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Genetic Evidence Implicating Multiple Genes in the MET Receptor Tyrosine Kinase Pathway in Autism Spectrum Disorder

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Abstract

A functional promoter variant of the gene encoding the MET receptor tyrosine kinase alters SP1 and SUB1 transcription factor binding, and is associated with autism spectrum disorder (ASD). Recent analyses of postmortem cerebral cortex from ASD patients revealed altered expression of MET protein and three transcripts encoding proteins that regulate MET signaling, hepatocyte growth factor (*HGF*), urokinase plasminogen activator receptor (*PLAUR*) and plasminogen activator inhibitor-1 (*SERPINE1*). To address potential risk conferred by multiple genes in the MET signaling pathway, we screened all exons and 5′ promoter regions for variants in the five genes encoding proteins that regulate MET expression and activity. Identified variants were genotyped in 664 families (2,712 individuals including 1,228 with ASD) and 312 unrelated controls. Replicating our initial findings, family-based association test (FBAT) analyses demonstrated that the *MET* promoter variant rs1858830 C allele was associated with ASD in 101 new families (P=0.033). Two other genes in the MET signaling pathway also may confer risk. A haplotype of the *SERPINE1* gene exhibited significant association. In addition, the *PLAUR* promoter variant rs344781 T allele was associated with ASD by both FBAT (P=0.006) and case-control analyses (P=0.007). The *PLAUR* promoter rs344781 relative risk was 1.93 (95% Confidence Interval [CI]: 1.12−3.31) for genotype TT and 2.42 (95% CI: 1.38−4.25) for genotype CT compared to genotype CC. Gene-gene interaction analyses suggested a significant interaction between *MET* and *PLAUR*. These data further support our hypothesis that genetic susceptibility impacting multiple components of the MET signaling pathway contributes to ASD risk.

Keywords

Genetic; Association; Plasminogen; PLAUR; uPAR; SERPINE1; Brain; Cerebral Cortex

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Introduction

Autism spectrum disorder (ASD) is a commonly diagnosed, behaviorally-defined, heterogeneous syndrome characterized by deficits in social interaction and communication and by repetitive behaviors with restricted interests [Centers for Disease Control and Prevention 2007]. The etiology of this complex disorder involves a strong genetic component, but environmental factors also are likely to impact ASD susceptibility and clinical heterogeneity [Persico & Bourgeron 2006; Geschwind & Levitt 2007; Gupta & State 2007]. We recently described significant association of the gene encoding the MET receptor tyrosine kinase with ASD in two independent family cohorts [Campbell et al 2006]. The ASD-associated *MET* allele is a promoter variant that alters binding of the transcription factors SP1 and PC4 (encoded by the *SUB1* gene), and decreases transcription of *MET* [Campbell et al 2006]. Analyses of postmortem tissue from the temporal lobe revealed decreased MET transcript and protein expression in individuals with ASD compared to matched controls [Campbell et al 2007].

The hepatocyte growth factor (*HGF*) gene encodes the activating ligand for the MET receptor. HGF is translated as an inactive precursor protein that requires cleavage for efficient binding to the MET receptor [Lokker et al 1992]. The activating cleavage of HGF is achieved most efficiently by the enzyme plasminogen activator (urokinase-type; uPA; gene symbol: *PLAU*) under conditions in which uPA binds to its receptor, the urokinase plasminogen activator receptor (uPAR; gene symbol: *PLAUR*). Activating cleavage of HGF can be suppressed by the plasminogen activator inhibitor-1 (PAI-1; gene symbol: *SERPINE1*). Together, these proteins regulate the activity of MET receptor tyrosine kinase signaling, and our recent microarray analyses of postmortem temporal lobe of individuals with ASD indicate that disrupted MET signaling may be common to ASD pathophysiology [Campbell et al 2007]. For example, we found that there is increased expression of the *HGF*, *PLAUR* and *SERPINE1* transcripts in ASD in postmortem cerebral cortex. The observation of disrupted expression suggests a general dysfunction of MET signaling in the cerebral cortex of individuals with ASD.

One interpretation of the observed transcriptional changes in postmortem cerebral cortex of individuals with ASD is that the decreased expression of MET results in secondary, compensatory changes in the expression of *HGF*, *PLAUR* and *SERPINE1*. An alternative hypothesis, tested here, is that variants within the *HGF*, *PLAUR* and *SERPINE1* genes contribute to ASD risk. We examined association of common alleles in the *HGF*, *PLAUR* and *SERPINE1* genes with ASD risk. In addition, we tested association of markers in the *SP1* and *SUB1* genes, the transcription factor protein products of which bind differentially to the ASD-associated *MET* promoter variant rs1858830. We further hypothesized that the known biological interactions among proteins involved in the activation of MET receptor tyrosine kinase signaling may predict gene-gene interactions in ASD. The results indicated association of the *PLAUR* and *SERPINE1* genes in ASD, as well as a significant gene-gene interaction between *MET* and *PLAUR*. These findings contribute to the genetic and pathophysiologic evidence that dysfunctional MET signaling contributes to ASD risk.

