UPPSALA
UNIVERSITET

MicroRNA target predictions in candidate genes for schizophrenia

Henrik Sperber

Degree project in biology, 2007
Examensarbete i biologi, 20p, 2007
Biology Education Centre, Uppsala University, and National University of Singapore, Bioinformatics Centre, 4 Kent Ridge Road, Singapore 119213
Supervisor: Martti Tammi

SUMMARY

A microRNA is a kind of relatively small RNA that binds to the untranslated regions of messenger RNAs, leading to translational repression. One microRNA can have several targets, and several different microRNAs can bind to the same messenger RNA. Being discovered relatively recently, many questions remain regarding what processes are regulated by microRNAs. So far it has been shown that microRNAs are involved with e.g. neural growth and new discoveries are constantly made.

Schizophrenia is a mental disorder on which a lot of research has been conducted. The results regarding the factors that lie behind the disorder remain elusive, but it is generally agreed that it is caused by a combination of both genetic and environmental factors. Many studies have been made in order to look for candidate genes and genomic regions related to schizophrenia. However, very little has been published where the approach was to investigate if microRNAs are involved. In this study I have looked for potential microRNA interactions that can be involved in schizophrenia.

I have collected candidate regions from schizophrenia linkage studies and looked for verified microRNA genes in those regions. From other studies on schizophrenia I have collected candidate genes as potential targets for the candidate microRNAs. I have run the gathered material through the target prediction program MicroTar which resulted in 1248 statistically significant predictions. The predictions are presented in a searchable SQL database and can be used for future studies on microRNAs related to schizophrenia. This study has resulted in a list of predictions, which can hopefully be looked into more carefully in future studies aiming for better understanding of what lies behind schizophrenia.

A study has shown that the microRNAs hsa-mir-29b and hsa-mir-29c are differentially expressed in the prefrontal cortex of schizophrenia patients (Perkins, D.O., Jeffries, C.D., Jarskog, L.F., Thomson, J.M., Woods, K., Newman, M.A., Parker, J.S., Jin, J.P. & Hammonds, S.M. 2007, Genome Biol 8:R27). The genes that code for these two microRNAs are located in one of the candidate regions for schizophrenia that I have found in the literature search, supporting the hypothesis that they play an important role in the onset of the disorder. For the two microRNAs target sites on the two genes zinc finger, DHHC-type containing 8 (*ZDHHC8*) and mal, T-cell differentiation protein (*MAL*) were predicted, with a p-value <0.05.

INTRODUCTION

MicroRNA

MicroRNA (miRNA) is a kind of regulatory RNA that has been discovered quite recently, first in *Caenorhabditis elegans* in 1993 (Lee *et al*. 1993) but later on also in a wide array of species (Lagos-Quintana *et al.* 2001). The mature miRNA is relatively short and inhibits the translation process by binding to messenger RNA (Kim and Nam 2006). Today one can only speculate regarding the importance of microRNAs, but there is evidence that they are involved with the onset of various diseases, immunoregulation, and neural growth (Bartel 2004, Cullen 2006, Hornstein and Shomron 2006, Mattick and Makunin 2006, Stark *et al*. 2005). There are plenty of speculations regarding how many microRNAs there are, but so far 533 experimentally verified human microRNAs are listed in miRBase (Griffiths-Jones *et al.* 2006).

MicroRNAs are transcribed in the nucleus by RNA polymerase II or III (Borchert *et al*. 2006, Kim and Nam 2006) as part of a long precursor called primary microRNA (pri-miRNA). One pri-miRNA can contain either one or several miRNAs (Cullen 2006). The length of the pri-miRNA is highly variable ranging from ~200 nucleotides in length up to several thousand (Cullen 2006, Du and Zamore 2005). The mature miRNA is ~22 nt in length and makes up the arm in an imperfect stem-loop sequence in an ~80 nucleotide precursor microRNA (Du and Zamore 2005, Kim and Nam 2006). The pri-miRNA is cleaved by a heterodimer consisting of the enzyme Drosha RNase III endonuclease and a cofactor called the DiGeorge syndrome critical region gene 8 (DGCR8) (Lee *et al*. 2003, Gregory *et al*. 2004). The cleaveage is performed at the base of the stem-loop and liberates a structure known as the microRNA precursor (pre-miRNA) (Han *et al*. 2004, Landthaler *et al*. 2004). The cleavage also results in a 5' and a 3' sequence which are generally degraded in the nucleus (Lee *et al*. 2002). The pre-miRNA is ~60-70 nucleotides in length and forms a hairpin structure with a 2 nucleotide 3' overhang (Basyuk *et al*. 2003, Lee *et al*. 2003). The pre-miRNA is then transported from the nucleus to the cytoplasm by a heterodimer consisting of the export receptor Exportin-5 (Exp5) and the GTP-bound form of its cofactor Ran (Ran-GTP) (Yi *et al*. 2003, Bohnsack *et al*. 2004). The heterodimer recognizes and binds the overhang and the adjacent stem and transports the pre-miRNA to the cytoplasm where the GTP-hydrolysis results in release of the pre-miRNA (Lund *et al*. 2004). In the cytoplasm the pre-miRNA is cleaved by the enzyme Dicer with its cofactor trans-activator RNA (tar)-binding protein (TRBP) (Chendrimada *et al*. 2005). Dicer binds the overhang and removes the terminal loop resulting in an imperfect ~22 nt miRNA/miRNA* duplex (Bernstein *et al*. 2001, Hutvagner *et al*. 2001). The miRNA enters the RNA-induced silencing complex (RISC), whereas the other strand is degraded (Bartel 2004, Du and Zamore 2005, Cullen 2006, Kim and Nam 2006). The choice of strand depends on thermodynamic stability, i.e. the strand whose 5' end is less stably paired is loaded into the RISC. The biogenesis is illustrated in Figure 1.

The miRNA directs RISC to mRNA strands with a complementary nucleotide sequence. Depending on how extensive the similarity is the mRNA will either be degraded (high similarity) or translational repression (partial similarity) will be induced (Kim and Nam 2006). A single miRNA may regulate hundreds of mRNAs with the only thing in common that they have sequences that are complementary to as few as six or seven bases of the miRNA. Therefore the impact of a single miRNA can be very high.

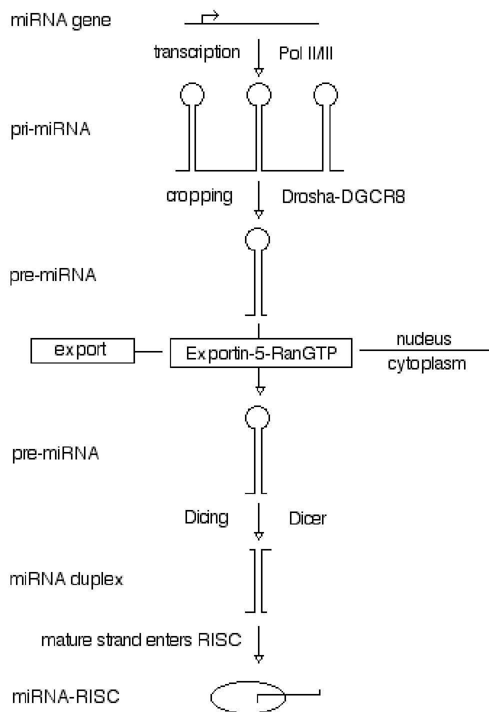

Figure 1. An overview of the miRNA biogenesis: The microRNA is transcribed by Pol II or III (Borchert *et al.* 2006, Kim and Nam 2006) resulting in a primary microRNA (pri-miRNA), the Drosha–DGCR8 complex crops the primary microRNA (Lee *et al.* 2003, Gregory *et al.* 2004) which results in a microRNA precursor stem-loop sequence (pre-miRNA), transportation of the microRNA precursor to the cytoplasm is performed by Exportin-5–RanGTP (Yi *et al.* 2003, Bohnsack *et al.* 2004), in the cytoplasm it is cleaved by Dicer (Chendrimada *et al.* 2005) before the mature microRNA enters the RNA-induced silencing complex (RISC) (Cullen 2006).

