Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2010 January 5; 153B(1): 332–335. doi:10.1002/ajmg.b.30984.

A Sequencing-Based Survey of Functional *APAF1* Alleles in a Large Sample of Individuals with Affective Illness and Population Controls

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Abstract

Rare apoptosis-promoting functional variants in the apoptosis protease activating factor 1 (*APAF1*) gene were recently reported to co-segregate with major depression in male members of families from Utah. In order to estimate the impact of these variants on risk for major depressive disorder (MDD) in the general population, we surveyed the frequency of the *APAF1* putative MDD risk alleles using re-sequencing in a large sample of northern European and European-American subjects, including a large number of males with MDD. The E777K and N782T *APAF1* variants previously described by Harlan et al. [Harlan et al. (2006) *Mol Psychiatry* 11(1):76–85] were found at low frequencies in affected individuals and population controls. The C450W and Q465R variants were not detected in any of the 632 subjects sequenced. These results show that the *APAF1* variants associated with risk for MDD in the Utah pedigrees are very rare in Northern European and European-American populations. In addition, the E777K and N782T variants were found at low frequencies both in patients and population controls, suggesting that these variants have limited impact on risk for MDD.

Keywords

apoptosis; apoptotic protease-activating factor 1; genetic predisposition; mood disorders; single nucleotide polymorphism

The lifetime prevalence of mood disorders, including major depressive disorder (MDD), can be as high as 19% [WHO International Consortium in Psychiatric Epidemiology, 2000], which highlights the importance of investigating their etiology and identifying novel biological mechanisms predisposing to MDD. According to linkage studies as well as family, twin, and adoption studies, there is ample evidence to suggest that major depression has a genetic component [Shih et al., 2004; Camp and Cannon-Albright, 2005]. A recent study linked the *APAF1* gene with depression in males [Shattuck et al., 2005], reporting that several non-synonymous *APAF1* variants co-segregated with depression in male family members in a Utah population. Further in vitro studies demonstrated that the variants promote apoptosome function [Harlan et al., 2006], thereby leading to premature death of neurons. Converging data from the linkage study and from in vitro experiments identified four non-synonymous substitutions, C450W, Q465R, E777K, and N782T, as novel risk alleles for MDD. Because the studies linking *APAF1* alleles with major depression were conducted in a fairly homogeneous population of northern European descent in Utah, it is important to investigate the frequency and impact on risk for depression of these alleles in other populations [Keen-Kim et al., 2006]. Thus, in a large cross-national sample of male subjects with mood disorder and population controls recruited in Russia, Finland, Norway, UK and the US, we sequenced exons in the three regions of *APAF1* containing the four missense variants previously linked with major depression and associated with promotion of apoptosome function. The goal of this study was to survey the frequency of these putatively rare variants in Northern European populations and estimate their impact on MDD risk at the population level. We hypothesized that we would observe an overabundance of these variants in depressed males compared to population control males.

Blood or buccal cell samples were obtained from 632 participants (Table I), including 317 male mood disordered subjects (264 with major depression, 38 with bipolar disorder, and 15 with depressive disorder not otherwise specified), and 16 Russian alcohol-dependent males without a history of depression. Of the 317 males diagnosed with a mood disorder, 210 were comorbid for substance abuse/dependence, anxiety disorder, or other disorders.

The Russian subjects were recruited at the Leningrad Regional Center for Addictions affiliated with Pavlov State Medical University. Psychiatric diagnoses in this population were established using clinical interviews administered by a trained psychiatrist under the supervision of EK. The Russian control population was composed of military personnel of the local subdivision who were participating in an epidemiologic study focusing on cardiac disease. These subjects were screened using non-structured clinical interviews to exclude the presence of psychiatric and substance use disorders at the time of study recruitment. The sample has been described in more detail in our earlier publications [Lappalainen et al., 2005, 2007].

