The genetic contribution of the NO system at the glutamatergic post-synapse to schizophrenia: further evidence and meta-analysis

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Abstract

NO is a pleiotropic signaling molecule and has an important role for cognition and emotion. In the brain, NO is produced by neuronal nitric oxide synthase (NOS-I, encoded by *NOS1*) coupled to the NMDA receptor via PDZ interactions; this protein-protein interaction is disrupted upon binding of NOS1 adaptor protein (encoded by *NOS1AP*) to NOS-I. As both NOS1 and NOS1AP were associated with schizophrenia, we here investigated these genes in greater detail by genotyping new samples and conducting a meta-analysis of our own and published data. In doing so, we confirmed association of both genes with schizophrenia and found evidence for their interaction in increasing risk towards disease. Our strongest finding was the *NOS1* promoter SNP rs41279104, yielding an odds ratios of 1.29 in the meta-analysis. As findings from heterologous cell systems have suggested that the risk allele decreases gene expression, we studied the effect of the variant on *NOS1* expression in human post-mortem brain samples and found that the risk allele significantly decreases expression of *NOS1* in the prefrontal cortex. Bioinformatic analyses suggest that this might be due the replacement of six transcription factor binding sites by two new binding sites as a consequence of proxy SNPs. Taken together, our data argue that genetic variance in *NOS1* resulting in lower prefrontal brain expression of this gene contributes to schizophrenia liability, and that *NOS1* interacts with *NOS1AP* in doing so. The NOS1-NOS1AP PDZ interface may thus well constitute a novel target for small molecules in at least some forms of schizophrenia.

Introduction

Nitric oxide (NO) is a gaseous messenger with atypical properties, acting in a pleiotropic manner by guanylyl cyclase activation and also direct nitrosylation of target proteins including CREB and thereby genomic effector mechanisms. In the brain, NO is produced by the neuronal isoform of nitric oxide synthase, NOS-I, which is encoded by the *NOS1* gene located on chromosome 12q24.2-.3. Approximately one percent of all neurons express NOS-I, with almost every neuron in the brain receiving input from a NOS-I positive

cell. However, the highest levels of NOS-I can be found in the cerebellum, cortex, basal ganglia, hypothalamus, and hippocampus. NOS-I occurs in various neuronal subtypes. Its most prominent functional interaction partner in excitatory neurons, e.g. in the cortex and the hippocampus, is the glutamatergic NMDA receptor. NOS-I is activated by calcium influx through the NMDA receptor and coupled to the site of action via the postsynaptic density, a protein scaffold comprising *inter alia* of the proteins PSD-93/-95, SHANK, and DLGAP. The so-called NOS1 adaptor protein NOS1AP (previously termed CAPON, carboxy terminal PDZ domain ligand of neuronal NO synthase) competes with PSD-93/-95 for NOS-I binding and has both a PDZ as well as an N-terminal phosphotyrosine binding (PTB) domain which allows it to connect NOS-I to synapsin, forming a ternary NOS-I – NOS1AP – synapsin complex (Jaffrey et al., 2002). Also, the NOS-I – NOS1AP complex can bind to RASD1 (also known as DEXRAS1) (Fang et al., 2000), which belongs to the superfamily of small GTPases and itself is activated by NO (Fang et al., 2000; Jaffrey et al., 2002). This is accomplished upon NOS1AP binding, resulting in S-nitrosylation of a cysteine residue. Another protein that interacts with NOS-I at this site is DYNLL1 (dynein, light chain, LC8-type 1), previously termed PIN (protein inhibitor of NOS-I), although specificity of this interaction and its mechanism were later questioned. Rather, it might function as part of the neuronal machinery serving the axonal transport of NOS-I, as it was shown to be part of the microtubule-associated motor protein dynein complexes and hence termed dynein light chain of 8 kDa (LC8, DLC1). It is thought to link the dynein complex to cargo molecules including NOS-I (Rodriguez-Crespo et al., 2001), DLGAP1, GluN3A and PSD-95 (Navarro-Lerida et al., 2004) supporting the notion that DYNLL1 serves as a transport adaptor vehicle for proteins which constitute the glutamatergic postsynaptic complex. This is underscored by data showing that DYNLL1 is also part of the above mentioned NMDA – PSD-95 – NOS-I – DLGAP1 complex, probably trafficking this complex along microtubules and actin cytoskeleton (Haraguchi et al., 2000).

Both NOS-I as well as NOS1AP have repeatedly been suggested to be involved in the pathogenesis of schizophrenia which relates to the “glutamatergic” theory of schizophrenia and the role of NO in mediating NMDA receptor-mediated signaling. While there are numerous studies on animal models or post-mortem findings, reviewed elsewhere (NOS: (Bernstein et al., 2011); NOS1AP: (Brzustowicz, 2008)), the genetic data shall be summarized here in brief as both the *NOS1* and the *NOS1AP* genes have been described as functional candidates for schizophrenia. Regarding *NOS1*, the majority of functional candidate gene

studies yielded positive results, although the ethnicity of the investigated samples as well as the tested SNPs were quite heterogeneous. Only one study (Fallin et al., 2005), conducted in Ashkenazi Jews, employed a family-based design and yielding positive results. The first case-control study was published in 2002 and tested a potentially functional SNP in the 3'UTR in a sample of 215 Japanese schizophrenic patients (Shinkai et al., 2002), also with positive outcome. Subsequently, our group conducted a mutation analysis, qRT PCR and haplotype analysis in Caucasian patients suffering from schizophrenia arguing that a functional promoter SNP (rs41279104), resulting in decreased expression of a reporter gene in cell culture experiments (Saur et al., 2004), is associated with disease (Reif et al., 2006a). Since then, six more case-control association studies on schizophrenia and *NOS1* were published in total (Cui et al., 2010; Nicodemus et al., 2010; Okumura et al., 2009; Riley et al., 2010; Tang et al., 2008; Wang et al., 2012). Four of those came from Asian populations (two Chinese (Tang et al., 2008) and two Japanese (Cui et al., 2010; Okumura et al., 2009)), with mixed results: while Cui and associates replicated the positive finding on rs41279104 (and also provided evidence for reduced NOS-I expression on the protein level in BA9 - part of the dorsolateral prefrontal cortex - for risk allele carriers), Okumura could not, although two other *NOS1* SNPs were significant (but did not survive correction for multiple testing). The same was true for the study by Wang (Wang et al., 2012) in China, where a SNP in intron 2 of *NOS1* was only nominally significant. In contrast, the other study from China (Tang et al., 2008) found significant evidence for an association of *NOS1* (5'UTR and intron 2) with schizophrenia as well. One Irish population did not provide evidence for an association of *NOS1* with schizophrenia, although only 4 SNPs were tested and did not include the previously significant rs41279104 (Nicodemus et al., 2010). Taken together, these association studies rather argue for an association of the 5' end of *NOS1* – especially the promoter region – with schizophrenia. Not surprisingly, a small Chinese study (n=198) on a CA-dinucleotide repeat in the 3'UTR of the gene yielded negative results (Liou et al., 2002).

However, *NOS1* is not only a functional candidate gene, but also a positional candidate gene as the *NOS1* locus is a hot spot for schizophrenia in linkage analyses (an overview can be gathered from Fig. 1 in (Reif et al., 2006)). Yet not only linkage studies, but also genome-wide association studies (GWAS) have suggested *NOS1* as a schizophrenia risk gene; with rs6490121 located in intron 10 of *NOS1* yielding a p-value of 9.82×10^{-6} in the study by O'Donovan and colleagues (O'Donovan et al., 2008), thus being the third best hit of

the GWAS. A follow-up of this SNP in an Irish sample of 1,021 cases did not yield positive results (Riley et al., 2010), however.

The most promising association findings were followed up in endophenotype experiments. The functional promoter SNP rs41279104 was tested in studies using event-related potentials and functional near-infrared spectroscopy (Reif et al., 2006; Reif et al., 2011), providing evidence for a prefrontal deficit in risk allele carriers. In addition, Kawohl and associates (Kawohl et al., 2008) demonstrated that rs41279104 risk allele carriers had decreased loudness dependence of auditory evoked potentials, which is a functional marker of serotonergic transmission, arguing for a connection between the NO and serotonin systems as also shown on the protein and the neuronal network (Kiss and Vizi, 2001) level. Also, the GWAS risk SNP rs6490121 was shown to be functional in respect to general intelligence, working memory and visual sensory processing as measured by the electroencephalogram event-related P1 response (Donohoe et al., 2009; O'Donoghue et al., 2012). Most of these effects could also be observed in healthy controls, and not only patients. Also, healthy risk allele carriers had a reduction in ventromedial prefrontal grey matter volume and altered activation of this and other structures during working memory tasks (Rose et al., 2012).

In addition to *NOS1*, the *NOS1AP* gene has consistently been suggested to be associated with schizophrenia. As a finding from linkage studies on Canadian families (having Celtic or German background), suggesting the *NOS1AP* locus chromosome 1q22 as a linkage hot spot, this gene came into focus in psychosis research as fine-mapping could narrow the critical region to this gene (Brzustowicz et al., 2004). In the following years, this group has provided further evidence that *NOS1AP* is implicated in schizophrenia pathogenesis, stemming from family-based genetic studies and suggesting a functional variant (Wratten et al., 2009), although another group examining a UK sample argued that rather the neighboring gene *UHMK1* underlies the linkage peak (Puri et al., 2007; Puri et al., 2006). Following up the studies of Brzustowicz and colleagues, both positive (Kremeyer et al., 2009; Miranda et al., 2006; Zheng et al., 2005) as well as negative (Fang et al., 2008; Nicodemus et al., 2010; Nicodemus et al., 2008) family-based and case-control studies were published. In addition, a mutation analysis suggested that rare coding variants in *NOS1AP* underlie obsessive-compulsive disorder and autism (Delorme et al., 2010). Furthermore, *NOS1AP* was

suggested to play a role in antipsychotic-mediated QT_c prolongation (Aberg et al., 2010) which is not surprising given its highly significant influence on the QT_c interval.

Due to these repeatedly described and promising associations of both *NOS1* and *NOS1AP* (as well as three other genes coding for components of the glutamatergic synapse which interact with NOS-I in a protein-protein manner, namely *DYNLL1*, *RASD1* and *SYN2*) with schizophrenia, we here tested in three samples whether we can confirm these findings and whether *NOS1* and *NOS1AP* interact in an epistatic manner, which is expected due to their physical interaction. We also aimed to back up this data by meta-analytic approaches as well as bioinformatic analysis, and finally we attempted to replicate the functionality of *NOS1* rs41279104 in human post-mortem brain tissue.

Experimental Procedures

Genotyped samples

We here extended a previously described sample (Reif et al., 2006) by genotyping more markers and adding a further 75 patients suffering from schizophrenia. In brief, a total of 270 unrelated patients (thereof 54% males; mean age 41±13 years) from the Lower Franconia area in Germany participated, which were ascertained as inpatients at the Department of Psychiatry and Psychotherapy, University of Würzburg. None of the subjects remitted completely during the course of the disease and thus the sample consists entirely of patients suffering from chronic schizophrenia, i.e. it is selected for severe cases; diagnostic evaluation was made by at least two experienced psychiatrists. The control sample consisted of 720 individuals (thereof 52% males; mean age 33±11 years), all healthy blood donors, hospital staff and volunteers stemming from the same catchment area as the patient group. A further 101 patients with schizophrenia were recruited from care centers in Umeå, Northern Sweden. All patients had at least two discharge diagnoses of schizophrenia as well as a life-time diagnosis of schizophrenia. Final diagnosis was determined by the consensus of two research psychiatrists, and only patients for whom full consensus was reached were included. Mean age of patients at the time of DNA sampling was 50 years. Control subjects (n=168, mean age 59 years) were recruited from a random population prospective longitudinal study in Umeå, and none of the controls had a life-time diagnoses of schizophrenia or any other psychotic disorder. The sex ratio was similar in the two samples: the schizophrenic patients consisted of 52% males, and the control subjects of 45%

males. Finally, 270 unrelated psychiatric patients (thereof 84 females) from Spain were included (mean age 39±11 years). Patients came from the psychiatric in-patient and out-patient units of the Mental Health Service 4 of the Clinical Hospital, University of Valencia, Spain. The retrospective clinical data collected from each patient were compared with the information provided from previous clinical reports and family members. Diagnoses were confirmed by a consensus meeting with the treating psychiatrist and one of the psychiatrists of the research group. Patients also had a minimum one-year evolution of the illness and were on antipsychotic treatment at evaluation time. The control group consisted of 360 healthy unrelated subjects of Spanish origin (thereof 124 females) with no history or familiar background of mental disorders (mean age 37±15 years).