MicroRNAs can be found anywhere in the genome, as a single miRNA far away from other genes or in clusters. However, about 50% of all miRNAs are expected to be found in introns of other genes (Mattick and Makunin 2006). MiRNAs derive from distinct genomic loci as opposed to siRNAs which often derive from e.g. mRNAs and transposons. MiRNA sequences are also almost always conserved in related organisms, whereas endogenous siRNA sequences are rarely conserved (Du and Zamore 2005).

Many miRNA prediction programs rely on evolutionary conservation, but there are also those who only search for the pre-miRNA structure, i.e. stem loop structures (Berezikov *et al.* 2006). Such a prediction is no evidence for an existing miRNA, so it has to be experimentally verified. The same applies to the prediction of miRNA targets. There are currently 763 experimentally verified targets in human listed in TarBase (Sethupathy *et al.* 2006), which can be compared to the number of predictions which can range up to over a thousand for one single miRNA.

Target prediction

MicroRNAs bind to the untranslated region (UTR) of an mRNA (Stark *et al.* 2005). In vitro tests have shown that microRNAs can bind to both the 5'UTR and the 3'UTRs, but target sites have only been verified in the 3'UTRs (Lytle et al. 2007, Kloosterman et al. 2004). The program MicroTar (Thadani and Tammi 2006) calculates the binding energy between a mature microRNA and an mRNA. First the folding of the mRNA is calculated, and then the free energy of the unbound version is calculated and compared with that of the bound version. If the bound version is energetically more favourable, then the mRNA is considered a potential target (Figure 2). In the mRNA input file the coding region is in upper case letters and the UTRs are in lower case. This enables the program to calculate the structure of the entire molecule, but only to do the target prediction on the UTRs.

In order to obtain p-values for the probability that the predictions have not occurred by chance the sequences must first be run through MicroTar again, but with the UTRs of the mRNA sequences shuffled using Altschul & Erickson trinucleotide shuffling (Altschul and Erickson 1985). This shuffling method conserves trinucleotides which keeps the sequence robust. Keeping the coding region of the mRNA unshuffled is important for the calculation of the secondary structure which is necessary for the calculation of the binding energies. By performing the target prediction against RNAs with shuffled sequences in the UTRs, the results can be used to calculate the probability that the same or a more favourable free energy is observed by chance. The statistical calculation is not performed by MicroTar. Instead the p-values are obtained using a MatLab script.

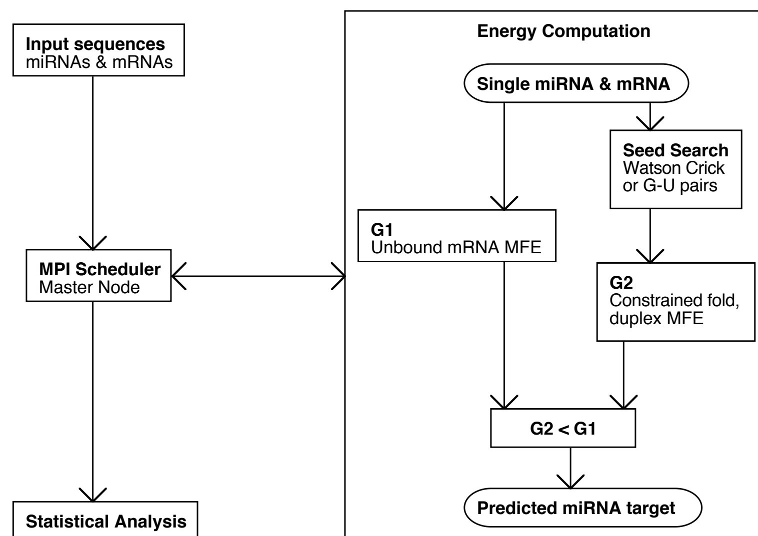

Figure 2. The target prediction program MicroTar requires input files with microRNA and messenger RNA sequences. The energies of the unbound messenger RNAs are calculated and compared to the energies of the bound versions, A target site is predicted if the bound version is energetically favourable. The results can be used for further statistical analysis, which is not performed by MicroTar.

Schizophrenia

Schizophrenia is a mental disorder affecting ~1% of the human population worldwide.
The factors that lie behind the onset of schizophrenia remain largely unclear, but most likely both environmental and genetic factors working together in a complex manner are contributors to the onset of the disease (Danese 2006, Van Den Bogaert *et al*. 2006). It is not known what genes would be involved, nor if, at all, there are actual genes involved, but there have been plenty of speculations regarding what processes are affected, e.g. the synthesis, release, uptake and metabolism of serotonin (Van Den Bogaert *et al*. 2006).

Many searches for candidate genes, loci, and retroviruses have been carried out that have led to many hypotheses about mechanisms involved in schizophrenia (Leonard and Freedman 2006). The findings, however, remain inconclusive.

At the time of the start of this project there were few, if any, published studies on whether miRNAs are involved with the onset of schizophrenia, but plenty of linkage studies had been performed (Danese 2006), resulting in a wide range of candidate regions. The likelyhood that a genetic region is linked to a trait is usually displayed with a LOD score (logarithm of odds, base 10). A LOD score is a measure of the likelyhood that genes or genetic markers are linked. This is expressed as the logarithm of the odds that an observed data set from one or several pedigrees is due to linkage rather than to independent assortment of nonlinked genes.

Basically the LOD score shows how likely it is that two specific genes or markers are inherited together. This can also be applied to the likelyhood of a trait such as schizophrenia being linked to a certain genetic marker.

A LOD score of 3 or higher is generally considered evidence for linkage, which means that the odds of the observed data set being due to linkage rather than to independent assortment of nonlinked genes is 1000 to 1 or higher. Even though the calculation can be performed on one pedigree, several pedigrees are generally needed to obtain a LOD score of 3.

After commencing the project, a study of expression of miRNAs in the prefrontal cortex of schizophrenia patients was published (Perkins *et al*. 2007).

Project aim

The aim of this project was to make predictions of interactions between candidate microRNAs and messenger RNAs related to schizophrenia. Candidate microRNAs were collected from candidate regions for schizophrenia in the genome. The candidate regions were gathered from linkage study reports. The data were presented in a database.

RESULTS

I went through the litterature for schizophrenia linkage studies and made a list of candidate regions for schizophrenia with a LOD score of 3 or higher. Then I checked in MiRBase (Griffiths-Jones 2004, Griffiths-Jones *et al.* 2006) what microRNAs could be found within the relevant regions and collected the microRNAs in a list of candidate microRNAs for schizophrenia. To the list I also added the microRNAs that had been found to be differently expressed in the prefrontal cortex of patients with schizophrenia compared to healthy controls. In total I listed 84 candidate microRNAs from chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 20 and X. A random selection of microRNAs along with the closest genomic marker are presented in Table 1.

There are many published linkage studies where certain genomic regions are found to be linked to schizophrenia. In a linkage study the entire genome is mapped with markers, and differences between healthy and ill subjects can be linked to certain markers. The linkage between a trait, e.g. schizophrenia, and a marker is represented by a LOD score. A LOD score of 3 or higher is considered evidence for linkage. In the candidate regions a microRNA was considered a candidate, if it was within a specified region with a LOD score higher than 3, or if the marker closest to the microRNA had a LOD score higher than 3.

Table 1. Random selection of microRNAs from candidate regions for schizophrenia in the genome.