The Finnish mood disordered subjects were recruited at the University of Oulu School of Medicine, Department of Psychiatry and were participants of an epidemiological study (STUDY-70) focusing on risks factors and outcomes of severe adolescent mental illness in Finland [Ilomaki et al., 2004; Makikyro et al., 2004]. Psychiatric diagnoses were established using the Schedule for Affective Disorder and Schizophrenia for School-Age Children-Present and Lifetime (K-SADS-PL) [Kaufman et al., 1997]. The Finnish population control subjects were survivors of subarachnoid hemorrhage (SAH) and had participated in a clinical trial at the University of Helsinki, Department of Neurosurgery testing the efficacy of enoxaparin on preventing post-hemorrhage vasospasm and improving cognitive recovery [Siironen et al., 2003; Vilkkki et al., 2004]. These control subjects were not formally evaluated for the presence or lifetime history of psychiatric disorders and thus can be considered at best non-screened population controls.

The Norwegian subjects were medically healthy college students recruited for a psychological study focusing on the effects of tryptophan depletion on mood and impulsivity in normal control subjects [Walderhaug et al., 2007]. British subjects were recruited at Oxford University and included both subjects with mood disorder and healthy controls [Bhagwagar et al., 2004, 2006]. Psychiatric interviews were conducted using the Structured Clinical Interview for DSM-IV (SCID-CV) [First et al., 1997]. The subjects recruited in the US were evaluated at the VA Connecticut Healthcare System, West Haven Campus and the Schools of Medicine of Yale and Brown Universities. The psychiatric diagnoses were determined using either SCID-IIIR, SCID-IV or Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA).

Exons 9, 10, and 17 of *APAF1* (<http://www.ensembl.org>; transcript ENST00000339433) containing the C450W, Q465R, E777K, and N782T substitutions and 150–400 bp of flanking introns were amplified using polymerase chain reaction (PCR) with *AmpliTaq* polymerase (PE Biosystems, Foster City, CA). PCR primers were selected using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Exon 9 was amplified using primers 5' TCAGGGCTTTAAGATAACCGTTT3' and 5' GGGGAAGCTAGGTGAAGGAC3' or with 5' CCTGTCTACAGTCCTGGTCATT3' and 5' AGGTGACAAGGCCATGAAAG3'. Exon 10 was amplified using 5' GCTGGGATTACAGGCGTGAG3' and 5' GAATTTTAAATGGGTCATCTT3'. Exon 17 was amplified using 5' TGTGGCATTTAATTTAGGAAAAA3' and 5' TGCCCAAATCACACAGCTA3'. Betaine (0.5 or 1 M) was added to PCR reactions as needed to enhance the specificity or yield of the amplification process.

Following PCR, the samples were sequenced using cycle sequencing at the Yale Keck Foundation Biotechnology Resource Laboratory. Sequence analysis was carried out on Applied Biosystems 3730 capillary instruments and reactions utilized fluorescently labeled dideoxynucleotides (Big Dye Terminators) and Taq FS DNA polymerase. Complete data from all three exons were available for 92% of samples, with data from at least two exons available for every sample. Single nucleotide polymorphisms (SNPs) were identified using Mutation Surveyor software (SoftGenetics, State College, PA).

Several Caucasian males were heterozygous for E777K or N782T (Table II). The E777K variant was found in one Caucasian North American male with major depression and in one psychiatrically screened Russian male control subject. The N782T variant was discovered in a total of 11 Caucasian males. Of these, six had been diagnosed with affective illness (either major depression or bipolar disorder) and five were population controls. Two novel non-synonymous variants were also found within the exons sequenced. Two males with bipolar I disorder were heterozygous for a novel T446S (A1336T) variant: a European-American and an African-American (who had a comorbid diagnosis of post-traumatic stress disorder). A novel E777D (G2331C) variant was found in a Russian male diagnosed with major depression and alcohol dependence. Two intronic mutations were discovered at 19,138 bp (T > C) and 51,231 bp (A > T) from the start codon.

