Genetic contribution to developing a major depressive disorder: Mendelian vs. Quantitative genetic influence?

Bachelorthesis
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Abstract

Major depressive disorder (MDD) is a disabling condition that negatively affects a person's family, work or school life, sleeping and eating habits, and general health. An increasing amount of the world's population is affected by it and MDD is thought to be the first leading cause of disability adjusted life years (DALY) in 2030. Twin studies have shown that there is a strong genetic component to this disease. MDD is a very complex disease and it is the result of many different genes interacting with one another. Current genetic research is trying to elucidate the mechanism underlying this disease, but there are many problems along the way. This thesis aims to identify these problems and try to think of ways on how to overcome them. This will be done by giving examples on current and previous research for different genetic approaches, Single gene and many genes. The examples are to illustrate what the merits of both approaches are but also where the bottlenecks lie. In conclusion there are a couple of bottlenecks. The first and major bottleneck is the poor diagnostic currently on psychiatric diseases. More objective factors to measure MDD's should be developed in order to box in this disease and to be able to compare humans to animals such as rodents. Another problem is the number of patients needed in order to find common variants using GWAS, large databases should be compiled combining all available patient data on MDD.

Introduction:

Major depressive disorder (MDD), also simply known as depression**,** is a mental disorder characterized by low moods throughout the day that are accompanied by low self-esteem and by loss of interest or pleasure in activities that would otherwise be enjoyed. MDD is a disabling condition that negatively affects a person's family, work or school life, sleeping and eating habits, and general health. Between 2-15 % of people develop a form of depression during their life time (Moussavi et al., 2007). Unipolar depressive disorder is thought to be the first leading cause of disability adjusted life years (DALY) by the year 2030, whereas it was at the third place in 2002. DALY is a way to quantify health loss by incorporating two factors, namely years lost due to premature death and years lived with the disease. (Mathers & Loncar, 2006).This analysis shows the gravity of the problem that is formed by MDD.

MDD is a result of two major factors, as is every phenotype: genetics and the environment. To determine what the relative contribution of each of these factors was Sullivan, Neale, & Kendler (Which year?) performed a meta-analysis including numerous familial studies on MDD. To be included in the analysis these studies needed to fulfill certain criteria to ensure they can be equally compared. A total of five familial studies was included in the analysis. These studies all showed major depression occurs more within families, therefor there has to be a heritable component to this disease.
The amount of heritability of MDD was estimated to be in the range of 31 and 42%, but the authors indicate that this is probably an underestimation because of poor diagnostic on depression.
This underestimation might also be due to the fact that a genetic vulnerability only reveals itself in the light of a stressful event for this individual (Sullivan et al., 2000). The amount of resilience to stress is not the only genetic factor of MDD. One study has shown that the genetic risk factors for major depression are positively correlated with the tendency to put oneself in situations that have a high risk on stressful life events (Kendler, Karkowski, & Prescott, 1999). It is important that we know which genes underlie this mechanism. This way we can predict if someone is more likely to develop a major depression and we could interfere before it is too late. We could, for instance, develop drugs that reduce the expression of these genes or the proteins they encode for.

Depression is a form of behavior, but behavior is not the results of a single gene as is the case with e.g. hair color. Behavior is a very complex phenomenon influenced by so many different factors. For example, sensory processing, emotion and motivation, neuronal development and plasticity are all essential for generating behaviors(Bendesky & Bargmann, 2011).
Two ways of looking at genetics can be distinguished. On one hand we have Mendelian genetics, one gene affects a trait and on the other quantitative genetics, many genes each contribute a small part in determining the quantitative aspect of a trait. Differences in these two types of genetic contributions are best understood in the light of the findings of two of the founding fathers of behavioral genetics: Seymour Benzer and Jerry Hirsh.

Seymour Benzer created random mutants in *drosophila melanogaster* by using mutagenesis. Because the rate of spontaneous mutations is very low, X-rays were used for mutagenizing the flies. X-ray mutagenesis increases the rate at which mutations occur by 20 to a 100 fold. This made it possible to induce random mutations in animals. Inbred lines were made out of them so that all of those flies had the same mutation. These mutants were then screened on deviating phenotypes (Bonini, 2008). By locating a mutation that caused a certain deviation in an animal's health or mental condition a piece of the genetic puzzle on how this phenotype comes to expression is revealed. Figure 1 gives a schematic representation of how mutagenesis is performed in the mouse. Later on the chemical N-Ethyl-N-Nitrosourea (ENU) was used to induce mutations. ENU creates mutations into spermatogonial stem cells in a rate of 10 times more than X-ray mutagenesis. The benefits of ENU are that it is easy to administer and male mice can create mutant progeny in a couple of months(Cordes, 2005).