Methods

Sample

Families recruited by the centers listed in Table 1 were used for this study. Clinical characterization has been described in detail previously [Campbell et al 2006;Weiss et al 2006]. The 664 ASD nuclear families (from 629 independent pedigrees) consist of the 539-family replication sample genotyped for the initial report of the *MET* gene [Campbell et al 2006], 24 AGRE families that overlapped the original sample (and were thus deleted from

the replication sample) in our initial report of the *MET* gene [Campbell et al 2006], and an additional 101 families from Tufts and Vanderbilt that were not genotyped previously for *MET*. The family sample genotyped here does not include the "original" sample in our initial report on *MET* [Campbell et al 2006], which consisted of 178 simplex families and 26 multiplex families. The control sample consisted of 312 unrelated, reportedly healthy Caucasians from the Sigma Human Random Control panel (n=192; Sigma-Aldrich, St. Louis, MO) and the Coriell Institute for Medical Research (n=120; Camden, NJ, USA). Among the 312 controls, 62 individuals from the Coriell Institute for Medical Research were included in our previous case-control analysis of the *MET* promoter variant rs1858830 [Campbell et al 2006]. All research was approved by the Vanderbilt University Institutional Review Board.

Screening for variants

Genomic DNA samples from 48 individuals with ASD were screened for variants in each exon and 2−3 kb of the 5′ putative promoter region of the *PLAUR*, *HGF*, *SERPINE1*, *SP1* and *SUB1* genes. Reveal™ (State College, PA) temperature gradient capillary electrophoresis was used to screen for variants. Amplicons identified as variant-positive were then directly re-sequenced to identify the variant.

SNP selection

For *MET*, only the ASD-associated functional promoter variant rs1858830 was genotyped. SNP selection for *PLAUR*, *HGF*, *SERPINE1*, *SP1* and *SUB1* was based on variants identified in the screen of exons and 5′ promoter regions, and additional markers were chosen based on position within the gene. Because the sample is >85% Caucasian, the HapMap "CEU" Caucasian panel was used to identify linkage disequilibrium patterns and markers with minor allele frequency >0.15.

SNP genotyping and quality control

Genotyping was performed using TaqMan™ SNP genotyping assays on the ABI Prism 7900HT and analyzed with SDS software as previously described [Campbell et al 2006]. SNP Genotyping Assays-On-Demand were obtained from Applied Biosystems (Foster City, CA). Genotyping was performed in a 384-well plate format using 3 ng genomic DNA. Quality control measures included seeding of each 384-well plate with 8−10 blank negative control wells and 20−30 duplicated positive control samples. Automated allele calls were made with SDS Data Collection software and reviewed by an experienced operator according to protocol.

Standard TaqMan™ SNP genotyping assays were reproducible and reliable for all markers in the *PLAUR*, *HGF*, *SERPINE1*, *SP1* and *SUB1* genes. A modified two-step PCR protocol, as previously described [Campbell et al 2006], was used to genotype *MET* variant rs1858830. Briefly, we generated a 652-bp amplicon including rs1858830 from genomic DNA for each sample, and then used the 652-bp amplicon as a template in TaqMan™ SNP genotyping assays [Campbell et al 2006]. The overall no-call rate was <5%.

All analyzed markers were in Hardy-Weinberg Equilibrium (HWE; P>0.05) in both the ASD families and the control sample. However, a marker in *SP1* intron 3, rs7300593, was not in HWE. Genotype for the rs7300593 marker was obtained for 2,581 (95%) of the 2,712-individual ASD family sample. Of the 2,581 genotyped individuals, 1,788 individuals were genotype TT (69%) and 793 individuals were genotype CT (31%); not a single individual with homozygous CC genotype was identified. These allele frequencies are consistent with those reported by dbSNP. The rs7300593 marker was therefore not used in association analyses.

Definition of linkage disequilibrium (LD) blocks

Haploview (version 3.2) was used to assess HWE and to define LD blocks. A single individual with ASD was randomly selected from each family using a random number generator to build trios suitable for Haploview analysis [Campbell et al 2006].