The major finding in this study is that functional *APAF1* variants previously reported to increase risk for major unipolar depression [Harlan et al., 2006] are rare in Northern European populations. Of the four functional variants described in the previous study, the N782T SNP was the most common variant (0.9%), detected at a low frequency in all European populations studied. Despite the relatively large sample of depressed males and controls in this study, no C450W or Q465R variants were found in any subject, suggesting that these are very rare in European populations. Lack of C450W or Q465R in our affected and population control samples makes it impossible to assess whether they increase risk for

depression. However, given their rarity, they will have only limited impact at the population level on susceptibility to depression.

These data do not support the role of E777K and N782T variants in susceptibility to depression. Although E777K and N782T variants were identified in 13 males, 6 of these were population controls. These observations translate to carrier frequencies of 2% and 4% among affected Caucasian males and Caucasian population control males in our sample, respectively. Although many of our population controls were not formally screened for the absence of major depression, the fact that the carrier frequency among population controls exceeds the frequency in the affected sample suggests that the E777K and N782T variants do not play a role in susceptibility to depression among European males. It is possible that the reported relationship between *APAF1* variants and MDD is reflective of linkage (and not truly linkage disequilibrium), unique to the Utah families, and the relationship cannot be established through an association analysis in other populations.

Two novel rare *APAF1* mutations were discovered in this study: the T446S and E777D SNPs. The latter variant is particularly intriguing as it alters the same residue as the previously described E777K variant. Although these two variants were found in affected males only, evaluation of the impact of these variants on susceptibility to depression is difficult considering their rarity and lack of prior reports on their associated risk.

In conclusion, we have provided preliminary data regarding large samples of subjects with and without diagnosis of mood disorder from populations likely to be related to the Utah population in which the rare non-synonymous *APAF1* variants were linked to depression. Our data do not support their role in susceptibility to depression. Future studies should evaluate the frequency of these variants among controls and mood-disordered subjects in each population to better describe the impact of these variants in each population. However, these kinds of studies will be difficult to conduct considering that a large number of samples in each population would be needed to definitely establish (or exclude) the impact of these variants on risk for MDD. Examining the influence of these *APAF1* variants on more proximal phenotypes, such as in vivo brain function and morphology, may be helpful for elucidating their effects on risk for mental illness in men and women.

Acknowledgments

Supported by Veterans Administration REAP Center "Genes, Neurodevelopment and Depression," and National Institute on Alcohol Abuse and Alcoholism (K08 AA 13732; Lappalainen).

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TABLE I

Nationality, Race, and Sex of Cases and Controls

	Cases				Controls			
	Caucasian		Other		Caucasian		Other	
	Male	Female	Male	Female	Male	Female	Male	Female
American	94	12	10	1	0	12	0	9
British	36	0	0	0	5	0	0	0
Finnish	46	0	0	0	53	52	0	0
Norwegian	0	0	0	0	39	43	3	0
Russian	147	0	0	0	70	0	0	0
Total	323	12	10	1	167	107	3	9

TABLE II

Mutations Identified in Participants

	C450W ^a	Q465R ^a	E777K ^a		N782T ^a		T446S ^b		E777D ^b	
			Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
American	0	0	1 ^c	0	4 ^d	0	2 ^f	0	0	0
British	0	0	0	0	0	1	0	0	0	0
Finnish	0	0	0	0	0	1	0	0	0	0
Norwegian	0	0	0	0	0	2	0	0	0	0
Russian	0	0	0	1	2 ^e	1	0	0	1 ^c	0
Total	0	0	1	1	6	5	2	0	1	0

^aNon-synonymous mutations described by Harlan et al. [2006].

^bNovel non-synonymous mutations identified in the present study.

^cMajor depression.

^dThree major depression and 1 bipolar I disorder.

^eMajor depression and alcohol dependence.

^fBipolar I disorder.