WDR46 is a Genetic Risk Factor for Aspirin-Exacerbated Respiratory Disease in a Korean Population

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Purpose: The human *WD repeat-containing protein 46* (*WDR46*; also known as *C6orf11*), located at the disease-relevant centromere side of the class II major histocompatibility complex region, is hypothesized to be associated with risk of aspirin-exacerbated respiratory disease (AERD) as well as a decline in forced expiratory volume in the first second (FEV1), an important diagnostic marker of asthma. **Methods:** To investigate the association between *WDR46* and AERD, five single-nucleotide polymorphisms (SNPs) were genotyped in 93 AERD cases and 96 aspirin-tolerant asthma controls of Korean ethnicity. Three major haplotypes were inferred from pairwise comparison of the SNPs, and one was included in the association analysis. Differences in the frequency distribution of *WDR46* SNPs and haplotype were analyzed using logistic and regression models via various modes of genetic inheritance. **Results:** Depending on the genetic model, the logistic and regression analyses revealed significant associations between *rs463260*, *rs446735*, *rs455567*, *rs469064*, and *WDR46_ht2* and the risk of AERD (P=0.007-0.04, P_{corr}=0.01-0.04) and FEV1 decline after aspirin provocation (P=0.006-0.03, P_{corr}=0.01-0.03). Furthermore, functional analysis *in silico* showed that the G>A allele of *rs463260* located in the 5' untranslated region potentially matched a nucleotide sequence within an upstream open reading frame of *WDR46*. **Conclusions:** These findings show for the first time that *WDR46* is an important genetic marker of aspirin-induced airway inflammation and may be useful for formulating new disease-management strategies.

Key Words: Aspirin exacerbated respiratory disease; WDR46; FEV1; haplotype; single-nucleotide polymorphism

INTRODUCTION

Aspirin-exacerbated respiratory disease (AERD) is characterized by asthma, the presence of polyps in nasal passages, and sensitivity to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs).[1] Although previous research has attributed AERD pathophysiology to inhibition of the cyclooxygenase-2 (COX-2) pathway, resulting in decreased synthesis of prostaglandin E_2 (PGE$_2$) and overproduction of cys-leukotrienes (cysLTs),[2] recent reports have suggested that several genes may play a predisposing role, implicating other pathways in AERD pathogenesis; these include major histocompatibility complex (MHC), class II, DP beta 1 (*HLA-DPB1*), solute carrier family 6 (neurotransmitter transporter, betaine/GABA) member 12 (SLC6A12), complementary component 6 (C6), D-tyrosyl-tRNA

deacylase (DTD1), and emilin/multimerin domain-containing protein 2 (EMID2).[3-7]

The MHC, a group of genes that determines the immune response to pathogens, is central to the activation of the innate

immune system. The human *WD repeat-containing protein 46* (*WDR46*, also called *C6orf11* and *BING4*; NM_005452) gene is located on chromosome 6p21.3 at the centromere side of the class II MHC region, and encodes a protein that is 610 amino acids in length[8] that shows homology to *Saccharomyces cerevisiae* and *Caenorhabditis elegans* proteins.[9] In particular, WDR46 contains a class I type aminotransferase pyridoxal phosphate attachment site motif, a class I aminoacyl-tRNA synthetase adenylate binding site motif, and G-beta (WD40) repeats found in the β-subunits of G-proteins.[10] A previous study of metastatic cancer patients reported high expression levels of WDR46 mRNA in melanoma cell lines, which correlated with lymphocyte recognition, resulting in secretion of additional cytokines. Furthermore, one of the MHC class II genes, *HLA-DPB1*, is a genetic marker for AERD in the Korean population, suggesting that *WDR46* may be a candidate gene for disease susceptibility. However, little of the structure of *WDR46* has been reported, and its function remains unknown.

Because genes at the centromere end of the MHC are often associated with several human diseases including asthma,[3] and recruitment and activation of T-lymphocytes with increased cytokine production has been implicated in aspirin-induced airway inflammation,[11,12] *WDR46* is hypothesized to be associated with the risk of AERD. Thus, we conducted an association analysis on a Korean asthma cohort.