Association analyses

Two complementary association analysis strategies were employed. Family-based association was used to determine transmission of genetic alleles from parents to children with ASD. Case-control analyses compared the genotype of individuals with ASD to a group of unrelated controls. Genotypic data from the case-control analyses were used for subsequent calculations of relative risk. Family-based single marker and haplotype association analyses were performed using the family-based association test (FBAT) [Horvath et al 2001] and haplotype-based association test (HBAT) [Horvath et al 2004] (FBAT version 1.7.2). All HBAT and FBAT analyses were performed using the additive model and the empirical variance ("-e" option) because linkage has been reported for the chromosomal regions containing these genes and because the empirical variance provides a more conservative estimate of association. HBAT was performed with minimum haplotype frequency set to 0.01. For case-control analyses, the single individual with ASD randomly chosen from each pedigree for Haploview analysis was used as the case. Case-control association analyses were carried out using a chi-squared test. Relative risk estimates were calculated as described previously [Campbell et al 2006].

Gene-gene interaction analyses

Gene-gene interaction analyses were performed with the 2-locus TDT method [Cordell et al 2004], which has been implemented as a Stata program "pseudocc" (http://www-gene.cimr.cam.ac.uk/clayton/software/stata; written by Dr. Clayton). For each case-parents trio, the program generated a "matched" case-control set consisting of a single case and one or more pseudo-controls, comprised of all other possible offspring two- locus genotypes given the parental genotypes. The resulting "matched" sets of two-locus genotypes were used for two conditional logistic regression analyses, one without interaction terms and the other with full interaction terms. To test for gene-gene interaction, a likelihood ratio test (with df=4) was carried out comparing these two regression analyses. The analyses were carried out in Stata v9.2.

Corrections for multiple comparisons

Appropriate corrections for multiple comparisons are an ongoing debate in human genetics. We report in the text uncorrected P values, permutation analysis P values, and P values following Bonferroni correction for the number of genotyped SNPs in each gene. Permutation testing of each marker with uncorrected significance was conducted with the "hbat –p" command in the FBAT package and >10,000 permutation cycles. Alternative corrections for multiple comparisons are possible.

Transcription assays

Two constructs containing 500-bp fragments of the *PLAUR* promoter corresponding to nucleotides −365 to +134 (transcription start site = +1) were cloned into the pGL4.10[luc2] luciferase reporter vector (Promega, Madison, WI). The two constructs differed only at the rs344781 locus (C or T at nucleotide −285). Luciferase assays were performed as previously described [Campbell et al 2006].

Results

Association of *MET* rs1858830 allele C

FBAT analysis of the 101 families not included in the original report of *MET* association replicated our initial findings [Campbell et al 2006], revealing a significant association of the rs1858830 C allele (T_{OBS}=73; T_{EXP}=61; P=0.033). In the entire 664-family sample, the *MET* promoter variant rs1858830 C allele was significantly associated with ASD (T_{OBS}=588; T_{EXP}=545; P=0.008) (Table 3). Stratification of the sample into multiplex and simplex families revealed that, similar to the initial report [Campbell et al 2006], association of the rs1858830 C allele was present in multiplex families (T_{OBS}=539; T_{EXP}=488; P=0.001) but not in simplex families (T_{OBS}=49; T_{EXP}=57; P=0.070). However, the sample of 90 simplex families tested here lacks statistical power necessary to draw conclusions.

Case-control analysis of a single randomly-selected individual from each of the newly-genotyped 101-pedigree ASD sample compared to the 250 controls not previously genotyped also indicated association of the *MET* rs1858830 C allele (χ^2=6.261; df=2; P=0.044). In the entire sample of 629 randomly-selected individuals with ASD from each pedigree compared to the 312 control individuals, the *MET* rs1858830 allele C was associated with ASD (χ^2=8.961; df=2; P=0.011) (Table 4). In the entire sample, relative risk was 1.76 (95% confidence interval [CI]: 1.19−2.62) for individuals of genotype CC and 1.59 (95% CI: 1.11−2.27) for individuals of genotype CG compared to individuals of genotype GG.

Screen for variants in the *HGF, PLAUR, SERPINE1, SP1* and *SUB1* genes

We screened 48 individuals with ASD for variants in each exon and 5′ promoter region of the *HGF, PLAUR, SERPINE1, SP1* and *SUB1* genes. This screen failed to identify novel variants. Rare non-synonymous variants were identified in *PLAUR* exon 3 (rs399145; Thr86Ala) and *SERPINE1* exon 2 (both rs6092 (Ala15Thr) and rs6090 (Val17Ile) (Table 2). A TaqMan™ assay designed to genotype the *PLAUR* rs399145 (Thr86Ala) failed to identify additional individuals polymorphic at this locus in 2,712 individuals from ASD families and 312 control individuals, which is consistent with the Caucasian minor allele frequency of 0.00 reported in dbSNP. The *SERPINE1* variant rs6090 was genotyped in subsequent association analyses (Table 3).

