-HTTLPR). As a result, the serotonin transporter gene promotor region exists as a “long” or “short” variant, although three additional but more rare variants have recently been discovered\textsuperscript{11}. Although these alleles all produce the same protein, the long allele (L-allele) is associated with a three times higher
activity than the shorter allele (S-allele) and produces significantly more serotonin transporters\textsuperscript{12}. The consequences of these different genetic variants are increasingly understood. For instance, it has been shown that in carriers of the S-allele, the amygdala was increasingly active to negative emotional stimuli\textsuperscript{13,14}. In addition, another study found that individuals carrying the L-allele have a significant bias towards positive information and selectively avoid negative information\textsuperscript{15}. Based on these studies, variation in the promotor region of 5-HTTLPR seems to be a promising candidate for individual differences in SWB. It is therefore no surprise that this gene was chosen in a study performed by De Neve and colleagues\textsuperscript{16,17}, who hypothesized that the L-allele of the 5-HTTLPR gene would be associated with a higher level of life satisfaction than the S-allele.

In their first study, genetic information was available from 2,574 individuals, including markers that identify alleles of the serotonin 5-HTT promotor gene. The included participants were asked to answer the following question: How satisfied are you with your life as a whole?, and response categories ranged from 1 (“very dissatisfied”) to 7 (“very satisfied”). By comparing allelic frequencies between low-scoring and high-scoring individuals, it was found that participants carrying the L-allele (i.e., the more efficient variant of the serotonin transporter gene) reported significantly higher levels of life satisfaction. Two independent samples were used to replicate this interesting finding\textsuperscript{17}. The first sample consisted of 3,460 individual’s that were genotyped using the European HAPMAP sample, which contain an alternative marker (rs2020933) associated with the transcription of serotonin transporters\textsuperscript{18–20}. Participants were asked to answer the following life-satisfaction question: Indicate where you think you belong between these two extremes: (1) satisfied with job or home life, or (2) ambitious, want change. Respondents could provide an answer on a seven-point scale and the coded scale was reversed so that higher values indicated greater life satisfaction. Although both, the DNA marker as the life satisfactory question were slightly different from the first study, a positive and statistically significant association between the more efficient A-allele of the rs2020933 marker and increased life satisfactory was found. The second sample extended the first discovery study, which was made possible due to a new release of genomic data ($N = 10,163$). Association models were identical to those described in the earlier mentioned discovery sample, but a significant association was no longer found between carriers of the L-allele and life satisfaction in this new sample nor in a pooled sample consisting of the discovery sample and the new released genomic data ($N = 12,391$).
Together, these results produce some mixed results indicating that more research is needed to test the hypothesis regarding the association between the 5-HTTLPR L-allele and life satisfaction. Moreover, the complete absence of any significant association between this gene variant and life satisfaction in the second replication study raises doubts whether this gene should be considered a suitable candidate gene for life satisfaction. Importantly, most molecular genetics studies have been studying this genetic polymorphism predominately in relation to depression and response to negative stimuli\textsuperscript{13,14,21}. Given that the absence of depression of negative emotion is not the same as happiness, the role of 5-HTTLPR in processes related to well-being is currently unclear\textsuperscript{22}, although some studies suggest that the 5-HTTLPR short allele may increase the positive response to supportive exposures.

\textit{Candidate genes: MAOA and happiness in women}

The second candidate gene study focused on a well-being phenotype, investigated the involvement of the monoamine oxidase A (MAOA) gene in modulating happiness\textsuperscript{23}. The MAOA gene is located on the X chromosome\textsuperscript{24} and encodes the MAOA enzyme, which metabolizes (i.e., rendering inactive) neurotransmitters such as norepinephrine (NE), serotonin (5-HT), and dopamine (DA)\textsuperscript{25}. Just as the serotonin transporter gene (5-HTTPLR), the MAOA gene possesses a VNTR polymorphism resulting in a short allele that is associated with lower-activity (L-allele) and a long allele which is associated with high activity (H-allele)\textsuperscript{26}. In some studies, the L-allele has been associated with maladaptive outcomes such as alcoholism\textsuperscript{27}, aggression\textsuperscript{28}, and antisocial behavior\textsuperscript{29}. Because of its putative involvement in mood regulation, it was hypothesized that the MAOA gene also plays a role in happiness. To test this hypothesis, 193 women and 152 men were assessed for the MAOA genotype. Happiness was assessed with the four-item Subjective Happiness Scale\textsuperscript{8,30} and responses were combined and averaged to provide a single continuous score, ranging from 1 to 7. Since MAOA is an X-linked gene, women can be classified as having high (HH), intermediate (LH), or low (LL) MAOA activity, whereas men can only be classified by having high (H) or low (L) activity, since males only have one X chromosome.