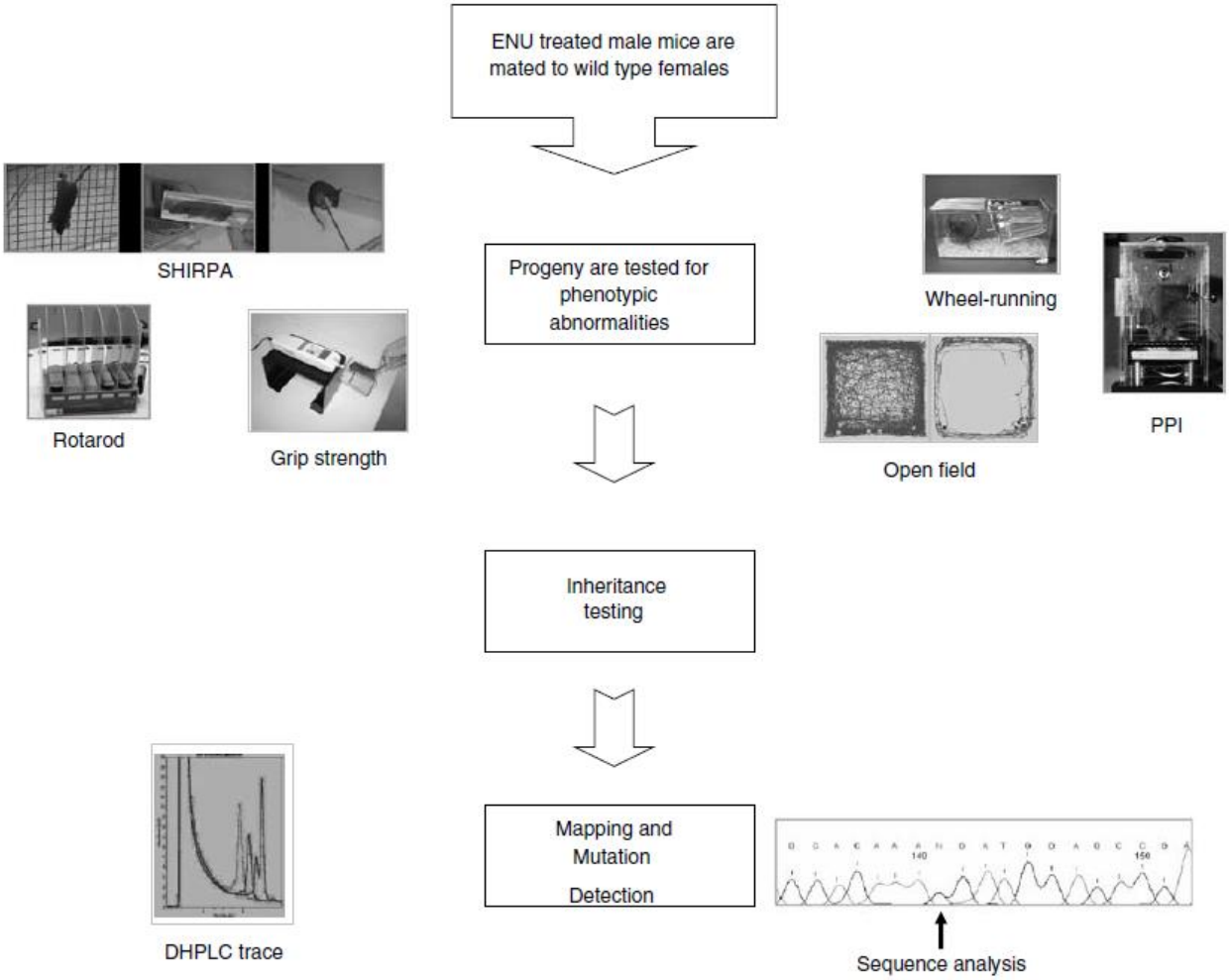


Figure 1 Screening for behavioural mutants. Flowchart illustrating the individual steps in screens for neurobehavioural mutants. Male mice are injected with ENU and mated to wild type females. Progeny are subjected to a battery of behavioural screening protocols and the phenotype of interest is confirmed through inheritance testing. Mutation detection follows through the generation of standard genetic backcross, positional cloning and candidate gene analysis(Godinho & Nolan, 2006).

The approach used by Benzer can be compared with Mendelian genetics, because one is looking at single genes that affect the behavior of an animal. The method used by Benzer is now commonly known as forward genetics. The biggest advantage of this approach is that no hypotheses about the molecular basis are required. Therefor this method is in no way biased and one cannot work towards a preferred outcome. "*By far the most important advantage of the forward genetic approach is the unbiased nature of inquiry, which requires no hypotheses regarding the molecular basis of the phenotype in question. Because of this, forward genetics has led to many new and unexpected discoveries* "(Moresco, Li, & Beutler, 2013) .

We also use reverse genetics, this way one knows what gene is altered and one looks if there is an effect on the phenotype. The benefits of using forward genetics are that genes found this way give insights in the mechanism of the resulting phenotype. Gradations of phenotype that result from mutations in different genes may suggest which genes are essential and which genes play a smaller role.

But this approach also has its limitations. If the effect of a gene on the phenotype is too small one will not be able to detect it and conclude that is has no effect at all. This way mediating genes, enhancers etc. will be left out. Another limitation to this approach or rather an opposition to it, is that these

induced mutations are not natural and very rarely, to never, observed in nature. Meaning that mutants induced this way would never survive in nature and that they would not tell us anything, because they simply do not exist(Greenspan, 2009). However, these mutants can tell us something about the mechanism in which these diseases work.

Another logistic problem is that one needs to mutate hundreds of animals for this mutagenesis to find a single mutant exhibiting deviating behaviors that might be interesting. The use of a model organism like *Drosophila Melanogaster* makes this easier. In mice, the amount of housing one would need is immense. As well as that the reproduction time of mice is much longer than that of *Drosophila*. But it is not known if *Drosophila* can be depressed and the link between humans and *Drosophila* is far less distinct then between mice and humans. Therefor rodents are still the best option for this kind of research. Mutagenesis in mice is mostly directed mutagenesis. By mutagenizing an area of interest. This does affect the "unbiased nature of inquiry" as stated by Moresco, Li, & Beutler. However, there are some institutions that perform these large ENU-mutagenesis screens. These are summarized in table 1.
Sadly, these institutions have not yet been able to find genes that are involved with MDD's.

Table 1 worldwide centers with ENU programs for behavioural mutant screens(Sullivan et al., 2000).