More common non-synonymous variants were identified in *HGF* exon 8 (rs5745687; Glu304Lys) and *PLAUR* exon 6 (rs2302524; Lys220Arg) (Table 2). The *HGF* marker rs5745687 was genotyped in subsequent association analyses (Table 3); however, *PLAUR* rs2302524 shows deviation from HWE in the HapMap CEU sample due to an absence of individuals with GG genotype [Consortium 2005], and was therefore not examined in these studies.

Rare synonymous variants were identified in *HGF* exon 6 (rs5745666; His237His), *SERPINE1* exon 3 (rs6091; Ala119Ala) and *SP1* exon 3 (rs3741651; Gln480Gln). Among non-coding variants were *PLAUR* exon 1 variant rs4251805, *PLAUR* 5′ promoter variant rs344781, *SERPINE1* 5′ untranslated region (UTR) variant rs2227635, *SERPINE1* 3′ UTR variant rs7242 and *SUB1* 5′ promoter SNP rs2008245 (Table 2).

Single Marker Association Analysis

PLAUR—The 5′ promoter variant rs344781 and six additional markers spanning the 24-kb *PLAUR* gene were analyzed (Figure 1). Family-based association test (FBAT) analysis indicated association of the *PLAUR* promoter variant rs344781 allele T (T_{OBS}=584; T_{EXP}=544; P=0.006; Table 3). The corresponding permutation P-value was 0.003 and the

result was significant after Bonferroni correction for the use of seven markers in the *PLAUR* gene (P=0.042). Association of the rs344781 allele T with ASD was also supported by case-control analysis (χ^2=9.952; df=2; P=0.007; Table 4). The relative risk was 1.93 (95% CI: 1.13−3.31) for genotype TT and 2.42 (95% CI: 1.38−4.25) for genotype CT compared to genotype CC.

SERPINE1—The exon 2 non-synonymous variant rs6090, the 3′ UTR variant rs7242 and three additional SNPs spanning the 12-kb *SERPINE1* gene were used in ASD association studies.

FBAT analysis of the entire 664-family sample revealed association with ASD diagnosis of the C allele at rs13238709, a marker immediately 3′ to the *SERPINE1* gene (allele frequency=0.564; T_{OBS}=624; T_{EXP}=592; P=0.048) (Table 3). The association of rs13238709 allele C remained significant in permutation test (P=0.044) but did not survive Bonferroni correction for multiple comparisons (P=0.216). Association of *SERPINE1* rs13238709 allele C was not supported by case-control analysis (χ^2=4.813; df=2; P=0.090) (Table 4).

HGF—We pursued association of the *HGF* gene using non-synonymous exon 8 SNP rs5745687 and eight other markers spanning the 68-kb *HGF* gene (Figure 1). FBAT analysis failed to identify any association with ASD (Table 3).

SP1—Four markers were chosen to genotype the 36-kb *SP1* gene (Figure 1). FBAT analysis of this 664-family sample failed to reveal significant association for any of the four analyzed *SP1* markers (Table 3).

SUB1—Promoter variant rs2008245 and three other markers were used to genotype the 16-kb *SUB1* gene (Figure 1). FBAT analyses revealed no significant single-marker association for *SUB1* (Table 3).

Haplotype-based association test (HBAT) analyses

Haplotype-based analyses were performed on the two genes with evidence of single-marker ASD association, *PLAUR* and *SERPINE1*. Analysis of intermarker linkage disequilibrium (LD) indicated a single 7-kb LD block near the 5′ end of the *PLAUR* gene consisting of markers rs344772, rs344780, rs344781 and rs344783 (Figure 1). Haplotype-based association test (HBAT) analysis of the 4-marker LD block indicated global significance among five haplotypes (χ^2=13.609; df=5; P=0.018) (Table 5). However, no individual haplotype was associated with ASD risk. HBAT analysis using the 2 markers that are in high LD (r^2=0.90), rs344780 and rs344781, indicated significant global transmission disequilibrium (χ^2=19.303; df=3; P =0.0002) and association of the C-T haplotype (frequency=0.742; T_{OBS}=897; T_{EXP}=860; P=0.010) (Table 5).

