Candidate Gene Analysis Identifies a Polymorphism in *HLA-DQB1* Associated With Clozapine-Induced Agranulocytosis

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Objective: Clozapine is considered to be the most efficacious drug to treat schizophrenia, although it is underutilized, partially due to a side effect of agranulocytosis. This analysis of 74 candidate genes was designed to identify an association between sequence variants and clozapine-induced agranulocytosis (CIA).

Method: Blood and medical history were collected for 33 CIA cases and 54 clozapine-treated controls enrolled between April 2002 and December 2003. Significant markers from 4 genes were then assessed in an independently collected case-control cohort (49 CIA cases, 78 controls).

Results: Sequence variants in 5 genes were found to be associated with CIA in the first cohort: *HLA-DQB1*, *HLA-C*, *DRD1*, *NTSR1*, and *CSF2RB*. Sequence variants in *HLA-DQB1* were also found to be associated with CIA in the second cohort. After refinement analyses of sequence variants in *HLA-DQB1,* a single SNP (single nucleotide polymorphism), 6672G>C, was found to be associated with risk for CIA; the odds of CIA are 16.9 times greater in patients who carry this marker compared to those who do not.

Conclusions: A sequence variant (6672G>C) in *HLA-DQB1* is associated with increased risk for CIA. This marker identifies a subset of patients with an exceptionally high risk of CIA, 1,175% higher than the overall clozapine-treated population under the current blood-monitoring system. Assessing risk for CIA by testing for this and other genetic variants yet to be determined may be clinically useful when deciding whether to begin or continue treatment with clozapine.

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Schizophrenia is associated with a great socioeconomic burden, as patients are often diagnosed early in life and many patients are insufficiently treated, remaining significantly disabled for many years.

Clozapine is considered by many to be the most efficacious antipsychotic agent for the treatment of chronic schizophrenia. Early studies found that clozapine was superior to typical antipsychotics in patients who had failed trials of multiple medications,[1,2] and it is also more efficacious than other atypical antipsychotics in refractory patients.[3,4] Clozapine is also associated with fewer substance abuse relapses,[5] reduced hospitalizations, decreased cost of treatment, and improved quality of life in the treatment of schizophrenia.[6]

Despite these benefits, clozapine prescriptions comprise only 2%–10% of the total antipsychotic market for schizophrenia in the United States.[7–11] One important factor that may contribute to this low utilization of clozapine is a rare but serious side effect, clozapine-induced agranulocytosis (CIA), a decrease in absolute neutrophil count (ANC) to ≤ 500 cells/mm^3. If left untreated, CIA may lead to increased susceptibility to systemic infection and is potentially lethal. Clozapine-induced agranulocytosis recurs with rechallenge of clozapine, often more rapidly and severely,[12] suggesting an immunologic mechanism of action, and thus readministration of clozapine following the development of CIA is not recommended. The risk of CIA has resulted in a US Food and Drug Administration–mandated requirement for blood monitoring in all patients prescribed clozapine in the United States. The implementation of this blood-monitoring system has successfully reduced the incidence of CIA to 0.4% from the 1.3% incidence observed in patients treated with clozapine in the absence of white blood cell count (WBC) monitoring.[13] Granulocytopenia (or leukopenia), defined as a WBC of < 3,500/mm^3 and ANC of < 2,000/mm^3, is observed in approximately 3% of clozapine-treated patients.[13]

An epidemic of clozapine-induced dyscrasias in Finland in the 1970s generated interest into whether epidemiologic or genetic factors could explain these cases.[14,15] Although pedigree analyses failed to make any strong genetic link to the condition at the time, subsequent research continued to explore the possibility of a genetic predisposition for clozapine-induced agranulocytosis. No association of cytochrome P450 enzyme variation with CIA has been reported, which is consistent with reports that CIA is not a dose-dependent side effect.[16,17] However, due to an observed association between Jewish ethnicity and incidence of CIA, genetic studies began to focus on the human leukocyte antigen (HLA) complex to distinguish these populations. One study, performed in a cohort of 31 cases of CIA, reported that 83% of these patients carried the *HLA-B38* marker.[18] These findings were more robust when a haplotype composed of 3 alleles, including *HLA-B38*, *HLA-DR4*, and *HLA-DQw3*, was examined selectively in Ashkenazi Jewish cases and controls, and 100% of cases were found to carry this haplotype compared to only 12% of controls.[18] Significant associations between CIA and HLA alleles or other major histocompatibility genes (HLA class III genes: tumor necrosis factor, heat shock protein 70) have been identified in numerous studies.[19–22]