MATERIALS AND METHODS

Study patients

Asthma patients enrolled in nine hospitals in Korea affiliated with the Asthma Genome Research Center were recruited. Diagnosis of asthma and tests measuring total immunoglobulin E (IgE), atopy, and individuals' reactions to inhalant allergens were performed. Oral aspirin challenge (OAC) was performed using increasing doses.[13,14] Briefly, patients with a history of aspirin hypersensitivity were given 30 mg orally. Respiratory and nasal symptoms, blood pressure, external signs (urticaria and angio-oedema), and FEV1 were documented every 30 minutes for 1.5 hours. In the absence of any symptom or sign suggesting an adverse reaction after 1.5 hours, increasing aspirin doses (60, 100, 300, and 400 mg) were administered until the patient developed a reaction; subsequently the same measurements were repeated hourly. Those with no history of aspirin hypersensitivity were started on 100 mg aspirin, which was increased to 200, 350, and 450 mg until a reaction developed. If no reaction occurred 4 hours after the final dose, the test was deemed negative. Aspirin-induced bronchospasm, reflected by a decline (%) in FEV1, was calculated as the pre-challenge FEV1 minus the post-challenge FEV1, divided by the pre-challenge FEV1. OAC reactions were categorized into three groups: (1) a 15% or greater decrease in FEV1 or nasal reactions (AERD), (2) less than a 15% decrease in FEV1 without naso-ocular or cutaneous reac-

tions (aspirin-tolerant asthma [ATA]), or (3) less than a 15% decrease in FEV1 with cutaneous reactions (aspirin-induced urticaria). Written informed consent was obtained from each patient, and the study protocol was approved by the Institutional Review Board of each participating hospital.

Selection and genotyping of single-nucleotide polymorphisms

Using the tagging approach, candidate single-nucleotide polymorphisms (SNPs) in *WDR46* were screened from the International HapMap database (version release #27; http://www.hapmap.org) based on linkage disequilibrium (LD) status in the Asian population (Han Chinese and Japanese) and location within the gene. Genotypes were derived by TaqMan assay[15] using an ABI prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). Evaluation of LDs between screened SNPs was carried out using the Haploview software,[16] and SNPs with a minor allele frequency (MAF) > 0.05 and those tagged because several polymorphisms had an LD > 0.98, were selected for association analysis.

Statistical analyses

The association between differences in genotype frequency distribution of *WDR46* variation and the risk of AERD and airway obstruction was analyzed according to previous methods.[7] The statistical power of single associations was determined using the Power for Genetic Association Analyses software,[17] and multiple testing corrections were calculated using the effective number of independent marker loci (Meff), that accounts for the eigenvalue spectral decomposition (SpD) of all of the genotypes represented in the correlation matrix extracted from the SNPSpD program.[18] Furthermore, functional analyses of *WDR46* SNPs were carried out in silico using the UTRScan program[19] and the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) splice site prediction software (http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2).

RESULTS

Categorization and clinical characteristics

Based on individual reactions to aspirin, the asthma patients (n=189) were stratified into 93 AERD cases (males, 32; females, 61) and 96 ATA controls (males, 24; females, 72) (Table 1). The aspirin-induced decrease in FEV1 (AERD, 23.61 vs. ATA, 0.94), positivity rate of aspirin side effects including external signs of urticaria and angioedema (AERD, 26.67% vs. ATA, 8.42%), and positive history of aspirin intolerance (AERD, 63.86% vs. ATA, 29.27%) were significantly different between cases and controls (*P* < 0.0001; Table 1). No significant differences for any other diagnostic factors were observed.

Distribution of *WDR46* variants

With high rates of concordance in duplicates (>99%), 5 *WDR46*

SNPs, *rs463260* in the 5' untranslated region (UTR), *rs466384* in the exon, and *rs446735*, *rs455567*, and *rs469064* in the introns (Figure A and Supplementary Table S1), were successfully gen-

Table 1. Clinical profiles of the study patients

	AERD (n = 93)	ATA (n = 96)	*P* value
Age, mean (range)	44.39 (17-73)	45.79 (15-77)	0.497
Onset age, mean (range)	38.01 (0-70)	37.99 (5-73)	0.995
BMI (kg/m²)	23.47 ± 3.18	24.41 ± 3.29	0.049
Peripheral eosinophil count*	80,734.1 ± 48,861.5	36,429.1 ± 33,900.2	0.060
FVC %, predicted	89.90 ± 14.74	87.76 ± 12.80	0.293
FEV1 %, predicted	86.63 ± 16.74	88.26 ± 17.04	0.509
PC20, methacholine (mg/mL)	4.23 ± 7.18	3.04 ± 4.27	0.193
Total IgE (IU/mL)	321.65 ± 623.31	309.54 ± 426.04	0.878
FEV1 decline after aspirin challenge	23.61 ± 14.48	0.94 ± 2.76	**0.001**
Sex (male/female)	32/61	24/72	0.156
Ex-smoker/current smoker (%)	15.63/9.38	6.45/12.90	0.219
Atopy rate (%)	61.46	56.99	0.532
Adverse reaction to aspirin (%)	26.67	8.42	**0.001**
Positive rate of nasal polyp (%)	63.86	29.27	**0.001**

