Association Strength Gene Ontology analysis

An alternative Gene Ontology analysis to help unravel the etiology of schizophrenia with Genome Wide Association study results.

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By N.R.Reinders

Supervised by Dr. Steven Bakker
Second examiner Dr. Jeroen Pasterkamp

Abstract

Schizophrenia is a psychiatric disorder marked by delusions, hallucinations, flat affect and disorganized thinking. After decades of research on schizophrenia its etiology remains elusive. Psychiatry, neuroimaging, molecular biology and other fields have provided several theories about schizophrenia that all seem to explain a part of the whole etiology. Because schizophrenia has a large heritable component, genetic studies such as genome wide association studies (GWAS) are frequently adopted to pinpoint genetic variance that increase the chance to develop schizophrenia. Identification of genetic variance that increases the chance to develop schizophrenia helps to understand the etiology of schizophrenia and can provide valuable targets for treatment. Despite the promising aspects of this approach, it holds many caveats and challenges. For example, GWAS on schizophrenia are hindered by large quantities of small effect common genetic variance that emerges from the heterogeneity, polygeneity and fuzzy diagnostical boundaries of schizophrenia. This creates 'noise' that makes it hard to find significant and replicable associations between genes and schizophrenia. Nonetheless, small effect common genetic variance from GWAS data can be used to identify biological mechanisms involved in schizophrenia, using appropriate statistical methods. One of those methods is called 'Gene Ontology analysis'. This analysis applies prior biological knowledge to GWAS results to identify pathways, biological processes, molecular processes and cellular components that are associated with the trait of interest.

This study has two aims: 1. To develop a Gene Ontology analysis that is especially suitable for GWAS by incorporating the association strength (AS) of each gene in the analysis. 2. To use this 'AS Gene Ontology analysis' on GWAS data and assess whether one or more of the current theories about the etiology of schizophrenia is particularly supported. First an introduction about schizophrenia is given and some popular theories about its etiology are described. Then the caveats and challenges of GWAS studies on schizophrenia are described followed by information on Gene Ontology analysis and arguments for its improvement in this study. This is followed by the methodology, results and conclusions of this study.

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1. Introduction

1.1 Schizophrenia in a nutshell

Schizophrenia (s*chizo*: 'split', *phrenia*: 'mind') was first described by Kurt Schneider as a mental disorder apart from 'dementia praecox' (*precocious madness*); a term used since 1891 for many psychiatric disorders. Before that time it was interpreted as being possessed by the devil or demons, having a connection to god or being paranormally gifted. Frequent occurring positive symptoms amongst schizophrenia patients are hallucinations, conspirative ideation, delusions and detachment from reality. Occurring negative symptoms are social withdrawal, flat affect, lack of motivation or abnormal social interaction. In addition there are cognitive symptoms like the inability to organize one's life or work sequentially and effectively. Schizophrenia is a heterogeneous disorder where prevalent symptoms vary considerably between patients. The onset of the symptoms occurs between late adolescence and early adulthood. Treatments for schizophrenia are limited. One of the first treatments of schizophrenia (and other psychiatric disorders) was lobotomy, implemented in 1935. Lobotomy is a procedure where the orbital frontal cortex (OFC) innervations with the thalamus and midbrain are severed. Despite severe side effects it was rewarded with the Nobel prize in 1949. Around the 1960's, symptom reducing drugs where developed that have their effect by blocking D2 receptors and thereby reducing dopamine transmission (Kapur & Mamo, 2003; Schotte et al., 1996). The first generation of these drugs had significant side effects, leaving the treated with, for example, motoric side effects. Although the next generation of antipsychotics that were developed around the 1990's have different side effects, their effectiveness is still limited.

1.2 The elusive etiology of schizophrenia

Schizophrenia remains a disorder that is difficult and costly to treat and has a large impact on the life of affected individuals and their social environment (Heider et al., 2009; Knapp, 2000; McEvoy, 2007). One of the reasons is that its etiology is not well understood. It is unclear how it develops and what underlies this development. It is known that environmental factors such as stress and trauma play a role and that proneness to develop schizophrenia is approximately 70-90% heritable (Farmer, McGuffin, & Gottesman, 1984; McGuffin, Farmer, Gottesman, Murray, & Reveley, 1984), indicating both genetic and environmental factors (McGuffin, 2004). Decades of research have led to several theories about the etiology of schizophrenia. Many of those theories are not mutually exclusive.

1.2.1 Myelin hypothesis:

Several lines of evidence suggest a major role of aberrant myelination in schizophrenia. Diffusor tension imaging and postmortem studies found indications of reduced myelin integrity in several brain regions of schizophrenia patients (Davis et al., 2003; Flynn et al., 2003). Furthermore, increased apoptosis and decreased density and number of oligodendrocytes (glial cells responsible for myelin sheath formation) have been repeatedly observed in schizophrenia patients (Hof et al., 2003; Segal, Koschnick, Slegers, & Hof, 2007; Takahashi, Sakurai, Davis, & Buxbaum, 2011). The latter effect is unlikely caused by antipsychotic medication entirely, because chronic antipsychotic exposure does not significantly affect oligodendrocyte number in monkeys (Konopaske et al., 2008). Further evidence of myelin aberrancies being causal for schizophrenia pathology comes from studies on disorders of myelin production, development and maintenance such as leukodystrophies or neoplasms syndrome; these disorders can induce symptoms indistinguishable from psychosis (Denier et al., 2007; Walterfang, Wood, Velakoulis, Copolov, & Pantelis, 2005). It is proposed that myelin aberrancies lead to the development of schizophrenia symptoms through affecting synaptic function and plasticity during brain development (Takahashi et al., 2011).

1.2.2 Dopamine hypothesis

It is well accepted that drugs antagonizing dopamine pathways, like anti-psychotics, relieve the positive symptoms of schizophrenia (Freedberg, Innis, Creese, & Snyder, 1979). Hence it was long though that dopamine hyperactivity was part of the etiology of schizophrenia (Baumeister & Francis, 2002; E. H. Simpson, Kellendonk, & Kandel, 2010). However, additional research has shown that this hypothesis needs refining, since it does not distinguish between different dopamine pathways being differentially affected (figure 1). In addition, some studies suggest that dopaminergic PFC (pre-frontal cortex) hypo-innervation leads to the cognitive and negative symptoms and that dopaminergic PFC hyper-innervation leads to the positive symptoms of schizophrenia (Ragland, Yoon, Minzenberg, & Carter, 2007). This led to the proposal that aberrant dopaminergic PFC innervation was secondary to the disrupted output of the 'lower-order' striatal and midbrain dopaminergic nuclei (E. H. Simpson et al., 2010). In any case, disregulation of the dopamine system does contribute to and even correlates with schizophrenia symptoms (Abi-Dargham et al., 2002; Goldberg, Weinberger, Berman, Pliskin, & Podd, 1987; Weinberger, 1987), confirming the role of the dopamine system in the occurrence of schizophrenia symptoms.

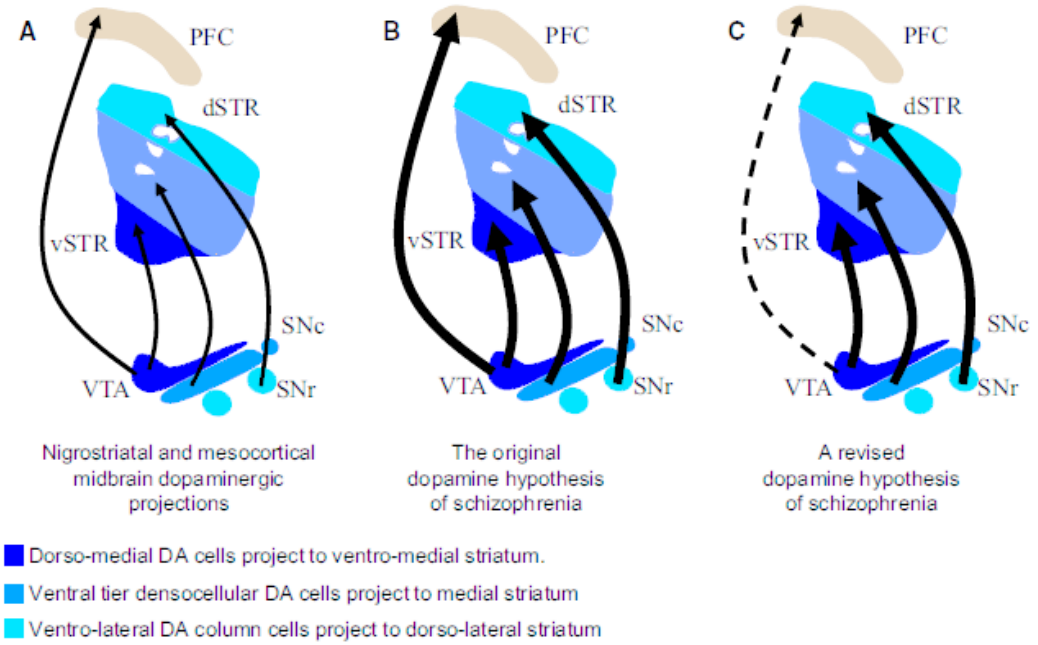

Figure 1. (A) Organization of the nigrostriatal and mesocortical midbrain dopaminergic projections. The dopaminergic midbrain neurons topographically project to the striatum but with an inverse dorsal-to-ventral organization. The mesocortical projections arise from the dorsal and medial dopamine cells. (B) The original dopamine hypothesis of schizophrenia. The original dopamine hypothesis proposed that a global hyperactivity of the dopaminergic projections in the brain may lead to the symptoms of schizophrenia. (C) The revised dopamine hypothesis of schizophrenia. The revised dopamine hypothesis proposed that a hyperactive nigrostriatal dopaminergic projection leads to positive symptoms but a hypoactive mesocortical projection is responsible for cognitive and negative symptoms. Adopted from (E. H. Simpson et al., 2010).

1.2.3 GABA hypothesis

Another prominent theory about the etiology of schizophrenia is a disregulated GABA systems (Hashimoto, Matsubara, & Lewis, 2010). Many postmortem findings show reduced GABA interneurons (Cotter et al., 2002) in frontal cortex tissue and reduced GABA re-uptake sites in the hippocampus, amygdala, basal ganglia and left temporal cortex in schizophrenia patients (M. D. Simpson, Slater, Deakin, Royston, & Skan, 1989). Some studies found an increase in GABAa receptors in the PFC, caudate nucleus and cingulate gyrus (Hanada, Mita, Nishino, & Tanaka, 1987) and increased activity of GABA producing enzymes in the PFC (Gluck, Thomas, Davis, & Haroutunian, 2002). A closely studied aspect of GABAergic system disregulation in schizophrenia patients focusses on GABA-chandelier cells in the PFC layer 3 (review: Lewis (2010)). The chandelier cells in PFC layer 3 communicate mainly with pyramidal cells that are situated in layer 3 and 4 of the PFC. The activity of pyramidal cells, most of the output of the PFC, is organized and synchronized by chandelier cells. Supposedly, during developmental periods in early adulthood, aberrancies in chandelier cells disregulate the normal development of pyramidal cells, leading to abnormal structuring of both cell types in the PFC. The aberrant structure would fail to produce the vigorous coordinated neuronal firing called gamma synchrony that is associated with cognitive control and working memory, processes that are impaired in schizophrenia patients (Minzenberg et al., 2010). The elegancy of this hypothesis is that it proposes a neural deficit underlying specific measurable phenotypes in patients.