Centre	*Examples of screening tests for behaviour phenotypes*	*Examples of present behaviour-related ENU- mutations*	Website
Mammalian Genetics Unit, Harwell, UK	Shirpa; learning and memory; wheel running activity; open field behaviour	Robotic, *Rob:* robotic motion; Spin cycle, *Scy*: heading bobbing, fits; Play68: long circadian period	http://www.mut.har.mrc.ac.uk/
CMHD, Toronto	Morris water maze; contextual & cued fear conditioning; pre-pulse inhibition	163-11-1: moves slowly, wobbles, quiet; S338-12-23: very small, shivering, memory outlier	http://www.cmhd.ca/
The Jakson Laboratory, Bar Harbor, ME	Clams (comprehensive lab animal monitoring system); acoustic startle response	NMF31: high threshold to seizures	http://nmf.jax.org/
TMGC, Tennessee	Open field activity; startle and pre-pulse inhibition; hotplate test; tails suspension test	22TNP: stress/behavioural despair; 22TNJ-2: hyper-activity in open field test;	http://www.tnmouse.org/ neuromutagenesis/
Northwestern University, Chicago	Body weight; hearing; elevated plus maze and open field behaviour; neuroendocrine response to stress; circadian ryhthmicity; vision	Overtime: lengthened free-running period; Timecourse, timeshare, half time: shortened circadian period	http://genome.northwestern.edu/neuro/
Norvatis Research, San Diego	Locomotor activity; learning and memory; circadian rhythms; anxiety	Not available	http://web.gnf.org/index.shtml
ORNL, Oak Ridge	Primary behaviour: observation; reaching; righting reflex; startle response; prepulse inhibition; habituation; locomotor activity; learning and memory	Baln2: shaking and head tilting behaviour; 6TNK: small, runted, juvenile lethality, cerebral histological abnormalities	http://lsd.ornl.gov/mgd/
Riken institute, Japan	Shirpa; home-cage activity; open field test; passive avoidance	M100934: total activity high; M100023: head tossing	http://www.gsc.riken.go.jp/Mouse/ main.htm
Taiwan	Locomotor activity; pentobarbital responsiveness	Not available	http://mmp.sinica.edu.tw/
University of Pennsylvania	Circadian ryhthmicity	Rab3a (*earlybird*)	http://www.neurogenome.org/lab/

Jerry Hirsch was focusing on quantitative genetics. Quantitative genetics quantifies the relative amount of contribution a given gene delivers to a certain phenotype(Miles & Wayne, 2008).
Jerry Hirsch showed that there was strong individual variability in behavior within a population. He selected lines of flies that had a high expression of certain behavior and low expression, these lines maintained this difference over the years. A problem Hirsch faced was that there were no genetic techniques yet developed to test which genes were responsible for this difference in behavior. He was only able to map the genes to a chromosome. When technique finally caught up with his ideas, he was able to map the genes responsible for the behavior he observed. (Roubertoux, 2008).
The research done by Jerry Hirsch formed the basis for modern quantitative trait loci (QTL) mapping in behavioural genetics. QTL mapping was first used to see if the genetic mechanism underlying a certain phenotype was caused by a few genes with large effects or by many genes with smaller effects. Figure 2 shows gives an explanation on how QTL mapping is performed.

