The *NDE1* Genomic Locus Affects Treatment of Psychiatric Illness through Gene Expression Changes Related to MicroRNA-484

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Abstract

Previous studies in a Finnish family cohort for schizophrenia found significant association with five genes within the DISC1 network; *DISC1*, *NDE1*, *NDEL1*, *PDE4B* and *PDE4D*. Here, in sub-samples of these families, we utilised gene expression and psychoactive medication use, in order to translate the role of the DISC1 network into biological consequences and potential treatment implications. Gene expression levels were determined in 63 individuals from 18 families, whilst prescription medication information has been collected over a ten year period for all 931 affected individuals. Replication of the observed changes was sought from the GTEx database and from a Finnish twin cohort ascertained for schizophrenia. We demonstrated that the *NDE1* SNP rs2242549 associates with changes in a large number of probes (n=2,908), and with 214 genes that could be replicated in independent cohorts. A significant number of the genes altered (347 out of 2,510, $p=3.0\times10^{-8}$) were predicted targets of microRNA-484, which is located on a 5' non-coding exon of *NDE1*. Variants within the *NDE1* locus also displayed significant association to early cessation of psychoactive medications, specifically a genotype by gender interaction with use of CYP2C19 metabolised medications. Furthermore, we demonstrated that miR-484 can affect the expression of CYP2C19 in a cell culture system. Thus, mutations at the *NDE1* locus may alter risk to mental illness, in part through functional modification of miR-484, and that such modification also alters treatment response to specific psychoactive medications, leading to the potential for the *NDE1* locus to be used in targeting of treatment for psychiatric illness.

Introduction

Disrupted in Schizophrenia 1 (*DISC1*) first came to light as a candidate gene for mental illness when it was discovered to be directly disrupted by a balanced t(1;11)(q42.2,q14.3) chromosomal translocation that segregated with schizophrenia and other major mental illness in a large Scottish family (1). Genetic studies of a Finnish family cohort ascertained for schizophrenia have consistently found evidence for the involvement of *DISC1* and genes encoding several of its major protein interaction partners, the DISC1 network, in this condition. Prior to the discovery of *DISC1*, a haplotype linked to schizophrenia that spanned from 1q32.2 to 1q41 in an internal sub-isolate of Finland had been reported (2). In 2001, a fine mapping study of chromosome 1 using families ascertained from all over Finland demonstrated that a *DISC1* intragenic marker, D1S2709, showed evidence for linkage to schizophrenia across the whole population, with the strongest evidence being from those families not originating from this internal sub-isolate (3). This Finnish family sample has since been expanded, enabling replication of the findings on chromosome 1 in a completely independent, but identically ascertained, sample, with linkage again being observed with a marker intragenic of *DISC1*, this time a single nucleotide polymorphism (SNP) (4). A follow-up study monitored the allelic diversity of the 1q42 region in these 498 nuclear Finnish families. This reported the identification of four restricted loci, named HEP haplotypes, which associated with a broad diagnostic criterion, consisting primarily of schizophrenia, but also including individuals affected with other schizophrenia spectrum diagnoses, as well as affective disorders (5). The most statistically robust of these was a haplotype, which consisted of two *DISC1* SNPs, rs751229 and rs3738401, the latter being a non-synonymous variant located in *DISC1* exon 2 (5). This haplotype was named the HEP3 haplotype in that original study (5). Multiple association studies for psychiatric (6-8), memory (9), cognitive (10) and neuroimaging (10) phenotypes have now also been reported for *DISC1* in Finnish cohorts.

A re-analysis of the Finnish families was performed so as to control for this observation of association at the *DISC1* locus. This genome-wide linkage scan conditioned on *DISC1* showed a peak of linkage at chromosome 16p close to the *NDE1* (Nuclear Distribution Element 1) gene (11), which encodes for an established interaction partner of the DISC1 protein (12-14). This linkage observation was followed up through association analysis at the *NDE1* locus, observing that a haplotype and its constituent SNPs associate with schizophrenia in the Finnish family cohort, in a gender dependent manner (11). Recently, variations at the *NDE1* locus have been identified to increase risk to schizophrenia in this Finnish family cohort through interaction with high birth weight (≥4000g), which is expected to be a proxy measure for multiple pre- and/or perinatal environments (15). Furthermore, *NDE1* is independently implicated in major mental illness through its presence at 16p13.11, which is subject to duplications in schizophrenia (16-19), as well as being directly implicated through rare SNPs in patients (20). The importance of this protein for neurodevelopment more generally has also been demonstrated in individuals with biallelic loss of the functional *NDE1* gene, leading to severe microcephaly phenotypes, sometimes described in conjunction with lissencephaly or hydrocephaly (21-24). Deletion of only one copy of the 16p13.11 region, meanwhile, has been associated with neurological conditions including autism and epilepsy (25-28).