All of the patients suffered from schizophrenic disorders according to ICD-10 (Germany) or DSM-IV (Spain, Sweden) criteria. None of the subjects showed significant neurological comorbidity, epilepsy, mental retardation, or other somatic disorders suggesting organic psychiatric disorder. Patients with substance-induced psychotic episodes were excluded from the study as well. Both patients as well as controls were of Caucasian ethnicity. Only patients and volunteers who gave written informed consent after oral as well as written explanation about scope and aim of the investigation were enrolled in the study. All studies complied with the Declaration of Helsinki and were approved by the respective local ethical committees; informed consent was obtained from all participating subjects.

Genotyping and SNP selection

SNPs in the *NOS1AP* and *NOS1* regions have previously been examined in case-control studies of schizophrenia {Brzustowicz, 2004 #252; Cui, 2010 #715; Nicodemus, 2010 #747; Okumura, 2009 #749; Puri, 2006 #755; Tang, 2008 #676; Zheng, 2005 #299}. To enable meta-analysis, we have selected 13 *NOS1AP* SNPs (rs1572495, rs1538018, rs945713, rs1415263, rs4306106, rs3924139, rs4145621, rs1508263, rs3751284, rs7521206, rs905721, rs348624, rs1964052) and 8 *NOS1* SNPs (rs3782206, rs3837437, rs499776, rs3782219, rs3782221, rs1879417, rs4767540, rs41279104) from previously published studies for further genotyping. Furthermore, *SYN2* SNPs have previously been analyzed in a family-based setting in schizophrenia {Saviouk, 2007 #784}; we have selected seven polymorphisms (rs598747, rs598704, rs308969, rs931676, rs3817004, ss35528972, rs3755724) for genotyping from this study, however, we did not carry out a meta-analysis due to the

different study types. For *RASD1* and *DYNLL1*, no publications reported on an associations with schizophrenia; we therefore selected a set of eight representative SNPs (*RASD1*: rs4924755, rs711352, rs2232841, rs2232838; *DYNLL1*: rs12857, rs3916065, rs787828, rs9788155) capturing the common allelic variation in these genes including the 5 kb upstream and 3 kb downstream regions with minimal genotyping effort. For SNP selection, we used the *Tagger* function implemented in Haploview 4.2 using HapMap CEU as reference population. Together, this resulted in 36 selected SNPs. Genomic DNA of all participants was extracted from venous blood by the standard methods. Subsequent SNP genotyping was performed with Sequenom's MassArray® system using the iPlex® chemistry following the MassArray® iPlex® standard operation procedure. Primer sequences can be found in Supplementary Table 1.

NOS1 *mRNA quantification*

For quantification of total *NOS1* RNA expression in human brain, a sample of human post-mortem brains, with post-mortem intervals (PMI) from 28 h up to 111 h (mean PMI 54.84 ± 16.63) was obtained from the Medical Research Council (MRC) Sudden Death Brain and Tissue Bank, Edinburgh. From 76 deceased individuals, aged between 16 and 74 (N = 76; female = 18, male = 58; mean age 48.55 ± 12.79), DNA and RNA from three brain regions (amygdala, forebrain and midbrain) were isolated, using the MELT^{TM} Total Nucleic Acid Isolation System (Applied Biosystem, AM Foster City, 1983) and stored at -80°C until use. RNA quality, measured with a Bioanalyzer (Agilent), revealed RNA integrity numbers (RIN) ranging from 1.5-2.0. Total RNA from forebrain, midbrain and the amygdala of human post-mortem brains was reversely transcribed by using the iScript™ cDNA synthesis kit (Bio-Rad, München, Germany) on 1 µg total RNA of each sample. cDNA was quantified in triplicates on a Bio-Rad CFX384 real-time PCR detection system, by applying the iQ™ SYBR green supermix from Bio-Rad and *NOS1*-specific QuantiTect Primer (QT00043372) from Qiagen in a 10 µl reaction volume. PCR conditions were 5 min at 95°C, 40 cycles of 10 s at 95°C, 30 s at 60°C, followed by a melting curve analysis with a gradient of 65°C to 95°C of 0.5°C per 5 s. Raw *NOS1* expression data were normalized by mean efficiencies obtained from LinRegPCR and normalization factors based on the three (of six investigated) most stable housekeeping genes (*GAPDH*, *TBP*, *SDHA*), defined by the geNorm software. To investigate the influence of rs41279104 on gene expression, we carried out ANOVAs and post-hoc t-tests on normalized

logarithmized *NOS1* expression values in genotypic and dominant models. Allele-specific changes of gene expression were examined with linear regression.

Statistical Analysis

Statistical analysis of genotype data was performed with PLINK version 1.07 and R version 2.10. Quality control required polymorphic variants with a minor allele frequency (MAF) above 1%, a call rate (CR) above 90% and that overall genotype frequencies did not deviate from Hardy-Weinberg equilibrium (HWE; χ^2 HWE p-value ≥ 0.05); thirty variants complied with these inclusion criteria. In all three samples (i.e., German, Swedish and Spanish), rs3837437 yielded a MAF below 0.01; rs2232841, rs2232838, rs3916065, rs4145621, rs931676 did not reach the CR threshold and were thus excluded from further analysis.

Single-marker associations were calculated by comparison of allele and genotype counts using Fisher´s exact tests. Calculations were performed in each sample separately to account for ethnic discrepancies; for joint analyses, samples were subjected to meta-analysis (see below). For multi-marker association, haplotype blocks were defined according to the solid spine method; inferred haplotype counts in groups were compared with 1-degree-of-freedom χ^2 tests. P-values from single marker and haplotype analyses were separately adjusted for the number of tests performed in each sample using the conservative Bonferroni correction. In the combined analysis of all three samples, we achieve a power of 66% and 62% to detect nominal significant SNPs and haplotypes, respectively, conveying a relative risk of 1.4 to develop schizophrenic disorder assuming a co-dominant model and a MAF of 0.05 (Power for Genetic Association version 2.0).

Meta-analysis

To obtain maximal information regarding the *NOS1* and *NOS1AP* variants tested in the present study, we performed a meta-analysis of the data (significant SNPs only) presented here together with all previous case-control genotyping efforts. To this end, a Pub Med search was carried out using the keywords "*(NOS1AP* OR *CAPON)* AND schizophrenia" as well as "*NOS1* AND schizophrenia", to identify all genetic studies on *NOS1AP* (n=24 retrieved studies) or *NOS1* (n=42 retrieved) and schizophrenic disorders. Titles and abstracts were scrutinized to exclude non-genetic studies, reducing the number of included studies to

15 for *NOS1AP* and 15 for *NOS1*. Of those, 9 studies on *NOS1AP* (Brzustowicz et al., 2004; Costain et al., 2010; Fang et al., 2008; Greenwood et al., 2011; Husted et al., 2010; Kremeyer et al., 2009; Miranda et al., 2006; Nicodemus et al., 2008; Wratten et al., 2009) and one on *NOS1* (Fallin et al., 2005), presented family-based, but not case-control data and were therefore not integrated in the meta-analysis for methodological reasons. The remaining studies on *NOS1AP* (n=6; (Aberg et al., 2010; Delorme et al., 2010; Nicodemus et al., 2010; Puri et al., 2007; Puri et al., 2006; Zheng et al., 2005)) and *NOS1* (n=14; (Cui et al., 2010; Donohoe et al., 2009; Kawohl et al., 2008; Nicodemus et al., 2010; O'Donoghue et al., 2012; Okumura et al., 2009; Reif et al., 2006; Reif et al., 2011; Riley et al., 2010; Rose et al., 2012; Shinkai et al., 2002; Silberberg et al., 2010; Tang et al., 2008; Wang et al., 2012)) reported on case-control association data and were scrutinized in greater detailed. For *NOS1AP,* two and for *NOS1,* 11 further studies had to be excluded from meta-analysis for the following reasons. For the *NOS1AP* gene: (Aberg et al., 2010) reported on cases only, while (Puri et al., 2007) investigated SNPs that were not included in our genotyping battery. For the *NOS1* gene: (Kawohl et al., 2008; O'Donoghue et al., 2012; Rose et al., 2012) analysed exclusively healthy participants. (Donohoe et al., 2009; Nicodemus et al., 2010; Riley et al., 2010) and (Shinkai et al., 2002) only presented data on SNPs which have not been genotyped for the present study; no SNP data could be obtained from the study of (Wang et al., 2012); and the study by (Silberberg et al., 2010) was excluded because of the rather small sample size (n=26) precluding meaningful interpretation of the data. Finally, (Reif et al., 2011) and (Reif et al., 2006), were excluded because the case samples of both studies overlapped with the sample described here (while the control sample was extended more than two-fold). Therefore, four studies on *NOS1AP* (n_{max}^{cases}=2406) and three studies on *NOS1* (n_{max}^{cases}=2006) were meta-analytically treated together with the data presented in this report yielding a sample power of 99% for *NOS1AP* and 98% for *NOS1* to detect SNPs associations, based on a relative risk of 1.4 and a MAF of 0.05.

The calculations for meta-analysis were performed using R version 2.10 along with the package metaphor version 0.5-7 using the “rma” command. For meta-analysis, we calculated odds ratios (ORs) as a measure for effect size and applied the Q-statistic to assess heterogeneity therein. Inconsistency across studies was quantified with the I^2 metric (I^2=Q-df/Q). The joint OR was determined as the weighted average of effect sizes entering the meta-analysis. When no heterogeneity was detected in the effect sizes, we applied fixed-

effects models, where the weights correspond to the inversed variances of the study ORs. In the presence of significant ($p<0.05$) heterogeneity, we applied random-effects models. Here, weights are initially calculated as in the fixed-effects model, but are then down-weighted by the degree of variance of effect sizes. Visual inspection of Funnel plots (Supplementary Figure 1) did not argue for the presence of publication bias.

Interaction analysis

Interaction analyses were calculated with PLINK version 1.07. SNPs with Bonferroni-resistant association and/or $p<0.05$ in the meta-analysis (see below) were subjected to pairwise interaction analysis, examining the genetic effect of one SNP in dependence of the genotype of the other SNP. The search for such epistatic effects was performed in two modes, namely in the whole case-control sample with the command “fast-epistasis” and in the case-only study subset by use of the additional command “case-only”. Calculations were performed separately in each study sample as well as in the combined sample to increase power.

Bioinformatic analysis

Analyses of SNPs were performed with tools that are contained in the GenEpi toolbox (http://genepi_toolbox.i-med.ac.at/). Annotation of SNPs in linkage disequilibrium (LD) with associated SNPs in a distance of 500 kb was retrieved from the SNAP website version 2.2. Differential transcription factor binding site (TFBS) predictions were made using the web-based tool MatInspector version 2.1. To indicate a SNP´s possible influence on splice junctions such as predictions of splice sites as well as binding sites for splicing regulatory elements (SREs, including Intronic Splicing Enhancer (IES) and Intronic Splicing Silencer (ISS)), the Human Splicing Finder software (HSF) version 2.4.1 was used.

Results

Single marker analysis

We calculated case-control single marker analyses for each of the three samples separately due to their different geographic origin. Genotypic, allelic as well as dominant models were used (Table 1). Of the 12 SNPs in *NOS1AP*, six were nominally associated in at least one of the samples, with rs945713 surviving Bonferroni correction in the German

sample. In the Swedish sample, this SNP displayed a trend towards association (p=0.064). Three of the seven examined *NOS1* SNPs were nominally associated in at least one sample; rs499776 survived Bonferroni correction in the Swedish sample and was nominally associated in the two other samples. No SNP associations were observed in *SYN2*, *DYNLL1* and *RASD1*.