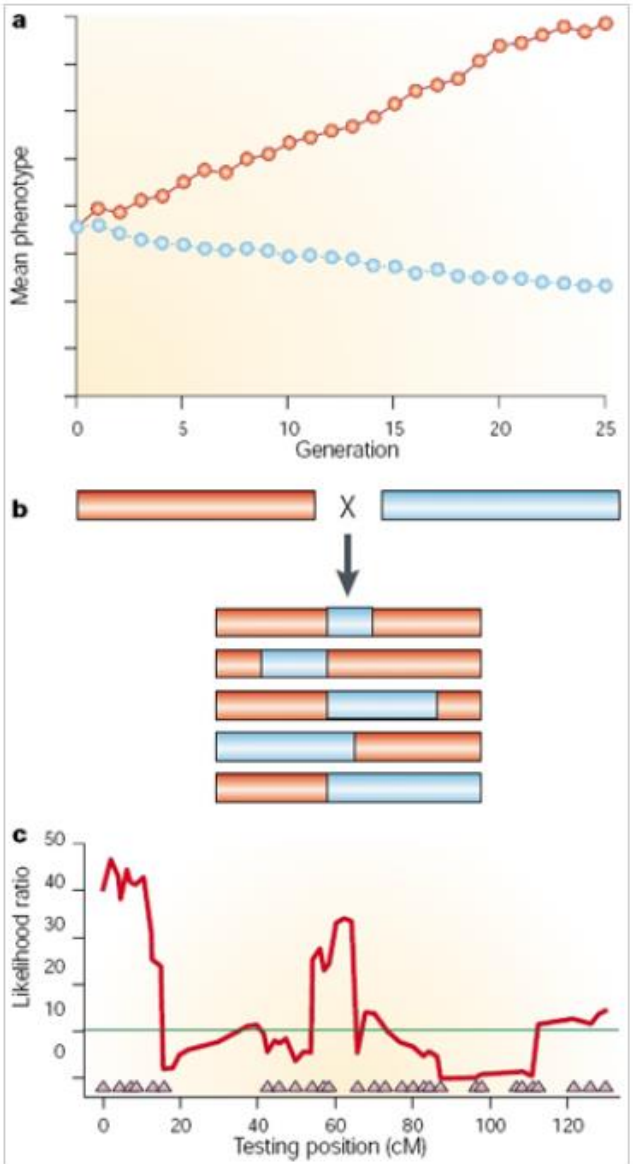


Figure 2. a) Quantitative trait locus (QTL) mapping requires parental strains (red and blue plots) that differ genetically for the trait, such as lines created by divergent artificial selection. b) The parental lines are crossed to create F1 individuals (not shown), which are then crossed among themselves to create an F2, or crossed to one of the parent lines to create backcross progeny. Both of these crosses produce individuals or strains that contain different fractions of the genome of each parental line. The phenotype for each of these recombinant individuals or lines is assessed, as is the genotype of markers that vary between the parental strains. c) Statistical techniques such as composite interval mapping evaluate the probability that a marker or an interval between two markers is associated with a QTL affecting the trait, while simultaneously controlling for the effects of other markers on the trait. The results of such an analysis are presented as a plot of the test statistic against the chromosomal map position, in recombination units (cM). Positions of the markers are shown as triangles. The horizontal line marks the significance threshold. Likelihood ratios above this line are formally significant, with the best estimate of QTL positions given by the chromosomal position corresponding to the highest significant likelihood ratio. Thus, the figure shows five possible QTL, with the best-supported QTL around 10 and 60 cM (Miles & Wayne, 2008).

Another way to find quantitative trait genes, is by using a genome wide association study (GWAS), rendered possible by the improvement in sequencing technologies allowing sequencing full genomes quickly and at low cost. A GWAS compares the allele frequencies of common single nucleotide variants with a certain quantitative trait. For example, height, this study can show one which common nucleotides are more associated with increased height. Another way to use GWAS is to compare a group of persons/animals, with a certain disease that one is interested in, with a control group. This way one can find common variants that are associated with the disease (Flint, 2013).

In this thesis examples will be given for both single gene (Mendelian) as well as multiple gene approach (quantitative genes). And what these studies have contributed to revealing the mechanisms of MDD's. The comparison of these two approaches might show what part of the genetic basis, shown by twin studies, is explained by Mendelian genetics and what part by quantitative genetics. The aim of this study is to show what methods should be used in further research to elucidate the genetic puzzle underlying MDD's.

Results:

Single gene mutations induced by mutagenesis (mendelian genetics):

One of the first mutagenesis screens in mice originates from 1994 and unintentionally uncovered a genetic link with depression. Chronobiologists sought out to discover genes involved in the mammalian circadian clock. This research originated from the breakthroughs that happened by the discovery of the *period* and *frequency* gene in respectively D*rosophila* and *Neurospora* (Konopka & Benzer, 1971) (Feldman & Hoyle, 1973). The general approach of any mutagenesis experiment is explained in figure 1. And that is exactly what they did in this research. They tested a total amount of 304 mice whose fathers had an ENU treatment.
For all of these rats they measured the total circadian period and found that for one of the rats this period was significantly longer then for all the others. Further crossings showed that this mutation was semi dominant. A linkage analysis using cross schemes was then used to map the *clock* gene. Mice that were homozygous for this mutation had a complete loss of circadian rhythm over a relatively short amount of time. (Vitaterna et al., 1994). The circadian clock is a very important regulator for our everyday lives. And the effects of the circadian system are notable throughout an animal's body in hormone release, organ function and so on. Variations in an animal's clock genes may contribute to the symptoms of seasonal affective disorder (SAD), such as winter depression, and a subgroup of major depressive diseases (Bunney & Bunney, 2000). In a study by (Benedetti et al., 2003) they showed that polymorphisms in the *Clock* gene in humans directly affects depression. At first they failed to confirm the hypothesis that the *Clock* gene influenced the mood fluctuations in bipolar disorder.They did however find that people with a single polymorphism, T to C on position 3111, have a higher recurrence rate of bipolar depressive disorder. This proves that the mammalian *Clock* gene plays a direct role in MDD's.

In 2000 another research group sought out to find new behavioural genes, including depression, by performing a large mutagenesis screen as performed in the previous study by Vitaterna et al., 1994. In this study however they did not look for a specific deviation of behavior as they did for the circadian rhythm. All the mice that were obtained by the mutagenesis were exposed to a series of behavioral tests designed to measure different kinds of behaviour, such as anxiety and various stress tests. Table 2 shows an overview of different tests that can be used to determine wheter an animal has depression or a form of depression.

Table 2. examples of animal models used in depression research. (Nestler et al., 2002)

Model	Main Features
Forced swim test	Antidepressants acutely increase the time an animal struggles in a chamber of water; lack of struggling thought to represent a state of despair.
Tail suspension test	Antidepressants acutely increase the time an animal struggles when suspended by its tail; lack of struggling thought of represent a state of despair.
Learned helplessness	Animals exposed to inescapable footshock take a longer time to escape, or fail to escape entirely, when subsequently exposed to escapable foot shock; antidepressants acutely decrease escape latency and failures.
Chronic mild stress	Animals exposed repeatedly to several unpredictable stresses (cold, disruption of light-dark cycle, footshock, restraint, etc.) show reduced sucrose preference and sexual behavior; however, these endpoints have been difficult to replicate, particularly in mice.
Social stress	Animals exposed to various types of social stress (proximity to dominant males, odors of natural predators) show behavioral abnormalities; however, such abnormalities have been difficult to replicate, particularly in mice.
Early life stress	Animals separated from their mothers at a young age show some persisting behavioral and HPA axis abnormalities as adults, some of which can be reversed by antidepressant treatments.
Olfacotry bulbectomy	Chemical or surgical lesions of the olfactory bulb cause behavioral abnormalities, some of which can be reversed by antidepressant treatments.
Fear conditioning	Animals show fear-like responses when exposed to previously neutral cues (e.g., tone) or context (cage) that has been associated with an aversive stimulus (e.g., shock).
Anxiety-based tests[a]	The degree to which aniamls explores a particular environment (open space, brightly lit area, elevated area) is increased by anxiolytic drugs (e.g., benzodiazepines).
Reward-based tests[b]	Animals show highly reproducible responses to drugs of abuse (or to natural rewards such as food or sex) in classical conditioning and operant conditioning assays.
Cognition-based tests[c]	The ability of animals to attend, learn, and recall is measured in a variety of circumstances.