The association of *NDE1* with schizophrenia has become a key piece of research in highlighting that the pathways in which DISC1 functions may also house genes that carry susceptibility altering variants, the DISC1 network hypothesis. Genetic association was therefore tested for 11 other DISC1 binding partners in the Finnish families ascertained for schizophrenia. Although SNPs and haplotypes from 6 other genes were initially observed to associate in a sub-sample of these families, only variants in *NDEL1* (NDE-like 1, a close paralogue of *NDE1*) and in the phosphodiesterases *PDE4B* and *PDE4D* replicated when the remaining families were tested in a second, distinct sample from the cohort (29).

One of the most important aspects of identifying genes that predispose to complex psychiatric traits is to be able to translate them, so as to improve our biological understanding and ultimately our treatment procedures for the disorders. This can be achieved through genetic studies in which, instead of using an end state diagnosis, alternative traits are employed that can measure biological or pharmacological aspects of the disorder. We have previously studied the effect of DISC1 pathway genetic variation on gene expression in a publicly available population cohort, with 528 genes being differentially expressed across 24 variants studied, of which 35 had pre-existing supporting evidence for a role in psychosis (30). Intriguingly, seven of these affected genes were noted to be targets for drugs for psychiatric illness, leading to the hypothesis that these *DISC1* pathway variants, through their action on gene expression, may alter treatment outcome for medications designed to target these genes (30). Here we take this study further, by utilising gene expression levels in case families where these DISC1 network genetic variants have been previously demonstrated to associate with schizophrenia, and by using data collected on how different psychoactive medications are used by the affected individuals in these families.

Results

Since we had previously studied gene expression changes related to these DISC1 network variants in a population cohort (30), we sought to confirm these in a family cohort ascertained for schizophrenia. Here we studied the effect of three of the variants used in the previous study that had been reported to significantly alter the expression levels of 86 genes (30). Eight of these 86 genes were observed here to also be significantly altered in their expression levels, including two probes for the *ST3GAL5* gene, and the probe for *TRIOBP* (Table S1) which has further been identified within brain aggregomes in post mortem brain samples of schizophrenia patients (31).

Next, we used a hypothesis-free approach to investigate the association between each of the variants and genome-wide gene expression changes. After correction to a 5% false discovery rate (FDR) no probes were significantly altered in expression levels in association with the *DISC1* haplotype HEP3, the *DISC1* SNP rs821616, or the *NDE1* SNPs rs4781678 and rs1050162. However, one variant within the *NDE1* gene locus was significantly associated with gene expression levels ($p \leq 0.05$, 5% FDR) of a large number of the 11,976 probes studied [rs2242549: $q<0.05$ for 2,909 probes (2542 genes); $p<0.05$ for 3,824 probes (3,314 genes)] in these Finnish families, in which the *NDE1* genotypes and the haplotype they constitute had initially been identified as risk factors for schizophrenia (11) (Table 1 and Table S1).

In order to replicate these observations, we pursued three lines of supporting evidence. Firstly, we noted that the *NDE1* SNPs rs2242549 and rs1050162 were in high linkage disequilibrium (LD) in the full family cohort $r^2=0.88$, and that 715 genes were significantly differentially expressed by both SNPs. Second, we utilised existing data from a twin cohort for schizophrenia from Finland, identifying 56 genes with replicable significant alteration in their expression levels related to rs2242549 (Table1 and Table S1). Lastly, we used the publicly available database GTEx, from which

we were only able to directly test 2,651 of the 3,314 genes identified, to confirm that 180 also display significant gene expression changes related to the *NDE1* SNP rs2242549 (Table1 and Table S1). Thus in total, 944 genes had supporting evidence from at least one additional source for their changes in expression related to the *NDE1* variant rs2242549, of which 77 genes had supporting evidence from more than one source.

In order to understand how such a large number of probes could be altered by a single genetic locus, we revisited the genomic annotations of the *NDE1* region, noting that since our original description of association between *NDE1* and schizophrenia (11), an additional non-coding 5' exon has been annotated to one splice variant of the *NDE1* gene, with this exon also containing the human microRNA miR-484. Specifically, this splice variant was the most highly expressed of the three transcripts encoding full length NDE1 in several cell culture systems (32). This microRNA has 16,027 predicted gene targets, of which 2,588 are predicted by six or more of the 12 independent prediction programs annotated by the miRWalk database (33). We used this level of consistency between prediction programs to determine the possibility of enrichment of targets for miR-484 within the genes whose expression changes are associated with the variant at the *NDE1* locus. These probes were indeed seen to be enriched for predicted targets of miR-484. This enrichment was also present for those genes altered by both *NDE1* SNPs, in those genes replicated in the independent Finnish twin cohort for schizophrenia, but not for those genes replicated by data from the GTEx database (Table 1), the only replication source studied here that is not derived from the Finnish population.