Among male participants, no differences in level of happiness were observed between carriers of the L-allele and H-allele variant of the MAOA gene. Interestingly, females carrying two L-alleles reported significantly higher level of happiness ($M = 5,83, SD = 0,75$) compared to females carrying both the L- and H-allele ($M = 5,50, SD = 1,00$) or carrying two H-alleles ($M = 5,30, SD = 0,97$), respectively, indicating that there seems to be a gender difference in the
association between MAOA genotype and perceived happiness.

However, these results should be interpreted with caution. First, there is inconsistency in the literature about the role of L-allele in behavior. As mentioned, different studies associate this variant with stress, aggressiveness, and antisocial behavior\textsuperscript{27-29}, although there are also studies suggesting that L-allele carriers are more susceptible to positive experiences\textsuperscript{31}. Therefore, more research is needed in order to understand the relation between the MAOA genotype and behavior more generally. Second, the main effect is based on a relatively small, probably underpowered sample size. In candidate gene studies, first findings often suggest a strong genetic effect that tends to be a lot weaker or no longer significant in replication studies. This is also known as the \textit{winners-curse} and replication of this study is therefore warranted.

\textbf{Genome complex trait analysis}

In summary, while linkage analysis is systematic but not powerful, candidate gene allelic association studies are the opposite—able to detect genes with small effect sizes but only with a priori knowledge. So far, using these two approaches, no convincing evidence emerged for the identification of specific genes associated with well-being. A valid question that could arise from these studies is whether we are currently able to explain any genetic variance in well-being at all. GCTA, a recently developed software tool, might provide an answer to this question\textsuperscript{32}. Rather than testing the association of any particular SNP, GCTA estimates the variance that is explained by multiple SNPs on a genome-wide basis. In other words, GCTA estimates how much of the variance in a trait can be accounted for by the genetic variance based on common SNPs, resulting in a heritability estimate based on molecular genetic data. Unlike twin studies, GCTA does not require a sample of related individuals and can therefore discard the assumption of environmental and genetic resemblance between relatives. Although the method is relatively new, it has already been successfully applied to several phenotypes, including height\textsuperscript{33}, intelligence\textsuperscript{34,35}, personality traits\textsuperscript{36}, and major depressive disorder\textsuperscript{37}.

\textit{Genome-wide complex trait analyses and well-being}

Recently, GCTA has been applied in order to estimate how much of the variance in well-being could be explained by shared SNPs in a pooled sample of \textasciitilde11,500 unrelated Swedish and Dutch individuals\textsuperscript{38}. Well-being was measured using the positive affect items (“During the past week, I was happy” and “During the past week, I enjoyed life”) from the Center for Epidemiology Studies Depression Scale (CES-D)\textsuperscript{39}. In addition to separate analyses for each item, scores of the two items were combined to generate an overall well-being measurement.
Using GCTA, it was estimated that 5–10% of the variance in well-being was accounted for by shared SNPs of unrelated individuals when measured with single-item well-being questions. It is likely that this estimation is attenuated by measurement error given that well-being, as measured in this study, was based on only two questionnaire items. Hence, a correction for measurement error of the well-being measures was applied, which raised the point estimate to the range of 12–18%. This estimation is in line with a large twin study yielding an additive genetic effect in the 10–20% range. Together, these results imply that SNPs, measured on existing platforms, do explain a significant proportion of the population variance in well-being. Therefore, future genome-wide large-scale efforts to search for SNPs associated with well-being are relevant and have potential for success.