Haplotype analysis

LD plots of the analyzed *NOS1AP* and *NOS1* regions are displayed in Figure 1. In the haplotype analysis, we found two associated haplotypes in *NOS1AP*'s block 1 in the Swedish sample (Table 2) containing SNPs that all were significant in the single marker analysis in this sample. Furthermore, in the German sample, a *NOS1* haplotype containing rs4767540 and rs41279104 was nominally significant (p=0.028), although not on the Bonferroni-adjusted level.

Meta-analysis

All *NOS1AP* (Table 3) and *NOS1* (Table 4, Figure 2) SNPs genotyped in the present study were subjected to a meta-analysis, thereby also incorporating results from previously published studies. The maximum number of investigated patients was 2,466 for *NOS1AP* and 2,006 for *NOS1*, respectively. Six *NOS1AP* SNPs displayed significantly heterogeneous genetic effects, but no significant pooled effect size was determined with random effects models (Tables 3 and 4). In contrast, effect sizes of all examined *NOS1* SNPs were homogeneous; three thereof conveyed a significant pooled genetic effect (Table 4, Figure 2): rs3782206, rs499776 and rs41279104, with the latter yielding the strongest signal conveying odds ratios of 1.29 (dominant model) and 1.25 (allelic model), respectively.

Interaction analysis

Epistatic effects were tested in pairwise combinations of SNPs rs945713 and rs499776 as well as rs3782206 and rs41279104, of which meta-analytic treatment predicted significant genetic effect sizes (see above). Neither the separate nor the combined case-control samples revealed a significant interaction. However, when considering cases only, a significant interaction between rs945713 and rs41279104 was found in the German (p=0.004) and the combined sample (p=0.012).

Assessment of rs41279104 function on mRNA level

Allele-specific mRNA quantification in human post-mortem prefrontal cortices revealed a significant linear reduction (beta=-0.087) of *NOS1* expression values per minor/risk T allele of rs41279104. Differential expression by genotype group showed significant association in the prefrontal cortex (ANOVA p=0.036, post hoc t-test p=0.012). *NOS1* expression in the amygdala or the midbrain was not found to be influenced by rs41279104 (data not shown).

Bioinformatic analysis of rs945713, rs3782206, rs499776 and rs41279104

For functional prediction of rs945713, rs3782206, rs499776 and rs41279104, high LD proxies ($r^2 \geq 0.9$ and D´=1) were searched within a distance of 500 kb (Suppl. Table 2). This search resulted for the *NOS1AP* variant rs945713 in six high LD proxies, all located in the second intron of the gene. The three *NOS1* SNPs rs41279104, rs499776 and rs3782206 had altogether 33 proxies of which six were located in the promoter region, four in the first and 23 in the second intron of *NOS1*. For the promoter SNP rs41279104, which was found to be most strongly associated with schizophrenia, no clear function was predicted, but the minor alleles of its four proxies rs900622, rs12316771, rs34731287 and rs12312120 replace six transcription factor binding sites (TFBS) for HOXC9, MSX1, ELF5, DLX1, SPZ1 and MAZR by two new binding sites for E2F and HMX3. As all predicted TFBS are expressed in the nervous system, we predict rs41279104 together with its four promoter proxies to have an effect on *NOS1* expression, which is in line with the significant differential mRNA level in forebrain (see previous section).

Moreover, we observed differences in counts of predicted splicing regulatory elements between major and minor alleles of associated intronic SNPs and their proxies. Specifically, the risk (minor) allele of the *NOS1AP* variant rs945713 replaces binding sites for two splicing enhancers (SE) by one splicing inhibitor (SI). Furthermore, the *NOS1* associated SNP rs3782206 and its proxies create three SI and 11 SE binding sites, but erase 6 SI and 16 SE binding sites in the presence of the minor risk allele (suppl. Table 1). Finally, the protective minor allele of rs499776 along with its two proxies rs570234 and rs1681506 decrease the predisposition towards schizophrenia by deletion of three binding sites for SEs (see Suppl. Table 2), which may repress alternative splicing of *NOS1* transcripts.

Discussion

Molecular effects of risk alleles

By investigating three discovery samples from Germany, Sweden and Spain, followed by meta-analysis of these and published data, we provide evidence that variants of genes coding for components of the NO system at the glutamatergic post-synapse interact to increase the risk towards schizophrenia. While neither *RASD1*, *DYNLL1* (which have not yet been specifically tested before) nor *SYN2* (which had some prior evidence; (Saviouk et al., 2007)) gave a significant signal, SNPs in *NOS1AP* and *NOS1* did; both genes in the present case-control studies and the latter also in the meta-analysis. These SNPs have been previously suggested to contribute to schizophrenia liability, and most interestingly, they also interacted in increasing disease risk. Odds ratios were in the expected range for common variants, although the *NOS1* promoter polymorphism rs41279104 conveyed a relatively high risk with an OR=1.3. Most interestingly, following initial studies arguing for an effect of this SNP on reporter gene expression in heterologous cell systems, we could extend this data here by showing that the risk allele resulted in lower *NOS1* expression in the prefrontal cortex which is in line with data showing reduced NOS-I immunohistochemical staining in the prefrontal cortex in risk allele carriers (Cui et al., 2010). To clarify molecular function of rs41279104 and six high LD proxy SNPs on *NOS1* expression in greater detail, we used several *in silico* approaches which revealed that the SNPs' minor alleles replace putative binding sites for transcription factors that are known to be expressed in the prefrontal cortex; this provides a possible mechanism how rs41279104 by means of its proxies may influence *NOS1* expression.

In contrast to our results, a study by Silberberg et al. (2010) did not detect any changes in *NOS1* expression in rs41279104 carriers. Importantly, in the study by Silberberg et al. (2010) samples from both healthy and schizophrenic subjects were used, with increased *NOS1* expression in patients with schizophrenia, regardless of genotype. In contrast, samples analyzed in our study were only obtained from healthy subjects. Therefore, it is possible that in the Silberberg et al. (2010) study increased NOS1 expression in schizophrenic patients is masking a possible reduction in NOS1 expression caused by rs41279104. Alternatively, this might be due to a mere power problem, inherent to this kind of studies where only a few risk allele carriers can be tested and overall sample size is

limited. Moreover, this could be a brain region-specific effect, or different LD structure in the tested population. Nevertheless we are confident that we are indeed picking up true molecular consequences of rs41279104 at least in Caucasians.

Interestingly, a number of studies have suggested that NO plays an important role in the biochemical and behavioral effects of the psychotomimetic NMDA-receptor antagonist phencyclidine (PCP) (Palsson et al., 2010), and a recent finding demonstrates that prefrontal NO/sGC signaling is important for the effects of PCP (Fejgin et al., 2008). However, preclinical and clinical data underscores that both an abnormal increase and a decrease in NO signaling can underlie schizophrenia-like deficits. Taken together, the present and previous findings indicate a dysregulated NO system as part of the pathophysiology of schizophrenia. Hence, pharmacological manipulation of NO activity may be a fruitful approach when trying to alleviate cognitive dysfunctions in schizophrenia.

NOS-I – NOS1AP interaction as a molecular mechanism in schizophrenia

NOS1 and NOS1AP tightly interact on the protein level and both proteins have repeatedly been associated with schizophrenia not only on the genetic level, as outlined above, but also coming from other lines of research. The NOS I protein carries an amino-terminal PSD 95/Discs large/Zonula occludens 1 (PDZ)-domain followed by a β finger encoding a PDZ-motif (-ETTF-). The PDZ-motif of NOS I interacts with the PDZ2 domain of PSD 95/ 93, thereby anchoring NOS I to the postsynaptic density and allowing proximity of NOS-I to NMDA receptors. This allows activity-dependent NO production by NOS-I by Calcium-influx through NMDA receptors. The PDZ-domain of NOS-I directly interacts with NOS1AP (Jaffrey et al., 1998), which alters the subcellular localization of NOS-I away from the post synaptic density, by binding to RASD1 (Fang et al., 2000) or Synapsin 1 (Jaffrey et al., 2002). The binding of NOS1AP to NOS-I directly competes with the interaction between NOS-I and PSD-95/-93, and overexpression of NOS1AP was shown to reduce the interaction between NOS-I and NOS1AP (Jaffrey et al., 1998). Blocking the PDZ-domain of NOS-I with small molecule inhibitors has been suggested as a possible therapeutic approach in the treatment of depression, a hypothesis that is supported by preclinical evidence (Doucet et al., 2013). Since we show that expression levels of NOS-I are reduced in rs41279104 risk allele carriers, and since elevated expression of NOS1AP interfering with post-synaptic targeting of NOS-I (Jaffrey et al., 2002; Jaffrey et al., 1998) is elevated in schizophrenic

patients (Xu et al., 2005), a comparable approach (i.e., blocking NOS-I – NOS1AP interaction with small molecules) might provide a feasible strategy for treatment at least in patients carrying this risk allele. In this context, it is also interesting to note that another SNP that was associated with schizophrenia in a family-based study (and therefore not included in our meta-analysis) produced elevated NOS1AP promoter activity in human cell lines (Wratten et al., 2009).

Human functional consequences of comprised prefrontal NOS functioning

Being stimulated by findings in *Nos1* knockout mice that feature cognitive deficits (Zoubovsky et al., 2011), studies on the differential influence of *NOS1* polymorphisms on human cognition provided compelling evidence for a role of this gene in prefrontal function. This was not only shown in patients, but rather also in healthy controls outlined in this section. For instance, we demonstrated (Reif et al., 2006) that the rs41279104 risk allele is associated with fewer errors and a reduced P300 latency in a continuous performance test (CPT) and argued that this polymorphism, leading to reduced *NOS1* expression in the prefrontal cortex, might raise efficiency for executive functions by reducing the signal to noise ratio. This is achieved via lower NO levels, which will result in less activation of neighboring neurons (a smaller "NO cloud", see (Kiss and Vizi, 2001)). We later reported an influence of another *NOS1* promoter polymorphism (*NOS1* ex1f-VNTR) on the same task. Performance during the CPT was measured with EEG and the resulting No-Go centroid, associated with activation of the anterior cingulate gyrus (ACC), was localized significantly more posterior in subjects carrying the risk allele of *NOS1* ex1f-VNTR, which also leads to lower *NOS1* expression. This was interpreted as a diminished ACC activation in risk allele carriers leading to impaired medial prefrontal functioning. This assumption was corroborated by data showing brain differential activation in *NOS1* ex1f-VNTR risk allele carriers in a working memory and a stop-signal task (Kopf et al., 2012).

Furthermore, another NOS1 polymorphism (rs6490121) was associated with working memory as homozygous carriers of the risk allele performed more poorly (Donohoe et al., 2009). This effect was also observed for verbal IQ measures. That same SNP was found to be associated with lower P1 visual evoked potentials elicited by a spatial working memory task in a high density EEG study (O'Donoghue et al., 2012). Carriers of the risk allele showed significantly lower P1 responses than non-carriers, pointing to a function of *NOS1* even in

early sensory processing. Finally, Rose and colleagues (Rose et al., 2012) used voxel based morphometry and showed that grey matter volume in the ventromedial prefrontal cortex is significantly reduced in risk allele carriers. They also conducted a spatial working memory test and demonstrated increases in the activation of fronto-parietal working memory networks and a failure to disengage regions of the default mode network for risk allele carriers. Taken together, present data strongly suggests that genetic variation in *NOS1* – mainly underlying reduced expression of the gene – leads to compromised cognitive functioning and differential prefrontal brain activity also in healthy individuals. This influence on neurocircuitry exerted by *NOS1* might well underlie the association of this gene with schizophrenia, where cognitive deficits are amongst the core symptoms of the disease.