We next explored the role that these variants may play in any pharmacological action, noting once again that, of the DISC1 network genes investigated (DISC1, NDE1 and PDE4B), it was the *NDE1* genotypes that significantly associated with early cessation of particular medications. Screening all of the medications frequently used within this cohort (a minimum of 15 instances of cessation by

three months during the ten-year period studied), we observed an association between *NDE1* rs4781678 genotype and early cessation of use of the antipsychotic levomepromazine (OR=4.13; 95%CI=1.72-9.91; p=0.00090). When analysed in interaction with gender, association was further observed across *NDE1* genotypes with early cessation of the use of diazepam and citalopram (Table S2). Both of these drugs share a common principal metabolising enzyme, CYP2C19 (34-37) (Table S3). We therefore followed up on these observations regarding singular drugs, by grouping the medications metabolised by the CYP2C19 enzyme in order to increase our power to detect any association through the increased number of observations. Since four out of seven of the drugs metabolised by CYP2C19 were selective serotonin reuptake inhibitors (SSRIs), we studied these as a separate group as well as further separated based on CYP2C19 metabolism. No significant interaction was observed for the grouping of all drugs metabolised by CYP2C19, however, a genotype by gender interaction was noted when all SSRIs were grouped together (rs2075512, OR=0.37; 95%CI=0.17-0.79; p=0.010). When all SSRI's metabolised by CYP2C19 were tested, this genotype by gender interaction became significant (OR=0.27 to 0.31; 95%CI=0.11 to 0.13–0.64 to 0.71; p=0.0030 to 0.0060) for almost all *NDE1* markers, while no interaction was noted for SSRI's not metabolised by CYP2C19 (Table 2 and Figure S2). We analysed the remaining drugs metabolised by CYP2C19 together as another grouping ("non-SSRIs metabolised by CYP2C19"). Interestingly, a significant interaction was observed (OR=3.33 to 5.82; 95%CI=1.44 to 2.25–7.25 to 15.0; p=0.0013 to 0.00030) for all *NDE1* markers (Table 2 and Figure S2). In this case, however, the gender effect is reversed, with SNPs being associated with cessation among females, in contrast to SSRIs metabolised by CYP2C19, for which SNPs were associated with cessation among males.

Human microRNA miR-484 is predicted to target a host of cytochrome P450 enzymes, however these were not detected in the gene expression analysis in the Finnish family cohort which was derived from whole blood. As a proof of principle experiment as to whether miR-484 could be mediating the

enzyme effect on medication, we analysed the effect on transfecting a mimic of the mature form of this microRNA into NLF human neuroblastoma cells. It could indeed be seen that protein levels of CYP2C19 were subtly, but significantly, up-regulated following miR-484 transfection, when compared to transfection with a negative control microRNA (Figure 1). In contrast, no effect on the expression of another principal metabolising enzyme of psychoactive medications, CYP2D6, was seen, indicating that this is a specific effect. This is in agreement with our findings that specifically those medications metabolised by CYP2C19 were affected by variants in the *NDE1* locus. Finally, we also confirmed that protein levels of TRIOBP-1 (also known as Tara), which is encoded by *TRIOBP*, a gene whose expression was associated with the *NDE1* variant rs2242549 in both our original study (30) and here, was affected in this system by miR-484. This indicates that, like CYP2C19, alterations in the expression of TRIOBP as the result of variation at the *NDE1* locus is likely to be driven principally by miR-484. Furthermore, *DISC1* is predicted to be a target of miR-484 by eight of the 12 programs collated by miRWalk, while *NDE1* is only predicted to be a target by one program. Thus in order to determine if these genes at the core of the DISC1 network hypothesis were also mediated in their expression by miR-484 we also studied them in the analysis of NLF human neuroblastoma cells. While the expression level of *DISC1* was too low to accurately quantify, the miR-484 mimic was seen to have a small but statistically significant effect, increasing NDE1 expression (Figure 1). It should be noted that in the GTEx database *NDE1* expression levels were strongly associated with the NDE1 SNP rs2242549 genotype (beta=0.24; Std err = 0.037; t-statistic=6.6; $p=2.3x10^{-10}$).