1.2.4 Synaptic pruning hypothesis

The excessive pruning of synapses is one of the earlier hypotheses about the etiology of schizophrenia, firstly proposed by Feinberg in 1982 (Keshavan, Anderson, & Pettegrew, 1994). Pruning of synapses during brain development leads to the fine mapped inter- and intra-regional connectivity of brain regions and is also thought to underlie many learning processes (Knafo, Libersat, & Barkai, 2005; Roberts, Roche, & Conley, 2005). Aberrant synaptic pruning is known to contribute to many different psychiatric disorders (Lin & Koleske, 2010; L. Q. Luo & D. D. M. O'Leary, 2005) and possibly schizophrenia symptoms (Hoffman & McGlashan, 1997). The developmental nature of schizophrenia, that seems indisputable (Karlsgodt et al., 2008), is in line with the pruning theory of schizophrenia (Keshavan et al., 1994) since tightly regulated pruning is essential for proper brain development (Low & Cheng, 2006). Signs of hyper pruning of synapses are seen in different regions and neuron types in the brains of schizophrenia patients (Glantz & Lewis, 2000; McGlashan & Hoffman, 2000). For example, abundant progressive reduction in neuropil, without large changes in neuron size or number, has been observed in the PFC (Pantelis et al., 2007; Selemon & Goldman-Rakic, 1999). Furthermore,

postmortem studies measured reduced expression of proteins related to synapse formation and maintenance (Chong et al., 2008; Karson et al., 1999). It is believed that various brain regions that show reduced volume in schizophrenia patients, supposedly due to hyper pruning, are linked to specific schizophrenia sub-syndromes within the current schizophrenia diagnosis (Nenadic, Sauer, & Gaser, 2010). The possibility that hyper pruning underlies the reduction in brain region volume makes this biological mechanism an interesting candidate for the etiology for schizophrenia.

1.3 Identification of the genetic contribution to schizophrenia

There is a significant genetic contribution to an individual's proneness to develop schizophrenia (estimated 70-90%) (Farmer et al., 1984; McGuffin et al., 1984). For this reason, a large body of research is dedicated to find genetic variance associated with schizophrenia. One approach is the so-called Genome Wide Association Study (GWAS). A GWAS uses a dense, genome-wide set of genetic markers to pick up genetic variance in a case and a control group. On each included subject a microarray is used to detect variations on a single nucleotide between groups or individuals, also called: Single Nucleotide Polymorphisms (SNPs). By use of a chi-square test it is calculated whether a SNP is significantly more prevalent in one group compared to the other; a p-value is assigned to each SNP. If the p-value is very low, the SNP was more prevalent in either the case or control group and is likely associated to the trait(s) of the case group. If a gene is strongly associated with a trait or disorder, one could state that the gene has a high 'Association Strength' (AS). Such association does not necessarily indicate functional involvement of the SNP, since it could be in 'linkage disequilibrium' with nearby causal genetic variance that was not tested. GWAS have recently identified multiple susceptibility variants of small effect in common disorders, including schizophrenia (Ripke et al., 2011).

There are several challenges and limitations of a GWAS analysis. A modern GWAS study commonly uses a set of up to one million SNPs. Still, SNP sets only capture a fraction of the total genomic variance (Couzin-Frankel, 2010). This way, much of the genomic variance falls outside the scope of a GWAS analysis, resulting in a high false-negative rate. On the other hand, using such a large number of SNPs demands a massive number of statistical tests to be performed leading to an unprecedented false-positive rate. This can be largely avoided by multiple comparison corrections, but this greatly reduces the statistical power of the analysis and therefore very high subject numbers are necessary to reach statistical significance.

Additional limitations concern GWAS on polygenetic and heterogenic disorders such as schizophrenia specifically. A heterogenic disorder has a large variety in symptoms between patients with the same diagnosis; in other words, no symptom occurs in all patients. The fuzzy diagnostical boundaries of a disorder such as schizophrenia, imposed by the descriptive diagnostic system (Rader, 2000), allow this heterogeneity within one patient group . When such a group is used in a GWAS, many sub-groups with different sets of symptoms receive the same diagnosis and are compared to controls as being one case group. However, each subgroup can have different genetic contributions. For this reason a GWAS on heterogeneous disorders is unspecific and detects many genetic variations with a small contribution to the disorder (Duan, Sanders, & Gejman, 2010).

Another factor contributing to large amounts of small effect genetic variance detected by a GWAS is the polygeneity of a disorder. A polygenic disorder means that the genetic contribution to a disorder originates from a large ensemble of small, common or rare genetic variants. Whether most of the polygeneity of schizophrenia originates from the fuzzy diagnostical boundaries or from the fact that schizophrenia symptoms do have a polygenetic causality, the polygeneity of schizophrenia remains a challenge. To illustrate this, figure 2 shows a graph of the results of a GWAS study on schizophrenia in the Chinese Han population. Even though only a few SPNs reach significance above the threshold, the -log10(p) values are scattered suggesting the presence of much common variance with small effect sizes that do not reach the -log10(p) threshold. There is convincing evidence that this variance might still play a relevant role in the development of the disorder of interest (Purcell et al., 2009).

One final limitation worth mentioning is the ethnic and regional genetic variation that interferes with GWAS studies. Common genetic variance can be more common in certain races or regions (Purcell et al., 2009). Furthermore, some variations might differ in their contribution to traits and symptoms between races and regions because of a different genetic background or different environmental factors. Differences between GWAS results on schizophrenia have been demonstrated between regions within the same race (Shi et al., 2011) and between GWAS studies on European, Asian of African populations (Purcell et al., 2009; Shi et al., 2011; Steinberg et al., 2011).

To tackle some of these challenges and limitations, researchers currently try to select fewer markers for analysis to decrease the demanded multiple comparison correction and focus on specific traits or symptoms to avoid the disadvantages of symptom heterogeneity and diagnostical boundaries (Greenwood et al., 2011;

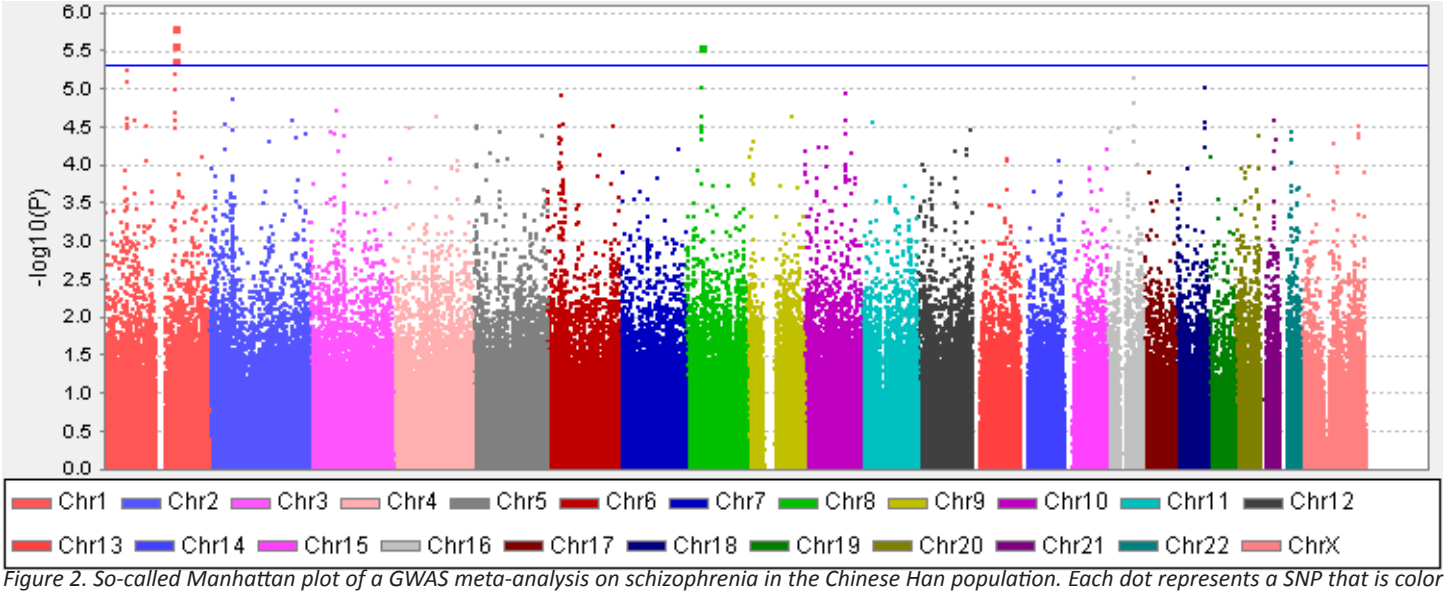

Figure 2. So-called Manhattan plot of a GWAS meta-analysis on schizophrenia in the Chinese Han population. Each dot represents a SNP that is color coded for its chromosome of origin. The x-axis shows the color associated with each chromosome and the y-axis shows the –log10(p) value of the SNPs. The blue bar indicates the significance threshold level. The –log(p) values are highly scattered, indicating the presence of many common genetic variations with a small effect size. Only 4 SNPs seem to reach above the significance threshold but many SNPs are just below. This is typical finding of a GWAS on polygenic disorders. Figure adopted from (Shi et al., 2011).

Morar et al., 2011). Several excellent reviews discuss the disadvantages of GWAS, e.g. (Pearson & Manolio, 2008).

Despite these discouraging arguments, GWAS analysis is still a valuable tool to pin point genes that have the 'least small' contribution to a polygenic disorder or symptom. By functional characterization of such genes, biologically and behaviorally (O'Tuathaigh et al., 2007; Waddington et al., 2007), one can still gather information about the etiology of a disorder or symptom.

1.4 Gene-ontology analysis; from GWAS to schizophrenia etiology

Fortunately, using genetic analyses, additional inferences about polygenic disorders with heterogeneous patient populations can be made based on GWAS results. Only a limited number of biological mechanisms are assumed to be involved in complex traits (Carlborg & Haley, 2004). Therefore, although there are many genes associated with polygenic disorders, there might still be only a few biological mechanisms or functions affected by those genes. How relevant biological mechanisms can be identified with GWAS data is exemplified for Crohn's disease by Kai Wang, Li, and Hakonarson (2010). Knowing which biological mechanisms or functions are associated with a disorder or trait is valuable information for the study on the etiology and treatment of disorders like schizophrenia. If the genes that are moderately associated with schizophrenia affect similar biological mechanisms or functions, it can be picked up by a Gene Ontology (GO) analysis. A GO-analysis takes advantage of prior biological knowledge about gene function and can detect functional commonality of genes in GWAS data.

A GO-analysis takes the most significantly associated genes from a GWAS study and categorizes them based on pathways, molecular functions, cellular components and biological processes using tools such as the 'protein analysis through evolutionary relationships' (PANTHER) classification system gene expression analysis tool (pantherdb.org, 2011). It examines if there are functional categories significantly overrepresented in a target list of genes (e.g. from GWAS) as compared to a reference list that represents the general population (for details, read section 2.3). There are several advantages of analyzing genetic association data this way:

1. Due to the disadvantages of GWAS (section 1.3), large quantities of small-effect genes associated with a disorder will be ignored. Such genes can still give useful information about the etiology of a polygenic disorder. They can help identify biological mechanisms that are relevant for a disorder and would otherwise not be noted by a GWAS.
2. Relevant biological mechanisms pinpointed by a GO-analysis are easier and perhaps more efficient targets for therapeutic interventions as opposed to just one gene/protein.
3. The GO classification database is widely used, making the results comparative across studies.
4. The GO classification database combines different gene databases and consortia to incorporate as much knowledge about genes and their properties for gene classification as possible. This results in a comprehensive reference list and up to date gene information.

More information about the PATHER tool (pantherdb.org) and gene ontology classification (geneontology.org) can be found in the method section and on the indicated websites.

1.5 Limitations of gene ontology analysis

Some limitations of the GO-analysis must be considered. The most important one would be the publication bias. Genes and functional categories that are frequently studied will be overrepresented in the database (and reference list). As a consequence, a gene that is studied thoroughly will occur in more functional categories and thoroughly studied functional categories will contain more genes. On the contrary, a relatively unknown gene will be underrepresented because its unconfirmed functions will not be in the database or reference list. Hence, the analysis is more sensitive to the well-studied genes and the power of well-studied functional categories is substantially higher than the lesser-studied functional categories.