Most of these tests are available in rats and mice; the tail suspension test is used in mice only.
[a]Examples include open field, dark-light, and elevated plus maze test.
[b]Examples include conditioned place preference, drug self-administration, conditioned reinforcement, and intra-cranial self-stimulation assays.
[c]Examples include test of spatial memory (Morris water maze, radial arm maze), working memory (T-maze), and attention (5 choices serial test).

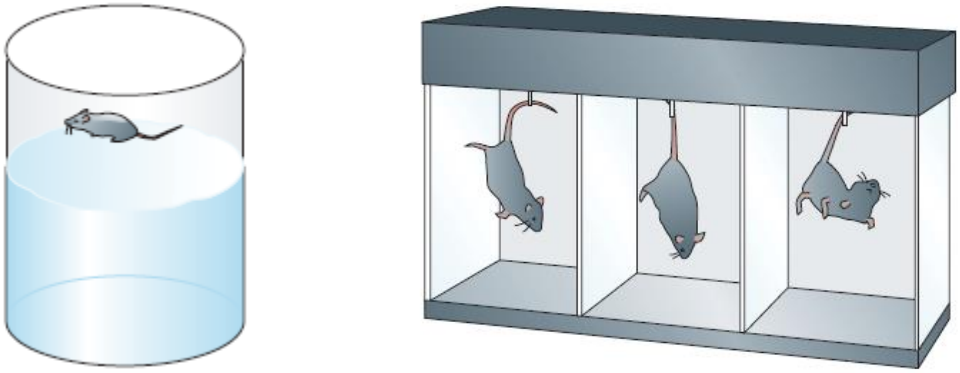
At the start of the experiment the young mice were observed for any abnormalities in morphology or simple behavior like hyper/hypoactivity. When the mice were 8-10 weeks old, formal tests were performed to see if there are any abnormalities in behavior to be found.
The first test was the prepulse inhibition (PPI) test. Here the mice are given a low intensity audio impulse before a high intensity audio impulse. The low intensity audio impulse lowers the startle reaction for the high intensity audio impulse. They measure the difference in startle response between the situation with a prepulse and without a prepulse. Patients that have schizophrenia show reduced prepulse inhibition, so abnormalities in this test might lead to pathways involved in psychosis.

Secondly they performed an open field test. This test is used to measure the degree of exploration of an animal. Exploration is a measure for fear and anxiety and these traits are potentially relevant to a broad range of behavioral and psychiatric conditions.

Thirdly a fear conditioning test. The test measures an animal's ability to remember the context in which it previously received a shock. Learning and memory deficits can also lead to psychiatric diseases such as schizophrenia.
Fourthly the tail flick test, this gives measure of pain perception and sensitivity. This test was used to complement the fear conditioning test because some animals respond stronger to the shock than others because of differences in pain perception.
Finally, spontaneous locomotor activity was used as a measure of motor function in a non- stressful environment. Abnormalities in motor function can impair the animal the move properly in the open field and fear conditioning tests. Also motor function is correlated to schizophrenia and also in animals that by whom psychosis was induced via drugs.
Eventually they found a mutation that had an influence on the PPI (Sayah, Khan, Gasperoni, & Smith, 2000). PPI was found to be deficient in patients that suffer from schizophrenia. In patients with mental disorders such as Gilles de la Tourette and obsessive compulsive disorder PPI was also found to be deficient, however there has been no scientific evidence to report a change in PPI as a result of depression. (Kohl, Heekeren, Klosterkötter, & Kuhn, 2013). However, in a study by Ophoff et al., 2013 a significant shared genetic base was shown for schizophrenia and depression.
So to conclude, Sayah et al., 2000 did find a mutation that affects the PPI. PPI has not been shown to be different in patients with a depression, however Ophoff et al., 2013 showed that there is a shared heritability between schizophrenia and MDD's therefor we cannot rule out PPI as having no effect on depression.
A different kind of approach that is more common in research is the one used by Smits et al., (2006). They combined the unbiased nature of mutagenesis with target directed mutations. Again ENU was used to create mutations, this time however in rats instead of mice, but they did not screen the whole genome of these rats to saturation. They only looked for rats that had mutations in genes of interest. These genes were already known to be important in certain behaviours, including depression and therefor investigated. They reported a total amount of 120 mutations in the regions of interest. One of the mutations found was a knock-out of the serotonin transporter (SERT/SLC6A4).(Smits et al., 2006) People that have one or two polymorphisms in the serotonin transporter have been reported to express more depressive symptoms as a result of stressful life events (Caspi et al., 2003).
So the mutation found by Smits et al. 2006 in the serotonin transporter could be a mechanism through which an animal can develop a depression. But in order to be sure further tests with mice/rats that have this knockout should be done to elucidate the entire effect.