Discussion

Here we have demonstrated that variations within genes encoding proteins of the DISC1 network of interaction partners can affect both gene expression levels and medication usage of psychoactive drugs used to treat major mental illnesses. Specifically, at the *NDE1* locus two SNPs are associated with replicable expression changes in a large number of genes, which in turn are significantly enriched for predicted targets of miR-484, a human microRNA encoded for within the 5' untranslated exon of the longest splice variant of *NDE1*. Expression of miR-484 would therefore be likely to be affected by the expression of this *NDE1* isoform. Indeed, the proximity of miR-484 to the start of the *NDE1* transcript makes it conceivable that they share a common promoter system. Interestingly, while orthologues of miR-484 are known to exist in multiple other species of mammal, to date the long *NDE1* splice variant which overlaps with it has only been described in humans (UCSC Genome Browser, December 2013 assembly (38)). It is also of note that a 1.45-fold increase in miR-484 has previously been reported in the superior temporal gyri of patients with schizophrenia (39).

This study forms a continuation of our prior work studying the effect of DISC1 network variants on gene expression changes in the general population, using publicly available data on the CEU (Utah residents with North and Western European ancestry) individuals (30). Yet here, in a study of Finnish families ascertained for schizophrenia, we were able to replicate the observed changes in expression of eight of the previously identified 86 genes. This included two probes for the *ST3GAL5* gene and a probe for *TRIOBP*, both of which are significantly altered in the expression in association with the *NDE1* SNP rs2242549. We also verified *TRIOBP* to be a target of miR-484, as expression of the TRIOBP-1 protein was increased by transfection of miR-484 in a neuroblastoma cell culture model. That the increased expression of *TRIOBP* in association with risk variants at the *NDE1* locus occurs specifically with this splice variant is of particular interest, as this splice variant has been previously observed to be within brain aggregomes in post mortem brain samples of schizophrenia patients (31).

Furthermore, TRIOBP-1 has been shown to be a protein interaction partner of NDEL1, a close paralogue of NDE1 (40).

When we tested for any gene expression alterations across the genome we identified a large number of probes and genes whose expression levels associated with variants at the *NDE1* locus, specifically with the SNP rs2242549. A large proportion of which (715 out of 3,314) were significantly altered by another *NDE1* SNP (rs1050162), which is in high LD with rs2242549. Yet replication attempts in independent cohorts, although providing validation for some genes (56 in an independent Finnish schizophrenia cohort and 180 using the GTEx database), did not provide unilateral confirmatory evidence. This lack of replication of both our previous observations and our new observations, in combination with the large quantity of new observations and their biological relevance through miR-484 to the *NDE1* locus, lead us to the conclusion that, although the variants studied here are common to many populations, their relationship to potential functional mutations at this locus, and their specific biological consequences associated with schizophrenia and gene expression changes may well be unique to this Finnish family cohort (41). This population difference may account for the lack of replication of most of the previously observed 86 genes in the CEU population (30), and the lack of enrichment for miR-484 targets in the GTEx database. This is consistent with the idea of *DISC1* variation playing a genetically heterogeneous role in the general incidence of schizophrenia, lacking common illness-associated variations which could be detected by genome-wide association studies (42,43) of global populations, and yet providing strong evidence for a role in the condition within specific populations and family studies (44). It should be noted that this study is based on a long line of evidence from prior studies of this Finnish family cohort ascertained for schizophrenia, in which linkage and association at the *DISC1* (3-10) and *NDE1* (11,15) loci, as well as replicated association at the *PDE4D* (29) locus have been observed with schizophrenia and related disorders. However, of all the DISC1 network genes, it is the *NDE1* locus at 16p13.11 that has been consistently observed in

the modern genome-wide association era, through studies of copy number variations (CNVs) associated with psychiatric and neurological disorders (16-19,24-28). Therefore, our observations here of specific variants at the *NDE1* locus highlight disruption of miR-484 also as a potential functional consequence of those CNVs. Thus, the results described here partially parallel recent findings that expression of mature miR-484 led to alterations in neural progenitor proliferation and differentiation, as well as behavioural changes in mice, thus implicating the microRNA in the phenotypes associated with 16p13.11 duplication (45). While NDE1 over-expression was not seen to have a gross effect on neuronal progenitor proliferation under similar circumstances, given the known severe neurological consequences which arise from biallelic disruption of the *NDE1* reading frame, in a manner which would not be anticipated to affect miR-484 (21, 22), there is still a potential role for NDE1 in the conditions associated with 16p13.11 duplication. Additionally, relatively mild phenotypic effects would be needed to explain the fact that while individuals with the duplication have an approximately 3-fold higher incidence of schizophrenia, most carriers of the CNV do not develop the condition (16-19). Nevertheless, it appears the vast majority of gene expression changes associated with the *NDE1* SNP rs2242549 here are likely to arise due to alterations in miR-484 and not *NDE1* function.

