Identifying Potential Candidate SNPs and Genes linked to Handedness for Future Study

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Abstract

The goal was to identify genes for future study that might be linked to handedness for potential candidate gene study. We were able to find 3 major genes through statistically analyzing SNP data within a Genome-wide association Statistical Analysis tool, PLINK. 27 SNPs were chosen based on P-values of below .001, further analysis was done to these 27 SNPs to figure out their gene locus. Using NCBI we found 21 SNPs were within a gene locus. A total of 16 genes were found from the 21 SNPs that were located within a gene locus. Three extra genes were examined since they were located in a gene of interest CTNNA2. The genes found were further screened for high expression in subcortical regions and that have been implicated in neural function or brain development. Out of the 17 genes determined, IRAK2, NRG1, and CTNNA2 adhered to the criteria for screening.

OBJECTIVE:

The overall goal is to identify genes for future study that might be linked to handedness for potential candidate gene study. We analyzed SNP data from the ADNI project with respect to handedness and identified SNPs with locally significant associations in genes with high expression in brain regions of interest that have been implicated in neural function.

METHODS:

Step 1: Identify candidate SNPs from ADNI project to use in Handedness study. The identification of candidate SNPs from ADNI project was done by using a Genome-wide association Statistical Analysis tool, PLINK. Using PLINK a basic association analysis was run on the ADNI data set, 620,901 SNPs collected from 37 subjects with self-reported hand preference as the phenotype. We omitted any SNPs with P-values > .001, which narrowed the number of SNPs to around a 1000. The P-value of below .001 allows the null hypothesis a . 1% possibility of being correct. The first 30 SNPs with lowest P-value were then chosen from this sett. A Manhattan plot using the computer program Haploview was created. Since there were so much data, the Manhattan plot would help to demonstrate stronger statistical significance of data points on the different chromosomes. The Manhattan plot (Figure 1) suggests loci on chromosomes 2, 5, 10, 22, 8 and 7. Three extra SNPs were obtained from the data set with p <.001 and these SNPs were found to be located within the LRRTM2 gene. The Specific genes picked from the SNPs were then checked for expression in the human brain using the Allen Brain database. The Allen Brain database was compiled from brain samples of 3 donors; 2 left-handed African Americans, and a cross-dominant Caucasian male. Analysis of expression of these genes in the basal ganglia (caudate nucleus, putamen, globes pallidus), substantia nigira, and sub thalamic nucleus.