MicroRNA	Chromosome	Location (Mb)	Closest genomic marker	Reference
hsa-mir-200b	1	10.9	D1S1612	Abecasis *et al*. 2004
hsa-mir-29c	1	206.0	D1S2891	Hovatta *et al.* 1999
hsa-mir-562	2	23.3	D2S427	Paunio *et al.* 2001
hsa-mir-28	3	189.9	D3S2418	Klei *et al*. 2005
hsa-mir-579	5	32.4	D5S111	Silverman *et al*. 1996
hsa-mir-146a	5	159.8	D5S422	Gurling *et al*. 2001
hsa-mir-597	8	9.6	D8S503	Gurling *et al*. 2001
hsa-mir-603	10	24.6	D10S582	Faraone *et al*. 1998
hsa-mir-100	11	121.5	D11S934	Gurling *et al*. 2001
hsa-mir-621	13	40.3	D13S894	Camp *et al*. 2001
hsa-mir-623	13	98.8	D13S174	Blouin *et al*. 1998
hsa-mir-626	15	39.8	D15S1042	Kohn *et al*. 2004
hsa-mir-627	15	40.3	D15S659	Kohn *et al*. 2004

The literature search also resulted in a list of 62 candidate genes for schizophrenia. A gene is a candidate if there is any reason to believe that it is involved with schizophrenia. This includes being located in a candidate region, or being expressed differently in schizophrenia patients than in healthy subjects. A random selection of candidate genes are listed in Table 2. The genes were found in a total of 168 splice forms in the Ensembl database (Hubbard et al. 2007).

Table 2. A random selection of candidate genes for schizophrenia.

Candidate gene	Reference
ADSS	Iwamoto and Kato 2006
AKT1	Lipska *et al*. 2006
APOBEC3B	Iwamoto and Kato 2006
CHI3LI	Zhao *et al.* 2007
COMT	Danese 2006, Lipska *et al*. 2006
DAOA	Danese 2006,
DATF1	Iwamoto and Kato 2006
DISC1	Danese 2006, Lipska *et al*. 2006
DISC2	Danese 2006,
DRD3	Crocq *et al*. 1992
FEZ1	Lipska *et al*. 2006
GNAL	Corradi *et al*. 2005
GRIN1	Begni *et al.* 2003
MTHFR	Muntjewerff, *et al*. 2005
PMX2B	Toyota *et al*. 2004
RTN4R	Sinibaldi *et al.* 2004
TAAR6	Duan *et al.* 2004
ZDHHC8	Chen, W.Y. *et al*. 2004

Running the 84 microRNAs against the 168 mRNA splice variants using MicroTar resulted in 6185 predicted targets. An example of a target prediction is shown in Figure 3. The statistical analysis with the shuffled sequences resulted in 1248 of the original 6185 predictions having a p-value of less than 0.05, suggesting they were real. Several times multiple targets were predicted for a single microRNA-mRNA pair, but the p-values were calculated only for each single prediction. Considering the uncertainty of how the microRNA-mRNA interaction actually works, it would be unreasonable to take the number of predicted interactions for a specific microRNA-mRNA pair into consideration in the calculation of the p-value. Also, there is very little known about how the gene silencing by microRNAs works, so the exact location of the binding site gives no clues to decide if the target prediction may be accurate. All the predicted targets were listed in a structured query language (SQL) database. SQL is a common language for managing databases. The database is available from the author upon request.

```
miRNA           : hsa-miR-29b
Query sequence: UAGCACCAUUUGAAAUCAGUGUU
miRNA Length    : 23
mRNA            : MAL
mRNA Length     : 1106


           610
           |
 mRNA      3'  ttgtggt  5'
 miRNA     5'  AGCACCA  3'
           |
           2

 mRNA Monomer Energy       : -399.12
 mRNA::miRNA Dimer Energy : -407.25
 Dimer-Monomer Difference : -8.13
```

Figure 3. Example of a target prediction. Hsa-mir-29b is run against *MAL* usign MicroTar. An interaction is predicted since the energy difference between the bound and the unbound form is negative (-8.13).

The microRNAs hsa-mir-29b and hsa-mir-29c are the only microRNAs found to be expressed at lower levels in the prefrontal cortex of schizophrenia patients than in that of healthy controls (Perkins *et al.* 2007). These microRNAs were also located in a candidate region. This made these microRNAs particularly strong candidates for being involved with schizophrenia. The target prediction resulted in predicted targets for hsa-mir-29b and hsa-mir-29c on the genes zinc finger, DHHC-type containing 8 (*ZDHHC8*) and mal, T-cell differentiation protein (*MAL*). For both hsa-mir 29b and hsa-mir-29c there was one statistically significant target prediction for *ZDHHC8* and one for all 3 spliceforms of *MAL*. *ZDHHC8* and *MAL* were selected as candidate genes based on genomic location. Their expression has not been studied in schizophrenia patients.

Tarbase (Sethupathy *et al.* 2006) was searched for any experimentally verified targets of the candidate microRNAs. Verified targets existed (Table 3), but among them there were no candidate genes for schizophrenia.

Table 3. TarBase results of target genes for candidate microRNAs.

MicroRNA	Gene	Direct Support	Reference
Hsa-mir-124	*MTPN*	*In vitro* reporter gene assay (Luciferase) and immunoblotting	Krek *et al*, 2005
Hsa-mir-124	*MAPK4*	Immunoblotting	Krek *et al*, 2005
Hsa-mir-15	*DMTF1*	*In vitro* reporter gene assay (Luciferase)	Kiriakidou *et al*, 2004
Hsa-mir-15	*BCL2*	*In vitro* reporter gene assay (Luciferase)	Cimmino *et al*, 2005
Hsa-mir-16	*CGI-38*	*In vitro* reporter gene assay (Luciferase)	Kiriakidou *et al,* 2004
Hsa-mir-16	*BCL2*	*In vitro* reporter gene assay (Luciferase)	Cimmino *et al*, 2005
Hsa-mir-32	*PFV-1*	*In vitro* antisense LNA inhibition of miRNA and *in vitro* reporter gene assay (GFP)	Lecellier *et al*, 2005
Hsa-mir-29b	*MCL1*	*In vitro* reporter gene assay (Luciferase)	Mott *et al*, 2007
Hsa-let-7a	*NF2*	*In vitro* reporter gene assay (Luciferase)	Meng *et al*, 2007

DISCUSSION

Quite a lot of research has been done on schizophrenia, so it is surprising that so little has been published on the involvement of microRNAs, especially considering that microRNAs have been found to be involved with neural growth (Stark *et al*. 2005). Hopefully the predictions that I have made can provide a good starting point for continued studies as the predictions need to be verified experimentally. After all, with only 763 verified microRNA targets in human (Sethupathy *et al*. 2006), and with little knowledge of how many more exist, it is difficult to know for sure how well a target prediction program works, making experimental verification necessary (Rajewsky 2006). Even if all the predictions would turn out to be correct that still gives no guarantee that the interactions have anything to do with schizophrenia since the candidate genes are only candidates and could potentially have nothing to do with schizophrenia. However, the results presented here are a step on the way in a field where little research has been conducted. There are very few other studies to compare any results with. But with 1248 statistically significant predictions for interactions there is a good chance that some essential components have been discovered, and the results provide an excellent foundation for planning new experiments, such as investigating how manipulated microRNA levels affect the expression of the predicted targets.

It was interesting that the study on expression of microRNAs in the prefrontal cortex of schizophrenia patients (Perkins *et al*. 2007) would include two microRNAs close to a genomic marker that had received a high LOD score in a linkage study for schizophrenia. Being suggested candidates by two studies, hsa-mir-29b and hsa-mir-29c should be worth further studying. It should of course be mentioned that most of the other microRNAs that were expressed differently in that study were located nowhere near any of the candidate regions. It is difficult to say if it is a negative result that the two different studies only support each other when it comes to two microRNAs, or if the reason for these two microRNAs being supported is that in fact they play a vital role and the others do not. It will therefore be interesting to see future studies on the involvement of microRNAs in schizophrenia and if they will get positive results for hsa-mir-29b and hsa-mir-29c as well.

After the project was finished some more studies were published in the field of microRNAs related to schizophrenia (Hansen *et al*. 2007, Beveridge *et al*. 2008, Carter *et al*. 2008, Stark *et al*. 2008), allthough the research in the field is still very limited. One study (Beveridge *et al*. 2008) found that hsa-mir-181b was upregulated in the temporal cortex of schizophrenia patients. Hsa-mir-181b was not part of my list of candidate microRNAs, nor was it found to be differentially expressed in the prefrontal cortex of schizophrenia patients (Perkins *et al*. 2007).