When the DISC1 network was studied with respect to treatment, the *NDE1* locus again demonstrated association, specifically in interaction with gender for drugs metabolised by CYP2C19. The microRNA miR-484 is predicted to target 46 different cytochrome P450 enzymes but not CYP2C19, although these predictions are not made by more than six of the prediction programs collated by miRWalk. However, their degree of expression in lymphocytes was too low to allow us to investigate potential changes in expression level. We were however, in a cell culture model, able to demonstrate that miR-484 is capable of increasing the expression of CYP2C19, while it did not alter expression of another major metabolising enzyme for psychoactive drugs, CYP2D6, in the same experimental

circumstances. This suggests that the mechanism through which the locus confers risk and alters medication usage is the same. In the case of medication use we used a dichotomous variable based on a cut-off of ceasing to use the prescribed medication after three months or less. This was designed to indicate that a treatment was either not considered to be working or having too severe side effects and was therefore stopped. Since the cell culture experiment showed that CYP2C19 protein expression is increased by miR-484, in the same way as TRIOBP-1, it can be hypothesised that the medications are more rapidly metabolised in individuals carrying these variants, leading to a reduced efficacy of those treatments. Interestingly, the genetic effects on medication are in interaction with gender, with the interaction leading to different consequences depending on both class of drug and gender. Males carrying the risk alleles had a higher probability of cessation of use for SSRIs metabolised by CYP2C19, while females carrying the risk alleles had an increased probability of cessation for non-SSRI drugs that are metabolised by CYP2C19. Although the mechanism for these gender dependent effects remains unclear at this time, it is noteworthy that the original association between the *NDE1* locus and schizophrenia in these families also displayed gender differences, being significant only in females (11). Taken together, however, this implies that one or more gender-specific effects act as modifying factors in conjunction with the *NDE1*/miR-484 locus, although this cannot be easily modelled in our cell culture system.

Here, through the identification of altered gene expression patterns that led to the functional implication of miR-484, which is coded on an untranslated exon of *NDE1*, we identified a means by which genetic variation in the DISC1 network can not only increase risk to major mental illnesses, but also how those same variants can alter treatment response to specific psychoactive medications through the regulation of their metabolising enzyme. This study has therefore provided new biological insight into psychiatric disorders to which novel medications could be designed, as well as suggesting

that knowledge of an individual's genotype within the *NDE1*/miR-484 locus may have potential value in the targeting of current therapies.

Materials and Methods

The principal samples used here are part of a larger study of schizophrenia in Finland, where Finnish patients with schizophrenia born between 1940 and 1976 were identified through the hospital discharge, disability pension, and the free medication registers (5,46). Close family members of each proband were then identified through the national population register, enabling the construction of pedigrees. The cohort now totals 458 families consisting of 498 nuclear families that contain 2,756 individuals, of which 2,059 have been genotyped. Of these genotyped individuals, 931 are classified as affected using criteria from the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (47). To study the alternative phenotypes used here, either a sub-set of families were used or all affected individuals, as stated below. In these studies, the principles recommended in the Declaration of Helsinki, and its amendments were followed. The study of the Finnish Schizophrenia Families has been approved by the Coordinating Ethics committee of the Hospital District of Helsinki and Uusimaa (434/E0, 05, with several amendments).

Gene Expression Data

A sub-sample of the Finnish families ascertained for schizophrenia was re-approached for collection of blood samples specifically for the extraction of RNA. RNA has been collected from 18 families consisting of 63 individuals with genome-wide gene expression measures assayed using Illumina Inc's HumanHT-12 v4 Expression BeadChip. The principal intention of this collection was to study the biological measures with which the *DISC1* haplotype HEP3 (5) associates in individuals both with and without a major mental illness diagnosis, on the assumption that the HEP3 haplotype is a surrogate for any potential functional variant to be identified at the *DISC1* locus. However, these families carry other variants in addition to the HEP3 haplotype. It is therefore possible to test the effect of other variants on genome-wide gene expression, as long as there are sufficient numbers of individuals that carry the allele of interest. Using a cut-off value for the minor allele homozygote

frequency of ≥10%, we selected five variants (*DISC1*: HEP3 haplotype and rs821616; *NDE1*: rs4781678, rs2242549, and rs1050162) with which to perform the analysis. Of the 48,212 probes on the chip, 11,976 were significantly detectable, at a threshold of $p \leq 0.01$ in more than 90% of individuals. These probes were processed using quantile normalisation followed by $\log_2$ transformation. Association analysis was performed in R (RStudio version 0.99.489) using the lme4 package (48), accounting for gender, age, affection status, and family affects. Test p-values were further corrected for false discovery rate (FDR) utilizing the Benjamini Hochberg algorithm at the 5% level. The raw anonymous data from this gene expression analysis has been deposited to the GEO database (GSE48072).