**Genome-wide association**

The main reason why genetic association studies were not performed on a genome-wide scale—until recently—is easy to explain: they were simply too expensive. To illustrate this, genotyping of 500,000 SNPs in 1,000 individuals would require 500 million analyses. In the beginning of the allelic association era, such an effort would have cost tens of millions of dollars. This is why allelic association studies were initially restricted to candidate genomic regions. Nowadays, however, whole-genome SNP panels can be genotyped across many samples for relative low costs, and more than nine thousand studies for a great diversity of disease or traits have been published, which led to the discovery and replication of several novel gene loci for many phenotypes. Such success was primarily restricted to medical and (some) psychiatric traits. However, a recent GWAS on educational attainment \( N = 126,559 \) yielded three genome-wide significant SNPs which replicated Rietveld et al. 42.

GWAS are an extension of the allelic association model used in candidate gene studies. The ultimate aim of the GWAS design is to capture all common genetic variations across the genome and test for associations between this genetic variation and a trait of interest. In other words, GWAS is explicitly designed to detect genetic variants under the common-disease common-variant (CDCV) model for complex traits or diseases. The CDCV hypothesis postulates that a significant proportion of the phenotypic variation in a population is largely due to many common variants of small effects, suggesting that association with a given trait is largely due to common variants. According to a recent review, GWAS have identified >100 loci for schizophrenia (SCZ), while only 20 loci for Alzheimer’s Disease (AD), eight loci for bipolar disorders (BIP), one locus for autism disorder spectrum (ADS), and none for
attention deficit hyperactivity disorder (ADHD), anorexia nervosa (AN), major depressive disorder (MDD), obsessive compulsive disorder (OCD), and Tourette’s syndrome (TS). The overt success for SCZ could be largely explained by the large sample size, which was achieved by the Psychiatric Genomic Consortium (PGC) which combined data from more than fifty studies (>35,000 cases compared with a maximum of ~17,000 cases for any other disorder). Importantly, sample size is only one of several factors to be considered in GWAS. Another significant influence is the distribution of the phenotype in the population, with studies focused on less frequent phenotypes having generally more power to detect SNPs 4,5. Higher ratings of well-being are likely to be more prevalent in the population than most psychiatric disorders. As a consequence, the sample size to detect SNPs associated with well-being should be bigger than any existing GWAS of psychiatric illness.

**GWAS and well-being**

To accomplish such an enormous sample size, a large genome-wide association meta-analysis on SWB is currently under way within the Social Sciences Genetic Association Consortium (SSGAC) (<http://www.ssgac.org>). This study consists of at least 34 cohorts and will include ~150,000 individuals, which will yield a power of approximately 50% to detect SNPs with a minor allele frequency (MAF) of 3%. After the discovery and replication phase, meta-analyses on positive affect and life satisfaction will be conducted in order to explain individual differences in SWB caused by genetic factors.

**The path between genes and behavior**

As already mentioned, quantitative behavioral genetic research consistently reports that heritable factors contribute substantially to the population variance in well-being 3, but these studies do not give us insight into the specific genomic regions that are responsible for these differences. Molecular genetic research, which aims at identifying the specific genes responsible for the heritability estimates of well-being, are showing mixed results. However, although present efforts are somewhat disappointing, we should not forget that molecular research pertaining to SWB is still in its infancy. In summary, results of significant heritability estimates suggest that DNA variation is involved in behavioral variation and we (as behavioral genetics) need to puzzle out which genomic regions are associated with these complex traits in order to understand the molecular mechanisms, which requires not only the identification of genomic regions associated with well-being but also investigation of how these regions affect complex behavior.
**Gene expression**

A possible way to bridge this gap is the study of gene expression as a mechanism underlying genetics associations with complex traits. The genes that are expressed or transcribed from genomic DNA—sometimes referred to as the transcriptome—represent the major determinants of cellular phenotype and function. Transcription of genomic DNA to produce messenger RNA (mRNA) is the first step in the process of protein synthesis, and differences in gene expression are responsible for both morphological and phenotypic differences as well as being indicative of cellular responses to environmental stimuli and perturbation. Unlike the genome, the transcriptome is highly dynamic and changes rapidly and dramatically in response to perturbations, or even during normal cellular events such as DNA replication and cell division\(^{46,47}\). With the development of micro-arrays, expression of all genes in the genome can be assessed simultaneously using RNA transcripts as an outcome measurement.