To date, the pathogenesis of CIA is unknown, and no reliable clinical or biologic markers of risk for CIA have been identified. While other studies[18,22–25] have suggested that specific HLA markers are associated with increased susceptibility to CIA, no specific HLA marker has consistently been associated with risk, perhaps secondary to small sample sizes, differing ethnic populations, and lack of experimental control for potential important confounding factors such as age, sex, and duration of exposure to clozapine. These studies were also hampered by the lack of availability of comprehensive genomic information, with technological limitations reducing the ability to comprehensively screen large numbers of genes for association with CIA.

The primary objective of this investigation was to identify and replicate a genetic marker predictive of CIA that could be used to assess a patient's risk of agranulocytosis. A case-control approach was applied in 2 large, independent cohorts of patients. Genetic variants in 74 candidate genes were analyzed in the discovery cohort, with replication of significant findings in a second, independent cohort.

METHOD

Subjects

Cohort I was recruited from sites within the United States, Russia, and South Africa. Clozapine-induced agranulocytosis cases (n = 33) were included if they were discontinued from clozapine treatment and had an ANC of less than 500 cells/mm^3. Controls (n = 54) were treated with clozapine at a minimum dose of 250 mg/d for at least 1 year with normal WBCs and ANCs. Cases and controls were recruited in an approximate 1:2 ratio to achieve a target of 99% power to detect genetic associations, with an odds ratio (OR) of 16, sensitivity and specificity both 80%, and $\alpha = .001$. All subjects were enrolled between April 2002 and December 2003. The protocol was approved by an institutional review board, and subjects provided informed consent.

Cases in Cohort II were identified by collaboration with the Drug Commission of the German Medical Association, through which adverse drug reactions, including clozapine-induced agranulocytosis, are reported. By contacting the reporting physician, blood samples from patients with CIA were collected for study. The cohort was composed of non-Jewish white patients of German descent diagnosed with schizophrenia. Clozapine-induced agranulocytosis cases (n = 49) were included if they were discontinued from clozapine treatment and had an ANC of less than 500 cells/mm^3. Controls (n = 78) were treated with clozapine at a minimum dose of 250 mg/d for at least 2 years with normal WBCs and ANCs. This cohort has been previously described elsewhere.[24–26]

Genotyping

Seventy-four candidate genes were selected based upon their role in clozapine pharmacodynamics and pharmacokinetics, their role in promyelocytic differentiation, or previous reports of association with CIA (eTable 1). Because CIA is a rare event that may be caused by a rare variant, sequencing was attempted for each gene in Cohort I for the full coding regions, intron-exon boundaries, and 500 to 1,000 bases upstream and downstream of the transcription start and stop. Over 75% of all targeted regions for most genes were successfully sequenced. The sequencing assays were developed on a subset of index repository subjects as described elsewhere.[27] Regions harboring polymorphisms (single nucleotide polymorphisms [SNPs]) from selected markers were sequenced in Cohort II (eTable 2; primers and polymerase chain reaction conditions in eTables 3 and 4).

Analyses

The sequence variations for each gene were phased into single- and multi-SNP markers for each subject using a combination of deductive and statistical logic.[28–30] The marker nomenclature used here includes the following: (1) "REC" for recessive, "DOM" for dominant; (2) the number of variants that comprise the marker; and (3) an identifier and allele for each polymorphism in the marker.