Across the 12-kb *SERPINE1* locus, intermarker LD was relatively high (r^2>0.30) with the exception of the exon 2 non-synonymous SNP rs6090 (Figure 1). We therefore performed haplotype analyses including all markers except rs6090. HBAT analysis indicated globally significant transmission disequilibrium (χ^2=16.215; df=4; P=0.003) and ASD association with the haplotype A-C-T-C (frequency=0.162; T_{OBS}=340; T_{EXP}=315; P=0.026) (Table 5). We repeated the haplotype-based analyses using only the high LD (r^2>0.89) 3′ markers rs2070682, rs7242 and rs13238709. Global transmission disequilibrium was observed (χ^2=13.020; df=2; P=0.001) and a common haplotype (C-T-C; frequency=0.540) was over-transmitted to individuals with ASD (T_{OBS}=874; T_{EXP}=839; P=0.031) (Table 5).

Stratification by multiplex and simplex families

We previously demonstrated that the *MET* 5′ promoter variant rs1858830 exhibited significant association in 478 multiplex but not in 265 simplex families [Campbell et al 2007]. We performed a similar analysis here for *PLAUR* and *SERPINE1*, though with a caveat that there are 539 multiplex families and only 90 simplex families in this sample, and thus less power to detect significant associations in the simplex family strata. All single-marker and haplotype-based associations with ASD for the *PLAUR* and *SERPINE1* genes reported here were enhanced in multiplex families and absent from simplex families (Figure 2). Association of the *PLAUR* promoter variant rs344781 allele T was specific to multiplex families (multiplex families: T_{OBS}=539; T_{EXP}=499; P=0.004; simplex families: T_{OBS}=45; T_{EXP}=45; P=0.891). HBAT analysis of the 4-marker *PLAUR* LD block rs344772-rs344780-rs344781-rs344783 indicated a global significance in the multiplex families (χ^2=11.872; df=5; P=0.037) but an absence of significance in the simplex families (χ^2=2.624; df=4; P=0.619) (Figure 2). No specific 4-marker *PLAUR* haplotype was associated with ASD in multiplex families. Stratification of the *PLAUR* 2-marker (rs344780-rs344781) LD block revealed a global transmission disequilibrium in the multiplex families (χ^2=19.441; df=3; P=0.0002) and association of the C-T haplotype (T_{OBS}=854; T_{EXP}=816; P=0.007), and no significant global transmission disequilibrium in simplex families (χ^2=1.022; df=2; P=0.600) (Figure 2).

In *SERPINE1*, association of the rs13238709 C allele was present in multiplex families (allele frequency=0.566; T_{OBS}=557; T_{EXP}=525; P=0.041) but not simplex families (allele frequency=0.557; T_{OBS}=67; T_{EXP}=67; P=0.917). The *SERPINE1* 4-marker LD block (rs2227631-rs2070682-rs7242-rs13238709) indicated global significance in the multiplex (χ^2=15.465; df=4; P=0.004) but not simplex (χ^2=2.715; df=3; P=0.438) families. The A-C-T-C haplotype (frequency=0.161) was over-transmitted to individuals with ASD in the multiplex families (T_{OBS}=309; T_{EXP}=288; P=0.043). The *SERPINE1* 3′ LD block (rs2070682-rs7242-rs13238709) also demonstrated transmission disequilibrium (χ^2=14.251; df=2; P=0.0008) and over-transmission of the C-T-C haplotype (frequency=0.540; T_{OBS}=808; T_{EXP}=772; P=0.019) in multiplex families but no evidence for association in simplex families (global χ^2=0.111; df=2; P=0.946).

Gene-gene interaction analyses

To further characterize the association of *MET*, *PLAUR* and *SERPINE1* and to test the hypothesis that genetic effects at these loci interact, we selected one marker from each of these three associated genes to test for allelic interactions. The markers were: *MET* promoter variant rs1858830, *PLAUR* promoter variant rs344781 and *SERPINE1* marker rs13238709. The two-locus transmission disequilibrium test (TDT) indicated a significant interaction between *MET* rs1858830 and *PLAUR* rs344781 (P=0.011; Table 6).

Functional analysis of the *PLAUR* promoter variant rs344781

Given the location of the ASD-associated *PLAUR* promoter variant rs344781 (285 bp upstream of the *PLAUR* transcription start site), we hypothesized that this region would be important for gene expression. A 500-bp fragment corresponding to nucleotides −365 to +134 (transcription start site = +1) of the *PLAUR* promoter increased transcription 6-fold in human embryonic kidney (HEK) and mouse neuronal SN56 cell lines compared to the promoterless vector (Figure 3). In this context, there was no difference in transcript levels between a construct containing the C allele and a construct containing the ASD-associated T allele at rs344781 in the epithelial cell line, but an increased induction with the T allele in the neural cell line (Figure 3).