Another limitation that amplifies the publication bias, is the premature state of the database that causes many genes to be 'unclassified'. Especially in the Pathway and Cellular component classifications, many genes are not yet present in any category (>85%, table 1), leaving those classification types especially prone to publication bias. The constant expansion of the gene database used for GO-analysis will gradually solve this problem as well as reduce the publication bias.

Furthermore, there is a high false negative rate. The SNP array used for the GWAS does not cover all genetic variation or all genes (Couzin-Frankel, 2010). All genetic variance that contributed to the trait of interest but was not covered by the SNP array, creates false negatives. The resulting rate of false negatives affects the gene list from the GWAS and therefor also the GO-analysis. Ways to reduce this false negative rate is to expand the SNP array itself and exclude the genes that are not captured by the SNP array from the GO-analysis.

Another increase in false negative rate comes from the selection of most significant SNPs from a GWAS study. By having to exclude the SNPs with a lower association with the trait of interest, cumulative contribution of small effect genetic variance is dismissed. This can neglect important contributions to disregulated mechanisms that underlie the trait of interest.

Finally, the input of the GO-analysis is limited to gene identity. However, for genetic association studies, additional information of the gene is important to determine what biological mechanisms are overrepresented in the GWAS data. Association strength, functional consequence and copy number, to name a few (Elbers et al., 2009; Kai Wang et al., 2010). Some of those parameters are useful specifically for GWAS data. Databases and logarithms are being tested and developed to allow the incorporation of such information (Elbers et al., 2009; Raychaudhuri et al., 2010).

1.6 The AS GO-analysis

This study explores a modified GO-analysis that addresses several limitations and aims to improve the statistical power of the GO-analysis. This modified GO-analysis (the AS GO-analysis) tackles the need to select only the most significant genes from a GWAS and incorporated additional information about the genes for the GO-analysis.

The current GO-analysis only uses the number of genes (in other words, all genes are assigned the value of 1) to compute the significance of over representation of functional categories in a given gene list. However, one can assume that genes with a high AS will have a higher a priori likelihood of truly causing an increased risk for a trait of interest than genes with a low AS; genes with a low AS may represent a chance finding, or a truly associated variant of minimal effect. Unfortunately, in the current GO-analysis, genetic variances of close to significance and far from significance have equal weights. Because GWAS results include much common small effect genetic variance of various effect sizes (varying AS), the current GO-analysis is a coarse approach to pinpoint relevant biological mechanisms. To tackle this problem, the current study explores the consequences of incorporating the AS in the GO-analysis.

The AS is incorporated by replacing the value of '1' of each gene by the AS value (for details read 2.4). This has an additional advantage next to the one mentioned above. Current GO-analysis is forced to select the genes from a GWAS with the highest AS because all genes have a value of 1. However, when replacing this value by the AS, all genes from a GWAS can be included in the analysis, giving a more thorough analysis of the results. Unfortunately, this advantage was not tested; a selection of genes had to be made to limit the data load.

Hypothetically this so-called 'AS GO-analysis', could give a better picture of what functional categories are involved in a target trait. To explore this hypothesis, the conventional and the AS GO-analysis are used on a GWAS study on schizophrenia subjects and the results are compared. Additionally, the results are interpreted with respect to some popular theories about the etiology of schizophrenia described in section 1.2.

2. Methods

2.1 Genome wide association study

2.1.1 Subjects

Data was collected from 1383 cases (877 men, 506 women) and 654 controls. Cases were referred to the Department of Psychiatry at the University Medical Center Utrecht from 1996 to 2007. Patients were identified through representative clinicians whose caseload was screened for inclusion criteria in selected representative geographical areas in the Netherlands. Subsequently, a group of patients presenting consecutively at these services either as outpatients or inpatients were recruited for the study. Controls were selected through a system of random mailings to addresses in the catchment areas of the cases. Eligible patients had to fulfill the following criteria: (1) age between 16 and 50, (2) meeting *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria for a nonaffective psychotic disorder (including schizophrenia, schizophreniform disorder, and schizoaffective disorder), (3) fluent in Dutch, and (4) able and willing to give written informed consent. Eligible healthy control subjects had to fulfill the criteria of (1) age between 18 and 50, (2) no lifetime psychotic disorder, (3) no first- or second-degree family member with a lifetime psychotic disorder, (4) fluent in Dutch, and (5) able and willing to give written informed consent.

2.1.2 Genotyping

Samples were genotyped at the University of California in Los Angeles using the Illumina HumanHap550 beadchip. Call rates were > 97% and all SNPs were in Hardy-Weinberg equilibrium in cases and controls ($p>0.05$).

2.2 Creation of the gene list for Gene Ontology analysis

From the list of 502905 SNPs, the SNPs in high LD (r2 0.25) where excluded leaving 125547 SNPs for analysis. To limit the data load, the threshold used to select SNPs out of this pool was set on $p\leq0.02$ leaving 2608 SNPs for the analysis. This threshold was based on (Purcell et al., 2009) using a similar SNP set to balance the amount of explained variance with the amount of SNPs used in this study. Because 'non-coding' regions around genes often regulate gene expression, SNPs located 10 kb up or downstream of genes were included. All other SNPs on non-coding regions were excluded, resulting in a list of 1106 SNPs with corresponding genes. Each of those genes was assigned the –LOG(p-value) (AS) of the corresponding SNP. 97 genes had 2 SNPs, 23 genes has 3 SNPs, 8 genes had 4 SNPs, 1 gene had 5 SNPs and 2 genes had 7 SNPs. Each gene with multiple SNPs was assigned the lowest p-value of those SNPs, resulting in 920 genes with one p-value each. 34 genes had to be excluded as they were not recognized by the PANTHER database, leaving a total of 886 genes for the GO-analysis.

2.3 Original PANTHER GO-analysis

The GO-analysis compared the gene list created from the GWAS data ('provided gene list') with a reference list. The reference list was provided by PANTHER and contained all genes in the database. Using the PANTHER (pantherdb.org) classification system, the provided gene list was categorized according to four different classification types, by pathway, molecular function, biological process and cellular components. For each classification type, the PANTHER classification system compares the number of genes in each category to a reference list of the human genome that was categorized identically. Binomial statistics were used to determine whether a category was significantly overrepresented in the GWAS data. Under the NULL hypothesis, genes in the uploaded list are sampled from the same general population as genes from the reference list, i.e. the probability of observing a gene from a particular category in the uploaded list is the same as in the reference list. Thus, in the conventional GO-analysis, when the number of genes in a category is not binomially distributed with the probability of observing that number of genes in the reference set ($p<0.05$), there is over- or underrepresentation of that category in the given gene list (e.g. from GWAS data). The supplementary method section (see also http://www.pantherdb.org/tips /tips_binomial.jsp #P-Value_calculated) includes a detailed description of the GO-analysis including the determination of the p-value of under- or overrepresentation of a category.

2.4 Association strength GO-analysis

In the AS GO-analysis, the p-values of each category is re-calculated after replacing the number of genes by their cumulative -log(p) values from the GWAS (see 'P-Value calculated by the Binomial statistic' of the supplementary methods). By doing so, the association strength (AS) of the genes is included in the GO-analysis. To improve the meaningfulness of the results, indiscriminate functional categories like 'Binding' were excluded so more refined categories such as 'Calcium channel binding' are highlighted. Additionally, functional categories containing >15% of the total classified genes in the reference list were excluded (table 1)**.** To maintain manageability of the data analysis, only the categories that showed

significant under of over representations of p<0.02 in the original GO-analysis were included in the AS GO-analysis. It should be noted that after all the exclusion criteria of functional categories, only one category was left in the cell component classification. For this reason the cell component classification was excluded.

Classification type	Unclassified	Classified	Total	% Unclassified	Max. genes
Pathway					15%
Reference list	17337	2574	19911	87%	386
This study	781	107	888	88%	
Biological Processes					
Reference list	6681	13230	19911	34%	1985
This study	256	632	888	29%	
Molecular Function					
Reference list	7622	12289	19911	38%	1843
This study	307	581	888	35%	
Cel Component					
Reference list	17808	2103	19911	89%	315
This study	784	104	888	88%	

Table 1. This table specifies the number of classified genes in each type of classification. 'Max. genes' indicates the maximum number of genes per category within a type of classification and is 15% of the total classified genes in a classification type. All categories with more genes in the reference list than 'Max. genes' were excluded from the analysis.

3. Results

The following section will present results of the preliminary assessment of the AS GO-analysis as well as the outcome of the AS GO-analysis of the pathway, biological processes and molecular function classification.

3.1 AS GO-analysis assessment

Figure 3 shows that p-values of the highest ranks are considerably lower in the AS GO-analysis than in the GO-analysis. However, the lower ranks are similar of even higher (in molecular function) in the AS GO-analysis.

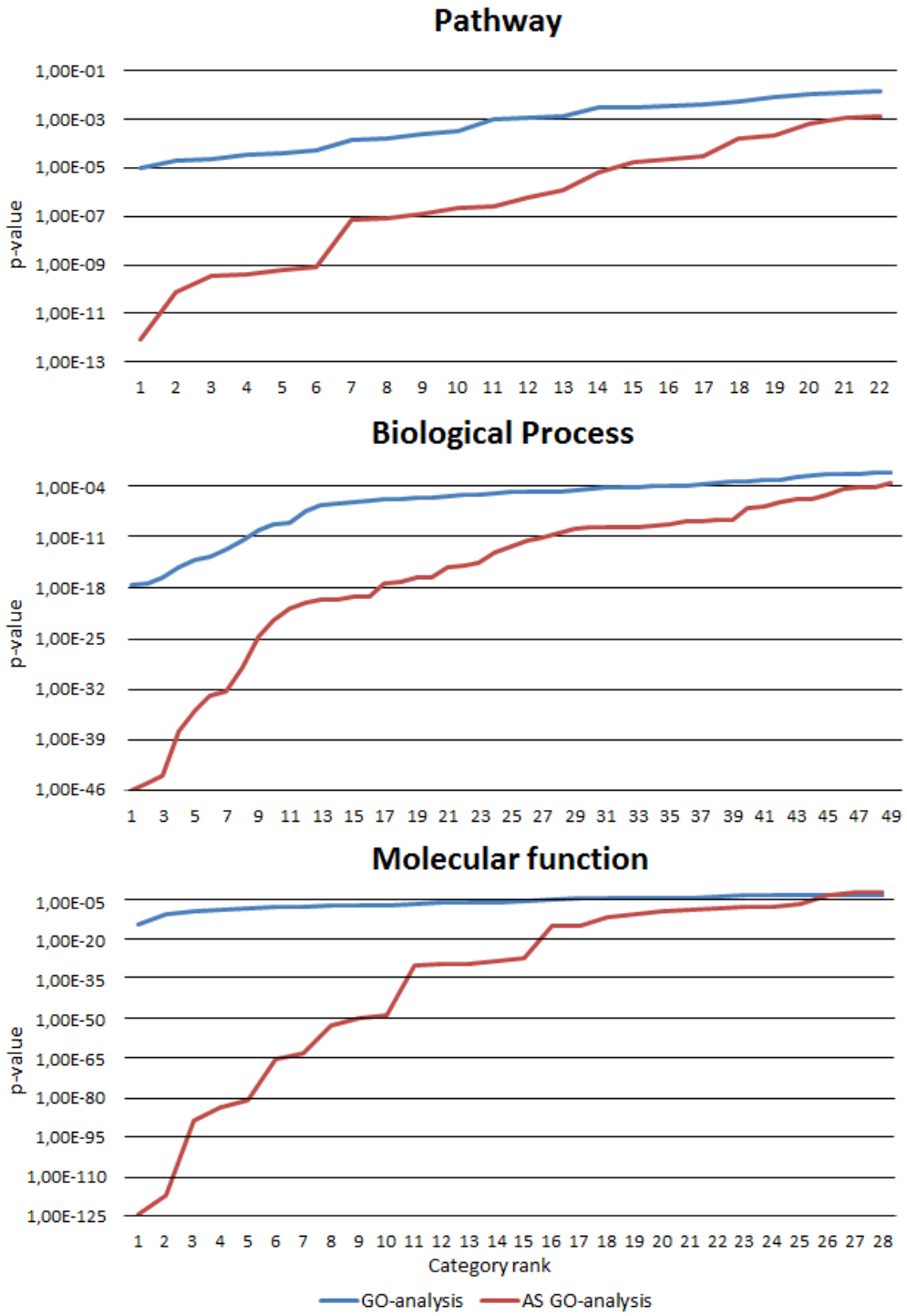

Figure 3. Graphs of each classification type that shows each category rank, irrespective of what category is assigned to it, plotted against its p-value. The blue line shows the results from the GO-analysis (Gene Ontology analysis), the red line shows the results from the AS GO-analysis (Association Strength GO-analysis). The graphs show that p-values of the highest ranks are considerably lower in the AS GO-analysis than in the GO-analysis and that the p-values of the lower ranks are similar or even higher in the AS GO-analysis.