In Cohort I, every nonredundant dominant or recessive marker including at least 1 but no more than 4 polymorphisms in each gene was tested for association with CIA case or control status. Polymorphisms and multi-SNP markers were excluded if their minor allele frequencies were < 1% and < 5%, respectively. An equal variance *t* test was used, which evaluates the null hypothesis of equal case frequency in marker-positive and marker-negative subsets, applied to the residuals of a linear fit of covariates (age, sex, and self-described race) to case status.

These tests provide *P* values that closely approximate those obtained in SAS using logistic regression (data not shown). Permutation testing was conducted within genes. For a gene with small nominal *P* values, up to 6,250 permutations of the residuals were retested against every marker to establish a null distribution of minimum *P* values for the collection of markers. Each marker's nominal *P* value was adjusted for within-gene multiple comparisons by measuring its quantile within the null distribution, resulting in an adjusted *P* value (adj-*P*). For many markers, another marker existed with a different set of polymorphisms that split the subjects into identical groups; these are termed *equivalent markers*.

Markers with an adj-*P* of ≤ .05 in Cohort I were assessed in Cohort II using logistic regression (SAS Version 8.2; SAS Institute Inc, Cary, North Carolina), with CIA status as the endpoint, the marker as the main term, and sex as a covariate. All *P* values in this second analysis were 1-sided. To adjust for multiple comparisons within and between genes, permutation testing (1,000 permutations) and the FDR (false discovery rate) method of Benjamini and Hochberg[31] (with the smallest permutation test *P* value from each gene) were conducted, respectively.

Further refinement analyses were conducted to identify a single marker that best explained the data. The equal variance *t* test, as described above, was applied to the combined white-only subjects of Cohorts I and II.

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Table 1. Cohort Demographics

Variable	Cohort I		Cohort II	
	CIA Cases (n = 33)	Controls (n = 54)	CIA Cases (n = 49)	Controls (n = 78)
Sex, n (%)				
Male	17 (52)	33 (61)	21 (43)	40 (51)
Female	16 (48)	21 (39)	28 (57)	38 (49)
Ethnicity, n (%)				
White	28 (85)	48 (89)	49 (100)	78 (100)
American Indian or Alaskan Native	4 (12)	4 (7)	0	0
Black or African American	1 (3)	2 (4)	0	0
Age,[a] median (range), y	36 (20–58)	35 (18–54)	NC[b]	NC[b]
Nadir ANC, median (range), cells/mm^3	237 (0–506)	NA	NC	NA
Diagnosis, n				
Schizophrenia/schizoaffective disorder	32	53	49	78
Bipolar disorder	1	1	0	0
Time to CIA, mean (SD), mo	8.9 (1.3)[c]	NA	2.5 (2.2)	NA

[a]Age at start of clozapine treatment. [b]Patient age at sample collection; median (range) cases: 45 (18–85) years, controls: 35 (18–82) years. [c]25 of 33 CIA cases in Cohort I had an onset of CIA < 6 months; range (0.5–62.6 mo).
Abbreviations: CIA = clozapine-induced agranulocytosis, NA = data not applicable, nadir ANC = lowest absolute neutrophil count, NC = data not collected to ensure anonymity.

Table 2. Genes With Significant Markers in Cohort I

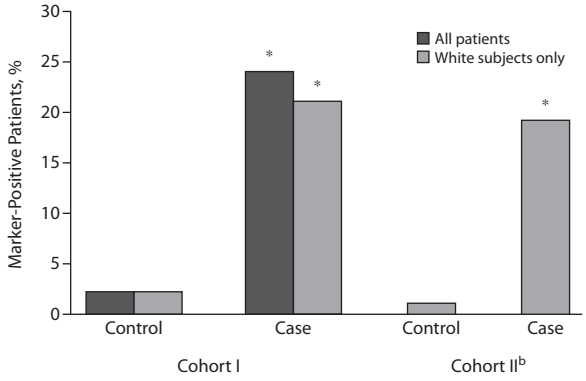
Gene	No. of Polymorphisms	No. of Haplotypes	No. of Markers	No. of Markers With Adjusted-*P* < .05	Minimum *P* Value	Minimum Adjusted-*P* Value
HLA-DQB1	52	72	8,374	22	.000002	.003981
CSF2RB	65	83	3,460	3	.00001	.01
DRD1	18	22	13	6	.0003981	.01
NTSR1	29	36	243	3	.0002	.0126
HLA-C	103	70	24,617	1	.000006	.031623