**Gene expression and well-being**

To date, there is only one gene expression study involving SWB\(^{48}\). In their study, the conserved transcriptional response to adversity (CTRA) gene expression profile was used as a molecular reference space in which to map potentially distinct biological effects of hedonic and eudaimonic well-being. Hedonic well-being represents the sum of an individual’s positive affective experience\(^9\), whereas eudaimonic well-being results from striving toward meaning and a noble purpose beyond simple self-gratification\(^0\). Although eudaimonic well-being is sometimes explained as a “deeper” form of well-being, these two forms are highly correlated\((r = .70)\) and tend to reciprocally influence one another\(^{51,52}\). CTRA is characterized by an increased expression of genes involved in inflammation (e.g., proinflammatory cytokines such as IL1B, IL6, IL8, and TNF), whereby genes involved in type I interferon antiviral responses (e.g., IF1-, OAS-, and MX-family genes) and IgG1 antibody synthesis (e.g., IGJ) are down-regulated. Activation of the CTRA response is associated with several pathological phenotypes, such as inflammatory mediated cardiovascular and neurodegenerative diseases, and impaired host resistance to viral infections\(^{53,54}\). Differential expression of the leukocyte CTRA was assessed in genome-wide transcriptional profiles of peripheral blood mononuclear cells (PBMCs) in 80 participants for whom hedonic and eudaimonic well-being was measured using the eight-item Short Flourishing Scale\(^5\).

It was shown that hedonic and eudaimonic well-being, although strongly correlated with each other, have divergent gene transcriptional correlates in human immune cells. Eudaimonic
well-being was associated with decreased expression of the CTRA transcriptome (e.g., less antiviral responses and antibody synthesis), whereas CTRA gene expression was significantly up-regulated in association with increasing levels of hedonic well-being (e.g., increase in proinflammatory cytokines). Although high levels of hedonic and eudaimonic well-being seem to have a divergent gene-expression profile in human immune cells, these results should be interpreted with caution and are subject to debate. The discussion focuses primarily on the fact that the complex analyses used in the study depend entirely on distinguishing the highly correlated constructs hedonic and eudaimonic well-being with a self-report measurement conducted in a small sample. The question that arises, therefore, is whether proper psychometric conditions could be met to see these two philosophic definitions of well-being as two independent constructs.

**Strengths, weaknesses, and opportunities**

*Strengths and weaknesses*

One of the most exciting directions for genetic research in well-being involves harnessing the power of molecular genetics to identify the specific genes responsible for the consistently reported, influence of genetics on well-being outcomes. The two major strategies for identifying genes associated with well-being are allelic association and linkage studies. Allelic association has a rather simple design and calculates the correlation between an allele and well-being. Linkage is—like association within families—tracing the co-inheritance of a DNA marker and well-being within families. A great strength of the linkage approach is that it systematically scans the genome with only a few hundred markers in order to test for violations of Mendel’s law of independent assortment between well-being and a DNA marker. However, in most complex traits, like well-being, it is likely that many genes with small effect sizes are involved. Therefore, using linkage is like using the Hubble telescope: it can scan planets in our galaxy (large QTL effects), but will go out of focus when trying to detect the Apollo landing sites on the moon (small QTLs). Furthermore, linkage studies are difficult to replicate, which was demonstrated in a review study by Altmüller et al., who found that many studies of the same disease were often showing inconsistency in their results.