A linkage study specifically designed to investigate the linkage of locations of microRNAs to schizophrenia came up with results suggesting hsa-mir-206 and hsa-mir-198 as candidates (Hansen *et al*. 2007). Also this time there was no support from my study for those particular microRNAs being involved with schizophrenia. However, hsa-mir-206 and hsa-mir-198 are predicted to bind to genes of the pathogenes causing influenza, rubella and polio (Carter *et al*. 2008). According to the same study maternal viral or parasitic infection during pregnancy is a risk factor for the subsequent development of schizophrenia in the adult offspring. The hypothesis that hsa-mir-206 and hsa-mir-198 are involved with shizophrenia by interacting with pathogens is interesting, but requires further supporting evidence.

Instead of looking at specific microRNAs one study looked at the correlation between schizophrenia susceptibility and downregulation of *Dgcr8* (Stark *et al*. 2008). There is a common microdeletion on chromosome 22, which shows a correlation with the incidence of emotional problems and schizophrenia. *Dgcr8,* which is important in the biogenesis of microRNAs, is located in that particular region on chromosome 22. The group generated mice with a similar chromosomal deficiency. The mice showed a significant difference in behavior compared to the wild type, as well as a decrease of *Dgcr8* in pre-frontal cortex and alterations in microRNA biogenesis.

It is still uncertain how microRNAs are involved with schizophrenia, but there is evidence supporting that they are at least involved. So far there are few but promising results that have been published in this new field. Hopefully more experiments will be carried out to examine further how microRNAs are involved with schizophrenia.

MATERIALS AND METHODS

Candidate microRNAs

A number of linkage studies on schizophrenia have been published over the years (Hamshere *et al.* 2006, Brzustowicz *et al.* 2000, Ekelund *et al*. 2000, Hamshere *et al*. 2005, Millar *et al*. 2000, Williams *et al*. 1999, Levinson *et al*. 2000, Gurling *et al*. 2001, Paunio *et al*. 2001, Camp *et al*. 2001, Fallin *et al*. 2003, Williams *et al.* 2003, Abecasis *et al*. 2004, Pimm *et al*. 2005, Arinami *et al*. 2005, Hovatta *et al*. 1999, Brzustowicz *et al*. 1999, Lerer *et al*. 2003, Sklar *et al*. 2004, Park *et al*. 2004, Saviouk *et al*. 2005, Lambert *et al*. 2005, Kohn *et al*. 2004, Klei *et al*. 2005, Faraone *et al*. 1998, Silverman *et al*. 1996, Straub *et al*. 1998, Stöber *et al*. 2002, Blouin *et al*. 1998, Moises *et al*. 1995). I have collected a number of articles through searches on PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and OMIM (http://www.ncbi.nlm.nih.gov/omim/). My approach was to read as many articles as time allowed. From the studies I looked closer at the markers with a LOD-score of 3 or higher. A LOD score of 3 or higher is considered evidence for linkage. The locations of the markers were verified using the database UniSTS (http://www.ncbi.nlm.nih.gov/sites/entrez?db=unists/), and any miRNAs close to the markers where found using MiRBase (Griffiths-Jones 2004, Griffiths-Jones *et al.* 2006), from where the mature sequences were retrieved in fasta format. A microRNA was considered close to a marker if it was within a specified region with a LOD-score of 3 or higher. In the cases where such a region was not specified a microRNA was selected if the marker closest to it had a LOD-score of 3 or higher.

Candidate messenger RNAs

I made a list of candidate genes for schizophrenia through a literature search (Rudduck *et al*. 1985, Crocq *et al*. 1992, Harrington *et al*. 1995, Arranz *et al*. 1998, Begni *et al*. 2003, Zhang and Brodt 2003, Chen, Q. *et al*. 2004, Chen, W.Y. *et al*. 2004, Duan, *et al.* 2004, Esau *et al*. 2004, Kiriakidou *et al*. 2004, Sinibaldi *et al*. 2004, Toyota *et al*. 2004, Corradi *et al*. 2005, Johnson *et al*. 2005, Lecellier *et al.* 2005, Muntjewerff *et al*. 2005, O'Donnell *et al*. 2005, Pimm *et al*. 2005, Hossain *et al*. 2006, Pekarsky *et al*. 2006, Reif *et al*. 2006, Volinia *et al*. 2006, Meng *et al*. 2007, Saunders *et al*. 2007, Wood *et al*. 2007, Zhao *et al*. 2007). A gene was considered a candidate if there was any reason to believe that it is involved with schizophrenia. The splice forms were retrieved from Ensembl (Hubbard et al., 2007). The sequences were obtained in FASTA format with the coding region in uppercase letters and the untranslated regions (UTR) in lower case. For the sequences that lack 3'UTRs a 594 base pair long flanking region was added, in lower case letters. The length was chosen since it was the median length of the mRNA sequences with existing 3'UTRs.

Verified Targets

Tarbase (Sethupathy *et al.* 2006), a database with experimentally verified microRNA targets, was systematically checked for verified targets of the candidate microRNAs. The references were checked to see if the target gene was considered a candidate gene for schizophrenia.

Target prediction

The target prediction was performed using MicroTar on a server with 24 central processing units (CPUs). The more CPUs, the faster the calculations can be performed. The input files were a list of FASTA formatted miRNA sequences from miRBase (Griffiths-Jones 2004, Griffiths-Jones *et al.* 2006) and a list of FASTA formatted mRNA sequences from Ensembl (Hubbard et al., 2007). 84 mature microRNA sequences were run against a total of 168 splice forms of 62 mRNAs.

In order to calculate the probability of a target prediction to occur by chance I needed to see how strong the target predictions were when the binding sites where randomised. To do this MicroTar was run again with altered input files to see what the probability is that a prediction would occur by chance. For this second run the microRNA input file remained the same, but in the mRNA input file the nucleotide sequences in the flanking regions and UTRs were shuffled using Altschul shuffling (Altschul and Erickson 1985) in order to make the binding sequence random, but keep the tertiary structure as intact as possible.

I extracted the negative normalised free energies (NNFEs) from the output data of the MicroTar run with the shuffled mRNA input file. A strong NNFE indicates a strong microRNA-mRNA interaction and accordingly a strong target prediction. I used the Matlab script on the NNFEs obtained from the second microTar run to compute an empirical cumulative distribution function and parameters 'b' and 'a' (named 'theta' and 'zeta' in Figure 4) as in equations (1) and (2) according to instructions from the MicroTar paper (Thadani and Tammi

2006). The empirical cumulative distribution function shows how likely it is for a target prediction to occur even if the UTRs are shuffled, i.e. it gives an estimate of how strong an NNFE has to be in order not to be considered predicted by chance.

The empirical cumulative distribution function is however not very easy to interpret in its original form so it can be converted to be easier to understand. The function can be described by two parameters: 'b' and 'a' for the original function, and 'm' and 'c' for the converted function. The parameters 'm' and 'c' in equations (1) and (2) are obtained from a transformation of the distribution function to a straight line on the form $y = mx + c$. The parameters 'm' and 'c' are never used in the calculations. They are only mentioned to clarify how the parameters 'b' and 'a' describe the empirical cumulative distribution function.

The purpose of this statistical analysis is to investigate the likelyhood of an interaction to be predicted by chance. The MatLab script was obtained from Rahul Thadani, the creator of MicroTar.

$$b = -1/m \quad (1)$$

$$a = cb \quad (2)$$

```
function [P, Y, x, theta, zeta] = empcdf(nnfe)
% Empirical CDF
%
samplesize = length(nnfe);
minimum = min(nnfe);
maximum = max(nnfe);
width = (maximum-minimum)/samplesize;
x = width:width:maximum;
Y = [];
for k = 1:length(x)
    Y(k) = length(nnfe(nnfe < x(k)));
end
Y = Y/samplesize;
figure, plot(x,Y,'.'), grid on;
P = polyfit(x,log(-log(Y)),1);
theta = -1/P(1);
zeta = P(2)*theta;
```

Figure 4. MatLab script for computing the parameters 'b' (theta) and 'a' (zeta) for the statistic calculations. The input data is the negative normalised free energies (nnfe) from the target predictions on mRNAs with shuffled untranslated regions.