3.2 Rank changes in the AS GO-analysis compared to the GO-analysis

Figure 4-6 show the selected categories ($p<0.02$ in the conventional GO-analysis, see section 2.4) in ranked order according to the AS GO-analysis. The blue lines indicate how their rank changed in the AS GO-analysis as compared to their rank in the conventional GO-analysis. It can be seen that in each classification a selection of categories undergo major rank changes. This implies that there is indeed a difference in average AS of genes within categories.

3.3 AS GO-analysis results

Tables 2-4 give an overview of all the results of the AS GO-analysis and the conventional GO-analysis. In section 4.3 the three most significantly overrepresented categories are discussed with respect to the etiology of schizophrenia.

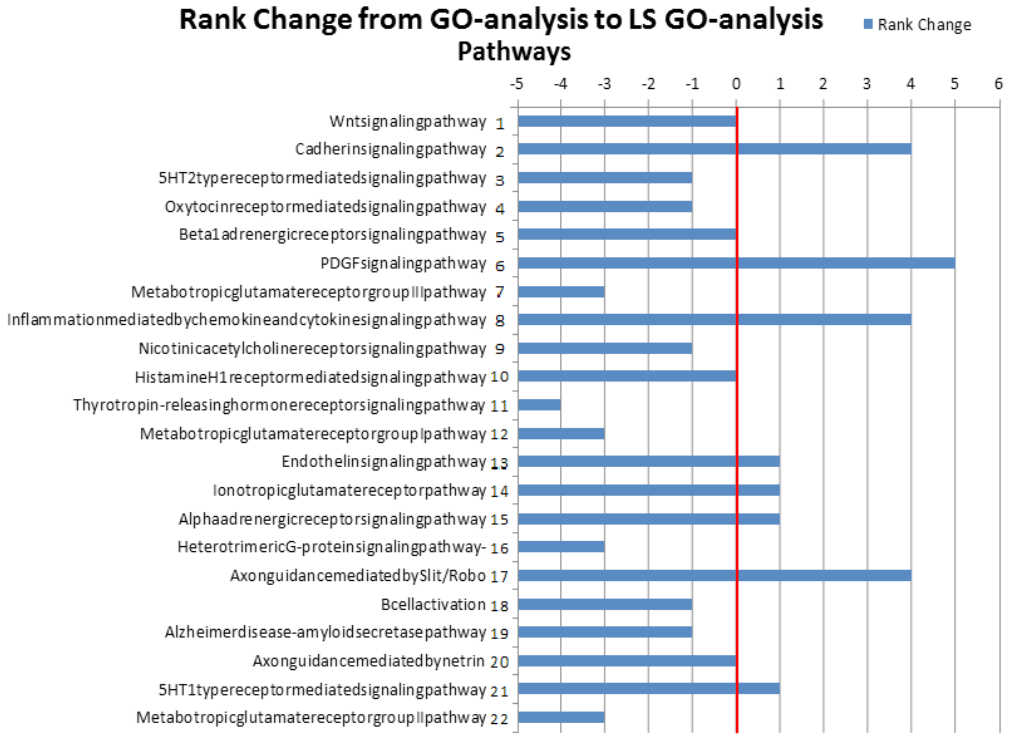

Figure 4. This graph shows the categories in the Pathway classification ranked by the AS GO-results. Rank one is the most significantly overrepresented category and 22 the least significantly overrepresented category. The horizontal blue lines indicate the change in rank as compared to the ranking of the conventional GO-analysis. For example, if the category on rank 5 shows a rank change of -4 it means that that category was on rank 1 in the conventional GO-analysis. The vertical red line highlights the point where the rank change equals zero and the category maintained its rank in the AS GO-analysis.

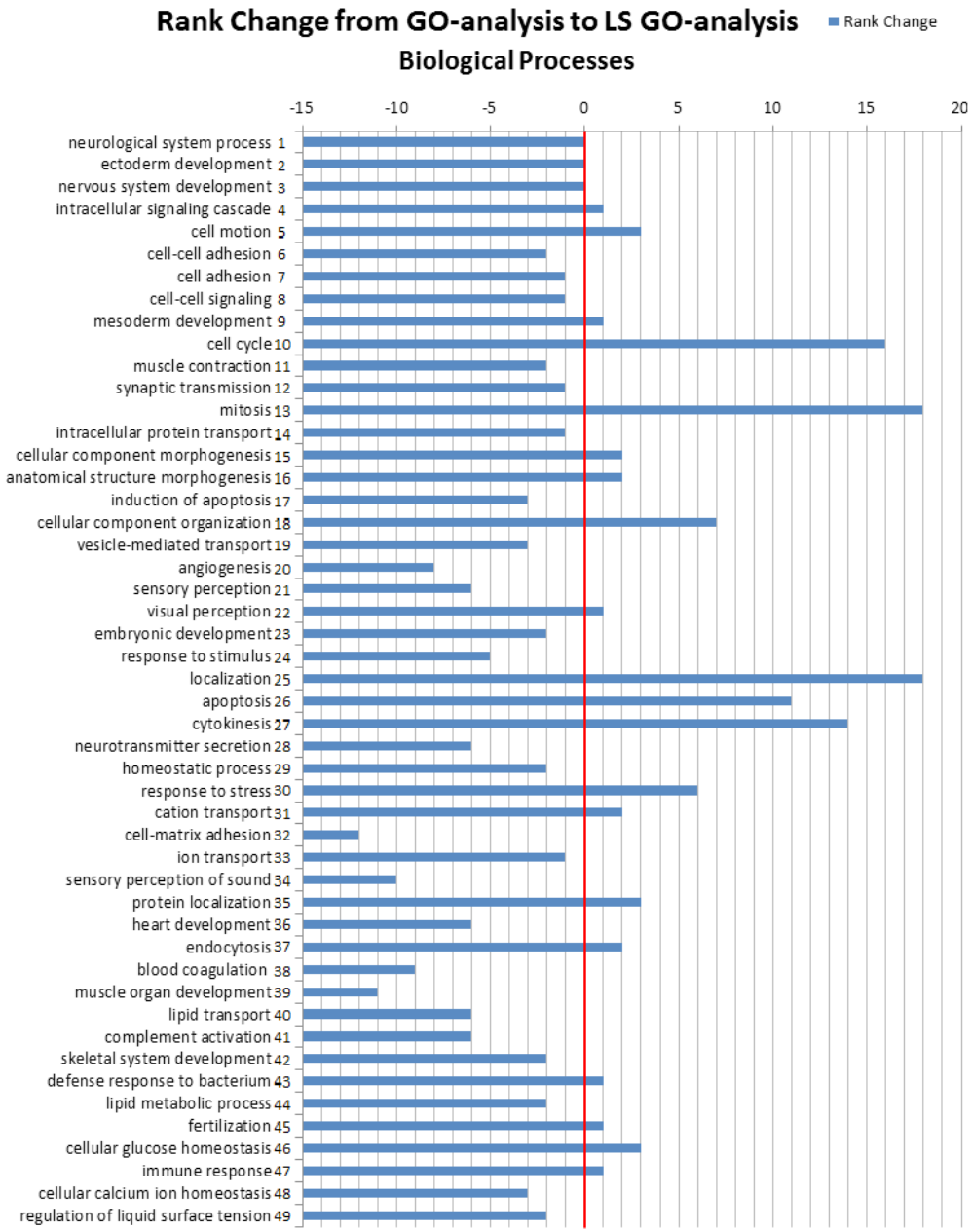

Figure 5. This graph shows the categories in the Biological Process classification ranked by the AS GO-results. Rank one is the most significantly overrepresented category and 49 the least significantly over-represented category. The horizontal blue lines indicate the change in rank as compared to the ranking of the conventional GO-analysis. For example, if the category on rank 5 shows a rank change of -4 it means that that category was on rank 1 in the conventional GO-analysis. The vertical red line highlights the point where the rank change equals zero and the category maintained its rank in the AS GO-analysis.

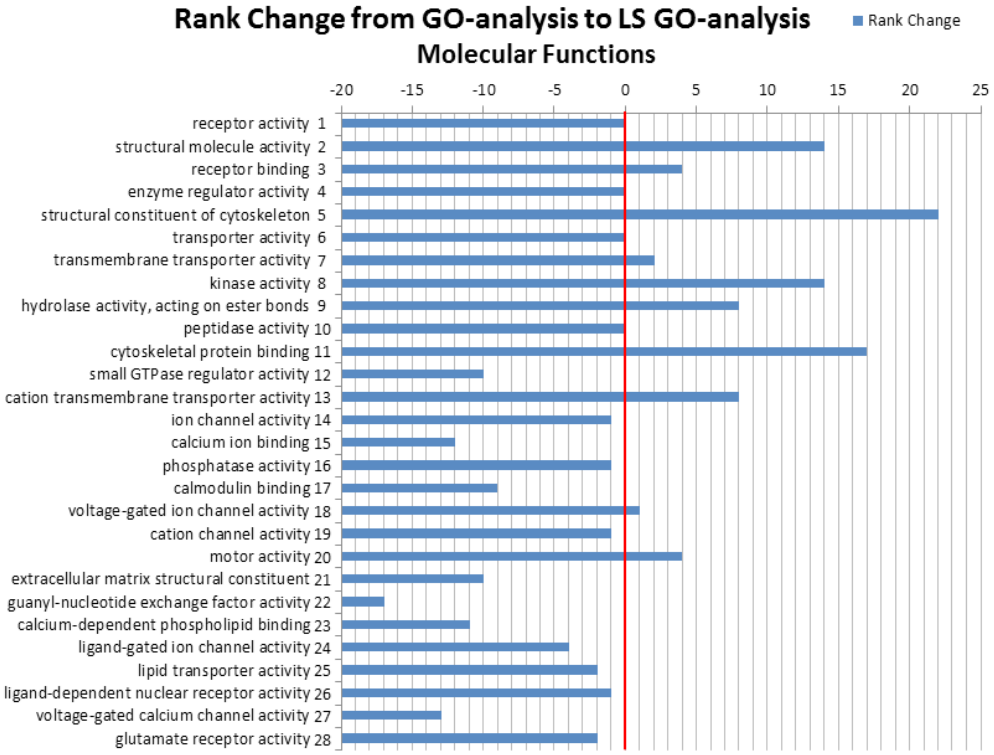

Figure 6. This graph shows the categories in the Molecular Function classification ranked by the AS GO-results. Rank one is the most significantly overrepresented category and 28 the least significantly over-represented category. The horizontal blue lines indicate the change in rank as compared to the ranking of the conventional GO-analysis. For example, if the category on rank 5 shows a rank change of -4 it means that that category was on rank 1 in the conventional GO-analysis. The vertical red line highlights the point where the rank change equals zero and the category maintained its rank in the AS GO-analysis.