In addition, if linkage can be compared to the Hubble telescope, candidate gene studies are more like a microscope with theoretically enough power to detect genes with small effect sizes. However, a major drawback of this approach is that these studies require the ability to predict functional candidate genes a priori—knowledge which is still limited despite our
increasing understanding of biochemical pathways and the etiology of quantitative traits. For instance, in existing candidate studies on well-being (5-HTTLPR and MAOA), the candidate gene of interest were chosen based on biological pathways that—at best—are only indirectly linked to well-being. Furthermore, just as with linkage, candidate genes studies are extremely difficult to replicate. Most likely, the failure of replication is due to the fact that the largest effect sizes of genes involved in complex traits are still much smaller than initially expected. In other words, the existing candidate gene studies on well-being were most likely underpowered to detect any genetic effects in the first place. In contrast, GWAS are hypothesis free and provide a relatively unbiased screening of the human genome, thereby enabling the discovery of previously unsuspected genetic variants. At time of writing, the upcoming GWAS (~150 K) on SWB is still in the pipeline, but it will be exciting to see the first results in the near future, which will hopefully bring us a step closer to the identification of genes associated with SWB.

**Opportunities**

As mentioned, the field of molecular genetics and well-being is still in its infancy, meaning that there are many opportunities left to unravel the genetic architecture of this increasingly popular topic. An interesting approach that recently generated much interest is polygenic score analysis. Polygenic scores are created based on the weighted sum of multiple alleles associated with the outcome of interest in a discovery sample. It is then tested whether the same score predicts the outcome in an independent replication sample. There is increasing evidence that a substantial proportion of the phenotypic variation might be better explained by a combination of multiple genetic variants rather than individual variants that often fail to reach significance in large GWAS studies. For instance, significant associations between polygenic scores and well-being would imply that a genetic signal is indeed present among the included markers. It would therefore be very interesting to construct such a polygenic risk score from the forthcoming GWAS meta-analysis on SWB.

Furthermore, and as mentioned at the very beginning of this chapter, well-being is influenced by both genetic and environmental factors. Despite a rich epidemiologic literature that is focused either on environmental and social influences or genetic factors, few studies to date have examined the dynamic interplay between genetics and environment in the prediction of well-being. It would therefore be very interesting to (1) investigate whether genetic factors (based on promising SNPs of the forthcoming GWAS on SWB) predict specific preferences
for particular (social) environment (gene–environment correlation), and (2) whether genetic factors predict different degrees of environmental sensitivity, including the sensitivity to positive exposures. To conclude, the field of molecular genetics is a scientific field that is constantly in development and changes very rapidly. Technical advances will ensure that we will be increasingly able to explain genetic variance that is associated with well-being.
Outline of this thesis

The overarching aim of this thesis is to increase knowledge on the causes of individual differences in well-being, and it relationship with related traits. This is achieved through a series of studies aimed at identifying genetic variants associated with well-being as well as identifying environmental influences through epigenetic measures.

Chapter 2 looks at the etiology of the association between well-being and depressive symptoms over the lifespan using data from over 43 thousand twins. It was shown that especially in adolescence and in young adults, the phenotypic correlation between well-being and depressive symptoms are explained by genetic effects, while in childhood genetic and environmental factors are equally important.

Chapter 3 reports the results of the first sufficiently powered GWAS of well-being, which identified three genome-wide significant associations. Additionally, we found the first two genetic variants associated with depressive symptoms and eleven genetic variants associated with neuroticism. Genetic correlations between these three traits revealed the existence of a large shared etiology.

Chapter 4 introduces two methods for multivariate genome-wide meta-analysis (GWAMA). We applied these methods to jointly analyze measures of well-being, depressive symptoms and neuroticism, collectively referred to as the well-being spectrum. We identified 319 genetic variants associated with this well-being spectrum. Moreover, extensive biological analyses including gene expression in brain tissue and single cells, showed that genes differentially expressed in the subiculum, the ventral tegmental area, and in GABAergic interneurons are enriched in their effect on the well-being spectrum.

Chapter 5 describes the results of the first epigenome-wide association study (EWAS) approach to identify differentially methylated sites with individual differences in well-being. Subjects in this study, were part of the longitudinal survey studies of the Netherlands Twin Register (NTR). We found that two CpG probes were genome-wide significant after Bonferroni correction. Gene ontology (GO) analysis highlighted enrichment of several central nervous system categories among higher-ranking methylation sites.
Chapter 6 examines epigenome-wide analyses of well-being through direct measurement (EWAS) and Mendelian Randomization (SMR). We found little correlation between the EWAS and SMR analyses suggesting that the associations we observed in our EWAS are mainly driven by processes other than pleiotropy or a direct causal effect of CpG methylation on well-being.