Limitations

A few limitations have to be considered in the interpretation of our data. First, the power of the discovery samples to detect effects was intermediate which might compromise the interaction analysis; as the meta-analytic study was well-powered, we aimed to overcome this issue. However, meta-analysis did not cover all previously significant SNPs so that further large-scale studies should incorporate a more extensive SNP panel also allowing for gene-based analyses. Ethnic differences in the investigated samples have also been taken into account, as different LD structures in different populations might obscure the linkage with "true" underlying risk variants. On the pathophysiological level, our data is at odds with three other studies arguing for unchanged (Cui et al., 2010) or even increased (Baba et al., 2004; Silberberg et al., 2010) *NOS1* expression in schizophrenia. As the study by Cui and associates also found reduced *NOS1* expression in risk allele carriers, one might rather assume that reduced *NOS1* expression is not to be found in schizophrenia as a whole but rather there is a genetically distinct schizophrenia sub-group that is characterized by compromised NO signaling, while other sub-groups might display compensatory up-regulation of *NOS1*. Such a genetic dissection of schizophrenia might lead to more meaningful insights into disease mechanisms than rather treating the disorder on the aggregate level which obscures biological findings.

Outlook

Taken together, from our data it appears that reduction of NOS-I expression as a consequence of genetic variation and especially in conjunction with increased NOS1AP

expression poses a risk factor for the development of schizophrenia. Further downstream, reduction of PSD-95/-93 associated NOS-I and consequently compromised NMDA – NO signaling might be the converging mechanism underlying at least some forms of psychosis. This puts NO pathways in the glutamatergic post-synapse central to the development of this disorder and calls for innovative pharmacological targeting of this protein complex. In line with these assumptions, a recent study demonstrated that a single dose of an NO donor was able to significantly reduce schizophrenia symptoms rapidly (4h) after infusion, and that this effect was detectable for almost 4 weeks (Hallak et al., 2013). Thus, genetic data informing about reduced *NOS1* expression might well provide information on which patients would best benefit from such an intervention in the sense of personalized medicine. According studies to either corroborate or reject this hypothesis might provide valuable insights in the role of NO in schizophrenia.

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Figures

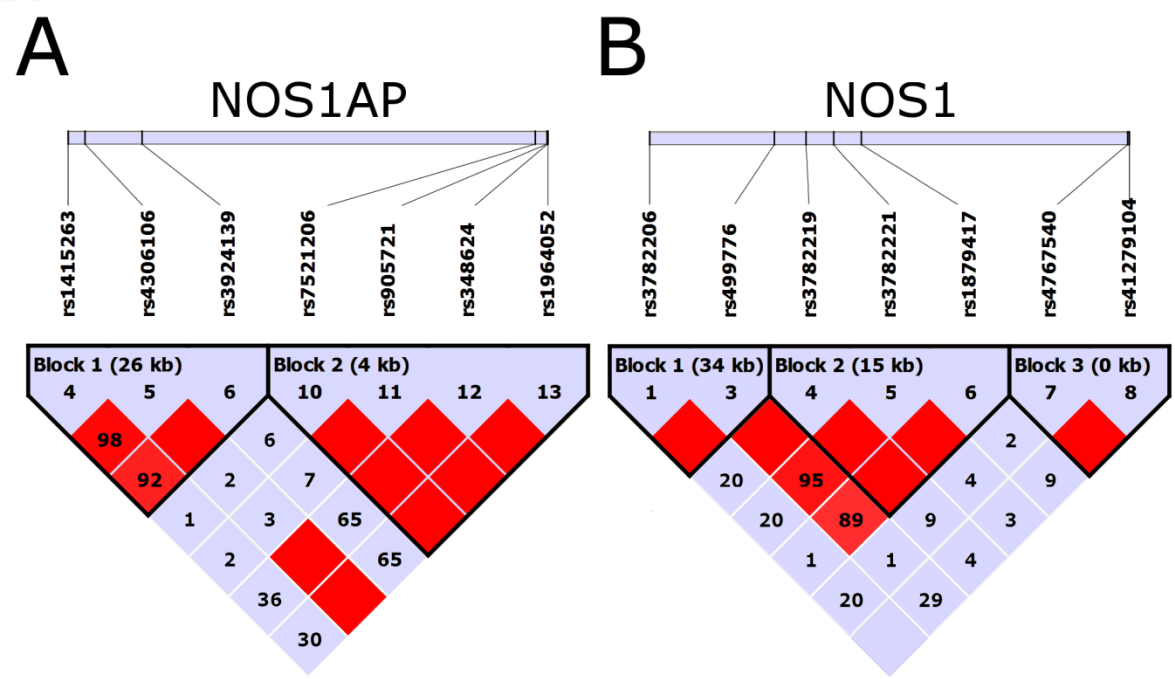


Figure 1: Linkage disequilibrium (LD) plots of *NOS1AP* **(A)** and *NOS1* **(B)**. LD is displayed as D' Haplotype blocks as defined by the solid spine method.

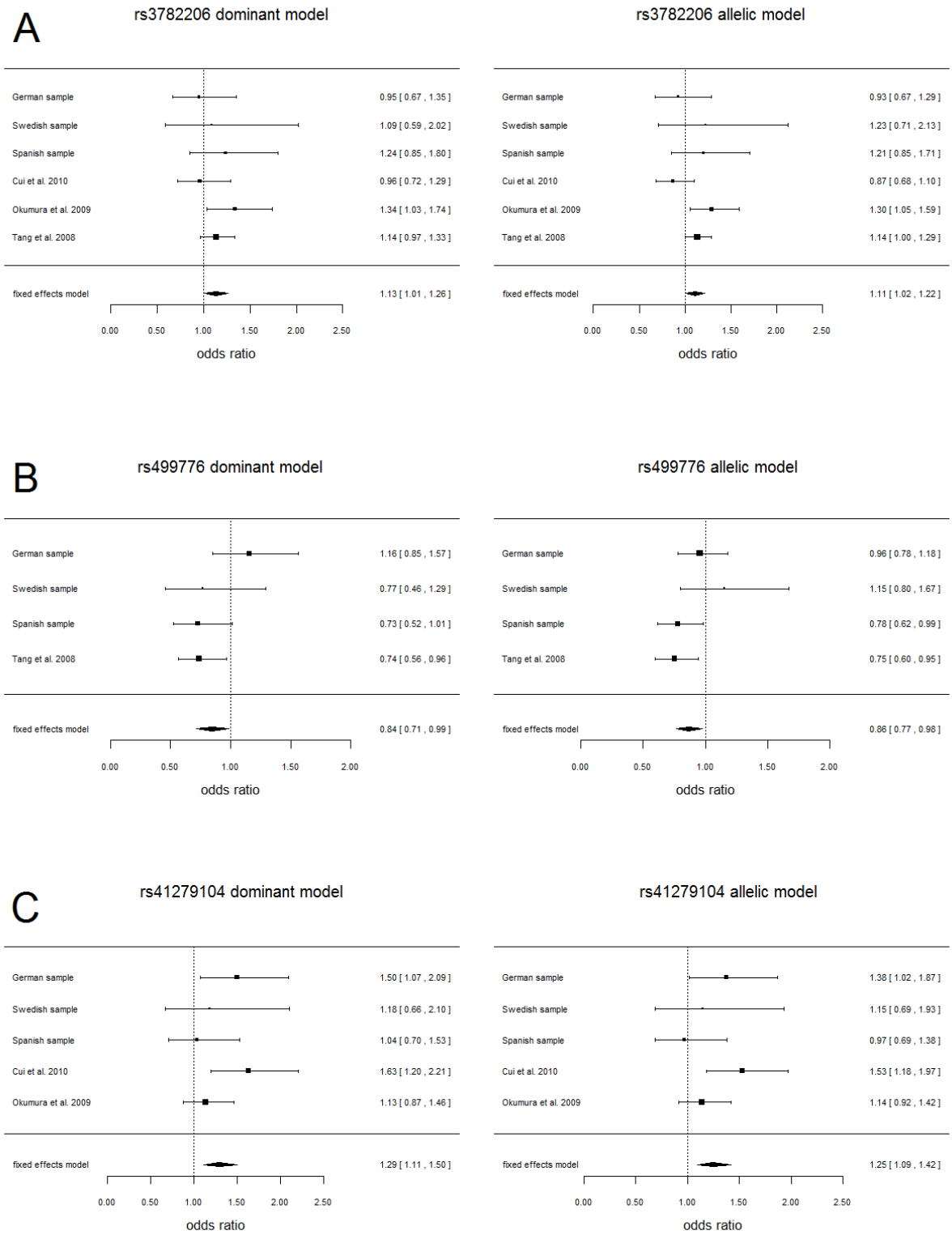


Figure 2: Forest plots of *NOS1AP* rs3782206 dominant and allelic model **(A)**, *NOS1* rs499776 dominant and allelic model **(B)** and *NOS1* rs41279104 dominant and allelic model **(C)**

Table 1: Association results for examined SNPs along with their chromosomal position, minor/major alleles, genotype and allele counts for cases and controls, the nominal p-value of Fisher´s exact tests of a genotypic, allelic and dominant model and the respective Bonferroni-corrected p-values for the German, Swedish and Spanish samples. Bold indicates SNPs with at least one significant p-value (p<0.05) in one or more of the three calculated models. Chromosomal positions were given according to the latest NCBI Genome assembly GRCh37.p5.

				German sample				Swedish sample				Spanish sample			
	Chromosomal	Allele		Number of Genotypes		Nominal	Bonferroni	Number of Genotypes		Nominal	Bonferroni	Number of Genotypes		Nominal	Bonferroni
SNP	Position (bp)	(d/D)	Association Model	Controls (n=720)	Cases (n=270)	P-value	P-value	Controls (n=168)	Cases (n=101)	P-value	P-value	Controls (n=360)	Cases (n=270)	P-value	P-value
NOS1AP (Capon); Chromosome 1:															
rs1572495	162099301	T/C	Genotypic n(dd/dD/DD)	7/108/587	3/39/213	0,936	1	1/22/140	0/11/89	0,735	1	2/56/284	4/41/223	0,552	1
			Allelic n(d/D)	122/1282	45/465	0,927	1	24/302	11/189	0,474	1	60/624	49/487	0,840	1
			Dominant n(dd+dD/DD)	115/587	42/213	1,000	1	23/140	11/89	0,571	1	58/284	45/223	1,000	1
rs1538018	162130481	C/G	Genotypic n(dd/dD/DD)	--	--	--	--	8/57/90	8/35/54	0,620	1	--	--	--	--
			Allelic n(d/D)	--	--	--	--	73/237	51/143	0,524	1	--	--	--	--
			Dominant n(dd+dD/DD)	--	--	--	--	65/90	43/54	0,794	1	--	--	--	--
rs945713	**162135670**	**C/T**	**Genotypic n(dd/dD/DD)**	**102/339/257**	**57/130/73**	**0,006**	**0,167**	36/71/49	12/48/37	0,101	1	63/162/118	49/139/79	0,413	1
			Allelic n(d/D)	**543/853**	**244/276**	**0,002**	**0,048**	143/169	72/122	0,064	1	288/398	237/297	0,415	1
			Dominant n(dd+dD/DD)	**441/257**	**187/73**	**0,012**	**0,326**	107/49	60/37	0,278	1	225/118	188/79	0,222	1
rs1415263	**162166043**	**T/C**	**Genotypic n(dd/dD/DD)**	107/333/262	43/134/78	0,155	1	**38/79/44**	**10/51/38**	**0,012**	**0,353**	71/162/108	50/138/77	0,532	1
			Allelic n(d/D)	547/857	220/290	0,102	1	**155/167**	**71/127**	**0,006**	**0,191**	304/378	238/292	0,954	1
			Dominant n(dd+dD/DD)	440/262	177/78	0,056	1	**117/44**	**61/38**	**0,074**	**1**	233/108	188/77	0,534	1
rs4306106	**162171994**	**A/G**	**Genotypic n(dd/dD/DD)**	36/217/442	14/93/151	0,337	1	**16/64/75**	**3/35/59**	**0,042**	**1**	29/139/175	16/118/132	0,419	1
			Allelic n(d/D)	289/1101	121/395	0,210	1	**96/214**	**41/153**	**0,018**	**0,536**	197/489	150/382	0,848	1
			Dominant n(dd+dD/DD)	253/442	107/151	0,154	1	**80/75**	**38/59**	**0,069**	**1**	168/175	134/132	0,744	1
rs3924139	**162192112**	**G/A**	**Genotypic n(dd/dD/DD)**	84/325/288	40/122/97	0,303	1	**33/79/45**	**7/49/41**	**0,004**	**0,128**	65/154/126	41/139/87	0,179	1
			Allelic n(d/D)	493/901	202/316	0,149	1	**145/169**	**63/131**	**0,003**	**0,087**	284/406	221/313	0,953	1
			Dominant n(dd+dD/DD)	409/288	162/97	0,299	1	**112/45**	**56/41**	**0,029**	**0,884**	219/126	180/87	0,347	1
rs1508263	**162279509**	**A/G**	**Genotypic n(dd/dD/DD)**	139/358/199	46/119/80	0,490	1	34/68/51	25/49/21	0,166	1	**69/176/97**	**30/142/94**	**0,007**	**0,187**
			Allelic n(d/D)	636/756	211/279	0,317	1	136/170	99/91	0,116	1	**314/370**	**202/330**	**0,006**	**0,155**
			Dominant n(dd+dD/DD)	497/199	165/80	0,255	1	102/51	74/21	0,063	1	**245/97**	**172/94**	**0,078**	**1**
rs3751284	**162313735**	**A/G**	**Genotypic n(dd/dD/DD)**	90/307/298	29/116/110	0,825	1	**30/65/67**	**8/54/38**	**0,022**	**0,653**	32/168/136	33/111/123	0,104	1
			Allelic n(d/D)	487/903	174/336	0,744	1	**125/199**	**70/130**	**0,457**	**1**	232/440	177/357	0,625	1
			Dominant n(dd+dD/DD)	397/298	145/110	0,941	1	**95/67**	**62/38**	**0,606**	**1**	200/136	144/123	0,185	1