Replication Samples

In order to replicate our gene expression based observations we used two independent cohorts. A sub-sample of an independent Finnish cohort of twins discordant for schizophrenia, and the GTEx database. For the twin cohort, information about recruitment and clinical evaluation of the participants in this study is described in detail elsewhere (49), and the gene expression data for this cohort has been reported previously (50). Briefly the participants are 18 schizophrenia patients, 18 co-twins, and 37 control twins who have provided blood samples for gene expression analysis (N=73). Genome wide gene expression was measured using the Illumina Human WG6 v3.0 chip. After quality control and data processing, identical to that used on the family data, 18,559 probes from this chip were able to be analysed. The association analysis was identical to that used in the family cohort, but additionally accounted for effects due to twin status. This analysis was only performed on the two *NDE1* SNPs rs2242549 and rs1050162. The GTEx database (51, www.gtexportal.org/home/) is a publicly available resource for exploring the correlation between genotypes and gene expression across multiple tissues and in a genome wide manner. This resource enables users to also test their own genotypes by gene expression, and this function was used to replicate only those genes identified

as significantly altered in their gene expression levels at a cut-off of $p \leq 0.05$. This was performed for findings for all SNPs studied in the families, but could not be used to study the *DISC1* haplotype's findings. All tests were only performed using the tissue definition of whole blood, so as to best match the source of the RNA used in the Finnish cohort studies.

MicroRNA Target Prediction and Enrichment Analysis

Through the miRWalk database (33) we obtained a comprehensive list of predicted 3'UTR targets for miR-484, based on the predictions of 12 different programs. A total of 16,027 genes have been putatively predicted to be targeted by miR-484 by at least one of the programs used in compiling this list, while no targets have been predicted by all 12 programs. In order to assess potential enrichment between the genes significantly altered in their expression levels and targets for miR-484, we restricted our analysis to gene targets predicted by at least six of the 12 programs. A gene list based on these 2,588 targets was uploaded into the Ingenuity Pathway Analysis (IPA) (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity), so as to enable enrichment analysis for those genes predicted to be targeted by miR-484.

Medication Data

Prescription medication data has been collected for all 931 affected individuals from the schizophrenia family cohort (52). Information on medication was obtained from the Finnish National Prescription Register of the National Social Insurance Institution, which had been found to highly correlate with self-reported medication use (52). Data was obtained for the period 1st January 1996 to 31st December 2005, inclusively. Medication periods were defined according to method 4 proposed by Mantel-Teeuwisse et al, multiplying defined daily dose by a factor of 1.1 and filling 15-day gaps between medication periods (53). In order to perform pharmacogenetic analysis this medication period data has been used to determine the probability of cessation of each drug. This was then

converted into a binary variable using three months as the cut-off. Only psychoactive medications with at least 15 instances of use for three months or less were taken for association analysis of the individual drugs, meaning that of the possible 101 psychoactive drugs only 24 were analysed. When multiple drugs were combined to study classes of medication, all medications for which data is available were used, regardless of individual frequency. The frequency of the genetic variants was restricted to those with a minor allele homozygote frequency of at least 5%. Therefore, seven variants from three DISC1 pathway genes were studied, *DISC1*: rs821616; *NDE1*: rs4781678, rs2242549, rs881803, rs2075512, and the haplotype of the four SNPs '*NDE1* Tag haplotype'; *PDE4B*: rs7412571. In order to account for the fact that some medication periods may come from the same individual, analysis of the medication usage used logistic regression with GEE-estimation (Generalized Estimating Equations), as utilized by the geepack-package (54) for the R statistical software (55).

Multiple Test Correction

All genetic variants to be analysed in this study have displayed prior evidence for involvement in the aetiology of schizophrenia in this cohort (5,9,11,29), with both *DISC1* and *NDE1* having displayed prior gender dependent effects (5,9,11). Therefore no multiple testing correction has been applied to correct across variants or gender interaction models, as they all can be considered hypothesis based. However, since we were screening alternative phenotypes in a hypothesis-free manner in order to further characterise these *a priori* variants, we have applied multiple test correction for the number of traits performed at each stage of the analysis.

In the case of the gene expression analysis in the Finnish family cohort a false discovery rate correction of 5% was integrated into the analysis protocol. Replication analysis was performed for all p-values$\leq$0.05 prior to false discovery correction. For the analysis of the individual medications (24

tests) and the groups of medications (five tests) p-values were presented unadjusted but only highlight those that would surpass the Bonferroni correction based thresholds, $p \leq 0.0021$ and $p \leq 0.01$ respectively, for these tests.