Discussion

The present study of genes encoding proteins involved in the regulation of MET signaling indicate that increased genetic risk for ASD may be conferred through multiple genes in this pathway. These data, together with our expression studies in postmortem brain, highlight a possible convergent pathophysiological etiology of ASD that may be mediated in part through the MET signaling cascade. The data reported here demonstrate a replication of the association of the *MET* promoter variant rs1858830 allele C with ASD in a 101-family subset that had not been tested previously. In addition, the analyses revealed association of the *PLAUR* promoter variant rs344781 T allele and a gene-gene interaction between *MET* and *PLAUR*. Our initial analysis indicates that the *PLAUR* 5′ fragment is functional and that the autism-associated T allele may enhance transcription compared to the C allele. This is consistent with the microarray analysis of postmortem tissue in which *PLAUR* expression is increased in ASD compared to matched controls [Campbell et al 2007]. Thus, there are at least two functional alleles that have the potential to alter MET signaling in ASD through their impact on gene transcription.

Further, analyses also indicated significant association for a haplotype of the *SERPINE1* gene, indicating that gene variants marked by the associated haplotype likely carry an allele conferring vulnerability to ASD. Future studies will be necessary to identify a functional variation in the putative *SERPINE1* risk haplotype. Overall, the association of three genes encoding proteins in the MET signaling pathway suggests that genetic susceptibility for ASD is conferred, in part, by multiple genes in the pathway.

The single marker associations of *PLAUR* and *SERPINE1* presented here would not survive stringent Bonferroni correction for each of the 29 markers genotyped in *PLAUR*, *SERPINE1*, *HGF*, *SP1* and *SUB1*. As with all initial reports of genetic association, the results suggesting association of *PLAUR* and *SERPINE1* should be considered provisional. Failure to replicate initially promising genetic association results has been common in ASD [Gupta & State 2007]. Among various possible reasons for non-replication, the propensity of association studies to generate false positive results must be considered [Ioannidis 2007; Sullivan 2007]. Thus the genetic association of *PLAUR* and *SERPINE1* reported here should be considered “tentative knowledge” and interpreted with caution [Sullivan 2007]. The absence of a replication sample is a limitation of this study, and independent replication will be necessary to establish specific *PLAUR* and *SERPINE1* variants as genetic risk factors for ASD.

Beyond genetic susceptibility, the functional integrity of the MET signaling system also is sensitive to environmental factors. This concept is supported by bioinformatics analyses that identified *PLAUR*, *SERPINE1* and *HGF* as genes active in immune response regulation, sensitive to environmental exposures, and within chromosomal regions previously implicated in ASD linkage studies [Herbert et al 2006]. Moreover, a recent cell biological study shows that chemically diverse toxicants reduce the expression of MET in oligodendrocyte progenitor cells, a result that is interpreted as the convergence of toxicant effects on oxidative status and the MET-regulating Fyn/c-Cbl pathway [Li et al 2007].

The initial report of *MET* association with ASD prompted our hypothesis that this signaling system could contribute to the multi-organ system involvement in subpopulations of individuals with ASD [Campbell et al 2006]. The finding regarding the ASD-associated *PLAUR* promoter variant rs344781 is consistent with this hypothesis. Behavioral phenotypes are the diagnostic basis of ASD, but subsets of patients with ASD also present with a diversity of gastrointestinal (GI) and immune symptoms [White 2003; Jyonouchi et al 2005; Valicenti-McDermott et al 2006]. The protein product of the *PLAUR* gene, like that of the

MET gene, participates in GI repair and immune responsiveness. *PLAUR* is expressed in luminal epithelial cells of the colon [Wang et al 2003], peripheral leukocytes and inflammatory-activated monocytes [Allgayer 2006]. Unlike *MET*, mice are viable following constitutive deletion of the gene orthologous to *PLAUR*. Our laboratory showed that in the developing forebrain, mice lacking *Plaur*, the gene encoding the urokinase plasminogen activator receptor (uPAR), exhibit secondary decreases in expression of both Hgf and Met. *Plaur*-deficient mice exhibited disrupted forebrain interneuron development, increased susceptibility to seizures, and anxiety and abnormal social behavior [Powell et al 2001; Powell et al 2003; Levitt et al 2004; Eagleson et al 2005; Levitt 2005]. Further, *Plaur* knockout mice are severely impaired in migratory capacity of granulocytes and monocytes toward inflammatory sites [Allgayer 2006], indicating that PLAUR participates in the inflammatory response to an immune challenge. We hypothesize that risk conferred by alleles at both *PLAUR* and *MET* in the individual with ASD may impact phenotypic expression of co-occurring medical complications associated with the psychiatric disorder.