rs7521206	162330927	G/C	Genotypic n(dd/dD/DD)	91/323/288	35/113/107	0,888	1	20/85/59	13/53/34	0,935	1	47/172/123	34/128/106	0,659	1
			Allelic n(d/D)	505/899	183/327	1,000	1	125/203	79/121	0,783	1	266/418	196/340	0,440	1
			Dominant n(dd+dD/DD)	414/288	148/107	0,824	1	105/59	66/34	0,791	1	219/123	162/106	0,400	1
rs905721	162335052	T/C	Genotypic n(dd/dD/DD)	94/322/282	38/116/104	0,868	1	18/80/59	13/51/33	0,811	1	49/171/122	33/130/105	0,605	1
			Allelic n(d/D)	510/886	192/324	0,790	1	116/198	77/117	0,573	1	269/415	196/340	0,342	1
			Dominant n(dd+dD/DD)	416/282	154/104	1,000	1	98/59	64/33	0,593	1	220/122	163/105	0,399	1
rs348624	162335256	T/C	Genotypic n(dd/dD/DD)	10/141/537	3/51/194	1,000	1	2/23/135	1/19/76	0,584	1	--	--	--	--
			Allelic n(d/D)	161/1215	57/439	0,935	1	27/293	21/171	0,352	1	--	--	--	--
			Dominant n(dd+dD/DD)	151/537	54/194	1,000	1	25/135	20/76	0,312	1	--	--	--	--
rs1964052	162335424	T/C	Genotypic n(dd/dD/DD)	10/145/545	3/55/195	0,948	1	2/25/136	1/19/79	0,777	1	2/70/268	5/53/203	0,394	1
			Allelic n(d/D)	165/1235	61/445	0,873	1	29/297	21/177	0,542	1	74/606	63/459	0,523	1
			Dominant n(dd+dD/DD)	155/545	58/195	0,792	1	27/136	20/79	0,508	1	72/268	58/203	0,765	1
SYN2; Chromosome 3:															
rs598747	12112010	C/T	Genotypic n(dd/dD/DD)	9/152/537	6/57/190	0,439	1	4/51/106	3/28/69	0,761	1	--	--	--	--
			Allelic n(d/D)	170/1226	69/437	0,390	1	59/263	34/166	0,726	1	--	--	--	--
			Dominant n(dd+dD/DD)	161/537	63/190	0,546	1	55/106	31/69	0,685	1	--	--	--	--
rs598704	12112053	C/T	Genotypic n(dd/dD/DD)	40/254/403	16/101/129	0,329	1	17/67/73	5/42/50	0,291	1	21/139/185	17/108/142	1,000	1
			Allelic n(d/D)	334/1060	133/359	0,182	1	101/213	52/142	0,232	1	181/509	142/392	0,896	1
			Dominant n(dd+dD/DD)	294/403	117/129	0,156	1	84/73	47/50	0,442	1	160/185	125/142	0,935	1
rs308969	**12177083**	**C/T**	**Genotypic n(dd/dD/DD)**	10/107/584	0/47/208	0,080	1	2/34/128	2/19/78	0,824	1	**3/70/269**	**0/40/228**	**0,049**	**1**
			Allelic n(d/D)	127/1275	47/463	0,928	1	38/290	23/175	1,000	1	**76/608**	**40/496**	**0,039**	**1**
			Dominant n(dd+dD/DD)	117/584	47/208	0,561	1	36/128	21/78	1,000	1	**73/269**	**40/228**	**0,046**	**1**
rs3817004	12195674	G/A	Genotypic n(dd/dD/DD)	0/42/656	2/17/236	0,098	1	0/16/148	0/8/92	0,826	1	1/23/316	1/22/245	0,770	1
			Allelic n(d/D)	42/1354	21/489	0,247	1	16/312	8/192	0,830	1	25/655	24/512	0,557	1
			Dominant n(dd+dD/DD)	42/656	19/236	0,455	1	16/148	8/92	0,826	1	24/316	23/245	0,542	1
ss35528972	12197255	T/A	Genotypic n(dd/dD/DD)	3/73/618	0/30/227	0,636	1	1/21/135	0/9/88	0,644	1	2/48/294	0/30/237	0,364	1
			Allelic n(d/D)	79/1309	30/484	0,912	1	23/291	9/185	0,263	1	52/636	30/504	0,205	1
			Dominant n(dd+dD/DD)	76/618	30/227	0,729	1	22/135	9/88	0,326	1	50/294	30/237	0,277	1
rs3755724	12200906	T/C	Genotypic n(dd/dD/DD)	71/294/327	26/103/120	0,959	1	19/84/54	11/51/36	0,932	1	29/149/149	33/102/127	0,158	1
			Allelic n(d/D)	436/948	155/343	0,910	1	122/192	73/123	0,779	1	207/447	168/356	0,900	1
			Dominant n(dd+dD/DD)	365/327	129/120	0,825	1	103/54	62/36	0,788	1	178/149	135/127	0,507	1
NOS1; Chromosome 12:															
rs3782206	117745089	T/C	Genotypic n(dd/dD/DD)	7/141/548	1/52/206	0,835	1	2/30/126	4/17/76	0,347	1	2/72/268	2/66/199	0,530	1
			Allelic n(d/D)	155/1237	54/464	0,681	1	34/282	25/169	0,478	1	76/608	70/464	0,288	1
			Dominant n(dd+dD/DD)	148/548	53/206	0,858	1	32/126	21/76	0,874	1	74/268	68/199	0,289	1

rs499776	**117779499**	**A/G**	**Genotypic n(dd/dD/DD)**	**123/331/243**	**32/145/82**	**0,038**	**1**	**12/90/55**	**20/37/40**	**0,002**	**0,046**	**58/166/119**	**34/121/113**	**0,117**	**1**
			Allelic n(d/D)	**577/817**	**209/309**	**0,714**	**1**	**114/200**	**77/117**	**0,452**	**1**	**282/404**	**189/347**	**0,038**	**0,997**
			Dominant n(dd+dD/DD)	**454/243**	**177/82**	**0,398**	**1**	**102/55**	**57/40**	**0,351**	**1**	**224/119**	**155/113**	**0,065**	**1**
rs3782219	117788240	T/C	--	--	--	--	--	4/55/100	6/23/68	0,080	1	--	--	--	--
			--	--	--	--	--	63/255	35/159	0,645	1	--	--	--	--
			--	--	--	--	--	59/100	29/68	0,278	1	--	--	--	--
rs3782221	117795881	A/G	Genotypic n(dd/dD/DD)	31/244/420	13/93/146	0,740	1	5/62/92	7/26/67	0,052	1	17/127/196	23/95/144	0,184	1
			Allelic n(d/D)	306/1084	119/385	0,455	1	72/246	40/160	0,512	1	161/519	141/383	0,203	1
			Dominant n(dd+dD/DD)	275/420	106/146	0,500	1	67/92	33/67	0,151	1	144/196	118/144	0,562	1
rs1879417	**117803515**	**T/C**	**Genotypic n(dd/dD/DD)**	**163/349/178**	**65/130/57**	**0,561**	**1**	**26/91/45**	**28/43/28**	**0,044**	**1**	72/169/97	51/132/84	0,702	1
			Allelic n(d/D)	**675/705**	**260/244**	**0,323**	**1**	**143/181**	**99/99**	**0,206**	**1**	313/363	234/300	0,416	1
			Dominant n(dd+dD/DD)	**512/178**	**195/57**	**0,350**	**1**	**117/45**	**71/28**	**1,000**	**1**	241/97	183/84	0,475	1
rs4767540	117877007	G/A	Genotypic n(dd/dD/DD)	160/324/213	64/122/71	0,638	1	28/76/51	17/42/36	0,688	1	67/160/118	47/129/89	0,817	1
			Allelic n(d/D)	644/750	250/264	0,352	1	132/178	76/114	0,577	1	294/396	223/307	0,861	1
			Dominant n(dd+dD/DD)	484/213	186/71	0,425	1	104/51	59/36	0,494	1	227/118	176/89	0,931	1
rs41279104	**117877485**	**T/C**	**Genotypic n(dd/dD/DD)**	**8/130/559**	**2/66/184**	**0,035**	**0,979**	**3/35/124**	**2/24/72**	**0,877**	**1**	9/64/268	4/55/209	0,567	1
			Allelic n(d/D)	**146/1248**	**70/434**	**0,041**	**1**	**41/283**	**28/168**	**0,596**	**1**	82/600	63/473	0,929	1
			Dominant n(dd+dD/DD)	**138/559**	**68/184**	**0,020**	**0,571**	**38/124**	**26/72**	**0,656**	**1**	73/268	59/209	0,921	1
DYNLL1 (PIN); Chromosome 12:															
rs12857	120933946	T/G	Genotypic n(dd/dD/DD)	21/202/471	11/64/174	0,374	1	7/47/103	4/34/59	0,679	1	17/101/186	11/76/170	0,452	1
			Allelic n(d/D)	244/1144	86/412	0,945	1	61/253	42/152	0,571	1	135/473	98/416	0,210	1
			Dominant n(dd+dD/DD)	223/471	75/174	0,579	1	54/103	38/59	0,502	1	118/186	87/170	0,253	1
rs787828	120937086	T/A	Genotypic n(dd/dD/DD)	86/296/320	27/128/99	0,081	1	20/70/70	10/41/48	0,722	1	51/155/136	47/121/100	0,644	1
			Allelic n(d/D)	468/936	182/326	0,325	1	110/210	61/137	0,442	1	257/427	215/321	0,375	1
			Dominant n(dd+dD/DD)	382/320	155/99	0,077	1	90/70	51/48	0,521	1	206/136	168/100	0,558	1
rs9788155	120938408	A/G	Genotypic n(dd/dD/DD)	68/292/338	23/105/131	0,845	1	16/65/76	12/38/46	0,867	1	22/123/197	26/101/141	0,242	1
			Allelic n(d/D)	428/968	151/367	0,538	1	97/217	62/130	0,768	1	167/517	153/383	0,116	1
			Dominant n(dd+dD/DD)	360/338	128/131	0,561	1	81/76	50/46	1,000	1	145/197	127/141	0,220	1
RASD1 (DEXRAS1); Chromosome 17:															
rs4924755	17397131	G/C	Genotypic n(dd/dD/DD)	60/280/363	16/100/137	0,515	1	7/48/108	1/33/65	0,340	1	33/133/175	31/110/127	0,561	1
			Allelic n(d/D)	400/1006	132/374	0,326	1	62/264	35/163	0,729	1	199/483	172/364	0,287	1
			Dominant n(dd+dD/DD)	340/363	116/137	0,510	1	55/108	34/65	1,000	1	166/175	141/127	0,369	1
rs711352	17397818	C/G	Genotypic n(dd/dD/DD)	56/261/378	13/92/144	0,300	1	7/43/108	1/30/67	0,319	1	24/124/189	25/107/136	0,351	1
			Allelic n(d/D)	373/1017	118/380	0,190	1	57/259	32/164	0,719	1	172/502	157/379	0,153	1
			Dominant n(dd+dD/DD)	317/378	105/144	0,373	1	50/108	31/67	1,000	1	148/189	132/136	0,218	1

Table 2: Association results for haplotypes examined in the German, Swedish and Spanish samples along with frequencies in cases and controls, nominal P-values of χ2-Tests in one degree of freedom and the Bonferroni-corrected p-values, corrected over all haplotypes. Bold indicates significant haplotypes p<0.05 in at least one sample.