Many genes by quantitative trait loci mapping (quantitative genetics):

In order to develop depression a person or animal has to experience one or more stressful life events. And the disease is often viewed as the inability to cope with these stressful life events. Therefor tests that have been developed do not measure depression but they measure the ability to cope with stressful events, see table 1. Two tests for measuring this are widely accepted. Firstly, the forced swim test (FST). In this test the mouse is introduced in a cylinder of water from which it cannot escape. Fig 1a. At first the mouse will try to escape but after a while it will give up and show more and more periods of immobility. A depressive mouse will give up on escaping sooner than a non-depressive mouse. This same principle goes for the tail suspension test (TST). Mouse are hung up from the tail in a manner they cannot free themselves from. Fig 1b. And again the amount of time before the mouse gives up on trying to escape is taken as a measure of depressiveness. Both of these tests show significant increase in results when the tested animals have received antidepressants and have therefor been accepted as a good measure for depression (Cryan & Holmes, 2005).



a b

Figure3: a: the forced swimming test, a mouse is introduced in a cylinder containing water from which it cannot escape. B: the tail suspension test. The mouse is hung up from the tail and is not able to free himself. In both test the amount of time spend

In a study performed by (Solberg et al., 2004) rats from the WKY and Fischer344 strain were bred reciprocally. And they used the approach as described in figure 2 to look for QTL's. They looked at the tendency of animals to be immobile, climbing or swimming. By use of genetic markers, the genomes of each of the animals was analysed and this way the researchers were able to find certain QTL's. They found three, seven and two QTL's for, respectively, climbing, immobility and swimming. By calculating the relative contribution of these QTL's they concluded that these had small effects with regard to this complex behaviour. More interestingly is that some QTL's that they found shared overlap with other QTL's previously reported to be involved with emotionality as well as with QTL's that are associated by linkage analyses with MDD's in humans. For further research they would need to increase the resolution and then be able to map some of the QTL's to a gene region and find out which gene corresponds with the locus. This could lead to new understandings in the mechanism of MDD's.

In research done by (Tomida et al., 2009), the mouse strain of CS was used. This strain shows several distinct phenotypes regarding the circadian behavioural rhythm. Previous studies showed that the circadian system is involved in various mental illnesses such as depression. This was the reason that they put these mice through a large battery of behavioural assays. Including the TST and FST. Other tests included the open-field, light-dark exploration, elevated plus-maze, prepulse inhibition, Y-maze. All of these tests are mentioned in table 1.
Surprisingly mice from the CS strain showed little to no immobility. This observation led scientists to perform a QTL analysis on the behaviour of immobility in the FST and TST. For this QTL analysis they crossed the CS strain with the B6 strain. Crossing schemes as previously described in figure 2 were set

up to find QTL's. After interval mapping several QTL's were discovered on chromosome 4 (FST) and on chromosome 5 (FST, TST).
Using more crossing schemes they were able to reduce the QTL interval on chromosome 5 and identified three genes lying within this interval. By measuring the expression levels of these three genes they were able to conclude that only the expression of gene *Usp46* differed between strains.
Results obtained suggested that *Usp*46 is involved in the GABAergic pathway, this is further supported by the fact that mice which lacked GABA-transporter subtype1 (GAT1) had a lower immobility time.
This involvement of the GABAergic system in depression was also found by (Miller, Schultz, Long, & Pletcher, 2010). They crossed an inbred strain for low immobility with two strains of high immobility in the TST. After whole genome interval mapping as done in the previously described research they found significant QTL's on chromosomes 4 and 6. Same as in the research of (Tomida et al., 2009) expression levels were measured to determine which genes might be involved. They found that expression of the *GABRA3* gene, which encodes for the GABAa receptor α3 subunit, was absent in the hippocampus of one of the two high immobility strains. Treatment with a positive modulator of the α3 subunit resulted in significantly lower TST immobility.
Research done by Sanacora et al., 2004 showed that the concentration of GABA in the occipital cortex of humans was reduced for patients with MDD. These findings show that the GABA system is an important target for the treatment and for further research of MDD's.

genome wide association studies:

Because of developments made in the field of sequencing, there now are more complex and faster ways of looking at quantitative genetics(GWAS) and Mendelian genetics. Both made possible by the improvement in sequencing technologies allowing sequencing full genomes quickly and at a low cost.
A GWAS is used to find common variants among a population for a certain trait. Or it can be used to find common variants by comparing a control group with a patients group that has the disease one is interested in.

The problem with a GWAS on a common disease is that one needs a lot of samples to get conclusive results. Because the disease is the results of many different genes interacting one would need to see a lot of samples with the same deviation to reach statistical significance. This is a problem that Shi et al., 2010 also ran in to. The samples for their study were taken out two other studies. Namely "genetics of recurrent early onset depression" (GenRed) I and II. This way they had a total of 1110 cases and 1636 controls. This number of subjects was not enough to give any statistical significant results. The top result was a SNP located in the SP4 transcription factor gene. The authors themselves also state that other GWAS' that did yield significant result had between 10000 and 20000 subjects(Shi et al., 2010). But research did give the incentive to look further in the SP4 gene. A recent study by (Chen et al., 2015) looked at different SNP's in the SP4 gene and their role in schizophrenia and MDD's. What they found was that one SNP of the SP4 gene was significantly associated with schizophrenia, but not with MDD's. However, the SP4 gene should not be ruled out for MDD's while MDD's and schizophrenia share a significant heritability. (Ophoff et al., 2013)

In comparison to the study by Shi et al., 2010 a very recent study claims to be the largest GWAS study to date on bipolar disorder(BP), a form of MDD. Previous GWAS' have identified some of the heritability of BP to identify more loci this study incorporated an amount of 9784 patients and 30471 controls. Four of the six found loci were reported before in other GWAS', but they did find two novel loci. The first loci, rs2517959, was located near the ERBB2 gene which encodes for a receptor tyrosine kinase. The second, rs12553324, located near the ELAVL2 gene which encodes a neuron-specific RNA binding protein. Next to the fact that they found two novel loci they also found confirmation for a lot of other loci that were previously reported by other GWAS'. But still the authors indicate that this number of subjects still is not enough to identify risk variants with a small effect size.