Table 3. Significance of *HLA-DQB1* Markers in Cohort II

Marker in *HLA-DQB1*[a]	*P* Value[b]	Adjusted-*P* Value
REC 3 5C, 18G, 21G	.002747	.01
REC 2 18G, 21G	.003905	.012
REC 3 18G, 19C, 21G	.006237	.019
REC 3 9G, 18G, 21G	.028432	.083
REC 3 4T, 21G, 50C	.088462	.234
REC 4 5C, 8T, 18G, 21G	.132099	.311
REC 2 21G, 49C	.176731	.391
REC 3 10C, 18G, 21G	.275536	.549

[a]Marker nomenclature: (1) model ("REC" for recessive, "DOM" for dominant), (2) the number of variants that comprise the haplotype, and (3) an identifier and allele for each polymorphism in the marker (eg, polymorphic site #5 in *HLA-DQB1* is nucleotide C). [b]Logistic regression, 1-sided *P* value.

Figure 1. *HLA-DQB1* 21G Marker Frequency[a]



[a]*HLA-DQB1* "REC 21G" frequency in Cohort I (adj-*P* = .0015), Cohort I white subset (adj-*P* = .0088), and Cohort II (adj-*P* = .00097). [b]Cohort II contains only white subjects.
*Fisher *P* case versus control: adj-*P* < .05.

RESULTS

Patient Characteristics

Demographic characteristics are presented in Table 1. Subjects in Cohort I were reasonably balanced by sex, ethnicity, and age. Most of the subjects were white (87%; 28 CIA cases and 48 controls). The median nadir ANC of CIA cases was 237 cells/mm^3. Subjects in Cohort II were all white patients and were balanced for sex.

Cohort I Analysis

There were 35 significant single- or multi-SNP markers in the following 5 genes (Table 2) with an adjusted *P* value less than .05: *HLA-DQB1*, *HLA-C*, *DRD1* (dopamine receptor D1), *NTSR1* (neurotensin receptor 1), and *CSF2RB* (β chain of colony-stimulating factor 2 receptor). The most significant markers in each of these genes were "REC 4 4T, 22G, 47G, 50C" in *HLA-DQB1*; "REC 3 9T, 24G, 36C" in *CSF2RB*; "REC 4 4C, 7T, 16C, 18G" in *DRD1*; "REC 4 1A, 8C, 17C, 25A" in *NTSR1*; and "DOM 4 3G, 14C, 42G, 73A" in *HLA-C*. The most significant single-SNP marker was found in *HLA-DQB1* ("SNP21," 6672G>C); raw *P* = .001116, adj-*P* = .0336, adjusted OR = 13.55. All significant markers in these 5 genes were assessed in Cohort II with the exception of those eliminated for (1) low population frequency, (2) comprising more polymorphisms than other markers in the gene, and (3) a poor *P* value in the white subset of Cohort I. The third criterion eliminated *HLA-C* from further analysis.

Table 4. Test Characteristics of *HLA-DQB1* "REC 21G"

	Marker Positive[a]		Marker Negative[a]							
	Cases	Controls	Cases	Controls	OR	Sensitivity,[b] %	Specificity,[c] %	Positive Predictive Value,[d,e] %	Negative Predictive Value,[d,f] %	1 – Negative Predictive Value,[d,g] %
Cohort I[h]	8	1	24	52	17.33	25.0	98.1	5.1	99.7	0.3
Cohort II[i]	9	1	38	71	16.82	19.1	98.6	5.2	99.7	0.3
Combined	17	2	62	123	16.86	21.5	98.4	5.1	99.7	0.3

[a]"REC 21G" is *HLA-DQB1* 6672G > C; marker positive is non-GG (GC or CC), marker negative is GG. [b]The probability of being marker positive, given that one is a case. [c]The probability of being marker negative, given that one is a control. [d]Assumed clozapine-induced agranulocytosis prevalence of 0.4%. [e]The probability of being a case, given that one is marker positive. [f]The probability of being a control, given that one is marker negative. [g]The probability of being a case, given that one is marker negative. [h]Genotypes are missing for 2 subjects, CRG1480 and CRG5562. [i]Genotypes are missing for 8 subjects (CRGRPA_005, CRGRPA_013, CRGRPA_025, CRGRPA_029, CRGRPA_045, CRGRPA_109, CRGRPA_119, and CRGRPA_120).