						German sample			Swedish sample			Spanish sample		
NOS1AP (Capon); Chromosome 1						**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block1:	**rs1415263**	**rs4306106**	**rs3924139**			**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
	T	**A**	**G**			0,24/0,21	0,206	1	**0,22/0,31**	**0,018**	**0,527**	0,28/0,29	0,796	1
	T	G	G			0,15/0,14	0,500	1	0,11/0,14	0,361	1	0,13/0,11	0,427	1
	C	G	G			0,02/0,02	0,822	1	0,01/0,02	0,182	1	--	--	--
	T	G	A			0,06/0,06	0,901	1	0,05/0,05	0,963	1	0,04/0,05	0,467	1
	C	**G**	**A**			0,56/0,60	0,130	1	**0,64/0,50**	**0,002**	**0,066**	0,55/0,55	0,984	1
						Frequencies	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block2:	**rs7521206**	**rs905721**	**rs348624**	**rs1964052**		**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
	C	C	T	T		0,12/0,12	0,944	1	0,11/0,09	0,575	1	0,12/0,11	0,546	1
	G	T	C	C		0,37/0,37	0,848	1	0,40/0,38	0,765	1	0,36/0,39	0,334	1
	C	C	C	C		0,52/0,53	0,889	1	0,51/0,54	0,537	1	0,52/0,5	0,577	1
SYN2; Chromosome 3:						**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block1:	**rs598747**	**rs598704**	**rs308969**	**rs3817004**	**ss35528972**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
	C	C	C	A	T	0,06/0,06	0,937	1	0,05/0,07	0,268	1	0,06/0,08	0,169	1
	C	C	T	G	A	0,04/0,04	0,358	1	0,05/0,05	0,739	1	0,04/0,04	0,564	1
	C	C	C	A	A	0,04/0,04	0,795	1	0,07/0,05	0,325	1	0,02/0,03	0,111	1
	C	C	T	A	A	--	--	--	0,02/0,02	0,729	1	0,15/0,12	0,118	1
	T	C	T	A	A	0,13/0,12	0,534	1	0,10/0,14	0,144	1	--	--	--
	T	T	T	A	A	0,74/0,77	0,314	1	0,74/0,68	0,174	1	0,73/0,74	0,936	1
NOS1; Chromosome 12:						**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block1:	**rs3782206**	**rs499776**				**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
	C	**A**				0,41/0,42	0,725	1	0,40/0,37	0,444	1	**0,34/0,04**	**0,032**	**0,895**
	T	G				0,11/0,11	0,807	1	0,13/0,11	0,482	1	0,12/0,10	0,260	1
	C	G				0,50/0,49	0,621	1	0,48/0,53	0,233	1	0,53/0,49	0,166	1
						Frequencies	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block2:	**rs3782219**	**rs3782221**	**rs1879417**			**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
	C	G	C			--	--	--	0,51/0,45	0,188	1	0,42/0,44	0,339	1

T	A	T	--	--	--	0,20/0,22	0,507	1	0,24/0,22	0,284	1
C	G	T	--	--	--	0,31/0,34	0,409	1	0,32/0,32	0,954	1
			Frequencies	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block3: rs4767540 rs41279104			**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
G	**T**		**0,15/0,11**	**0,028**	**0,722**	0,16/0,13	0,469	1	0,12/0,12	0,955	1
G	C		0,35/0,36	0,565	1	0,26/0,30	0,285	1	0,3/0,31	0,825	1
A	C		0,52/0,55	0,394	1	0,6/0,58	0,637	1	0,58/0,57	0,865	1
DYNLL1 (PIN); Chromosome 12:			**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block1: rs12857 rs787828 rs9788155			**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
G	A	A	0,29/0,31	0,520	1	0,33/0,31	0,684	1	0,28/0,24	0,118	1
G	T	G	0,36/0,34	0,279	1	0,31/0,35	0,363	1	0,4/0,38	0,363	1
T	A	G	0,18/0,18	0,859	1	0,21/0,20	0,680	1	0,18/0,22	0,102	1
G	A	G	0,18/0,19	0,698	1	0,16/0,16	0,837	1	0,13/0,16	0,183	1
RASD1 (DEXRAS1); Chromosome 17:			**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**	**Frequencies**	**Nominal**	**Bonferroni**
Block1: rs4924755 rs711352			**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**	**Case/Control**	**P-Value**	**P-Value**
G	C		0,24/0,27	0,171	1	0,17/0,19	0,640	1	0,29/0,25	0,140	1
G	G		0,03/0,02	0,685	1	--	--	--	0,03/0,04	0,305	1
C	G		0,75/0,72	0,222	1	0,84/0,82	0,640	1	0,68/0,71	0,306	1

Table 3: Meta-analysis of 12 NOS1AP variants. Table shows all SNPs along with their minor/major alleles, cases and control counts for the genotypic and dominant model as well as the nominal p-values for each sample and the total sample. Further are given p-values for heterogeneity and odds ratios plus p-values of the fixed effect and random effect model. Bold indicates SNPs with at least one significant p-value (p<0.05).

SNPs	**rs1572495**		**rs1538018**		**rs945713**		**rs1415263**		**rs4306106**		**rs3924139**		**rs1508263**		**rs3751284**		**rs7521206**		**rs905721**		**rs348624**		**rs1964052**	
Alleles (d/D)	**T/C**		**C/G**		**C/T**		**T/C**		**A/G**		**G/A**		**A/G**		**A/G**		**G/C**		**T/C**		**T/C**		**T/C**	
Test	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**
German sample (Controls: n=720; Cases: n=270)																								
Controls	122/1282	115/587	--	--	543/853	441/257	547/857	440/262	289/1101	253/442	493/901	409/288	636/756	497/199	487/903	397/298	505/899	414/288	510/886	416/282	161/1215	151/537	165/1235	155/545
Cases	45/465	42/213	--	--	244/276	187/73	220/290	177/78	121/395	107/151	202/316	162/97	211/279	165/80	174/336	145/110	183/327	148/107	192/324	154/104	57/439	54/194	61/445	58/195
P-value	*0,927*	*1,000*	--	--	*0,002**	*0,012*	*0,102*	*0,056*	*0,210*	*0,154*	*0,149*	*0,299*	*0,317*	*0,255*	*0,744*	*0,941*	*1,000*	*0,824*	*0,790*	*1,000*	*0,935*	*1,000*	*0,873*	*0,792*
Swedish sample (Controls: n=168; Cases: n=101)																								
Controls	24/302	23/140	73/237	65/90	143/169	107/49	155/167	117/44	96/214	80/75	145/169	112/45	136/170	102/51	125/199	95/67	125/203	105/59	116/198	98/59	27/293	25/135	29/297	27/136
Cases	11/189	11/89	51/143	43/54	72/122	60/37	71/127	61/38	41/153	38/59	63/131	56/41	99/91	74/21	70/130	62/38	79/121	66/34	77/117	64/33	21/171	20/76	21/177	20/79
P-value	*0,474*	*0,571*	*0,524*	*0,794*	*0,064*	*0,278*	*0,006*	*0,074*	*0,018*	*0,069*	*0,003*	*0,029*	*0,116*	*0,063*	*0,457*	*0,606*	*0,783*	*0,791*	*0,573*	*0,593*	*0,352*	*0,312*	*0,542*	*0,508*
Spanish sample (Controls: n=360; Cases: n=270)																								
Controls	60/624	58/284	--	--	288/398	225/118	304/378	233/108	197/489	168/175	284/406	219/126	314/370	245/97	232/440	200/136	266/418	219/123	269/415	220/122	--	--	74/606	72/268
Cases	49/487	45/223	--	--	237/297	188/79	238/292	188/77	150/382	134/132	221/313	180/87	202/330	172/94	177/357	144/123	196/340	162/106	196/340	163/105	--	--	63/459	58/203
P-value	*0,840*	*1,000*	--	--	*0,415*	*0,222*	*0,954*	*0,534*	*0,848*	*0,744*	*0,953*	*0,347*	*0,006*	*0,078*	*0,625*	*0,185*	*0,440*	*0,400*	*0,342*	*0,399*	--	--	*0,523*	*0,765*
Nicodemus et al., 2010 (Controls: n=487; Cases: n=415)																								
Controls	94/646	81/288	140/476	126/182	296/422	243/116	309/429	248/121	--	--	--	--	--	--	263/469	214/152	--	--	--	--	106/516	92/219	--	--
Cases	55/585	53/267	152/392	132/140	238/394	193/123	242/388	201/114	--	--	--	--	--	--	234/400	202/115	--	--	--	--	67/485	62/214	--	--
P-value	*0,014*	*0.075*	*0,041*	*0.065*	*0,181*	*0,073*	*0,194*	*0,351*	--	--	--	--	--	--	*0,707*	*0,161*	--	--	--	--	*0,018*	*0,050*	--	--
Delorme et al., 2010 (Controls: n=286; Cases: n=296)																								
Controls	--	--	--	--	--	--	--	--	--	--	--	--	--	--	61/91	50/26	--	--	189/309	147/102	34/283	31/128	34/283	31/128
Cases	--	--	--	--	--	--	--	--	--	--	--	--	--	--	30/58	24/20	--	--	213/353	169/114	74/500	69/218	74/500	69/218
P-value	--	--	--	--	--	--	--	--	--	--	--	--	--	--	*0,353*	*0,222*	--	--	*0,914*	*0,873*	*0,343*	*0,270*	*0,343*	*0,270*
Puri et al., 2005 (Controls: n=450; Cases: n=450)																								
Controls	81/797	--	207/631	--	321/539	--	331/549	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Cases	65/715	--	182/598	--	294/486	--	270/502	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