Recent analysis of postmortem brain tissue from ASD cases and controls supports our multi-gene hypothesis related to MET signaling [Campbell et al 2007]. The reduced expression of MET in temporal lobe samples from individuals with ASD was accompanied by increases in transcript expression of *PLAUR*, *SERPINE1* and *HGF*. The analyses of transcription in a heterologous cell-based assay of the promoter variants in *MET* and *PLAUR* are consistent with the expression data for each gene. We hypothesize that the impact of genetic risk imposed by the ASD-associated alleles of *MET* and *PLAUR* may be accompanied by adaptive changes by other members of the signaling cascade. There also may be further convergence downstream of MET activation, as our data are consistent with reported analysis of ASD cases in which there are rare functional mutations in the *PTEN* gene [Butler et al 2005; Herman et al 2007]. PTEN negatively regulates signaling through AKT, a primary downstream target of MET. Further, the protein product of the ASD-associated *ITGB3* gene, β3-integrin, directly interacts with PLAUR, and PLAUR-integrin interactions regulate cell adhesion and migration [Xue et al 1997; Tarui et al 2001; Degryse et al 2005; Weiss et al 2006]. Future studies will test the hypothesis that the MET signaling cascade may be a primary target of gene-environment interactions, involving multiple components that contribute to the core behavioral and co-occurring medical conditions in individuals with ASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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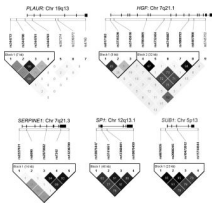

Figure 1.
Gene structure, genotyped markers and linkage disequilibrium (LD; in r^2) block structure of the five genes involved in regulation of MET receptor expression and signaling. Each gene is represented 5′>3′. Exons are indicated by boxes.

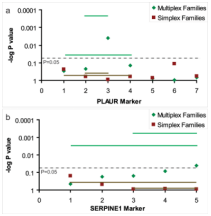

Figure 2.

Associations of *PLAUR* and *SERPINE1* with ASD in multiplex families. Plotted are -$\log_{10}$ P values for over-transmitted alleles (points) and global LD block analyses (lines). (a) The *PLAUR* promoter variant rs344781 allele T (marker 3; P=0.004), the rs344772-rs344780-rs344781-rs344783 LD block (markers 1−4; P=0.037) and the rs344780-rs344781 LD block (markers 2−3; P=0.0002) demonstrated significant association in multiplex, but not simplex, families. (b) The *SERPINE1* marker rs13238709 allele C (marker 5; P=0.041), the rs2227631-rs2070782-rs7242-rs13238709 LD block (markers 1, 3, 4 and 5; P=0.004) and the rs2070782-rs7242-rs13238709 LD block (markers 3−5; P=0.0008) were associated with ASD in multiplex, but not simplex, families.

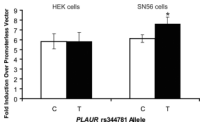

Figure 3.

Functional analysis of the *PLAUR* promoter variant associated with ASD. Insertion of a 500-bp *PLAUR* promoter fragment containing either variant of rs344781 increased transcription ~6-fold compared to the promoterless vector control following transfection of human embryonic kidney (HEK) or mouse neuronal (SN56) cell lines. The T allele-containing fragment exhibits greater induction in the neuronal cell line compared to the C allele (P=. 044, paired t-test), but no difference in the epithelial cell line. Error bars represent SEM (n=5).

Table 1

Description of ASD family sample.

Center	Individuals with ASD	Multiplex Pedigrees	Simplex Pedigrees
AGRE Consortium	631	284	28
Iowa	164	66	19
Stanford	257	115	16
Tufts-Vanderbilt	176	74	27
Total	**1,228**	**539**	**90**

Table 2

Variants identified in a screen of the *HGF*, *PLAUR*, *SERPINE1*, *SP1* and *SUB1* genes in 48 individuals with ASD.

Gene	Marker	Location	Amino acid
PLAUR	rs344781	Promoter	Non-coding
PLAUR	rs4251805	Exon 1	Non-coding
PLAUR	rs399145	Exon 3	Thr86Ala
PLAUR	rs2302524	Exon 6	Lys220Arg
SERPINE1	rs2227635	5′ UTR	Non-coding
SERPINE1	rs6092	Exon 2	Ala15Thr
SERPINE1	rs6090	Exon 2	Val17Ile
SERPINE1	rs6091	Exon 3	Ala119Ala
SERPINE1	rs7242	3′ UTR	Non-coding
HGF	rs574666	Exon 6	His237His
HGF	rs5745687	Exon 8	Glu304Lys
SP1	rs3741651	Exon 3	Gln480Gln
SUB1	rs2008245	Promoter	Non-coding

Table 3

Family-based association test (FBAT) analyses.