Pathway classification									
Category	Genes in database	Genes in provided list	Expected number of genes in the provided list	LS of the genes in the provided list	Expected LS of the genes in the provided list	p-value	p-value original GO analysis	Rank	Rank original GO analysis
Wnt signaling pathway	317	33	14	76	31	8,28E-13	1,06E-05	1	1
Cadherin signaling pathway	147	19	7	44	14	7,86E-11	5,19E-05	2	6
5HT2 type receptor mediated signaling pathway	69	13	3	28	07	3,58E-10	1,99E-05	3	2
Oxytocin receptor mediated signaling pathway	60	12	3	26	06	3,91E-10	2,32E-05	4	3
Beta2-adrenergic receptor signaling pathway	44	10	2	22	04	6,60E-10	3,85E-05	5	5
PDGF signaling pathway	159	17	7	44	15	8,41E-10	1,04E-03	6	11
Metabotropic glutamate receptor group III pathway	73	13	3	25	07	7,72E-08	3,52E-05	7	4
Inflammation mediated by chemokine and cytokine signaling pathway	283	25	13	58	27	9,01E-08	1,24E-03	8	12
Nicotinicacetylcholine receptor signaling pathway	97	14	4	29	09	1,26E-07	1,62E-04	9	8
Histamine H1 receptor mediated signaling pathway	47	9	2	19	05	2,41E-07	3,25E-04	10	10
Thyrotropin-releasing hormone receptor signaling pathway	62	11	3	22	06	2,53E-07	1,42E-04	11	7
Metabotropic glutamate receptor group I pathway	36	8	2	16	03	6,24E-07	2,61E-04	12	9
Endothelin signaling pathway	91	11	4	26	09	1,25E-06	3,09E-03	13	14
Ionotropic glutamate receptor pathway	54	8	2	18	05	6,93E-06	3,36E-03	14	15
Alpha-adrenergic receptor signaling pathway	32	6	1	13	03	1,73E-05	3,48E-03	15	16
Heterotrimeric G-protein signaling pathway	134	15	6	30	13	2,24E-05	1,29E-03	16	13
Axon guidance mediated by Slit/Robo	20	4	1	10	00	2,90E-05	1,30E-02	17	21
B cell activation	82	10	4	20	08	1,65E-04	4,39E-03	18	17
Alzheimer disease-amyloid secretase pathway	71	9	3	18	07	2,17E-04	5,26E-03	19	18
Axon guidance mediated by netrin	30	5	1	10	03	7,23E-04	1,19E-02	20	20
5HT1 type receptor mediated signaling pathway	44	6	2	12	04	1,51E-02	1,51E-02	22	22
Metabotropic glutamate recepto rgroup II pathway	51	7	2	13	05	8,75E-03	8,75E-03	23	19

Table 2: Overview of the results of the AS GO-analysis and the conventional GO-analysis for the Pathway classification. 'Genes in database' is the total number of genes assigned to the category in the reference list. 'Genes in provided list' is the total number of genes assigned to a category from the gene list provided by the GWAS study (section 2.2). 'Expected number of genes in the provided list' is the number of genes in the category that are expected to be in the provided gene list according to the reference list. 'AS of the genes in the provided list' is the cumulative –log(p) value of all genes in the provided list in the category. 'Expected AS of the genes in the provided list' is the expected cumulative –log(p) value of the genes in the category according to the reference list. 'p-value' is the significance of the overrepresentation of the category according to the AS GO-analysis. 'p-value of the original GO-analysis' is the significance of the overrepresentation of the category according to the conventional GO-analysis. 'Rank' is the rank of the category according to the AS GO-analysis and 'Rank original GO-analysis' is the rank of the category according to the conventional GO-analysis.

Biological Process classification									
Category	Genes in database	Genes in provided list	Expected number of genes in the provided list	LS of the genes in the provided list	Expected LS of the genes in the provided list	p-value	p-value original GO analysis	Rank	Rank original GO analysis
neurological system process	1954	173	87	394	189	1,27E-46	3,00E-18	1	1
ectoderm development	1426	139	64	319	138	9,28E-46	6,25E-18	2	2
nervous system development	1258	126	56	292	122	1,31E-44	3,78E-17	3	3
intracellular signaling cascade	1568	139	70	321	152	1,40E-38	1,13E-14	4	5
cell motion	964	93	43	228	93	1,03E-35	6,04E-12	5	8
cell-cell adhesion	799	91	36	198	77	1,55E-33	1,08E-15	6	4
cell adhesion	1333	123	59	274	129	5,91E-33	3,27E-14	7	6
cell-cell signaling	1331	120	59	265	129	9,82E-30	3,30E-13	8	7
mesoderm development	1528	121	68	278	148	2,65E-25	8,61E-10	9	10
cell cycle	1840	119	82	310	178	4,40E-23	3,17E-05	10	26
muscle contraction	448	54	20	116	43	2,36E-21	1,25E-10	11	9
synaptic transmission	594	62	26	137	57	1,13E-20	1,39E-09	12	11
mitosis	635	50	28	142	61	3,07E-20	1,10E-04	13	31
intracellular protein transport	1646	117	73	276	159	3,28E-20	5,08E-07	14	13
cellular component morphogenesis	1121	84	50	208	108	7,72E-20	3,52E-06	15	17
anatomical structure morphogenesis	1121	84	50	208	108	7,72E-20	3,52E-06	16	18
induction of apoptosis	358	39	16	94	35	6,68E-18	5,85E-07	17	14
cellular component organization	1443	98	64	242	140	1,00E-17	2,84E-05	18	25
vesicle-mediated transport	1160	87	52	204	112	4,82E-17	2,26E-06	19	16
angiogenesis	408	45	18	100	39	4,91E-17	5,69E-08	20	12
sensory perception	708	61	32	140	68	1,13E-15	1,33E-06	21	15
visual perception	412	39	18	97	40	1,67E-15	1,48E-05	22	23
embryonic development	481	44	21	106	47	5,50E-15	9,78E-06	23	21
response to stimulus	1798	121	80	268	174	1,53E-13	4,52E-06	24	19
localization	107	12	5	40	10	8,45E-13	3,71E-03	25	43
apoptosis	966	67	43	162	93	4,27E-12	3,12E-04	26	37
cytokinesis	238	22	11	61	23	1,10E-11	1,37E-03	27	41
neurotransmitter secretion	346	35	15	76	33	4,67E-11	1,05E-05	28	22
homeostatic process	142	19	6	42	14	2,97E-10	3,30E-05	29	27
response to stress	500	41	22	95	48	3,81E-10	2,00E-04	30	36
cation transport	621	49	28	111	60	3,92E-10	1,23E-04	31	33
cell-matrix adhesion	173	23	8	47	17	4,06E-10	5,69E-06	32	20
ion transport	739	56	33	126	71	4,38E-10	1,16E-04	33	32
sensory perception of sound	123	18	5	38	12	6,37E-10	1,68E-05	34	24
protein localization	41	8	2	21	04	1,05E-09	6,10E-04	35	38
heart development	365	34	16	74	35	2,48E-09	6,94E-05	36	30
endocytosis	575	43	26	102	56	2,99E-09	9,02E-04	37	39
blood coagulation	325	32	14	68	31	3,28E-09	4,10E-05	38	29
muscle organ development	478	42	21	89	46	3,49E-09	3,83E-05	39	28
lipid transport	246	25	11	52	24	1,64E-07	1,71E-04	40	34
complement activation	162	19	7	39	16	2,57E-07	1,78E-04	41	35
skeletal system development	490	38	22	82	47	1,02E-06	9,30E-04	42	40
defense response to bacterium	73	9	3	22	07	3,47E-06	6,25E-03	43	44
lipid metabolic process	1119	72	50	154	108	3,51E-06	1,42E-03	44	42
fertilization	170	15	8	36	16	1,18E-05	1,09E-02	45	46
cellular glucose homeostasis	72	8	3	19	07	8,89E-05	1,69E-02	46	49
immune response	756	47	34	104	73	1,38E-04	1,57E-02	47	48
cellular calcium ion homeostasis	51	7	2	15	05	1,52E-04	8,75E-03	48	45
regulation of liquid surface tension	19	4	1	08	02	5,52E-04	1,10E-02	49	47

Table 3: Overview of the results of the AS GO-analysis and the conventional GO-analysis for the Biological process classification. 'Genes in database' is the total number of genes assigned to the category in the reference list. 'Genes in provided list' is the total number of genes assigned to a category from the gene list provided by the GWAS study (section 2.2). 'Expected number of genes in the provided list' is the number of genes in the category that are expected to be in the provided gene list according to the reference list. 'AS of the genes in the provided list' is the cumulative –log(p) value of all genes in the provided list in the category. 'Expected AS of the genes in the provided list' is the expected cumulative –log(p) value of the genes in the category according to the reference list. 'p-value' is the significance of the overrepresentation of the category according to the AS GO-analysis. 'p-value of the original GO-analysis' is the significance of the overrepresentation of the category according to the conventional GO-analysis. 'Rank' is the rank of the category according to the AS GO-analysis and 'Rank original GO-analysis' is the rank of the category according to the conventional GO-analysis.

Molecular Function classification									
Category	Genes in database	Genes in provided list	Expected number of genes in the provided list	LS of the genes in the provided list	Expected LS of the genes in the provided list	p-value	p-value original GO analysis	Rank	Rank original GO analysis
receptor activity	1808	152	81	337	175	1,96E-125	3,67E-14	1	1
structural molecule activity	1488	97	66	236	144	6,21E-118	1,38E-04	2	16
receptor binding	1233	95	55	216	119	2,87E-89	2,33E-07	3	7
enzyme regulator activity	1187	96	53	237	115	3,25E-84	2,19E-08	4	4
structural constituent of cytoskeleton	1003	62	45	159	97	1,16E-81	6,90E-03	5	27
transporter activity	942	78	42	172	91	7,64E-66	2,04E-07	6	6
transmembrane transporter activity	897	73	40	163	87	9,72E-64	9,50E-07	7	9
kinase activity	695	49	31	116	67	4,38E-53	1,40E-03	8	22
hydrolase activity, acting on ester bonds	667	50	30	118	65	3,28E-50	3,40E-04	9	17
peptidase activity	717	62	32	148	69	6,54E-49	9,66E-07	10	10
cytoskeletal protein binding	396	29	18	70	38	4,36E-30	7,55E-03	11	28
small GTPase regulator activity	495	56	22	136	48	1,61E-29	5,51E-10	12	2
cation transmembrane transporter activity	402	33	18	75	39	3,23E-29	7,90E-04	13	21
ion channel activity	426	41	19	94	41	6,55E-28	6,13E-06	14	13
calcium ion binding	454	51	20	113	44	2,70E-27	4,13E-09	15	3
phosphatase activity	241	27	11	66	23	7,07E-15	1,92E-05	16	15
calmodulin binding	252	31	11	69	24	1,43E-14	7,34E-07	17	8
voltage-gated ion channel activity	162	18	7	41	16	4,61E-11	4,88E-04	18	19
cation channel activity	158	18	7	41	15	4,39E-10	3,66E-04	19	18
motor activity	121	13	5	30	12	7,86E-09	3,70E-03	20	24
extracellular matrix structural constituent	147	21	7	44	14	2,61E-08	4,95E-06	21	11
guanyl-nucleotide exchange factor activity	160	26	7	67	15	5,42E-08	3,40E-08	22	5
calcium-dependent phospholipid binding	137	20	6	43	13	1,08E-07	6,05E-06	23	12
ligand-gated ion channel activity	112	14	5	32	11	1,61E-07	6,62E-04	24	20
lipid transporter activity	92	11	4	26	09	1,53E-06	3,35E-03	25	23
ligand-dependent nuclear receptor activity	46	7	2	14	04	4,74E-03	5,13E-03	26	25
voltage-gated calcium channel activity	31	9	1	36	03	5,51E-02	1,44E-05	27	14
glutamate receptor activity	25	5	1	12	02	8,21E-02	5,71E-03	28	26

Table 4: Overview of the results of the AS GO-analysis and the conventional GO-analysis for the Molecular function classification. 'Genes in database' is the total number of genes assigned to the category in the reference list. 'Genes in provided list' is the total number of genes assigned to a category from the gene list provided by the GWAS study (section 2.2). 'Expected number of genes in the provided list' is the number of genes in the category that are expected to be in the provided gene list according to the reference list. 'AS of the genes in the provided list' is the cumulative –log(p) value of all genes in the provided list in the category. 'Expected AS of the genes in the provided list' is the expected cumulative –log(p) value of the genes in the category according to the reference list. 'p-value' is the significance of the overrepresentation of the category according to the AS GO-analysis. 'p-value of the original GO-analysis' is the significance of the overrepresentation of the category according to the conventional GO-analysis. 'Rank' is the rank of the category according to the AS GO-analysis and 'Rank original GO-analysis' is the rank of the category according to the conventional GO-analysis.