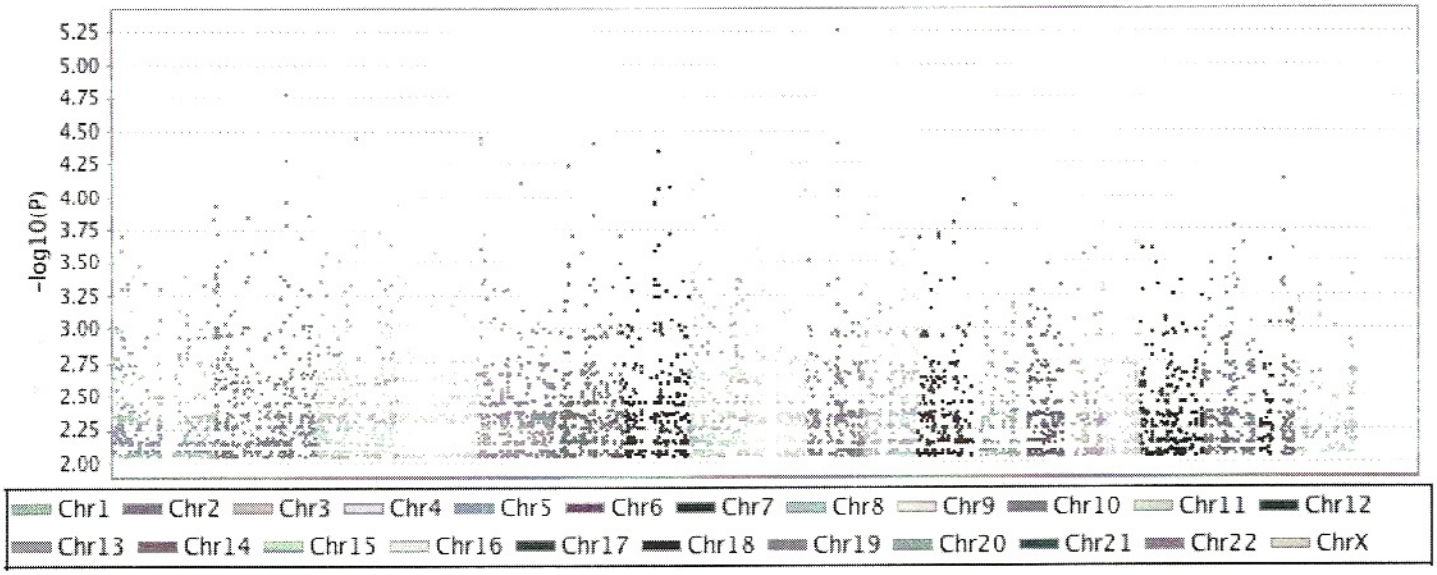

Figure 1. Manhattan plot of 5233 most significant SNP

Step 2: Determine Genes of selected SNPs and expression in specific brain structures. The SNP data was compiled from 37 individuals of which 20 were males and 17 were females. The determination of genes was done by using the National Center for Biotechnology Information (NCBI) database on SNPs. The top 30 SNPs were reduced further since many of the SNPs were not within a gene locus.

BACKGROUND

The human brain is a sophisticated symmetrical structure that functions asymmetrically. Handedness is a one of the most obvious results of this functional asymmetry. Studies using magnetic source imaging have

shown that the cortical representation of the right hand is larger than the one of the left hand in right-handers, and vice versa in left-handers (1). There is a correlation between handedness and hemispheric control of language; In more than 95% of right-handed men and 90% of right-handed women, language and speech were localized to the brain's left hemisphere. Though in left-handed people, the incidence of left-hemisphere language dominance has been reported as 73% (2).

Approximately 90% of the population prefers the right hand for manual activities, while 10% prefers the left hand. The development of handedness is thought to be influenced by genetic factors. There are two genetic models for handedness, the mode proposed by Marian Annett and the model proposed by Chris McManus. The model by Marian Annett proposes that there is a gene, right-shift (RS) gene, whose specific duty is to down-regulate speech control in the right hemisphere while at the same time up-regulate it in the left, and handedness is a consequence of the left side being up-regulated (3). The model of Chris McManus is based on Mendelian genetics; handedness is controlled by 2 alleles, D (dextral) and C (chance), having a D allele will increase our chance for being right-handed while having a C allele will increase your chance of being left-handed (4).

In 1991 an observational study was published in which a group of researchers using ultrasound observed, 75 fetuses at 15 weeks till 37 weeks were observed to have a marked bias for sucking thumb of the right hand (5). A follow up study by the same group was conducted on the 75 fetuses that had matured into their teens; 60 fetuses that preferred to suck their right thumb, all were indeed right-handed as teenagers, but 15 that preferred the left thumb, 10 were left-handed and 5 were right-handed as teenagers. Twin studies and single individuals have shown that left-handedness is more likely to occur in twins at a higher frequency (6) and that there is very little difference in the frequency between left-handedness in monozygotic and dizygotic twins (7). A recent twin study on the heritability of handedness found that there is 25% chance of variance due to additive genetic effect, and in the other 75% chance of variance, no common environmental influence was detected (8).

In 2007 a gene, LRRTM1 was found to be associated paternally with relative hand skill (9). In the study the researchers found significant association of the haplotype upstream of LRRTM1 with a quantitative measure of human handedness in a set of dyslexic siblings, when the haplotype was inherited paternally ($P = 0.00002$) (9). All these findings suggest that there are genetic factors that lead to specific handedness of an individual.

RESULTS

From the initial analysis of the ADNI data set, 27 SNPs were chosen using Genome-wide association Statistical Analysis tool, PLINK. The 27 SNPs were chosen based on P-values of below .001, further analysis was done to these 27 SNPs to figure out their gene locus. Out of the 27 SNPs chosen, 21 SNPs were located within a gene locus with many SNPs having similar genes. A total of 16 genes were found from the 21 SNPs that were located within a gene locus. Another set of SNPs rs7579935, rs1368919, rs2916492 were examined since they are located within the gene, CTNNA2 that also contains LRRTM1. The 21 SNPs will be used for future study using the iPLEX Sequenom massARRAY.