Chapter 7 provides a comprehensive review to investigate how much evidence there is for a conceptual overlap between subjective well-being (SWB) and psychological well-being (PWB). We found that SWB and PWB are related constructs that are likely domains of a general factor well-being. However, while the constructs are related, they are not interchangeable and can be distinguished both conceptually and biologically.

Chapter 8 reports the first two genetic variants associated with eudaimonic well-being as well as six genetic variants associated with hedonic well-being. Moreover, a large shared genetic etiology between both measures was observed indicated by the large genetic correlation and similar patterns of genetic correlations with related traits (e.g. depressive symptoms, personality, and loneliness).

Chapter 9 expands the interrelation among well-being, neuroticism, and depressive symptoms which we refer to as the 3-phenotype well-being spectrum (3-WBS). Based on polygenic scores and genetic correlation analyses of multiple related traits, we found that self-rated health as well as loneliness are important aspects influencing well-being.

Chapter 10 concludes with a reflection on the current state of the field of molecular genetics of well-being and provides a perspective on promising ways for future endeavors in the field.
References


**Table 10.1 Overview of molecular genetic studies involved in subjective well-being**  
NA = not available; VNTR = variable-number tandem repeat; 5-HTT = serotonin transporter; 5-HTTLPR = serotonin-transporter-linked polymorphic region; MAOA = Monoamine oxidase A; α = Cronbach’s alpha; GREML = genomic-relatedness-matrix restricted maximum likelihood; CES-D = Center for Epidemiologic Studies Depression scale; CTRA = conserved transcriptional response to adversity; PBMC = peripheral blood mononuclear cells

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<tr>
<th>Author</th>
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<th>Design</th>
<th>N Markers</th>
<th>Gene of Interest</th>
<th>Measurement Well-being</th>
<th>Main Conclusion Study</th>
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<td>711 families</td>
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<td>α: .86</td>
<td>Chromosome 1: LOD: 2.37 (p = .209)</td>
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<td>Candidate Gene</td>
<td>7 genetic markers</td>
<td>VNTR</td>
<td>Single Item measuring Life Satisfaction</td>
<td>Individuals with the more efficient version of 5-HTTLPR respond</td>
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<td>(including 5-HTTLPR allele markers)</td>
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<td></td>
<td>polymorphism marker for 5-HTT 528 bp (more efficient) 484 bp (less efficient)</td>
<td>“How satisfied are you with your life as a whole?” 5-point scale α: NA</td>
<td>significant higher levels of satisfaction (p = .001)</td>
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<td>Rs2020933 marker “A” allele more efficient VNTR polymorphism marker for 5-HTT 528 bp (more efficient) 484 bp (less efficient)</td>
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<td>Chen et al., 2013</td>
<td>345</td>
<td>Candidate Gene (MAOA gene)</td>
<td>NA</td>
<td>VNTR MAOA gene promoter (3.5–4 repeats: high activity) (3 repeats: low activity)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>N</td>
<td>Study Factor</td>
<td>Sample Size</td>
<td>CES-D Scale positive affect subscale</td>
<td>Fraction of variance in SWB explained by common polymorphisms</td>
<td></td>
</tr>
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</tr>
<tr>
<td>Rietveld et al., 2013</td>
<td>~11,500</td>
<td>GREML</td>
<td>&gt;500,000 SNPS</td>
<td>NA</td>
<td>5–10% and 12–18% after correction for measurement error.</td>
<td></td>
</tr>
<tr>
<td>Fredrickson et al., 2013</td>
<td>84</td>
<td>Gene expression</td>
<td>NA</td>
<td>CTRA related gene set</td>
<td>Short Flourishing Scale</td>
<td>Hedonic well-being was associated with increased expression of proinflammatory genes ($p = .0047$). In contrast, eudaimonic well-being was associated with CTRA down-regulation ($p = .0045$).</td>
</tr>
</tbody>
</table>

**CES-D Scale positive affect subscale**
- Items: 2
- $\alpha: .85$

**Short Flourishing Scale**
- Items 8
- $\alpha: .87$

**CES-D Scale**
- Items: 20
- $\alpha: .85$