4. Discussion

This study explored a modified Gene Ontology analysis that is adapted to suit the analysis of GWAS. This AS GO-analysis uses the AS of genes in a functional category instead of the number of genes to identify over represented functional categories in a given gene list from a GWAS. The AS GO-analysis was released on a GWAS study on schizophrenia patients and some characteristics of the analysis were explored.

The characterization of the AS GO-analysis led to some interesting observations. The observations were indicative of improvements in the AS GO-analysis as compared to the conventional GO-analysis and suggest that the AS GO-analysis is an interesting method worthy of further validation and testing. The first part (4.1-4.3) of the discussion will elaborate on this.

The result of the AS GO-analysis of the GWAS data from schizophrenia subjects highlighted some particular theories about the etiology of schizophrenia and supported several others. This will be described in the second part (4.4 and 4.5) of the discussion.

4.1 Sensitivity and specificity of the AS GO-analysis

When comparing the p-value of each rank of the AS GO-analysis with that of the conventional GO-analysis, it seems that the top ranks were assigned lower p-values and the lower ranks higher p-values (figure 3). However, because of the limited selection of categories (see section 2.4), only the Molecular Function classification contained sufficient ranks to show this effect. Figure 3 shows that the lowest ranks in the Molecular Function classification only, have higher p-values in the AS GO-analysis as compared to the conventional GO-analysis. The Pathway and Biological Process classification did show trends that suggest a similar outcome for low ranks excluded from the analysis.

This is an interesting observation that could suggest that the AS GO-analysis is more specific (lower ranks have a higher p-value) and sensitive (ranks have a lower p-value) in identifying significant overrepresented categories. To be conclusive about the specificity and sensitivity of the AS GO-analysis compared to the conventional GO-analysis, further testing is crucial. A larger range of ranks should be used to map the changes in p-value of the higher and lower ranks. Section 4.3 and 4.6 discuss more options for further assessment of the AS GO-analysis.

4.2 Rank changes

The above discussed results are indicative for improved specificity and sensitivity of the AG GO-analysis compared to the conventional GO-analysis. This implies that in the AS GO-analysis the categories that are more relevant for the etiology of schizophrenia have a heightened rank and that the categories that are less relevant for the etiology of schizophrenia are lowered in rank as compared to the conventional GO-analysis. A challenge to support this property of the AS GO-analysis in this study is that it is uncertain which categories are more or less relevant to schizophrenia.

Many categories in the AS GO-analysis changed ranks considerably as compared to the conventional GO-analysis (figure 4-6). The following section will elaborate on the largest rank changes (table 5). The thresholds for the largest rank changes was set in consideration of the number of categories and their theoretical significance. For the Pathway classification the categories with a rank change of >3 or <-3 were selected. For the Biological process classification the categories with a rank change of >11 or <-9 were selected. For the Molecular Function classification the categories with a rank change of >10 or <-10 were selected. For more information about the categories and their associated genes, go to 'http://www.

Top rank changes			
Category	**Rank Change**	**Old rank**	**New rank**
Pathway			
cadherin signaling pathway	4	6	2
PDGF signaling pathway	5	11	6
axon guidance mediated by Slit/Robo	4	21	17
inflammation mediated by chemokine and cytokine signaling pathway	4	12	8
thyrotropin-releasing hormone receptor signaling pathway	-4	7	11
Biological Process			
cell cycle	16	26	10
mitosis	18	31	13
localization	18	43	25
cytokinesis	14	41	27
cell-matrix adhesion	-12	20	32
sensory perception of sound	-10	24	34
muscle organ development	-11	28	39
Molecular Function			
structural molecule activity	14	16	2
structural constituent of cytoskeleton	22	27	5
kinase activity	14	22	8
cytoskeletal protein binding	17	28	11
calcium ion binding	-12	3	15
guanyl-nucleotide exchange factor activity	-17	5	22
calcium-dependent phospholipid binding	-11	12	23
voltage-gated calcium channel activity	-13	14	27

Table 5. Table depicting the categories with large rank changes selected for discussion. For the Pathway classification the categories with a rank change of >3 or <-3 were selected. For the Biological process classification the categories with a rank change of >11 or <-9 were selected. For the Molecular Function classification the categories with a rank change of >10 or <-10 were selected. The inclusion criteria for the categories included in this table was made to limit the number of categories to be discussed but to include the most important ones (i.e. highest rank changes and theoretical significant functional categories).

pantherdb.org/panther/prowler.jsp'.

4.2.1 Pathway classification

In the pathway classification the 'cadherin' and 'PDGF (Platelet Derived Growth Factor) signaling pathway', 'Slit/Robo mediated axon guidance' and 'chemokine/cytokine mediated inflammation signaling pathway' had the highest positive rank changes. The 'cadherin signaling pathway' plays a role in brain development, including astrocyte development (Schnadelbach, Ozen, Blaschuk, Meyer, & Fawcett, 2001) but also inflammatory processes and is involved in the recovery from hypoxia induced fetal brain disorganization (Herr, Herr, Lee, Noguchi, & Chun, 2011). The 'PDGF signaling pathway' is known for its role in angiogenesis, but also acts as a prominent mitogen for glial cells (Heldin, 1992). Slit and Robo proteins are involved in axon guidance during neural development (Mastick et al., 2010; Ypsilanti, Zagar, & Chedotal, 2010). The role of the 'chemokine/cytokine inflammation signaling' category lies in initiating inflammatory responses. Compared to the conventional GO-analysis, the AS GO-analysis highlights neural brain development (Cadherin, PDGF, Slit/Robo) and inflammatory processes as suspects for the etiology of schizophrenia.

One pathway was substantially lower in rank in the AS GO-analysis as compared to the conventional GO-analysis: the 'Thyrotropin-releasing hormone receptor signaling pathway'. This signaling pathway regulates the release of hormones from the thyroid gland. Exact functional consequences of thyroid hormones are not yet defined but seem to involve metabolism and cell growth and affect cells of the entire body. Interestingly, it also increases prolactin release from the pituitary gland. This release is inhibited by dopamine and antipsychotics (via dopamine) and is used as indicator of changing dopamine activity in response to antipsychotic agents (Volavka et al., 2004). However, besides being used as biomarker for the effects of antipsychotics on dopamine transmission, it was never described as having a causal role in schizophrenia.

4.2.2 Biological process classification

In the biological process classification the categories 'cell cycle', 'mitosis', 'localization' and 'cytokinesis' had the most prominent positive rank changes. Despite the exclusion of categories with many genes, these are fairly uninformative processes as they perform many functions. 'Cell cycle' regulation is crucial for a nicely timed cell division that results in two cells. 'Mitosis' is specifically involved in the condensation of DNA during cell division and cytokinesis is the separation of the cytoplasm during cell division, genes in this category likely overlaps with those of the cell cycle category. Disruption of any of these functions in neurons can affect brain development. Specific theories of schizophrenia cannot be assigned to these categories, but they do highlight the developmental nature of schizophrenia. 'Localization' is the process where any cell, protein, organelle or RNA is brought or maintained in a position. 'Localization' can be involved in both developmental and day to day functioning of any cell and is also crucial during cell division. Compared to the conventional GO-analysis, the AS GO-analysis highlights the developmental processes of cell division (likely neuron division) as being involved in schizophrenia development. The 'Localization' category extends towards day-to-day cell functioning; it is hard to assign that function to a particular schizophrenia theory.

'Cell-matrix adhesion', 'sensory perception of sound' and 'muscle organ development' were lowered ten or more ranks in the AS GO-analysis. 'Cell-matrix adhesion' processes facilitate the attachment of a cell to the surrounding matrix in order to fix the cell in its position. Genes, like DISC 1, that are known as susceptibility genes for several psychiatric disorders, affect cell-matrix adhesion (Hattori et al., 2010). However, the role of this process in schizophrenia was overestimated in the conventional GO-analysis according to the AS GO-analysis. Sensory perception of sound and muscle development seem justly lowered in rank since these categories focus on the development of muscle organs and the perception of real sounds; hearing and muscle development are intact in schizophrenia patients. Impairments in these functions unlikely lead to disturbance of body experience or auditory hallucinations or any schizophrenia symptoms (http://www.pantherdb.org/panther/prowler.jsp) (Rader, 2000).

4.2.3 Molecular Function

In the molecular function classification the categories 'structural molecule activity', 'structural constituent of cytoskeleton', 'kinase activity' and 'cytoskeletal protein binding' were significantly increased in rank. Especially the first three categories acquired a considerably high rank (2, 5 and 8 respectively). The categories 'structural molecular activity' and 'structural constituent of cytoskeleton' seem particularly involved in the structural integrity of cells and synapses. Impairments in these process could affect the development, pruning, maintenance and re-structuring of neurons and synapses, as stable neuronal structural integrity is critical for these processes to be exerted properly (L. Luo & D. D. O'Leary, 2005). 'Kinase activity' regulates the transfer of phosphates groups between proteins and is involved in energy transfer and regulation of protein activity. This is a crucial process for all cells during development and 'online' functioning and is therefore hard to link to a process that contributes specifically to schizophrenia symptoms. Impaired 'cytoskeletal protein binding' can have structural and functional consequences to a cell because most

proteins in this category play a role in structural integrity of a cell or in protein/RNA transport to and from the soma. In short, AS GO-analysis substantially highlighted biological processes that ensure proper structural integrity of a cell, but also proper 'online' functioning of a cell.

The AS GO-analysis on the molecular process classification made 'calcium ion binding', 'guanyl-nucleotide exchange factor activity', 'calcium-dependent phospholipid binding' and 'voltage-gated calcium channel activity' lower in rank substantially. Strikingly all these processes are involved in calcium ion related functions. 'Guanyl-nucleotide exchange factor activity' promotes the production of cGMP that allows cGMP gated calcium channels to open. Calcium has a major role in pre-synaptic neurotransmitter release and synaptic plasticity. Rapid influx of calcium in a post-synapse stimulates long term potentiation and regulates gene transcription of the target neuron. In addition, many cell and synapse adhesion molecules rely on strong calcium bonds. Alterations in the latter molecules could significantly compromise processes, such as pruning and axon guidance that underlie brain development. Especially calcium ion binding and guanyl-nucleotide exchange factor activity were high in rank and considerably overestimated by the conventional GO-analysis. In summary, although theoretically these calcium processes could be involved in schizophrenia, the AS GO-analysis suggests that there overrepresentation in the GWAS gene list was overestimated by the conventional GO-analysis.