To finally get the p-values ($P[Z \geq g_n]$) for all target predictions the parameters 'b' and 'a' were used in equation (3) along with the negative normalised free energies (g_n) of each prediction on the unshuffled mRNA sequences.

$$P[Z \geq g_n] = 1 - \exp(-\exp((a - g_n)/b)) \quad (3)$$

Explaining it in a more simple manner the NNFEs from the mRNA sequences with shuffled flanking regions and UTRs are used as data input in a MatLab script (Figure 4) to obtain the parameters 'b' and 'a'. The parameters 'b' and 'a' are used in equation (3) to obtain a p-value for each target prediction where the flanking regions and UTRs of the mRNA have not been shuffled.

The predictions were put together in an SQL database in the structure of three tables, with microRNAs, mRNAs, and the predicted interactions. The database can be obtained from the author.

ACKNOWLEDGMENTS
I would like to thank my supervisor Martti for giving me this interesting field of research, as well as a pleasant working environment making this semester a truly rewarding and appreciated one. Rahul, the creator of MicroTar has also been a tremendous help to me explaining how the programs work and helping me whenever my proficiency in UNIX was too weak. Björn, my brother for helping me with SQL. I would finally like to thank my dear friend George, whom I lived with in Singapore, for help with practicalities and all kinds of support.

REFERENCES

Abecasis, G.R., Burt, R.A., Hall, D., Bochum, S., Doheny, K.F., Lundy, S.L., Torrington, M., Roos, J.L., Gogos, J.A. & Karayiorgou, M. 2004. Genomewide scan in families with schizophrenia from the founder population of Afrikaners reveals evidence for linkage and uniparental disomy on chromosome 1. Am J Hum Genet 74: 403-17

Altschul, S.F. & Erickson, B.W. 1985. Significance of nucleotide sequence alignments: A method for random sequence permutation that preserves dinucleotide and codon usage. Mol Biol Evol 2: 526-38

Arinami, T., Ohtsuki, T., Ishiguro, H., Ujike, H., Tanaka, Y., Morita, Y., Mineta, M., Takeichi, M., Yamada, S., Imamura, A., Ohara, K., Shibuya, H., Ohara, K., Suzuki, Y., Muratake, T., Kaneko, N., Someya, T., Inada, T., Yoshikawa, T., Toyota, T., Yamada, K., Kojima, T., Takahashi, S., Osamu, O., Shinkai, T., Nakamura, M., Fukuzako, H., Hashiguchi, T., Niwa, S.I., Ueno, T., Tachikawa, H., Hori, T., Asada, T., Nanko, S., Kunugi, H., Hashimoto, R., Ozaki, N., Iwata, N., Harano, M., Arai, H., Ohnuma, T., Kusumi, I., Koyama, T., Yoneda, H., Fukumaki, Y., Shibata, H., Kaneko, S., Higuchi, H., Yasui-Furukori, N., Numachi, Y., Itokawa, M., Okazaki, Y., Japanese Schizophrenia Sib-Pair Linkage Group. 2005. Genomewide high-density SNP linkage analysis of 236 Japanese families supports the existence of schizophrenia susceptibility loci on chromosomes 1p, 14q, and 20p. Am J Hum Genet 77: 937-44

Arranz, M.J., Munro, J., Owen, M.J., Spurlock, G., Sham, P.C., Zhao, J., Kirov, G., Collier, D.A. & Kerwin, R.W. 1998. Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine. Mol Psychiatry 3: 61-6

Bartel, D.P. 2004. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. Cell 116: 281-297

Basyuk, E., Suavet, F., Doglio, A., Bordonne, R., Bertrand, E. 2003. Human let-7 stem-loop precursors harbor features of RNase III cleavage products. Nucleic Acids Res 31: 6593-7

Begni, S., Moraschi, S., Bignotti, S., Fumagalli, F., Rillosi, L., Perez, J. & Gennarelli, M. 2003. Association between the G1001C polymorphism in the GRIN1 gene promoter region and schizophrenia. Biol Psychiatry 53: 617-9

Berezikov, E., Cuppen, E. & Plasterk, R.H. 2006. Approaches to microRNA discovery. Nat Genet 38 Suppl: S2-7

Bernstein, E., Caudy, A.A., Hammond, S.M., Hannon, G.J., 2001., Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409: 363-6

Bernstein, E. & Allis, C.D. 2005. RNA meets chromatin. Genes Dev 19:1635-55

Beveridge, N.J., Tooney, P.A., Carroll, A.P., Gardiner, E., Bowden, N., Scott, R.J., Tran, N., Dedova, I., Cairns, M.J. 2008. Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. Hum Mol Genet 17: 1156-68

Blouin, J.L., Dombroski, B.A., Nath, S.K., Lasseter, V.K., Wolyniec, P.S., Nestadt, G., Thornquist, M., Ullrich, G., McGrath, J., Kasch, L., Lamacz, M., Thomas, M.G., Gehrig, C., Radhakrishna, U., Snyder, S.E., Balk, K.G., Neufeld, K., Swartz, K.L., DeMarchi, N., Papadimitriou, G.N., Dikeos, D.G., Stefanis, C.N., Chakravarti, A., Childs, B., Housman, D.E., Kazazian, H.H., Antonarakis, S. & Pulver, A.E. 1998. Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. Nat Genet 20: 70-3

Bohnsack, M.T., Czaplinski, K., Gorlich, D. 2004. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10: 185-91

Borchert, G.M., Lanier, W. & Davidson, B.L. 2006. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 13: 1097-101

Brzustowicz, L.M., Honer, W.G., Chow, E.W., Little, D., Hogan, J., Hodgkinson, K. & Bassett, A.S. 1999. Linkage of familial schizophrenia to chromosome 13q32. Am J Hum Genet 65: 1096-103

Brzustowicz, L.M., Hodgkinson, K.A., Chow, E.W., Honer, W.G., Bassett, A.S. 2000. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. Science 288: 678-82

Camp, N.J., Neuhausen, S.L., Tiobech, J., Polloi, A., Coon, H. & Myles-Worsley, M. 2001. Genomewide multipoint linkage analysis of seven extended Palauan pedigrees with schizophrenia, by a Markov-chain Monte Carlo method. Am J Hum Genet 69: 1278-89

Carter, C.J. 2008. Schizophrenia Susceptibility Genes Directly Implicated in the Life Cycles of Pathogens: Cytomegalovirus, Influenza, Herpes simplex, Rubella and Toxoplasma gondii. Schizophr Bull, doi:10.1093/schbul/sbn054

Chen, Q., He, G., Qin, W., Chen, Q.Y., Zhao, X.Z., Duan, S.W., Liu, X.M., Feng, G.Y., Xu, Y.F., St Clair, D., Li, M., Wang, J.H., Xing, Y.L., Shi, J.G. & He, L. 2004. Family-based association study of synapsin II and schizophrenia. Am J Hum Genet 75: 873-7

Chen, W.Y., Shi, Y.Y., Zheng, Y.L., Zhao, X.Z., Zhang, G.J., Chen, S.Q., Yang, P.D. & He, L. 2004. Case-control study and transmission disequilibrium test provide consistent evidence for association between schizophrenia and genetic variation in the 22q11 gene ZDHHC8. Hum Mol Genet 13: 2991-5

Chendrimada, T.P., Gregory, R.I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K., Shiekhattar, R. 2005. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436: 740-4

Cimmino, A., Calin, G.A., Fabbri, M., Iorio, M.V., Ferracin, M., Shimizu, M., Wojcik, S.E., Ageilan, R.I., Zupo, S., Dono, M., Rassenti, L., Alder, H., Volinia, S., Liu, C.G., Kipps, T.J., Negrini, M., Croce, C.M. 2005. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 102: 13944-9