In 2013 large group of researchers sought out to find linkage between different MDD's as mentioned before in the QTL section. To do this they took data from almost every GWAS done on MDD's by the Psychiatric research consortium. In this study they took h^2_{SNP} (SNP heritability) value as an estimate of the total variance in liability to disease explained by SNPs together. 'Genetic variation is estimated when case-case pairs and control-control pairs are, on average, more similar across the genome than case-control pairs'(Ophoff et al., 2013).
Results are shown in figure 4.

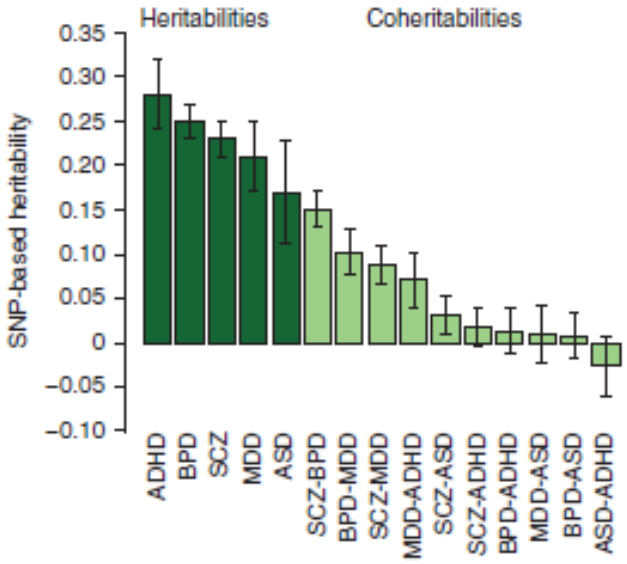


Figure 4: Evidence for genome-wide pleiotropy between psychiatric disorders. Proportion of variance in liability (SNP-based heritability) and proportion of covariance in liability between disorder (SNP-based coheritability) for five major psychiatric disorders. The 95% error bars represent the estimates ± 1.96 s.e. SCZ, schizophrenia; MDD, major depressive disorder; BPD, bipolar disorder(Ophoff et al., 2013).

They showed that there is a correlation between certain MDD's, with the strongest genetic correlation between bipolar disorder and Schizophrenia and secondly between bipolar disorder and major depression disorder. The authors do however state that by taking data from a lot of different studies the ways of how these data are acquired and sample sizes also vary between these studies. The h^2_{SNP} is expected to be an unbiased factor, but because of the difference in sample sizes the errors on some diseases might be larger due to smaller sample size. Nevertheless, this study shows that these psychiatric disorders cannot be seen as separate diseases, because there is a definite genetic link between some of them(Ophoff et al., 2013). This shared genetic background of these diseases show why present diagnostics should be revised, because they make a clear distinction between these diseases while this cannot be seen as black and white.

As stated before forward genetics is an unbiased approach for identifying genes involved in depression or other biological phenotypes. Recent technological advances allow for the fast genotyping of mutagenized mice/rats. The process of mapping these genes was the biggest challenge for many years. This made this method of testing very tedious and costly work. But due to the development of massively parallel DNA sequencing, with this method thousands-millions of DNA fragments can be sampled at the same time (Moresco et al., 2013). Figure 5 shows how technological advances have improves the time and cost it takes to map a certain mutation.