4.2.4 Rank changes and schizophrenia etiology

Does the diverging categorical ranking of the AS GO-analysis compared to the conventional GO-analysis change the support of the GO-analysis for theories about the etiology of schizophrenia? Results of the AS GO-analysis laid more emphasis on developmental processes such as cell division, axon guidance, glial cell growth, synaptic plasticity and pruning. This confirms the developmental nature of schizophrenia and could indicate an increased support for the synaptic pruning and myelination hypothesis of schizophrenia (Keshavan et al., 1994). The heightened ranking of cadherin and PDGF signaling pathways points to an increased support for the myelin hypothesis of schizophrenia. Cadherin and PDGF signaling is involved in glial cell development, including astrocytes that are responsible for myelination of axons (Heldin, 1992; Schnadelbach et al., 2001). However, some functions like kinase activity and cytoskeletal transport were also highlighted. Impairments in such processes could undermine normal 'online' neuronal functioning and could be supportive for the dopamine and GABA theory of schizophrenia.

Interestingly, 'inflammation mediated by chemokine and cytokine signaling pathway' was also highlighted in the AS GO-analysis as compared to the conventional GO-analysis. It has been suggested that gestational insults such as hypoxia or viral infections increase the risk of schizophrenia (Ballon, Dean, & Cadenhead, 2008; Brixey, Gallagher, McFalls, & Parmelee, 1993; Brown & Derkits, 2010). Impaired inflammatory processes could affect the recovery or prevention of neural damage induced by such events.

As expected, the AS GO-analysis lowered some irrelevant categories considerably. Categories such as 'sensory perception of sound' and 'muscle development' unlikely contribute to the development of schizophrenia and can be considered noise. These categories focus on the 'development of muscle organs' and the 'perception of sounds'. Impairments in these functions unlikely leads to disturbance of body experience or auditory hallucinations or any other schizophrenia symptoms (with the possible exception of Bonnet syndrome symptoms (Hughes, 2012)). The signaling pathway of the thyroid release hormone was also lowered in rank in the AS GO-analysis. Thyroid hormone release was always assumed to be secondary to schizophrenia and this could be the reason why it is lowered in rank. However, the fact that this category was overrepresented in the GWAS results could indicate a more causal role for this pathway in schizophrenia than previously thought.

Surprisingly, molecular functions that involve calcium dependent functions were also substantially lowered in rank. Proper regulation of calcium bindings and concentrations is crucial for the functioning of synaptic plasticity, pruning, signal transfer and more. Theoretically, impairments in these processes can lead to disturbed brain function and could thereby contribute to schizophrenia symptoms. The fact that the AS GO-analysis devalues the role of impaired calcium functions in the development of schizophrenia implies that its role was overestimated by the conventional GO-analysis.

Unfortunately, it cannot be determined whether some categories are justly lowered in rank. Some rank changes, like muscle development and perception of sound, are in line with the current research about schizophrenia, but others like calcium function and metabotropic glutamate receptor groups 1-3 (not discussed) are not. Before applying significance to these rank changes in light of the etiology of schizophrenia, the AS GO-analysis needs further validation. If indeed the AS GO-analysis is more specific and sensitive, it would be valuable tool to guide current research in the right direction.

4.3 Limitations of the AS GO-analysis

Besides the limitations mentioned in section 1.5 there are additional limitation concerning the AS GO-

analysis and this study specifically.

The advantages of the AS GO-analysis have theoretical support, but are difficult to support statistically. The main reason for this is the lack of a golden standard. The conventional GO-analysis is used on diseases where the etiologies (i.e. the involved functional categories) are not well-defined. This poses a conundrum concerning the validation of deviant results of the AS GO-analysis compared to the conventional GO-analysis. Comparing the two GO-analysis by using data from studies on diseases where the etiology as well as the genetic factors are better defined, would facilitate the qualitative comparison of the two GO-analysis. It would allow better assessment of the sensitivity and specificity of the AS GO-analysis.

The scope of this study enforced some unfortunate limitations as well. To ensure manageability of the data load, a selection of SNPs ($p<0.02$) was used. However, one of the advantages of the AS GO-analysis is that, unlike in the GO-analysis, there is no need to select only the SNPs with the highest AS. Using all or most SNPs could increase the quality of the AS GO-analysis. Additionally, the consideration to select the SNP with the lowest p-value on a gene with multiple SNPs allowed a bias of gene size and SNP coverage. To eliminate these biases, a valid method to assign a single p-value to a gene with multiple SNPs is crucial.

4.4 AS GO-analysis and the etiology of schizophrenia

While many plausible theories about the etiology of schizophrenia have been proposed, the biological causes of developing schizophrenia remain elusive. In the following section, the results of the AS GO-analysis will be discussed with respect to leading theories about the etiology of schizophrenia. The results of the AS GO-analysis pointed to certain functional categories affected by genetic variance that increase the risk of schizophrenia development. This chapter looks at these functional categories and their relation to literature on schizophrenia to assess what theories about the etiology of schizophrenia (discussed in 1.2) is most supported by the AS GO-analysis.

To limit the discussion about the AS GO-analysis results, only the three most overrepresented categories of each classification are discussed. For additional information about the other categories, see the PANTHER prowler (http://www.pantherdb.org/panther/prowler.jsp).

4.4.1 Pathway classification

4.4.1.1 Rank 1: The wnt signaling pathway

The ‘wnt signaling pathway’ was the most significantly overrepresented category in the pathway classification in both the classical and AS GO-analysis. This is not surprising because wnt signaling is involved in brain development, regulation of synaptogenesis, synapse specification and determination of cell fate (Freyberg, Ferrando, & Javitch, 2010). Disregulation of ‘wnt signaling pathways’, and pathways that interact with wnt signaling is thought to be associated with schizophrenia (Freyberg et al., 2010; Lovestone, Killick, Di Forti, & Murray, 2007). It is shown that the expression of proteins from the ‘wnt signaling pathway’ are altered in hippocampal regions of schizophrenia patients (Cotter et al., 1998; Miyaoka, Seno, & Ishino, 1999). Furthermore, SNPs in the gene encoding the wnt receptor ‘frizzled 3’ have been associated with schizophrenia in a Chinese population (Yang et al., 2003; Zhang et al., 2004), although this could not be replicated in Japanese (Zhang et al., 2004) or German subjects (Reif et al., 2007), or in this study. Considering the role of wnt signaling in establishing synaptic connections, it supports the pruning hypothesis. An increase of faulty synaptic connections could be compensated by additional pruning, which is common in schizophrenia patients (Glantz & Lewis, 2000; McGlashan & Hoffman, 2000). Additionally, Wnt signaling delays oligodendrocyte maturation in the spinal cord through β-catenin (Feigenson, Reid, See, Crenshaw, & Grinspan, 2009; Shimizu et al., 2005) and seems crucial for myelinogenesis (Tawk et al., 2011). Hence it is very likely that disregulated wnt signaling can affect the myelinisation of axons and its timing during brain development.

It seems that the ‘wnt signaling pathway’ supports the myelin and pruning hypothesis. However, it cannot be ignored that disregulated neuronal and synaptic network formation can lead to secondary aberrant dopamine or GABA systems as well.

4.4.1.2 Rank 2: The cadherin signaling pathway

The second in rank of the pathway classification was the ’cadherin signaling pathway’. Cadherin pathways facilitate cell recognition and cell adhesion. The Cadherin pathway plays a role in several psychiatric disorders like bipolar disorder (Pedrosa et al., 2010) and autism (K. Wang et al., 2009). There are signs of its involvement in schizophrenia although not very strong (Bray et al., 2002; S. M. Singh, Castellani, & O’Reilly, 2010), except for (Pedrosa et al., 2010). Considering the broad consequences that impaired cell recognition and cell adhesion can have on brain development, it is difficult to assign a specific hypothesis of schizophrenia to this category. Knockdown of N- and beta- cadherin delays the myelination of axons in rats (Lewallen et al., 2011) and inhibiting these cadherin’s negatively affects Schwann cell proliferation in cell culture, thus giving fairly strong evidence for this category’s involvement in myelination. Conditional knockdown of E-cadherin in rodents reduces the formation of GABA synapses specifically (Fiederling, Ewert, Andreyeva,

Jungling, & Gottmann, 2011), implying that this category could support the GABA hypothesis as well. And lastly, cadherin protein Fat3 affects neurite pruning (Deans et al., 2011), thus giving the possibility that the category supports the pruning theory. Although there currently is no strong evidence of this category having a role in dopamine neurons, the possibility cannot be dismissed.

There is a likely possibility that specific alteration in this pathway affect the brain in different ways. What specific functions are affected could depend on the SNP allele an individual carries in his or her cadherin genes. This would also explain why certain proteins in cadherin pathways are associated with different psychiatric disorders and neuron morphologies. Therefore it seems that this category supports all hypotheses except for the dopamine hypothesis, for no reports of direct associations between cadherin proteins and aberrant dopamine systems were found. Impaired dopamine function could still be a secondary effect of impaired cadherin signaling.

4.4.1.3 Rank 3: The 5HT2 type receptor mediated signaling pathway

The third category in the pathway classification was the '5HT2 type receptor mediated signaling pathway'. Interestingly, several effects on mood, cognition and psychotic symptoms of atypical antipsychotic drugs seem, at least in part, attributed to their affinity for 5HT2 type receptors (Meltzer & Massey, 2011). 5HT2 receptors are mostly excitatory receptors distributed on most projection areas of 5HT neurons and can provide auto-inhibition (5HT2b receptor) on 5HT pre-synapses. The receptors were shown to modulate physical responses to mental states and multiple cognitive processes. The 5HT2a receptor is known to induce anti-inflammatory processes (Yu et al., 2008), suggesting a role in the recovery from gestational insults such as hypoxia (Ballon et al., 2008). Genetic or pharmacological inactivation of the 5HT2b receptor in mice results in impaired vascular proliferation and remodeling, pulmonary vasoconstriction and elastase activity upon hypoxia (Callebert et al., 2006; Launay et al., 2002). This is additional support for a role of this category in the recovery from gestational insults such as hypoxia. 5-HT2c receptor has a potential role in the cognitive adaptations to stress and anxiety. It is involved in activating the hypothalamic-pituitary-adrenal axis (Heisler et al., 2007) and modulates dopamine release in several meso-cortical and nigro-striatal dopaminergic pathways (Alex, Yavanian, McFarlane, Pluto, & Pehek, 2005). Impairments in the 5HT2c receptor could affect the cognitive adaptation to stressful events (possibly by modulating dopamine release) and thereby increase the chance that such an event triggers psychosis (Holtzman, Shapiro, Trotman, & Walker, 2012; Mizrahi et al., 2011).

The '5HT2 type receptor mediated signaling pathway' supports a role for impaired inflammatory processes as being involved in the etiology of schizophrenia. During gestation, it can affect the recovery of hypoxia (5HT2a receptor) but could also play a role in the protection against viruses (5HT2b receptor) associated with schizophrenia (Whitford et al., 2012). Furthermore, considering the role of the 5HT2c receptor, impaired adaptations to stress could be related to a dysfunctional '5HT2 type receptor mediated signaling pathway' and result in an increased risk for stress induced psychosis. In summary, none of the theories discussed in chapter one is supported by this system. Instead, protection from gestational insults and resilience for psychosis inducing events ('stress resilience') could be associated with this category.

4.4.2 Biological process classification

The results of the biological process classification are slightly ambiguous. The main reason for this is that the categories of the highest ranks are very broad and contain multiple sub categories. However, by looking into these sub categories, some interesting observations can be made.