Cohort II Analysis

The best raw *P* values for markers in *CSF2RB*, *DRD1*, and *NTSR1* were .19, .30, and .42, respectively (data not shown). Therefore, these genes were not further analyzed. The 20 *HLA-DQB1* markers selected for replication were reduced to 8 unique markers because some markers that were highly correlated in Cohort I were perfectly correlated in Cohort II. Two very similar markers in *HLA-DQB1* were significant after adjustment (Table 3), with FDR-adjusted *P* values of .01 and .012. The more simple of the 2 markers is "REC 2 18G, 21G" (6657G, 6672G).

Refinement Analysis

To use the full power of the available samples, all markers in *HLA-DQB1* were analyzed in the combined subjects of Cohorts I (white subset) and II. The marker with the smallest *P* value in this analysis was "REC 2 18G, 21G" (6657G, 6672G), as was found in the analysis of Cohort II alone. Each SNP in this marker was individually examined for its contribution to the overall significance. Both SNPs were in Hardy-Weinberg equilibrium in all populations tested. Each SNP was significantly correlated with CIA in Cohort I (white subset), with adjusted *P* values of .0014 and .0088 for "REC 18G" and "REC 21G," respectively. However, in Cohort II, only "REC 21G" was significant (adj-*P* = .00097; "REC 18G" adj-*P* = .22). This analysis is supportive of "REC 21G," not "REC 18G," as a risk factor for CIA. There is a significantly higher frequency of "REC 21G" in CIA cases compared to controls, and the effect of "REC 21G" is consistent in 2 independent cohorts (Figure 1). In contrast, "REC 18G" fails to achieve significance in the second cohort. Genotyping of "REC 21G" (6672G>C) identifies a small group, ~ 2%, of clozapine-treated patients, with an OR of 16.9 (95% CI, 3.57–109; *P* < .001) of developing CIA compared with patients of any other genotype (Table 4). Clinical test characteristics for genotyping "REC 21G" can be found in Table 4.

DISCUSSION

We have demonstrated an association between CIA and a single SNP in *HLA-DQB1* (6672G>C or "REC 21G") based on analyses of 2 case-control populations. "REC 21G" (6672G) is an intronic SNP, suggesting that it may be linked with other causative variants. It is perfectly correlated with another SNP ($r^2 = 1$), which results in a G to A change at position 6874 from the transcription start site. These SNPs have not been previously described; therefore, no functional information is available. Further research is warranted, including HLA typing, assessment of additional variants in the region, and the addition of more case and control subjects as well as assessment of the allele frequency in healthy control populations. A high resolution HLA typing analysis of this population is currently underway.

These data are consistent with previous genetic studies implicating other variants in *HLA-DQB1* in risk for CIA. Previous analyses of the subjects in Cohort II revealed an association between HLA-DQB1*0502, as well as other HLA markers, and risk of CIA.[24] The HLA-DQB1*0502 marker was also identified by Yunis and colleagues[22] to be significantly associated with CIA, specifically in non-Jewish subjects, whereas HLA-DQB1*0302 was associated with CIA in Ashkenazi Jewish subjects. In a third small study, the HLA-DQB1*0201 marker was found in 100% of 13 CIA and 5 granulocytopenia cases and only 54% of controls.[23] However, these studies also report statistically significant associations with other HLA alleles, and there has largely been a lack of replication between studies, including some conflicting reports.[32] Differing study methodologies, sample sizes, and specific alleles tested are likely causes of variance among the findings in these studies. Ongoing HLA typing studies are expected to determine the correlation, if any, between the results presented here and previously published findings.