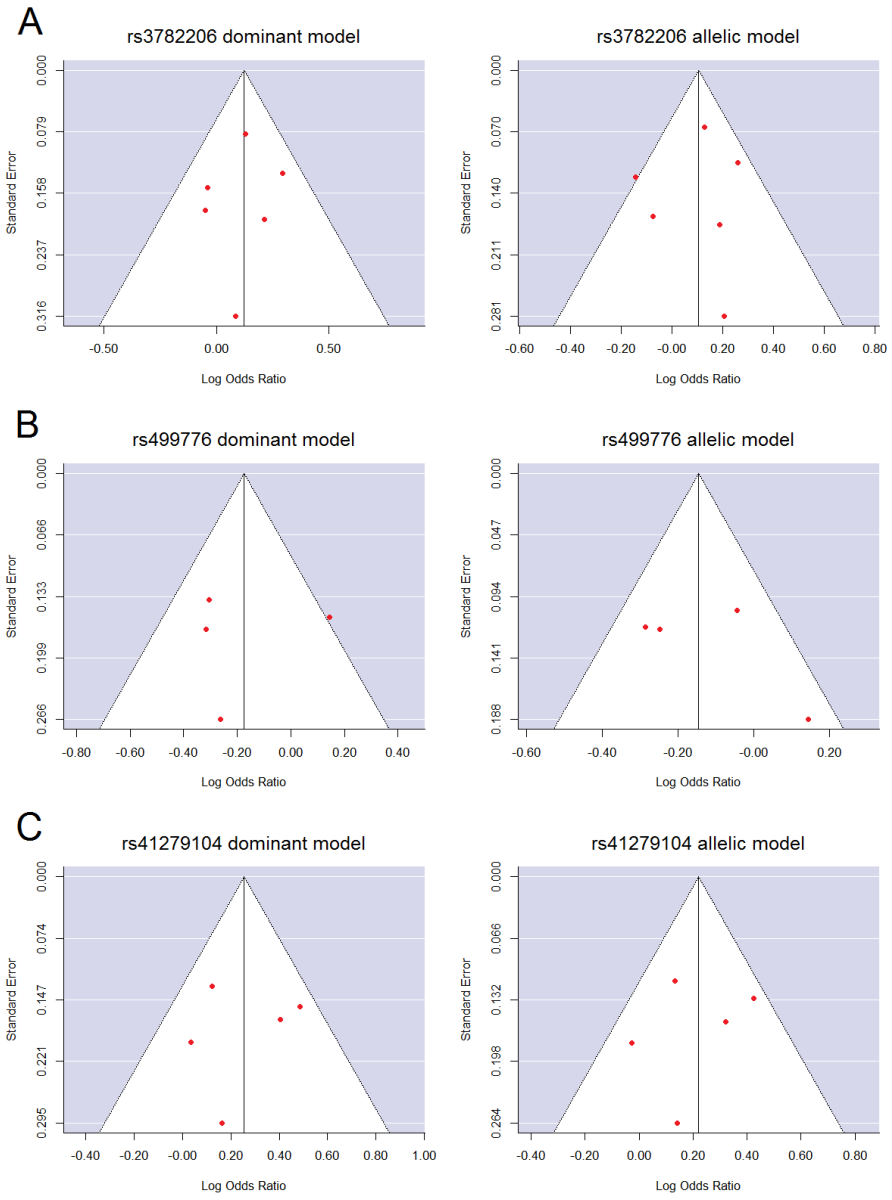
P-value	*0,522*	--	*0,520*	--	*0,878*	--	*0,266*	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Zheng et al., 2005 (Controls: n=941; Cases: n=664)																								
Controls	359/1523	324/617	--	--	1354/528	868/73	882/1000	660/274	--	--	--	--	--	--	1115/767	776/165	--	--	961/921	716/225	346/1536	319/622	--	--
Cases	253/1075	228/436	--	--	959/369	609/55	660/668	489/175	--	--	--	--	--	--	784/544	544/120	--	--	700/628	521/143	169/1159	155/509	--	--
P-value	*0,990*	*0,969*	--	--	*0,870*	*0,702*	*0,114*	*0,191*	--	--	--	--	--	--	*0,905*	*0,781*	--	--	*0,357*	*0,265*	*$2*10^{-5}$*	*$5*10^{-6}$*	--	--
Total (Controls: n=3412; Cases: n=2406)																								
Controls	740/5174	601/1916	420/1344	191/272	2945/2909	1884/613	2528/3380	1698/809	582/1804	501/692	922/1476	740/459	1086/1296	844/347	2283/2869	1732/844	896/1520	738/470	2045/2729	1597/790	674/3843	618/1641	302/2421	285/1077
Cases	478/3516	379/1228	385/1133	175/194	2044/1944	1237/367	1701/2267	1116/482	312/930	279/342	486/760	398/225	512/700	411/195	1469/1825	1121/526	458/788	376/247	1378/1762	1071/499	388/2754	360/1211	219/1581	205/695
Heterogeneity:																								
P-value	0.301	--	0.138	--	0.008	--	0.008	--	0.026	0.06	0.003	0.033	0.015	0.035	0.891	0.340	0.732	0.731	0.270	0.712	0.009	0.004	0.906	0.854
Cochran-Mantel-Haenszel Metaanalysis:																								
Fixed effect	0,91	--	1,09	--	1,04	--	1,00	--	0,98	1,04	0,99	1,06	0.88	0.87	0,97	0,99	0,97	0,94	1,09	1,04	0,78	0,77	1,12	1,12
P-value	*0.142*	--	*0.316*	--	*0.426*	--	*0.983*	--	*0.819*	*0.706*	*0.911*	*0,877*	*0.086*	*0.237*	*0.541*	*0.858*	*0.710*	*0.579*	*0.064*	*0.633*	*0.0004*	*0.001*	*0.295*	*0.307*
DerSimonian and Laird Metaanalysis:																								
Random effect	0,90	--	1,11	--	1,23	--	1,04	--	0,92	0.98	0.90	0,97	0,93	0.95	0,97	0,98	0,97	0,94	1,10	1,04	0,88	0,89	1,11	1,12
P-value	*0.144*	--	*0.397*	--	*0.836*	--	*0.596*	--	*0.599*	*0.903*	*0,565*	*0,877*	*0.648*	*0.812*	*0.527*	*0.830*	*0.684*	*0.543*	*0.098*	*0.611*	*0.366*	*0,502*	*0.275*	*0.285*

*significant after Bonferroni correction ($p<0.05$)

Table 4: Meta-analysis of 7 NOS1 variants. Table shows all SNPs along with their minor/major alleles, cases and control counts for the genotypic and dominant model as well as the nominal p-values for each sample and the total sample. Further are given p-values for heterogeneity and odds ratios plus p-values of the fixed effect and random effect model. Bold indicates SNPs with at least one significant p-value (p<0.05).

SNPs	**rs3782206**		**rs499776**		**rs3782219**		**rs3782221**		**rs1879417**		**rs4767540**		**rs41279104**	
Alleles (d/D)	**T/C**		**A/G**		**T/C**		**A/G**		**T/C**		**G/A**		**T/C**	
Test	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**	**d/D**	**d+/d-**
German sample (Controls: n=720; Cases: n=270)														
Controls	155/1237	148/548	577/817	454/243	--	--	306/1084	275/420	675/705	512/178	644/750	484/213	146/1248	138/559
Cases	54/464	53/206	209/309	177/82	--	--	119/385	106/146	260/244	195/57	250/264	186/71	70/434	68/184
P-value	*0,681*	*0,858*	*0,714*	*0,398*	--	--	*0,455*	*0,500*	*0,323*	*0,350*	*0,352*	*0,425*	*0,041*	*0,020*
Swedish sample (Controls: n=168; Cases: n=101)														
Controls	34/282	32/126	114/200	102/55	63/255	59/100	72/246	67/92	143/181	117/45	132/178	104/51	41/283	38/124
Cases	25/169	21/76	77/117	57/40	35/159	29/68	40/160	33/67	99/99	71/28	76/114	59/36	28/168	26/72
P-value	*0,478*	*0,874*	*0,452*	*0,351*	*0,645*	*0,278*	*0,512*	*0,151*	*0,206*	*1,000*	*0,577*	*0,494*	*0,596*	*0,656*
Spanish sample (Controls: n=360; Cases: n=270)														
Controls	76/608	74/268	282/404	224/119	--	--	161/519	144/196	313/363	241/97	294/396	227/118	82/600	73/268
Cases	70/464	68/199	189/347	155/113	--	--	141/383	118/144	234/300	183/84	223/307	176/89	63/473	59/209
P-value	*0,288*	*0,289*	*0,038*	*0,065*	--	--	*0,203*	*0,562*	*0,416*	*0,475*	*0,861*	*0,931*	*0,929*	*0,921*
Cui et al., 2010 (Controls: n=377; Cases: n=343)														
Controls	197/557	164/213	--	--	302/448	247/128	347/403	270/105	--	--	--	--	132/622	119/258
Cases	161/525	146/197	--	--	289/397	236/107	303/383	232/111	--	--	--	--	168/518	147/196
P-value	*0,244*	*0,800*	--	--	*0,474*	*0,402*	*0,425*	*0,203*	--	--	--	--	*0,001*	*0,002*
Okumura et al., 2009 (Controls: n=519; Cases: n=542)														
Controls	641/1879	554/706	--	--	1121/1399	866/394	441/597	344/175	--	--	--	--	182/856	165/354
Cases	645/1663	544/610	--	--	950/1358	745/409	486/598	369/173	--	--	--	--	212/872	187/355
P-value	*0,049*	*0,118*	--	--	*0,020*	*0,030*	*0,276*	*0,532*	--	--	--	--	*0,231*	*0,349*
Tang et al., 2008 (Controls: n=480; Cases: n=480)														

Controls	218/718	186/282	204/742	185/288	404/540	315/157	458/428	332/111	498/430	366/98	185/751	168/300	--	--
Cases	266/676	221/250	162/784	152/321	409/525	319/148	493/407	355/85	497/449	365/108	188/740	168/296	--	--
P-value	*0,014*	*0,026*	*0,015*	*0,025*	*0,664*	*0,607*	*0,191*	*0,040*	*0,625*	*0,527*	*0,790*	*0,922*	--	--
Total (Controls: n=2624; Cases: n=2006)														
Controls	1321/5281	1158/2143	1177/2163	965/705	1890/2642	1487/779	1785/3277	1432/1099	1629/1679	1236/418	1255/2075	983/682	583/3609	533/1563
Cases	1221/3961	1053/1538	637/1557	541/556	1683/2439	1329/732	1582/2316	1213/726	1090/1092	814/277	737/1425	589/492	541/2465	487/1016
Heterogeneity:														
P-value	0.166	0.557	0.139	0.114	0.209	0.151	0.524	0.128	0.299	0.597	0.764	0.722	0.245	0.256
Cochran-Mantel-Haenszel Metaanalysis:														
Fixed effect	1,11	1,13	0,86	0.84	0.94	0.92	1,07	1,07	1,01	0.98	1,03	1,03	1,25	1,29
P-value	***0.024***	***0.030***	***0.020***	***0.039***	*0.182*	*0.195*	*0.161*	*0.425*	*0.826*	*0.849*	*0.700*	*0.754*	***0.001***	***0.001***
DerSimonian and Laird Metaanalysis:														
Random effect	1,10	1,13	0,87	0.84	0.96	0.94	1,07	1,07	1,02	0.98	1,03	1,03	1,25	1,29
P-value	*0.152*	***0.028***	*0.112*	*0.147*	*0.524*	*0.542*	*0.157*	*0.153*	*0.781*	*0.802*	*0.677*	*0.727*	***0.006***	***0.005***


A
rs3782206 dominant model
Standard Error
0.000
0.079
0.158
0.237
0.316
-0.50
0.00
0.50
Log Odds Ratio
rs3782206 allelic model
Standard Error
0.000
0.070
0.140
0.211
0.281
-0.60
-0.40
-0.20
-0.00
0.20
0.40
0.60
0.80
Log Odds Ratio
B
rs499776 dominant model
Standard Error
0.000
0.066
0.133
0.199
0.266
-0.80
-0.60
-0.40
-0.20
0.00
0.20
0.40
Log Odds Ratio
rs499776 allelic model
Standard Error
0.000
0.047
0.094
0.141
0.188
-0.60
-0.40
-0.20
-0.00
0.20
Log Odds Ratio
C
rs41279104 dominant model
Standard Error
0.000
0.074
0.147
0.221
0.295
-0.40
-0.20
-0.00
0.20
0.40
0.60
0.80
1.00
Log Odds Ratio
rs41279104 allelic model
Standard Error
0.000
0.066
0.132
0.198
0.264
-0.40
-0.20
0.00
0.20
0.40
0.60
0.80
Log Odds Ratio

Supplementary Figure 1: Funnel plots of *NOS1AP* rs3782206 dominant and allelic model **(A)**, *NOS1* rs499776 dominant and allelic model **(B)** and *NOS1* rs41279104 dominant and allelic model **(C).**

Supplementary Table 1: Primer sequences in 5'-3' direction used for Sequenom's MassArray® system.