Cell Culture and Western Blotting

In order to determine whether miR-484 had the potential to affect the expression of selected proteins in a human cell based system, NLF neuroblastoma cells (Children's Hospital of Philadelphia) were grown in RPMI 1640 media supplemented with 10% foetal calf serum and 2mM L-Glutamine (all from Thermo Fischer Scientific) and then transfected with 50nM of either a mimic of mature miR-484 (QIAgen, Sy-hsa-miR-484) or a negative control microRNA (QIAgen, AllStars Negative Control microRNA) using Lipofectamine 2000 (Thermo Fischer Scientific) according to manufacturer's instructions. After 48 hours, cells were lysed using PBS/1% Triton X-100/20mM $MgCl_2$ containing protease inhibitor cocktails and DNaseI. Lysates were then Western blotted and proteins detected using the following antibodies: anti-α-actin (Sigma, A2066), anti-CYP2C19 (Novus Biologicals, NBP1-19698), anti-CYP2D6 (Abnova, H00001565-B01P), anti-DISC1 (14F2, developed in house and described previously (56)), anti-NDE1 (ProteinTech, 10233-1-AP), anti-TRIOBP (Sigma, HPA019769) and anti-α-tubulin (Sigma, T9026). IRDye secondary antibodies were used (LI-COR) and the signal visualised and quantified using an Odyssey CLx infrared imaging system (LI-COR) and associated software. All membranes were probed with secondary antibodies alone first to ensure specificity of signal. The ratio of each antibody signal to the actin antibody signal was used to negate any anomalies in sample loading and total protein concentration. Mean fold-changes between the control and miR-484-treated samples were calculated from 7-8 internal replicates. Three independent experiments were performed and the results compared by two-tailed paired Student's t-test.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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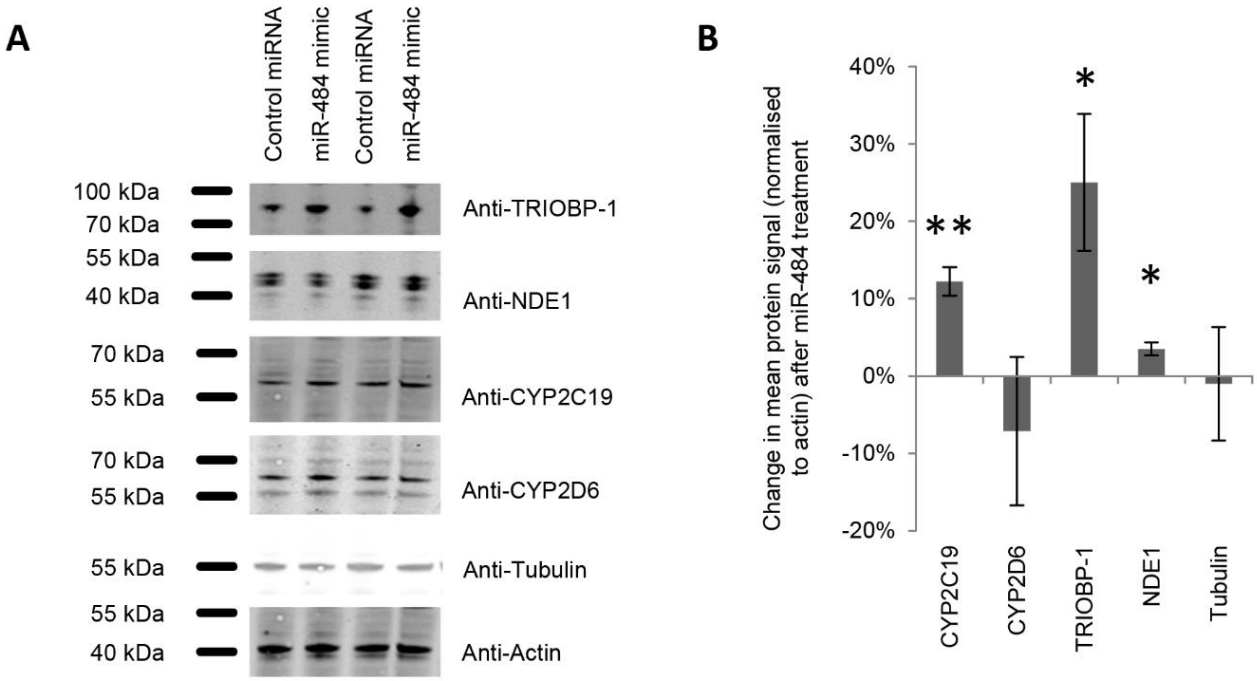
Legends to Figures

Figure 1: The effect of a miR-484 mimic on protein expression in human NLF neuroblastoma cells. a) Sample Western blots showing levels of six proteins in the lysates of cells which had been transfected with either a mimic of miR-484 or with a negative control microRNA. b) Quantification of three independent experiments, each comprising 7-8 internal replicates. All proteins were normalised to actin. *: $p < 0.05$, **: $p < 0.01$.