A considerable strength of the current study was the ability to sequence each candidate gene in order to capture the maximum genetic variation. The polymorphism that provided the greatest signal in this study was previously unknown in the published literature and databases, and thus has not previously been assessed for association with CIA. These data reaffirm the importance of comprehensively assessing the variation within genes in sufficiently large populations of subjects in order to fully examine the genetic contribution to rare phenotypes.

A second strength of this study was the availability of an independent cohort with a virtually identical phenotype. The presence or absence of CIA is an objective criterion that relies on a laboratory test, and thus measurement variance between cohorts is minimized. This is in contrast to other pharmacogenetic work in psychiatry, in which outcome measures such as clinical response to treatment are used,

and assessments or measurements may vary markedly. The risk of type I error has been much discussed with genetic association studies, and the utilization of a second cohort in this study mitigates against this possibility. Finally, this represents the largest study conducted to date of this rare adverse drug event.

The present study presents some limitations, above all the possibility of a false-positive finding, despite the use of a replication cohort.[33] Furthermore, it was not possible to include all clinical factors possibly modulating CIA occurrence, such as concomitant medications.[34] One additional limitation of this study is the lack of exploration of other possible hereditary factors such as copy number variation, methylation, and epistatic effects that could be explored in future studies. Lastly, the majority of patients examined in this study were white; therefore, it remains possible that there may be differences in the frequency of the polymorphism and its clinical impact in other populations. The relationship between this polymorphism and the incidence of clozapine-induced agranulocytosis should be explored in other populations in future studies.

Pharmacogenetic associations for rare but serious adverse events have been demonstrated for other HLA variants. The HLA-B*5701 allele has been repeatedly shown to be predictive of hypersensitivity reactions following treatment with the antiretroviral drug abacavir.[35–38] Hypersensitivity reactions due to treatment with nevirapine, a nonnucleoside reverse transcriptase inhibitor, also used in the treatment of HIV, may be linked to expression of HLA-DRB1*0101.[39] Similarly, an association between HLA-B*1502 and Stevens-Johnson syndrome induced by carbamazepine, indicated for the treatment of epilepsy and bipolar disorder, has been identified in a Han Chinese cohort,[40] although it is unclear whether this finding extends to other ethnicities.[41,42] The US Food and Drug Administration has recently modified the product labeling for carbamazepine to recommend genetic testing for Asian patients.

In summary, these studies have identified a strong association between genetic variation in *HLA-DQB1* and risk for clozapine-induced agranulocytosis. This sequence variant (6672G>C) in *HLA-DQB1* can identify a subset of patients with an exceptionally high risk of CIA (5.1% positive predictive value), 1,175% higher than the overall clozapine-treated population under the current blood-monitoring system (0.4%).[13] With a prevalence of schizophrenia of 1% and an estimated population of more than 305 million in the United States (6.7 billion worldwide), approximately 67,000 patients with schizophrenia in the United States are predicted to carry the higher risk genotype (1.3 million worldwide). Assessing risk for CIA by testing for this and other genetic variants yet to be determined may be clinically useful when deciding whether to begin or continue treatment with clozapine.

Future research could lead not only to safer treatment of schizophrenia with clozapine but also to alterations in the blood-monitoring requirements of clozapine prescription. Increasing the safe utilization of this uniquely effective drug may in turn lead to substantial improvement in the long-term outcome and in the health care costs of treating patients with this devastating and disabling illness.

Drug names: abacavir (Ziagen), carbamazepine (Carbatrol, Equetro, and others), clozapine (FazaClo, Clozaril, and others), nevirapine (Viramune).

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Supplementary material: Supplementary eTables available at PSYCHIATRIST.COM.