Corradi, J.P., Ravyn, V., Robbins, A.K., Hagan, K.W., Peters, M.F., Bostwick, R., Buono, R.J., Berrettini, W.H. & Furlong, S.T. 2005. Alternative transcripts and evidence of imprinting of GNAL on 18p11.2. Mol Psychiatry 10: 1017-25

Crocq, M.A., Mant, R., Asherson, P., Williams, J., Hode, Y., Mayerova, A., Collier, D., Lannfelt, L., Sokoloff, P., Schwartz, J.C., et al. 1992. Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. J Med Genet 29: 858-60

Cullen, B.R. 2006. Viruses and microRNAs. Nat Genet 38 Suppl: S25-30

Danese, A. 2006. A public health genetic approach for schizophrenia. Epidemiol Psichiatr Soc 15: 185-93

Du, T. & Zamore, P.D. 2005. microPrimer: the biogenesis and function of microRNA. Development 132: 4645-52

Duan, J., Martinez, M., Sanders, A.R., Hou, C., Saitou, N., Kitano, T., mowry, B.J., Crowe, R.R., Silverman, J.M., Levinson, D.F. & Gejman, P.V. 2004. Polymorphisms in the trace amine receptor 4 (TRAR4) gene on chromosome 6q23.2 are associated with susceptibility to schizophrenia. Am J Hum Genet 75: 624-38

Ekelund, J., Lichtermann, D., Hovatta, I., Ellonen, P., Suvisaari, J., Terwilliger, J.D., Juvonen, H., Varilo, T., Arajärvi, R., Kokko-Sahin, M.L., Lönnqvist, J. & Peltonen, L. 2000. Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. Hum Mol Genet 9: 1049-57

Esau, C., Kang., X., Peralta, E., Hanson, E., Marcusson, E.G., Ravichandran, L.V., Sun, Y., Koo, S., Perera., R.J., Jain., R., Dean, N.M., Freier, S.M., Bennett, C.F., Lollo, B. & Griffey, R. 2004. MicroRNA-143 Regulates Adipocyte Differentiation. J Biol Chem 279: 52361-5

Fallin, M.D., Lasseter, V.K., Wolyniec, P.S., McGrath, J.A., Nestadt, G., Valle, D., Liang, K.Y. & Pulver, A.E. 2003. Genomewide linkage scan for schizophrenia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 10q22. Am J Hum Genet 73: 601-11

Faraone, S.V., Matise, T., Svrakic, D., Pepple, J., Malaspina, D., Suarez, B., Hampe, C., Zambuto, C.T., Schmitt, K., Meyer, J., Markel, P., Lee, H., Harkavy Friedman, J., Kaufmann, C., Cloninger, C.R. & Tsuang, M.T. 1998. Genome scan of European-American schizophrenia pedigrees: results of the NIMH Genetics Initiative and Millennium Consortium. 81: 290-5

Gregory, R.I., Yan, K.P., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., Shiekhattar, R. 2004. The Microprocessor complex mediates the genesis of microRNAs. Nature 432: 235-40

Griffiths-Jones, S. 2004. The microRNA Registry. Nucleic Acids Res 32: D109-11

Griffiths-Jones, S., Grocock, R.J., van Dongen, S., Bateman, A. & Enright, A.J. 2006. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 34: D140-4

Gurling, H.M., Kalsi, G., Brynjolfson, J., Sigmundsson, T., Sherrington, R., Mankoo, B.S., Read, T., Murphy, P., Blaveri, E., McQuillin, A., Petursson, H. & Curtis, D. 2001. Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. Am J Hum Genet 68: 661-73

Hamshere, M.L., Bennett, P., Williams, N., Segurado, R., Cardno, R., Norton, N., Lambert, D., Williams, H., Kirov, G., Corvin, A., Holmans, P., Jones, L., Jones, I., Gill, M., O'Donovan, M.C., Owen, M.J. & Cradddock, N. 2005. Genomewide linkage scan in schizoaffective disorder: significant evidence for linkage at 1q42 close to DISC1, and suggestive evidence at 22q11 and 19p13. Arch Gen Psychiatry 62: 1081-8

Hamshere, M.L., Williams, N.M., Norton, N., Williams, H., Cardno, A.G., Zammit, S., Jones, L.A., Murphy, K.C., Sanders, R.D., McCarthy, G., Gray, M.Y., Jones, G., Holmans, P., O'Donovan, M.C., Owen, M.J. & Craddock, N. 2006. Genome wide significant linkage in schizophrenia conditioning on occurrence of depressive episodes. J Med Genet 43: 563-7

Han, J., Lee, Y., Yeom, K.H., Kim, Y.K., Jin, H., Kim, V.N. 2004. The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev 18: 3016-27

Hansen, T., Olsen, L., Lndow, M., Jakobsen, K.D., Ullum, H., Jonsson, E., Andreassen, O.A., Djurovic, S., Melle, I., Agartz, I., Hall, H., Timm, S., Wang, A.G., Werge, T. 2007. Brain expressed microRNAs implicated in schizophrenia etiology. PloS ONE 2: e873

Harrington, C.R., Roth, M., Xuereb, J.H., McKenna, P.J. & Wischik, C.M. 1995. Apolipoprotein E type epsilon 4 allele frequency is increased in patients with schizophrenia. Neurosci Lett 202: 101-4

Hornstein, E. & Shomron, N. 2006. Canalization of development by microRNAs. Nat Genet 38 Suppl: S20-4

Hossain, A., Kuo, M.T. & Saunders, G.F. 2006. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Mol Cell Biol 26: 8191-201

Hovatta, I., Varilo, T., Suvisaari, J., Terwilliger, J.D., Ollikainen, V., Arajärvi, R., Juvonen, H., Kokko-Sahin, M.L., Väisänen, L., Mannila, H., Lönnqvist, J. & Peltonen, L. 1999. A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. Am J Hum Genet 65: 1114-24

Hubbard, T.J., Aken, B.L., Beal, K., Ballester, B., Caccamo, M., Chen, Y., Clarke, L., Coates, G., Cunningham, F., Cutts, T., Down, T., Dyer, S.C., Fitzgerald, S., Fernandez-Banet, J., Graf, S., Haider, S., Hammond, M., Herrero, J., Holland, R., Howe, K., Johnson, N., Kahari, A., Keefe, D., Kokocinski, F., Kulesha, E., Lawson, D., Longden, I., Melsopp, C., Megy, K., Meidl, P., Ouverdin, B., Parker, A., Prlic, A., Rice, S., Rios, D., Schuster, M., Sealy, I., Severin, J., Slater, G., Smedley, D., Spudich, G., Trevanion, S., Vilella, A., Vogel, J., White, S., Wood, M., Cox, T., Curwen, V., Durbin, R., Fernandez-Suarez, X.M., Flicek, P., Kaspryk, A., Proctor, G., Searle, S., Smith, J., Ureta-Vidal, A. & Birney, E. 2007. Ensembl 2007. Nucleic Acids Res 35: D610-7

Hutvagner, G., McLachlan, J., Pasquinelli, A.E., Balint, E., Tuschl, T., Zamore, P.D. 2001. A cellular function for the RNA interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293: 834-8

Iwamoto, K. & Kato, T. 2006. Gene expression profiling in schizophrenia and related mental disorders. Neuroscientist 12: 349-61

Jacob, F. & Monod, J. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol. 3:318-56

Johnson, S.M., Grosshans, H., Shingara, J., Byrom, M., Jarvis, R., Cheng, A., Labourier, E., Reinert, K.L., Brown, D. & Slack, FJ. RAS is regulated by the let-7 microRNA family. Cell 120: 635-47

Kim, V.N., Nam, J.W. 2006. Genomics of microRNA. Trends Genet 22: 165-73

Kiriakidou, M., Nelson, P.T., Kouranov, A., Fitziev, P., Bouyioukos, C., Mourelatos, Z. & Hatzigeorgiou, A. 2004. A combined computational-experimental approach predicts human microRNA targets. Genes Dev 18: 1165-78