Tables

Table1: Number of probes and genes significantly altered by variants in the DISC1 network, how they replicate across different cohorts, and how they overlap with the predicted targets of miR-484.

		Observations				Gene Replications			
Gene	Variant	Probe p≤0.05	Gene p≤0.05	Probe q≤0.05	Gene q≤0.05	Neighbouring Variant	Hennah & Porteous (29)	Finnish SCZ Twins	GTEx (Whole Blood)
DISC1	Haplotype HEP3	432	419	0	0	na	na	na	na
	rs821616	1332	1265	0	0	na	1	na	49
NDE1	rs4781678	340	327	0	0	na	1	na	18
	rs2242549	3824	3314	2908	2542	715	6	56	180
	rs1050162	1071	985	0	0	715	na	13	60
Enrichment for predicted targets of mircoRNA-484 *									
	rs2242549		2.2×10^{-13}		3.0×10^{-8}	1.2×10^{-3}		1.4×10^{-3}	5.5×10^{-2}
	rs1050162		5.6×10^{-4}			1.2×10^{-3}		ns	1.5×10^{-1}

* Targets predicted by 6 out of 11 prediction tools summarised by miRWalk were uploaded to Ingenuity Pathway Analysis (IPA), thus enabling a test for enrichment when the subsequent probe/gene lists were studied.
na = value was not applicable as it was not tested
ns = value was not significant nor returned by IPA

Table 2: Results for the association of *NDE1* variants with groups of medications based on their metabolism by the CYP2C19 enzyme and/or Selective Serotonin Reuptake Inhibitor (SSRI) class status, showing the p-values and odds ratios (and 95% confidence intervals) for the interaction model. P-values below 0.01 are below the Bonferroni correction threshold for the five groups tested.

***NDE1* Variant**	**SNP p-value**	**SNP*Gender p-value**	**OR (95% CI)**
All psychoactive drugs metabolised by CYP2C19 (N=510-581, no of instances: 843-945)			
rs4781678	0.79	0.90	0.97 (0.55 - 1.69)
rs2242549	0.56	0.74	1.10 (0.64 - 1.86)
rs881803	0.29	0.15	1.53 (0.86 - 2.73)
rs2075512	0.64	0.79	0.93 (0.53 - 1.63)
Haplotype	0.35	0.73	1.10 (0.63 - 1.93)
SSRIs (N=357-402 , no of instances: 496-563)			
rs4781678	0.31	0.033	0.43 (0.20 - 0.93)
rs2242549	0.92	0.031	0.46 (0.23 - 0.93)
rs881803	0.41	0.29	0.67 (0.32 - 1.40)
rs2075512	0.97	**0.010**	**0.37 (0.17 - 0.79)**
Haplotype	0.87	0.029	0.42 (0.19 - 0.91)
SSRIs metabolised by CYP2C19 (N=282-318, no of instances: 357-395)			
rs4781678	0.42	**0.003**	**0.27 (0.11 - 0.64)**
rs2242549	0.47	**0.006**	**0.30 (0.13 – 0.70)**
rs881803	0.51	0.064	0.44 (0.19 - 1.05)
rs2075512	0.39	**0.005**	**0.31 (0.13 - 0.70)**
Haplotype	0.95	**0.006**	**0.30 (0.13 - 0.71)**
SSRIs not metabolised by CYP2C19 (N=123-145, no of instances: 142-169)			
rs4781678	0.48	0.71	1.36 (0.27 - 6.87)
rs2242549	0.30	0.58	1.37 (0.45 - 4.23)
rs881803	0.58	0.23	2.24 (0.60 - 8.38)
rs2075512	0.10	0.88	0.88 (0.19 - 4.13)
Haplotype	0.66	0.77	1.25 (0.27 - 5.76)
Non-SSRIs metabolised by CYP2C19 (N=318-355, no of instances: 411-459)			
rs4781678	0.24	**0.0051**	**3.33 (1.44 - 7.73)**
rs2242549	0.62	**0.0013**	**3.42 (1.61 - 7.25)**
rs881803	0.63	**0.00030**	**5.82 (2.25 - 15.0)**
rs2075512	0.92	**0.0064**	**3.63 (1.44 - 9.17)**
Haplotype	0.22	**0.00030**	**4.93 (2.07 - 11.76)**

p-values and their respective ORs that are below the Bonferroni threshold are in bold.
Medications included in each group analysis can be found in Figure S2, and Table S3.