SNP ID	Primary PCR Primer 1	Primary PCR Primer 2	Extend Primer
rs12857	ACGTTGGATGCTTAGATGCGCCACGGTTTC	ACGTTGGATGGGTGCTAGCACAGCTCAGG	tTTTCGGTAGCGACGGTA
rs3916065	ACGTTGGATGAAAGCGCCACCTAGCAACG	ACGTTGGATGCAGGAACCACACAGAAGGG	ggaacCGGTATCTAAGATCAGGGAGC
rs787828	ACGTTGGATGGCAGTCACCTAGCTGCTTAG	ACGTTGGATGGGTCCTGACTTGTAGTTAGC	tttccCCTAGCTGCTTAGAAATCCC
rs1415263	ACGTTGGATGCTAAATGGTGAGCCCCAATG	ACGTTGGATGTGGGTTGGAAGGCAACATAC	accTTAGGCATTCCCAATTCCTTTATC
rs4145621	ACGTTGGATGCCATCACCCCTTGATAAAAG	ACGTTGGATGGACTTAAATCCAACTCTTC	TGCTGTGGTTTGATGGAGAA
rs348624	ACGTTGGATGCCTTGTTCTGCAGCAAAAGC	ACGTTGGATGACCAGTTGGCTGCTGAGG	gctgaAGCAAAAGCTGATGCAC
rs9788155	ACGTTGGATGGACAGATCATCCGTTCTGAG	ACGTTGGATGTGAAGCCCACAGGGAATTTG	GTTCTGAGTAGGGCTTAA
rs1572495	ACGTTGGATGCTTCAGAGGATGCAGATTTG	ACGTTGGATGCCCTGCCTAAATATCCTTTG	ATGCAGATTTGAGCTGAGCC
rs1538018	ACGTTGGATGCCAACTCTGAACTTAGGATG	ACGTTGGATGACTTTCTCCCTAAGTGGCCC	GGATGAAAAGGAGAACAATGA
rs945713	ACGTTGGATGCTGTGTGACACTCCCATTTG	ACGTTGGATGGATAACTTCAGTTTTTTGAC	TCCCATTTGGATATCCCAAAG
rs3751284	ACGTTGGATGTGCAGATGGCCAGGAAGATG	ACGTTGGATGACAGAAGCCGCAGTGCCTAC	CAGGAAGATGGAGAGAG
rs905721	ACGTTGGATGCCTCCTCTGGAATGATAAGC	ACGTTGGATGATGCCCTCCACATTCAGTTC	ccTGATAAGCCCAGATGCC
rs1964052	ACGTTGGATGCTGGAATATAGGGGTAGGTC	ACGTTGGATGAAGGCTCTGGAAAGAGTGTC	cAGAAAGCACCACCAAAAACTTA
rs4306106	ACGTTGGATGTATCGCTTTCAGGGTCAAGG	ACGTTGGATGGAAGAAGAAGAATGAATTTC	ctcacAAAACTCAGTAAAGCTACCC
rs3924139	ACGTTGGATGCAGTGTTGTATACAATGCGG	ACGTTGGATGAACAACTGCCTGTGCTCAAG	aaAATGCGGTATATAATATCTAACA
rs1508263	ACGTTGGATGGGGACAGCCGTTTAGTTAC	ACGTTGGATGTGATCTCACTATTAAGTTG	agggcGTTACTTGGTAGTGAAAGAAA
rs7521206	ACGTTGGATGGCCGTAGTGTCACATCACTC	ACGTTGGATGCTGTGTTGTCTTTGGCAATG	cacgCTGTGCCACATCACTGA
rs4924755	ACGTTGGATGCAGAGAGATTGGTGTTCTGG	ACGTTGGATGTGTAGAGCCCCATCCCCCTT	ttTGCCTTAGCCATGAGAC
rs711352	ACGTTGGATGGACACGAACAAAACCTTACC	ACGTTGGATGAAACCAAATAAAGCAATAAC	cccctGTGTTTATACTGTGTGTGT
rs2232841	ACGTTGGATGGTTAAGTCAAATCCAACGGC	ACGTTGGATGGATCGCCGGGAGGGGAGAC	AATCCAACGGCCCGGTGCGCCCC
rs2232838	ACGTTGGATGGCCAAAGGCAGAGCAAGCG	ACGTTGGATGCTCGGGCTAGGCTGGGCT	gttTGCCCAGATCCTGGGAG
rs4767540	ACGTTGGATGGCTTTAGGGTTTCCACTCTG	ACGTTGGATGAGGCTTAGAGTCCCAGACAG	TTCCACTCTGCCCTCAT
rs1879417	ACGTTGGATGCTCTACTCGGCCTTCAAGTC	ACGTTGGATGGAAGAGGGACATGCAGAGTG	GCCTTCAAGTCTTAGCG
rs3782221	ACGTTGGATGCTTAACCACATTCCAAGCCC	ACGTTGGATGGGGTGTCTTATGACAAGACT	ccaCGCACAGACCCACAGAACCTGAGT
rs3782219	ACGTTGGATGTCCAGGGCATTGCAACTTAG	ACGTTGGATGCACCTCCTCAATTAACTGGG	tgcacCTTAGCCTGCAAATTGTAG
rs499776	ACGTTGGATGCTACATACCTGCCCCATTGC	ACGTTGGATGCAAACCCTGGTTTTTCTAGC	GCCCCATTGCTGTAAAT
rs3837437	ACGTTGGATGTGAAGAATGTTGTTAGGTGC	ACGTTGGATGCTGGGTGACAGAGCAAGAAC	ggaAATGTTGTTAGGTGCTTTTTTT

rs3782206	ACGTTGGATGCTACACACACAAAAGTCTTTC	ACGTTGGATGAGTAAGGAAGGCTGGGTAAC	ccctgTAAATATGCAACTAAATGTCCT
rs598747	ACGTTGGATGCATGAAGAAGATTTGGCACC	ACGTTGGATGTCAGCAGCACCAGGTGCTC	cttGCACCACACCTTCTACA
rs598704	ACGTTGGATGTGAGTTGCGTGTGGCCCCG	ACGTTGGATGTCTTCTCTCTGTTGGCCTTG	AGGAGCACCTGGTGCTGCTGAC
rs308969	ACGTTGGATGGCTAATGGACCTGAAAGAGC	ACGTTGGATGCTAGCCTGAGATTTGATCCT	ccCCTAGAGTATAAATCCTCCCA
rs931676	ACGTTGGATGCACTTTCCACCCATGGCTTG	ACGTTGGATGTTGCAGTATGCAGGCCTCC	GGCTTGTCACAGAAGTT
rs3817004	ACGTTGGATGTCCCAGCCAAAGTGTAAGTA	ACGTTGGATGCTGCACTGTATGAAGTTGGG	CAGCCAAAGTGTAAGTACTTTGAG
rs35528972	ACGTTGGATGTGCTAGGCATGGAGGTACAG	ACGTTGGATGCACCCTCAGTTAGACTTGAC	ctaatGGGAAATGAGGAAACATG
rs3755724	ACGTTGGATGTTGGAGCCTCATAAATAGGG	ACGTTGGATGGAATGAGACGAGCTACTCAG	cccagCTCATAAATAGGGCATTGAAA
rs41279104	ACGTTGGATGAAGGCTTGGCCTCCCAACC	ACGTTGGATGTTTAATTGACACCAGGTGGC	cccCCTCCCAACCCAGCAGAGCC

Supplementary Table 2: Functional prediction of *rs945713, rs3782206, rs499776 and rs41279104* (in bold and highlighted grey) and their proxy SNPs in high linkage disequilibrium ($r^2 \geq 0.8$ and D´=1). All Transcription Factors (TFs), shown in table have a core similarity of 1 and a matrix similarity of 0.85 and more. Changes in Splicing Regulatory Elements (SREs), like Splicing Enhancers (Srp40, SF2/ASF, SC35, SRp55, 9G8, Tra-ß) and Splicing Inhibitors (hnRNP A1) with a match similarity of at least 65.0 are shown for minor and major alleles.

Associated SNP	Proxy SNP	Position (bp)	Alleles (d/D)	location	Transcription Factor (TF)	
					Minor Allele (d)	Major Allele (D)
NOS1AP; chromosome 1						
rs945713	rs10918796	162133343	C/A	Intron	SF2/ASF	9G8,hnRNP A1
rs945713	rs10918797	162133602	A/G	Intron	9G8	SF2/ASF
rs945713	rs6427656	162134638	G/A	Intron	-	-
rs945713	**rs945713**	**162135670**	**G/A**	**Intron**	**hnRNP A1**	**9G8,Tra2-β**
rs945713	rs11581189	162136904	C/T	Intron	SRp55	SC35
rs945713	rs11805598	162140336	A/G	Intron	9G8,Tra2-β,hnRNP A1	-
rs945713	rs7549718	162140862	A/G	Intron	Tra2-β,9G8	-
NOS1; chromosome 12						
rs3782206	rs7961147	117738879	T/C	Intron	Tra2-β	
rs3782206	rs12578810	117738948	T/C	Intron	Tra2-β	SF2/ASF, 9G8, hnRNP A1
rs3782206	rs12810591	117739450	C/T	Intron	-	SRp40,hnRNP A1
rs3782206	rs12811583	117739942	C/T	Intron	-	SRp55
rs3782206	rs3825103	117740509	C/T	Intron	-	9G8, hnRNP A1
rs3782206	rs12811676	117741229	A/G	Intron	-	SRp40, SC35, SF2/ASF
rs3782206	rs12812274	117742714	A/G	Intron	-	-
rs3782206	rs6490124	117743269	C/A	Intron	9G8	-
rs3782206	rs7139134	117743766	T/C	Intron	Tra2-β	hnRNP A1
rs3782206	rs11068444	117744743	G/A	Intron	SRp55	hnRNP A1
rs3782206	rs11068445	117744929	A/G	Intron	hnRNP A1	SRp40
rs3782206	**rs3782206**	**117745089**	**T/C**	**Intron**	**-**	**-**
rs3782206	rs35555584	117746177	C/A	Intron	SF2/ASF	SC35, hnRNP A1
rs3782206	rs7310618	117747306	G/C	Intron	SRp55	-

rs3782206	rs7299154	117747395	G/T	Intron	-	SF2/ASF
rs3782206	rs10850809	117747441	C/G	Intron	hnRNP A1	SF2/ASF
rs3782206	rs11068447	117747687	T/C	Intron	9G8	SC35
rs3782206	rs35736046	117747885	G/T	Intron	-	Tra2-β
rs3782206	rs12824048	117748042	T/C	Intron	9G8	-
rs3782206	rs36020061	117748156	C/G	Intron	SRp55	-
rs3782206	rs9658309	117748304	T/A	Intron	-	-
rs3782206	rs9658308	117748410	G/A	Intron	-	-
rs3782206	rs9658297	117750695	T/C	Intron	hnRNP A1	9G8
rs3782206	rs35320403	117752761	C/G	Intron	SRp55	SRp40
rs499776	rs816293	117762699	G/C	Intron	-	-
rs499776	rs570234	117770982	G/T	Intron	-	Tra2-β
rs499776	rs576881	117772835	G/A	Intron	-	-
rs499776	rs1681506	117775578	C/T	Intron	-	SRp40
rs499776	**rs499776**	**117779499**	**A/G**	**Intron**	**-**	**SRp55**
rs41279104	rs900622	117875158	C/T	Upstream	E2F	-
rs41279104	rs900623	117875160	T/G	Upstream	-	-
rs41279104	**rs41279104**	**117877485**	**T/C**	**Upstream**	**-**	**-**
rs41279104	rs12316771	117879236	C/T	Upstream	-	HOXC9,MSX1,ELF5,DLX1
rs41279104	rs34731287	117880958	A/C	Upstream	-	SPZ1,MAZR
rs41279104	rs12312120	117881133	T/C	Upstream	HMX3	-
rs41279104	rs17618096	117882250	C/G	Upstream	-	-