We further screened for SNPs of potential functional relevance by screening for SNPs located in genes with high expression in subcortical regions and that have been implicated in neural function or brain development. To locate these specific regions we used the Allan Brain Atlas database, from which we were able to see expression of these genes. Out of the 17 genes determined, IRAK2, NRG1, and CTNNA2 adhered to the criteria stated above. The chosen SNPs, their corresponding gene, p-value and chromosome are shown in figure 2.

NRG1 or Neuroligins has been linked having great importance in brain development, since mutations in the gene have been found to be linked to autism, schizophrenia, and mental retardation (10). NRG1 like LRRTM1 has been found to bind to Neurexin-1beta and induce excitatory synapses (11). NRG1 has been found to have high expression in the left sub thalamic nucleus, substantia nigira, and putamen. NRG1 shows varied expression in the Globus Pallidus external and internal, and Caudate nucleus.

IRAK2 or interleukin-1 receptor-associated kinase 2 is linked signaling pathway through up-regulating IL-1 (12). IL-1 also has been found to up-regulate NF-kappaB which is linked to growth of neural processes in

developing peripheral and central neurons (13). Expression of IRAK2 is high in Putamen, and Globus Pallidus internal and external, but low expression is found within the Thalamic nucleus, Caudate Nucleus, and Sub thalamic Nucleus.

CTNNA2 or Alpha-Catenin 2 up-regulates cell-cell and cell-matrix interactions in tissues. CTNNA's contain within them an antisense orientation of the gene LRRTM1which has been linked to handedness (14). LRRTM1 or Leucine-rich repeat transmembrane neuronal protein is expressed during the development of specific forebrain structures, and thus could influence neuronal differentiation and connectivity (9) CTNNA2 contains bidirectional promoters that are shared with LRRTM2. Therefore expression of LRRTM2 could influence expression of CTNNA2 (14). CTNNA2 seems to be widely expressed within all structures of the brain, possibly due to its important role of helping in cell-cell and cell-matrix interaction.

SNP	P- Value	Gene	CHISQ	BP	Chromosome
rs548216	5.49E-06	KCNMA1	20.66	78616033	10
rs17554	1.63E-05	CYBRD1	18.58	172111615	2
rs9313195	3.87E-05	ADCY2 (specific for brain)	16.94	7590575	5
rs10762752	3.87E-05	KCNMA1	16.94	78644806	10
rs10118127	4.56E-05	KDM4C	16.62	6841118	9
rs13009270	5.24E-05	CYBRD1	16.36	172110783	2
rs6458325	5.77E-05	PTK7	16.18	43223648	6
rs11706450	6.84E-05	IRAK2	15.85	10252866	3
rs4458837	7.45E-05	NRG1	15.69	32423925	8
rs5761524	7.45E-05	ASPHD2	15.69	25166008	22
rs1692821	8.62E-05	CTSB	15.42	11737397	8
rs7875140	8.87E-05	COL5A1	15.36	136753593	9
rs1436090	8.87E-05	KCNMA1	15.36	78627240	10
rs7792974	0.0001098	MAGI2	14.96	78482887	7
rs3806563	0.000111	CYBRD1	14.94	172085179	2
rs985175	0.000112	MAGI2	14.92	78503986	7
rs231394	0.0001161	SH3BP2	14.85	2806426	4
rs3885617	0.0001161	METTL21C	14.85	102144443	13
rs724395	0.0001393	PID1	14.51	229619810	2
rs764469	0.0001393	PID2	14.51	229620900	2
rs1012105	0.0001393	KCNC1	14.51	17726331	11
rs7579935	0.0009647	CTNNA2	10.89	79796211	2
rs1368919	0.0004026	CTNNA2	12.52	79939942	2
rs2916492	0.0001448	CTNNA2	14.44	79939983	2

Figure 2. Chosen SNPs for study

FUTURE STUDY:

In the future study we would like to explore prevalence of these SNPs in a larger sample of about 100 subjects, who have undergone an EEG. The EEG will gather brain activity data while subject attempts a task that would be related to handedness and language tasks. The 100 samples of DNA will be run on a MassARRAY, iPLEX Sequenom. The iPLEX Sequenom would assay for the 24 SNPs suggested above. The massARRAY data will help us to understand the prevalence of the SNPs in our subject population. Using the EEG data we could compare SNP gene data to resting states and excited states, or even workings of the language centers of the brain.

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