Klei, L., Bacanu, S.A., Myles-Worsley, M., Galke, B., Xie, W., Tiobech, J., Otto, C., Roeder, K., Devlin, B. & Byerley, W. 2005. Linkage analysis of a completely ascertained sample of familial schizophrenics and bipolars from Palau, Micronesia. Hum Genet 117:349-56

Kloosterman, W.P., Wienholds, E., ketting, R.F. & Plasterk, R.H. 2004. Substrate requirements for let-7 function in the developing zebrafish embryo. Nucleic Acids Res 32: 6284-91

Kohn, Y., Danilovich, E., Filon, D., Oppenheim, A., Karni, O., Kanyas, K., Turetsky, N., Korner, M. & Lerer, B. 2004. Linkage disequlibrium in the DTNBP1 (dysbindin) gene region and on chromosome 1p36 among psychotic patients from a genetic isolate in Israel: findings from identity by descent haplotype sharing analysis. Am J Med Genet B Neuropsychiatr Genet 128: 65-70

Krek, A., Grün, D., Poy, M.N., Wolf, R., Rosenberg, L., Epstein, E.J., MacMenamin, P., da Piedade, I., Gunsalus, K.C., Stoffel, M., Rajewsky, N. 2005. Combinatorial microRNA target predictions. Nat Genet 37: 495-500

Kwek, K.Y., Murphy, S., Furger, A., Thomas, B., O'Gorman, W., Kimura, H., Proudfoot, N.J. & Akoulitchev, A. 2002. U1 snRNA associates with TFIIH and regulates transcriptional initiation. Nat Struct Biol 9: 800-5

Lagos-Quintana, M., Rauhut, R., Lendeckel, W., Tuschl, T. 2001. Identification of novel genes coding for small expressed RNAs. Science 294: 853-8

Lambert, D., Middle, F., Hamshere, M.L., Segurado, R., Raybould, R., Corvin, A., Green, E., O'Mahony, E., Nikolov, I., Mulcahy, T., Haque, S., Bort, S., Bennett, P., Norton, N., Owen, M.J., Kirov, G., Lendon, C., Jones, L., Jones, I., Holmans, P., Gill, M. & Craddock, N. 2005. Stage 2 of the Wellcome Trust UK-Irish bipolar affective disorder sibling-pair genome screen: evidence for linkage on chromosomes 6q16-q21, 4q12-q21, 9p21, 10p14-p12 and 18q22. Mol Psychiatry 10: 831-41

Landthaler, M., Yalcin, A., Tuschl, T. 2004. The human DiGeorge syndrome critical region gene 8 and Its D. melanogaster homolog are required for microRNA biogenesis. Curr Biol 14: 2162-7

Lecellier, C.H., Dunoyer, P., Arar, K., Lehmann-Che, J., Eyquem, S., Himber, C., Saïb, A. & Voinnet, O. 2005. A cellular microRNA mediates antiviral defense in human cells. Science 308: 557-60

Lee, Y., Jeon, K., Lee, J.T., Kim, S., Kim, V.N. 2002. MicroRNA maturation: stepwise processing and subcellular localization. EMBO J 21: 4663-70

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S. 2003. The nuclear RNase III Drosha initiates microRNA processing. Nature 425: 415-9

Leonard, S. & Freedman, R. 2006. Genetics of Chromosome 15q13-q14 in Schizophrenia. Biol Psychiatry 60: 115-22

Lerer, B., Segman, R.H., Hamdan, A., Kanyas, K., Karni, O., Kohn, Y., Korner, M., Lanktree, M., Kaadan, M., Turetsky, N., Yakir, A., Kerem, B. & Macciardi, F. 2003. Genome scan of Arab Israeli families maps a schizophrenia susceptibility gene to chromosome 6q23 and supports a locus at chromosome 10q24. Mol Psychiatry 8: 488-98

Levinson, D.F., Holmans, P., Straub, R.E., Owen, M.J., Wildenauer, D.B., Gejman, P.V., Pulver, A.E., Laurent, C., Kendler, K.S., Walsh, D., Norton, N., Williams, N.M., Schwab, S.G., Lerer, B., Mowry, B.J., Sanders, A.R.,

Antonarakis, S.E., Blouin, J.L., DeLeuze, J.F. & Mallet, J. 2000. Multicenter linkage study of schizophrenia candidate regions on chromosomes 5q, 6q, 10p, and 13q: schizophrenia linkage collaborative group III. Am J Hum Genet 67: 652-63

Lipska, B.K., Mitkus, S.N., Mathew, S.V., Fatula, R., Hyde, T.M., Weinberger, D.R. & Kleinman, J.E. 2006. Functional genomics in postmortem human brain: abnormalities in a DISC1 molecular pathway in schizophrenia. Dialogues Clin Neurosci 8: 353-7

Lund, E., Guttinger, S., Calado, A., Dahlberg, J.E., Kutay, U. 2004. Nuclear export of microRNA precursors. Science 303: 95-8

Lytle, J.R., Yario, T.A. & Steitz, J.A. 2007. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5'UTR as in the 3'UTR. Proc Natl Acad Sci U S A 104: 9667-72

Mattick, J.S. & Makunin, I.V. 2006. Non-coding RNA, Hum Mol Genet, doi:10.1093/hmg/ddl046

Meng, F., Henson, R., Wehbe-Janek, H., Smith, H., Ueno, Y. & Patel, T. 2007. The microRNA let-7a modulates interleukin-6-dependent STAT-3 survival signaling in Malignant human cholangiocytes. J Biol Chem 282: 8256-64

Millar, J.K., Wilson-Annan, J.C., Anderson, S., Christie, S., Taylor, M.S., Semple, C.A., Devon, R.S., Clair, D.M., Muir, W.J., Blackwood, D.H. & Porteous, D.J. 2000. Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet 9: 1415-23

Moises, H.W., Yang, L., Kristbjarnarson, H., Wiese, C., Byerley, W., Macciardi, F., Arolt, V., Blackwood, D., Liu, X. & Sjögren, B. 1995. An international two-stage genome-wide search for schizophrenia susceptibility genes. Nat Genet 11:321-4

Mott, J.L., Kobayashi, S., Bronk, S.F., Gores, G.J. 2007. Mir-29 regulates Mcl-1 protein expression and apoptosis. Oncogene 26: 6133-40

Muntjewerff, J.W., Hoogendoorn, M.L., Kahn, R.S., Sinke, R.J., Den Heijer, M., Kluijtmans, L.A. & Blom, H.J. 2005. Hyperhomocysteinemia, methylenetetrahydrofolate reductase 677TT genotype, and the risk for schizophrenia: a Dutch population based case-control study. Am J Med Genet B Neuropsychiatr Genet 135: 69-72

O'Donnell, K.A., Wentzel, E.A., Zeller, K.I., Dang, C.V. & Mendell, J.T. 2005. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 435: 839-43

Park, N., Juo, S.H., Cheng, R., Liu, J., Loth, J.E., Lilliston, B., Nee, J., Grunn, A., Kanyas, K., Lerer, B., Endicott, J., Gilliam, T.C. & Baron, M. 2004. Linkage analysis of psychosis in bipolar pedigrees suggests novel putative loci for bipolar disorder and shared susceptibility with schizophrenia. Mol Psychiatry 9: 1091-9

Paunio, T., Ekelund, J., Varilo, T., Parker, A., Hovatta, I., Turunen, J.A., Rinard, K., Foti, A., Terwilliger, J.D., Juvonen, H., Suvisaari, J., Arajärvi, R., Suokas, J., Partonen, T., Lönnqvist, J., Meyer, J., Peltonen, L. 2001. Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. Hum Mol Genet 10: 3037-48

Pekarsky, Y., Santanam, U., Cimmino, A., Palamarchuk, A., Efanov, A., Maximov, V., Volinia, S., Liu, C.G., Rassenti, L., Calin, G.A., Hagan, J.P., Kipps, T. & Croce, C.M. 2006. Tcl1 Expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. Cancer Res 66: 11590-3