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Supplementary Material

Article Title: Candidate Gene Analysis Identifies a Polymorphism in HLA-DQB1 Associated With Clozapine-Induced Agranulocytosis

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eTable 1. Candidate Gene List

Gene Symbol	Gene Name
ADRA2C	adrenergic, alpha-2C-, receptor
ADRB1	adrenergic, beta 1 receptor
ADRB2	adrenergic, beta 2 receptor
ALB	Albumin
ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta
ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
BAX	BCL2-associated X protein
BCL2L1	BCL2-like 1
BIK	BCL2-interacting killer (apoptosis-inducing)
CHRM3	cholinergic receptor, muscarinic 3
CHRM4	cholinergic receptor, muscarinic 4
CHRM5	cholinergic receptor, muscarinic 5
CSF2	colony stimulating factor 2 (granulocyte-macrophage)
CSF2RA	colony stimulating factor 2 receptor, alpha
CSF2RB	colony stimulating factor 2 receptor, beta
CSF3	colony stimulating factor 3
CSF3R	colony stimulating factor 3 receptor
CYBA	cytochrome b-245, alpha polypeptide
CYBB	cytochrome b-245, beta polypeptide
CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2

CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19
CYP2C9	cytochrome P450, family 2, subfamily C, polypeptide 9
CYP2D6	cytochrome P450, family 2, subfamily D, polypeptide 6
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4
CYR61	cysteine-rich, angiogenic inducer, 61
DRD1	dopamine receptor D1
DRD2	dopamine receptor D2
DRD3	dopamine receptor D3
DRD4	dopamine receptor D4
DRD5	dopamine receptor D5
FKBP1	FK506 binding protein 1A
FPR1	formyl peptide receptor 1
HGF	hepatocyte growth factor
HLA-C	major histocompatibility complex, class I, C
HLA-DQB1	major histocompatibility complex, class II, DQ beta 1
HLA-DRA	major histocompatibility complex, class II, DR alpha
HRH1	histamine receptor 1
HRH2	histamine receptor 2
HRH4	histamine receptor 4
HSPA1A	heat shock 70kDa protein 1A
HSPA1B	heat shock 70kDa protein 1B
HSPA1L	heat shock 70kDa protein 1-like
HTR2A	serotonin receptor 2A

HTR7	serotonin receptor 7
IL1R1	interleukin 1 receptor, type I
IL2	interleukin 2
IL2RA	interleukin 2 receptor, alpha
IL3	interleukin 3
IL3RA	interleukin 3 receptor, alpha
ITGAL	integrin, alpha L
ITGB2	integrin, beta 2
LAMR1	ribosomal protein SA (RPSA)
LEP	Leptin
LEPR	leptin receptor
MCL1	myeloid cell leukemia sequence 1
MET	met proto-oncogene; hepatocyte growth factor receptor
MPO	Myeloperoxidase
MTATP6	mitochondrially encoded ATP synthase 6
NCF2	neutrophil cytosolic factor 2
NCF4	neutrophil cytosolic factor 4
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
NQO2	NAD(P)H dehydrogenase, quinone 2
NTSR1	neutotensin receptor 1
P2RX1	purinergic receptor P2X, ligand-gated ion channel, 1
P2RX7	purinergic receptor P2X, ligand-gated ion channel, 7
P2RY2	purinergic receptor P2y, G-protein coupled, 2

PLAB	growth differentiation factor 15 (GDF15)
RAC2	ras-related C3 botulinum toxin substrate 2
SKI	v-ski sarcoma viral oncogene homolog (avian)
TACR1	tachykinin receptor 1
TNF	tumor necrosis factor
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A
TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B
TXNIP	thioredoxin interacting protein

eTable 2. Markers Selected for Evaluation in Cohort II

GENE	MARKER[a]
CSF2RB	REC 4 11T, 46G, 50G, 87G
	REC 4 11T, 46G, 50G, 86G
	REC 3 11T, 46G, 50G
	REC 4 11T, 44G, 46G, 86G
	REC 4 11T, 44G, 46G, 87G
	REC 3 11T, 44G, 46G
DRD1	REC 3 5C, 19C, 21G
	REC 3 21G, 22G, 50C
	REC 2 18G, 21G
	REC 2 21G, 50C
	REC 4 4T, 21G, 22G, 50C
	REC 3 18G, 21G, 49C
	REC 4 8T, 21G, 22G, 50C
HLA-DQB1	REC 4 5C, 21G, 22G, 50C
	REC 3 8T, 18G, 21G
	REC 3 10C, 18G, 21G
	REC 2 21G, 49C
	REC 4 19C, 21G, 22G, 50C
	REC 4 9G, 21G, 22G, 50C
	REC 3 4T, 21G, 50C