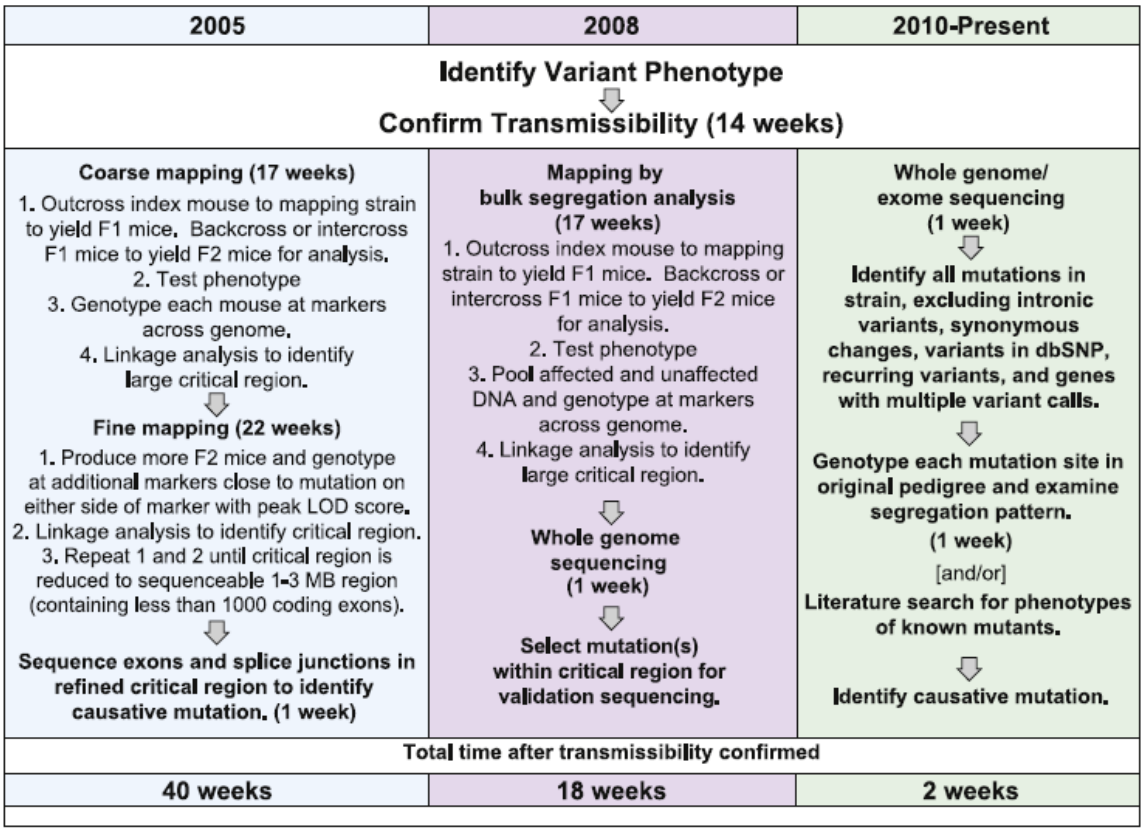


Figure 5 Workflow typical in 2005, 2008, and currently for identifying a mutation responsible for a variant phenotype. Estimated time requirements are indicated for each major step. Left panel: Around 2005, identifying a mutation causative for a particular phenotype by geneticmapping and capillary sequencing of critical region coding sequences commonly required approximately 1 year. Center panel:More efficient mapping by bulk segregation analysis (BSA) and massively parallel genome sequencing were implemented for mutation finding beginning around 2008 and reduced by approximately 55% the time needed to find a causative mutation (counting the time fromconfirmation of transmissibility). Right panel: Identification of causative mutations without genetic mapping has recently been demonstrated, made possible by the high accuracy of massively parallel sequencers and the use of multiple data filters to exclude false-positive mutation calls. This process results in the identification of only a few mutations per strain, which can be tested for linkage with the affected phenotype. Rapid mutation finding by BSA and/ormassively parallel sequencing permits the simultaneous investigation of manymore phenotypes than was previously possible. In every case, confirmation of causality depends on knowledge of the effect of a second mutant allele or on transgenic rescue of the mutant phenotype with the wild-type allele. (Moresco et al., 2013)

Discussion:

First of all, all of these studies show that doing research on depression brings along many difficulties. Research on depression is made especially hard by the fact that there is no good definition of depression. Diagnosis of depression is being done using questionnaires developed by psychiatrists rather than biologists. The difference between these two would be that a biologist looks more at objective markers that can be measured and quantified, that way one can imply a categorical system for different severities of the disease. Current questionnaires are based on clusters of symptoms and characteristics observed in patients that do not necessarily describe a single disorder, but rather reflect common final pathways of different patho-physiological processes. Also there is a large degree of depression, from mild to severe. Yet, the diagnostics do not imply a categorical system of severity.
These factors in the diagnosis of the disease are the first problem in depression studies (Hasler, Drevets, Manji, & Charney, 2004). How can a study represent good data if the diagnosis of the disease was wrong in the first place?
In rat and mouse studies diagnosis of depression is even harder. The tests that are used to screen for Depression in rodents are validated by the fact that the animals score better when they are given antidepressants. This way of validating a test is the wrong way around one might say. Normally patients are given a drug and if the drug works it is a drug for said disease. But now we say this drug improves performance so this patient has said disease. Yet it has never been ruled out that this drug can also have an effect in other diseases or conditions. But they have been widely accepted as the tests to use (Nestler et al., 2002). Table 2 gives an overview of these tests.
For future research there should be more attention for these tests and for developing a good classification system of depressive symptoms. Also these symptoms should be able to be measured objectively and not with questionnaires. If symptoms can be measured objectively it would also be much easier to link human and rodent research on depression because they use the same factors defining depression.
In the results a lot of different techniques on how to study genetics have been explained as well as some of the findings that resulted of them. But which techniques should be used in the future?
Since depression is such a complex disease and because there are many degrees of depression it suggests that it is a quantitative phenotype. The GWA studies however require so many samples that they would not be a very feasible technique in the future. Simply because one cannot house so many animals or find that many patients in the same age category and region, to exclude other effects, that statistical significance is reached. Take for instance the research by Hou et al., 2016, they claim to have the biggest sample size for BP, more or less 40000 subjects, so far and still they say that this sample size was not enough to find small effect risk variants. They do not however speculate on what the sample size should be in order to find these small effect genes. QTL mapping is an older technique but is still a good way do look at the relative contribution of genes as shown in the QTL section. Mutagenesis on itself would be a good way, but was not really an option a few years ago due to the amount of work is takes to map the different genes. But because of the development of massive parallel sequencing the bottleneck has shifted. Now the time it takes to sequence is no longer the problem, but rather the creation of new genotypes to sequence. This makes the forward genetics approach a good way to continue research on depression in the future. Because the effects of different mutations result in a different amount of expression of the phenotype depression something can also be inferred about the relative contribution of a gene.

Another technique that is entirely state of the art might prove its use in this field of research.
Prokaryotes have a system that can be used to facilitate site-specific DNA cleavage, the CRISPR (clustered regularly interspaced short palindromic repeats)/CAS system. Researchers have modified this system so that it can be used in animals and humans as well. This makes it possible to change the DNA sequence wherever one wants in whatever one wants. This might result in major breakthroughs in genetic research (Le Cong et al., 2013).

So to conclude new insights are needed in the way psychiatric diseases are diagnosed and the way testing for these diseases is done in animal models. If the diseases are not clearly biologically defined many genes that affect entirely different phenotypes might be associated with depression.
And for the future of genetic research on depression forward genetics using the massive parallel sequencing technique would be the way to go. Hopefully this way we can identify the genes that underlie depression. And when we know which genes are responsible, for different severities of depression, it will be possible to screen for these genes and identify risk groups before they develop a depression. The CRISPR technique proves big potential to be able to “repair” these genes that lead to depression. If the gene causing the depression is localized in a single spot it is thinkable that a patient can be treated with the CRISPR technique fixing the gene in this region. If the gene causing the depression is not localized to a single spot, then new drugs need to be developed that target the mechanism of action and not the final symptoms of the disease. This can also be made possible if we have a better understanding of the mechanism underlying depression.

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