Gene	Marker	Allele	Freq[a]	Inf Fam[b]	T_{OBS}[c]	T_{EXP}[d]	Z	P
MET	**rs1858830**	**C**	**0.562**	**255**	**588**	**545**	**2.639**	**0.008**
PLAUR	rs344772	T	0.593	271	561	549	0.716	0.474
PLAUR	rs344780	C	0.752	227	580	565	1.056	0.291
PLAUR	**rs344781**	**T**	**0.745**	**223**	**584**	**544**	**2.742**	**0.006**
PLAUR	rs344783	C	0.529	238	480	460	1.294	0.196
PLAUR	rs397374	A	0.752	194	466	464	0.175	0.861
PLAUR	rs2239372	G	0.523	269	504	495	0.570	0.569
PLAUR	rs4760	T	0.148	125	141	136	0.555	0.579
SERPINE1	rs2227631	A	0.570	277	595	577	1.087	0.277
SERPINE1	rs6090	A	0.018	31	31	26	1.058	0.290
SERPINE1	rs2070682	C	0.557	284	598	577	1.317	0.188
SERPINE1	rs7242	T	0.562	284	601	576	1.585	0.113
SERPINE1	**rs13238709**	**C**	**0.564**	**289**	**624**	**592**	**1.981**	**0.048**
HGF	rs917183	A	0.507	243	444	440	0.295	0.768
HGF	rs3735520	C	0.535	244	464	460	0.281	0.779
HGF	rs5745616	G	0.760	198	474	470	0.301	0.763
HGF	rs7804865	G	0.215	190	244	239	0.420	0.675
HGF	rs4732404	T	0.210	191	231	227	0.335	0.737
HGF	rs5745687	A	0.068	70	75	69	0.848	0.396
HGF	rs1800793	A	0.197	182	240	219	1.724	0.085
HGF	rs6467866	G	0.805	176	420	420	0.021	0.983
HGF	rs5745752	G	0.717	204	456	455	0.062	0.950
SP1	rs10876447	G	0.850	134	346	332	1.417	0.156
SP1	rs3741651	A	0.836	176	502	484	1.431	0.152
SP1	rs12368491	G	0.830	178	483	471	0.967	0.333
SP1	rs10876450	T	0.835	185	525	510	1.194	0.233

Gene	Marker	Allele	Freq[a]	Inf Fam[b]	T_{OBS}[c]	T_{EXP}[d]	Z	P
SUB1	rs6876035	T	0.378	272	430	422	0.517	0.605
SUB1	rs2008245	G	0.611	270	577	568	0.589	0.556
SUB1	rs10472812	T	0.611	268	580	571	0.569	0.569
SUB1	rs11744644	C	0.344	232	355	350	0.345	0.730

[a] Freq, the frequency of the associated allele

[b] Inf Fam, number of informative families

[c] T_{OBS}, transmissions observed; equivalent to the “S” statistic in FBAT

[d] T_{EXP}, transmissions expected; equivalent to “E(S)” statistic in FBAT

Table 4

Case-control analyses.

Gene	Marker	Status	n	Allele1	Allele2	Freq 1/1	Freq 1/2	Freq 2/2	χ^2	P
MET	rs1858830	Case	596	C	G	0.320	0.515	0.164	8.961	0.011
		Control	300	C	G	0.271	0.484	0.245		
PLAUR	rs344781	Case	588	C	T	0.051	0.371	0.578	9.952	0.007
		Control	296	C	T	0.101	0.304	0.595		
SERPINE1	rs13238709	Case	619	C	T	0.307	0.512	0.181	4.813	0.090
		Control	288	C	T	0.375	0.441	0.184		

Table 5

Haplotype-based association test (HBAT) analyses.

Gene	Markers in LD Block	Haplotypes	Global χ^2	Global P	ASD-associated Haplotype	Haplotype Frequency	Haplotype-specific P
PLAUR	$_4{}^a$	5	13.609	0.018	None	-	-
PLAUR	$_2{}^b$	3	19.303	0.0002	C-T	0.742	0.010
SERPINE1	$_4{}^c$	4	16.215	0.003	A-C-T-C	0.162	0.026
SERPINE1	$_3{}^d$	2	13.020	0.001	C-T-C	0.540	0.031

[a] *PLAUR* markers rs344772, rs344780, rs344781 and rs344783

[b] *PLAUR* markers rs344780 and rs344781

[c] *SERPINE1* markers rs2227631, rs2070682, rs7242 and rs13238709

[d] *SERPINE1* markers rs2070682, rs7242 and rs13238709

Table 6

Gene-gene interaction analyses.

Gene Marker - Gene Marker	2-locus TDT (P)
MET rs1858830 - *PLAUR* rs344781	0.011
MET rs1858830 — *SERPINE1* rs13238709	0.782
PLAUR rs344781 — *SERPINE1* rs13238709	0.755