	REC 4 5C, 8T, 18G, 21G
	REC 3 9G, 18G, 21G
	REC 3 5C, 18G, 21G
	REC 3 4T, 21G, 42C
	REC 4 10C, 18G, 21G, 45I
	REC 4 5C, 18G, 21G, 49C
	REC 3 18G, 19C, 21G
NTSR1	REC 3 8C, 17C, 25A

[a] Marker nomenclature: 1) model ("REC" for recessive, "DOM" for dominant); 2) the number of variants that comprise the haplotype; and 3) an identifier and allele for each polymorphism in the marker (eg, polymorphic site #5 in *HLA-DQB1* is nucleotide C)

eTable 3. Primer Sequences, Cohort II

GENE	DIRECTION	TAIL [a,b]	SPECIFIC SEQUENCE [b]	SIZE OF PRODUCT (bp)
CSF2RB	Forward	TGTAAAACGACGGCCAGT	TCTCTCTCTCTCGGAGCTGTTGG	533
	Reverse	AGGAAACAGCTATGACCAT	GCACTGGGGAGCACTCTTCC	
	Forward	TGTAAAACGACGGCCAGT	CCTGGCACATAAGAGACCTCAGC	575
	Reverse	AGGAAACAGCTATGACCAT	AACAGGGAAACTGAGTCACAGAACC	
DRD1	Forward	TGTAAAACGACGGCCAGT	CTGACCTTTGGGGTTCATACGG	464
	Reverse	AGGAAACAGCTATGACCAT	CCTTAAAGTCCATCCTCCTTCAGC	
	Forward	TGTAAAACGACGGCCAGT	TTTGAGCAGGCCCCACTACC	461
	Reverse	AGGAAACAGCTATGACCAT	CAACCTCACTCACAAGGGTTCC	
	Forward	TGTAAAACGACGGCCAGT	CCACTTCCCTGGCTGAGACC	546
	Reverse	AGGAAACAGCTATGACCAT	CTAAGAGGGTTGAAAATGCCTTCC	
	Forward	TGTAAAACGACGGCCAGT	CCTTCTGCATTGATTCCAACACC	504

	Reverse	AGGAAACAGCTATGACCAT	CTTTTGGGATGAGCATGTGTGG	
HLA-DQB1	Forward	TGTAAAACGACGGCCAGT	GCATCAAGCTGAAGTTCTGTG	529
	Reverse	AGGAAACAGCTATGACCAT	GCTTCTCTTGAGCAGTCTGAGG	
	Forward	TGTAAAACGACGGCCAGT	AACCACGGGTTCTGGAGCTAGG	571
	Reverse	AGGAAACAGCTATGACCAT	AGGCTCTGCAGCGACTTCTTCC	
	Forward	TGTAAAACGACGGCCAGT	CTCCTGAACTTGTGTTGACTGAGC	549
NTSR1	Reverse	AGGAAACAGCTATGACCAT	GAGGGATGGAGTGCAGAATGG	
	Forward	TGTAAAACGACGGCCAGT	TCCCCAAACAACCATTCTCAAGG	530
	Reverse	AGGAAACAGCTATGACCAT	CTGCCTGACCCCCATCAAGG	

[a] For sequencing, used tail primer. [b] For PCR Amplification, used specific + tail primer. M13F and M13R tail sequences used for sequencing.

eTable 4. PCR Conditions, Cohort II

PCR STEP	TIME (min)	TEMP. (°C)
1	2:00	97
2	0:15	97
3	0:45	70
4	0:45	72
5	Repeat Steps 2-4: 23x. [a]	
6	0:15	97
7	0:45	55
8	0:45	72
9	Repeat Steps 6-8 22x.	
10	5:00	72

[a] Decrease annealing temp 0.7°C per cycle.