Perkins, D.O., Jeffries, C.D., Jarskog, L.F., Thomson, J.M., Woods, K., Newman, M.A., Parker, J.S., Jin, J.P. & Hammonds, S.M., 2007, microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder, Genome Biol 8:R27

Pimm, J., McQuillin, A., Thirumalai, S., Lawrence, J., Quested, D., Bass, N., Lamb, G., Moorey, H., Datta, S.R., Kalsi, G., Badacsonyi, A., Kelly, K., Morgan, J., Punukollu, B., Curtis, D. & Gurling, H. 2005. The Epsin 4 gene on chromosome 5q, which encodes the clathrin-associated protein enthoprotin, is involved in the genetic susceptibility to schizophrenia. Am J Hum Genet 76: 902-7

Rajewsky, N. 2006. microRNA target predictions in animals. Nat Genet 38 Suppl: S8-13

Reif, A., Herterich, S., Strobel, A., Ehlis, A.C., Saur, D., Jacob, C.P., Wienker, T., Topner, T., Fritzen, S., Walter, U., Schmitt, A., Fallgatter, A.J. & Lesch, K.P. 2006. A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function. Mol Psychiatry 11: 286-300

Rudduck, C., Beckman, L., Franzen, G. & Lindström, L. 1985. C3 and C6 complement types in schizophrenia. Hum Hered 35: 255-8

Saunders, M.A., Liang, H. & Li, W.H. 2007. Human polymorphism at microRNAs and microRNA target sites. Proc Natl Acad Sci U S A 104: 3300-5

Saviouk, V., Chow, E.W., Bassett, A.S. & Brzustowicz, L.M. 2005. Tumor necrosis factor promoter haplotype associated with schizophrenia reveals a linked locus on 1q44. Mol Psychiatry 10: 375-83

Sethupathy, P., Corda, B., & Hatziegeorgiou, A.G. 2006. TarBase: A comprehensive database of experimentally supported animal microRNA targets. RNA 12:192-97

Silverman, J.M., Greenberg, D.A., Altstiel, L.D., Siever, L.J., Mohs, R.C., Smith, C.J., Zhou, G., Hollander, T.E., Yang, X.P., Kedache, M., Li, G., Zaccario, M.L. & Davis, K.L. 1996. Evidence of a locus for schizophrenia and related disorders on the short arm of chromosome 5 in a large pedigree. Am J Med Genet 67: 162-71

Sinibaldi, L., De Luca, A., Bellacchio, E., Conti, E., Pasini, A., Paloscia, C., Spalletta, G., Caltagirone, C., Pizzuti, A. & Dallapiccola, B. 2004. Mutations of the Nogo-66 receptor (RTN4R) gene in schizophrenia. Hum Mutat 24: 534-5

Sklar, P., Pato, M.T., Kirby, A., Petryshen, T.L., Medeiros, H., Carvalho, C., Macedo, A., Dourado, A., Coelho, I., Valente, J., Soares, M.J., Ferreira, C.P., Lei, M., Verner, A., Hudson, T.J., Morley, C.P., Kennedy, J.L., Azeveco, M.H., Lander, E., Daly, M.J. & Pato, C.N. 2004. Genome-wide scan in portuguese island families identifies 5q31-5q35 as a susceptibility locus for schizophrenia and psychosis. Mol Psychiatry 9: 213-18

Stark, A., Brennecke, J., Bushati, N., Russell, R.B. & Cohen, S.M. 2005. Animal MicroRNAs Confer Robustness to Gene Expression and Have a Significant Impact on 3'UTR Evolution. Cell 123: 1133-1146

Stark, K.L., Xu, B., Bagchi, A., Lai, W.S., Liu, H., Hsu, R., Wan, X., Pavlidis, P., Mills, A.A., karayiorgou, M., Gogos, J.A. 2008. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. Nat Genet 40: 751-60

Straub, R.E., MacLean, C.J., Martin, R.B., Ma, Y., Myakishev, M.V., Harris-Kerr, C., Webb, B.T., O'Neill, F.A., Walsh, D. & Kendler, K.S. 1998. A schizophrenia locus may be located in region 10p15-p11. Am J Med Genet 81: 296-301

Stöber, G., Seelow, D., Rüschendorf, F., Ekici, A., Beckmann, H. & Reis, A. 2002. Periodic catatonia: confirmation of linkage to chromosome 15 and further evidence for genetic heterogeneity. Hum Genet 111:323-30

Thadani, R. & Tammi, M.T. 2006. MicroTar: predicting microRNA targets from RNA duplexes. BMC Bioinformatics 7 (Suppl 5): S20

Torrey, E.F., Leweke, M.F., Schwarz, M.J., Mueller, N., Bachmann, S., Schroeder, J., Dickerson, F. & Yolken, R.H. 2006. Cytomegalovirus and schizophrenia. CNS Drugs 20: 879-85

Toyota, T., Yoshitsugu, K., Ebihara, M., Yamada, K., Ohba, H, Fukusawa, M., Minabe, Y., Nakamura, K., Sekine, Y., Takei, N., Suzuki, K., Itokawa, M., Meerabux, J.M.,. Iwayama-Shigeno, Y., Tomaru, Y., Shimizu, H., Hattori, E., mori, N. & Yoshikawa, T. 2004. Association between schizophrenia with ocular misalignment and polyalanine length variation in PMX2B. Hum Mol Genet 13: 551-61

Tsuang, M.T., Taylor, L. & Faraone, S.V. 2004. An overview of the genetics of psychotic mood disorders. J Psychiatr Res 38: 3-15

Van Den Bogaert, A., Del-Favero, J. & Van Broeckhoven. 2006. Major affective disorders and schizophrenia: a common molecular signature? Hum Mutat 27: 833-53

Volinia, S., Calin, G.A., Liu, C.G., Ambs, S., Cimmino, A., Petrocca, F., Visione, R., Iorio, M., Roldo, C., Ferracin, M., Prueitt, R.L., Yanaihara, N., Lanza, G., Scarpa, A., Negrini, M., Harris, C.C. & Croce, C.M. 2006. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 103: 2257-61

Williams, N.M., Rees, M.I., Holmans, P., Norton, N., Cardno, A.G., Jones, L.A., Murphy, K.C., Sanders, R.D., McCarthy, G., Gray, M.Y., Fenton, I., McGuffin, P. & Owen, M.J. 1999. A two-stage genome scan for schizophrenia susceptibility genes in 196 affected sibling pairs. 8: 1729-39

Williams, N.M., Norton, N., Williams, H., Ekholm, B., Hamshere, M.L., Lindblom, Y., Chowdari, K.V., Cardno, A.G., Zammit, S., Jones, L.A., Murphy, K.C., Sanders, R.D., McCarthy, G., Gray, M.Y., Jones, G., Holmans, P., Nimgaonkar, V., Adolfson, R., Osby, U., Terenius, L., Sedvall, G., O'Donovan, M.C. & Owen, M.J. 2003. A systematic genomewide linkage study in 353 sib pairs with schizophrenia. Am J Hum Genet 73:1355-67

Wood, L.S., Pickering, E.H. & Dechairo, B.M. 2007. Significant support for DAO as a schizophrenia susceptibility locus: examination of five genes putatively associated with schizophrenia. Biol Psychiatry 61: 1195-9

Yi, R., Qin, Y., Macara, I.G., Cullen, B.R. 2003. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17: 3011-6

Zhang, D. & Brodt, P. 2003. Type 1 insulin-like growth factor regulates MT1-MMP synthesis and tumor invasion via PI 3-kinase/Akt signaling. Oncogene 22: 974-82

Zhao, X., Tang, R., Gao, B., Shi, Y., Zhou, J., Guo, S., Zhang, J., Wang, Y., Tang, W., Meng, J., Li, S., Wang, H., Ma, G., Lin, C., Xiao, Y., Feng, G., Lin, Z., Zhu, S., Xing, Y., Sang, H., St Clair, D. & He, L. 2007. Functional Variants in the promoter region of Chitinase 3-like 1 (CHI3L1) and susceptibility to schizophrenia. Am J Hum Genet 80: 12-8