4.4.2.1 Rank 1: neurological system processes

The most significantly overrepresented category in the biological process classification was 'neurological system processes'. This category has three sub-categories, 'neuronal action potential propagation', 'neurotransmitter secretion' and 'sensory perception'. These categories are all involved in electrical and chemical signal transduction and modulation and not so much in neuron/synapse formation and brain development *. For this reason it is likely that variations in this category modulate 'online' traits of electrical and synaptic communication present pre- and post-morbid. Such traits have been identified in schizophrenia (Bloemen et al., 2011; Fusar-Poli et al., 2010; Howes et al., 2011; Mizrahi et al., 2011). Considering the non-developmental profile of this category, it is primarily supportive for the GABA and dopamine hypothesis.
** There might however be an indirect effect of neurotransmission and neural activity on neuron connectivity and maturation (Baho & Di Cristo, 2012).*

4.4.2.2 Rank 2: ectoderm development

The category on the second rank of the biological process classification is one of a developmental nature. 'Ectoderm development' is a process that regulates the development of the ectoderm cell layer of the embryo. These cells will develop into the nervous system after embryonic development; this category points to processes taking place pre-natally. So far, only pre-natal insults that involve immunoreactive processes have been linked to an increased risk on schizophrenia (Brown & Derkits, 2010; Fruntes & Limosin, 2008). But it cannot be dismissed that

other event during ectoderm development can lead to neural properties that increase the chance of developing schizophrenia later in life. However, with a category with such broad and significant roles in brain development, it cannot be predicted which aberrancies will lead to impairments in brain development that increase the risk for schizophrenia later in life. Therefor this category does not support a particular hypothesis, but does emphasize the developmental nature of schizophrenia, starting prenatally.

4.4.2.3 Rank 3: nervous system development

The 'nervous system development' category was on the third rank in the biological process classification. This category involves the processes that facilitate neural development after the ectoderm development (e.g. post-embryonic stages). Being a temporal extension of 'ectoderm development', the list of involved genes overlaps considerably with the 'ectoderm development' category. Therefor the same things can be said about this category: this category does not support a particular hypothesis, but does emphasize the developmental nature of schizophrenia.

4.4.3 Molecular function classification

4.4.3.1 Rank 1: receptor activity

The category 'receptor activity' was the most overrepresented category in the molecular function classification. The category is quite ambiguous as it encompasses any receptor that changes the activity of its cell. This includes receptors in immune cells, cell communication, kinase activity and GABA/Glutamate receptors. Of all sub-categories of 'receptor activity', the categories 'ligand-dependent nuclear receptor activity' (rank 26) and 'glutamate receptor activity' (rank 28) (table 4) were significantly overrepresented according to the criteria of this study. 'Ligand-dependent nuclear receptor activity' remains an ambiguous category as it plays a role in every cell with an active nucleus. The category 'glutamate receptor activity' is more interesting as glutamate systems have been proposed to play a role in psychosis (Egerton, Fusar-Poli, & Stone, 2012). However, glutamate is an excitatory neurotransmitter and is the most prominent neurotransmitter in the nervous system. It is present in most brain areas and affects neurotransmission of all brain systems. Aberrant glutamate systems could relate to most existing theories about the etiology of schizophrenia (Egerton et al., 2012), for this reason it is hard to assign a particular theory about the etiology of schizophrenia to this category. However, a possibility that the glutamate system could play a role in schizophrenia has a good foundation. Abnormal glutamate levels, effects of glutamate neurotransmission on dopamine neurons (Bloemen et al., 2011; Gluck et al., 2002) and expression and localization of proteins regulating glutamate transmission (Eastwood & Harrison, 2005; Watis, Chen, Chua, Chong, & Sim, 2008) have been observed in schizophrenia subjects or subjects with an ultra-high risk of psychosis. However, depending on the glutamate related aberrancy observed, different systems are affected, each supporting various theories of the etiology schizophrenia (Eastwood & Harrison, 2005; Gluck et al., 2002; Hayashi-Takagi et al., 2010; Watis et al., 2008). Despite the broad involvement of glutamate in neural systems, it has been proposed as a target for the treatment of schizophrenia (Chaki & Hikichi, 2011). The category 'glutamate receptor activity' seems to support all theories discussed in this study or could be an additional factor involved in the etiology of schizophrenia.

4.4.3.2 Rank 2: structural molecule activity

The category on the second rank of the molecular function classification was 'structural molecule activity'. This category has four sub categories, 'extracellular matrix structural constituent', 'structural constituent of cytoskeleton', 'structural constituent of myelin sheath' and 'structural constituent of ribosome'. The 'structural constituent of cytoskeleton' category reached the fifth rank in the AS GO-analysis, the other sub categories fell outside the inclusion criteria of this study. Structural constituents of the cytoskeleton are important for proper maintenance of synapses and axons but also for the propagation and guidance of growth cones. Impairments in this function could compromise synapse formation and integrity. The category 'extracellular matrix structural constituent' is important for cell adhesion and therefor for synapse maintenance as well. The category 'structural constituent of myelin sheath' is interesting because it directly relates to myelination, clearly supporting the myelin hypothesis. The role of the 'structural constituent of ribosome' with respect to schizophrenia is too broad and unpredictable to elaborate on in this study. Is short, it seems this category further emphasizes the developmental role of the etiology of schizophrenia and supports the pruning and myelin hypothesis.

4.4.3.3 Rank 3: receptor binding

The category 'receptor binding' was ranked third in the molecular function classification. Of the four sub-categories, none fell into the selection criteria. Receptor binding plays a role in all biological mechanisms underlying all theories about schizophrenia discussed above. This category is too ambiguous to support any particular hypothesis.

4.5 Summary

The preliminary exploration of the AS GO-analysis showed promising results. The AS GO-analysis is a auspicious candidate for further study, validation and development. When fully developed, this method can extend the interpretation of GWAS results considerably.

Of all classifications, the pathway classification seems to have given the best results. Each functional category was easy to put in perspective of the theories of etiology of schizophrenia. However, this could be partly due to the high percentage of unclassified genes and consequently high publication bias.

A summary of the top three ranked categories per classification and the theories they support is summarized in table 6, the rationale behind it is discussed in section 4.4. The pruning and myelin hypothesis are the most supported theories about the etiology of schizophrenia. Following these theories in the search for therapeutical targets to prevent the development of schizophrenia could prove fruitful. However, all theories received some support from the AS GO-analysis. This can be explained by several non-mutually exclusive premises:

1. Schizophrenia is an ensemble of several sub-disorders that each have a different combination of disregulated biological mechanisms with different underlying genetic risk factors.

2. The development of schizophrenia is caused by interplay between genes and environment. Specific environmental factors need to be combined with specific genetic risk factors in order to result in the development of schizophrenia. Hence, genetic risk factors that affect several biological mechanisms can contribute to schizophrenia development depending on the environmental factors.

3. The risk to develop schizophrenia only increases when multiple biological mechanisms are affected to a certain extent. The pathological combination of affected biological mechanisms is variable. That would explain the absence of an affected biological mechanism (e.g. myelination, synapse formation and pruning or dopamine innervation) in all patients.

In order to rule out one or more of these premises, several challenges must be overcome. Schizophrenia has to be sub-categorized in terms of psychological and physiological symptoms. Currently the DSM IV (Rader, 2000) only provides course descriptions of a few sub-categories of schizophrenia in terms of psychological symptom. Ever since schizophrenia was described scientist tried to classify patient sub-groups on psychological symptoms with limited success (Berner, 1997; Peralta Martin & Cuesta Zorita, 1994).

Classification of subgroups in terms of physiological symptoms is also challenging. Many physiological symptoms, such as synapse number, cannot be measured in living individuals. EEG and MRI provided only course sub-groups classifications with limited importance to the treatment of schizophrenia (Simon et al., 2010; E. H. Simpson et al., 2010; M. M. Singh, Kay, & Opler, 1987). Another factor that obstructs the classification of sub-groups is the possibility that a symptom sub-group has several possible etiopathologies, and that an etiopathology can show different symptom sub-groups. Because of this, schizophrenia needs to be approached from both the physiological and the psychological front in order to reach a valid and useful classification of schizophrenia sub-groups.

Summary of the support for theories about the etiology of schizophrenia					
	Pruning	**Myelin**	**GABA**	**Dopamine**	**Gestational insult**
Pathway classification					
Wnt signaling pathway	+	+	-	-	-
Cadherin signaling pathway	+	+	+	-	-
5HT2 type receptor mediated signaling pathway	-	-	-	-	+
Biological process classification					
neurological system process	-	-	+	+	-
ectoderm development	N/A	N/A	N/A	N/A	N/A
nervous system development	N/A	N/A	N/A	N/A	N/A
Molecular function					
receptor activity	+	+	+	+	+
structural molecule activity	+	+	-	-	-
receptor binding	N/A	N/A	N/A	N/A	N/A
Total categories supporting the theory	4	4	3	2	2

Table 6: Summary of the support for the different theories about the etiology of schizophrenia according to the AS GO-analysis. The different theories are described in section 1.2. The theory 'Gestational insults' is described in section 4.2.4 and in (Ballon et al., 2008; Brown & Derkits, 2010).

4.6 Future study

Further qualitative assessment of the AS GO-analysis is needed. The absence of a golden standard in this study is regrettable. Because of the unknown etiology of schizophrenia it is hard to determine whether a category in the AS GO-analysis is justly lowered in rank compared to the conventional GO-analysis. Using a trait with better defined etiology such as hypercholesterolemia (Jansen, van Wissen, Defesche, & Kastelein, 2002), systemic sclerosis (Martin, Bossini-Castillo, & Martin, 2012) or body length (Visscher, McEvoy, & Yang, 2010) to compare the AS GO-analysis with the GO-analysis will aid in interpreting the rank changes. Additional useful data for validating the AS GO-analysis could come from the field of plant genetics. Molecular and genetic research on plants led to comprehensive gene-ontology lists (see http://www.arabidopsis.org/tools/bulk/go/) that are frequently adopted to interpret the abundance of GWAS on plant traits (Huang et al., 2011). This would allow a more valid assessment of the specificity and sensitivity of the AS GO-analysis.

GO-analysis can be further developed to be suitable for GWAS and its sequel, whole-genome sequencing. Besides including all genes from a GWAS in the AS GO-analysis (section 1.6), additional parameters could be included. Parameters such as functional consequences of SNPs or copy number variation could increase statistical power, specificity and sensitivity to the GO-analysis on GWAS results.

It is a matter of time until the GO-analysis reaches considerable higher quality because of the ever-expanding gene database. From the fact that in some categories, only ±30% of the genes are classified and that ±98% of the genome is coined 'non-coding' DNA, it can be said that the GO-analysis is in its infantile state. When more genes and 'non-coding' DNA regions are functionally characterized and classified, the strength of the GO-analysis will grow substantially and the publication bias will lower. This is a promising prospect for GO-analysis.

A possible extension of the GO-analysis, specifically for GWAS, is to create a 'reference list' from the control group. By applying regression analysis, the AS of SNPs or CNVs with quantifiable traits/symptoms can be determined in the case and control group. The cumulative -log(p) of the AS of each category can be calculated for the control group ('reference list') and the case group ('case list'). Finally, the same binomial statistical method used for the AS GO-analysis can be applied to compare each category of the case list to the reference list.

When GO-ontology analysis for GWAS data has developed to its adult stage and psychiatric disorders can be classified in terms of both symptoms and underlying biological mechanism, a GO-analysis could even be a valuable tool for the prevention and treatment of any polygenic disorder. A microarray of all significant SNPs and a corresponding GO-analysis can be performed on an individual to identify at risk biological systems and calculate an individual's risk to develop a disorder. This could allow better prediction of disease and disease course and allows treatment methods to be selected according to the specific disregulated biological mechanism in an individual. This would be therapy directed towards prevention, early intervention and individually adjusted treatments.

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In the end, this project turned out far too large for a thesis. However, I decided to pursue this project anyway and the amount I learned by pursuing this project grew proportionately. I have no regrets and I am satisfied with the results. I truly believe that my design of the AS GO-analysis holds an important step towards a Gene Ontology analysis suited for GWAS data.

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