Values are mean ± SE.
AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; BMI, body mass index.
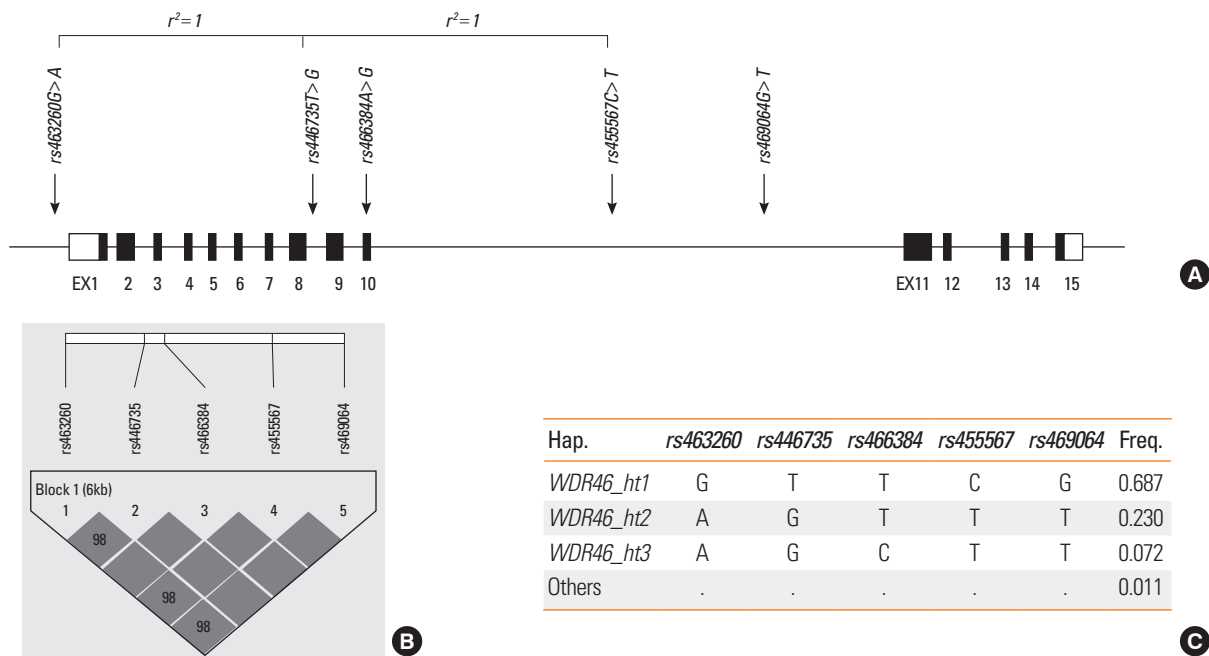*Blood eosinophil (%): 6.29 ± 5.80 (AERD group), 4.88 ± 4.19 (ATA group).

otyped. The genotype counts and MAFs of each SNP are summarized in Supplementary Table S1. Furthermore, none of the variants deviated from the Hardy-Weinberg Equilibrium (HWE). Pairwise comparison of the genotyped SNPs showed a tight LD in the study population (Figure B) and produced three major haplotypes (MAF > 0.05; Figure C). However, because *WDR46_ht1* and *WDR46_ht3* were equivalent to some SNPs, only *WDR46_ht2* was included in the association analysis.

Association analysis of *WDR46* variants with a risk for AERD and FEV1 decline

The results of logistic analysis controlling for age, sex, smoking status, atopy, and body mass index (BMI) revealed that the majority of the analyzed *WDR46* variants (*rs463260*, *rs446735*, *rs455567*, *rs469064*, and *WDR46_ht2*) were associated with a risk for AERD (P = 0.007-0.04, odds ratio [OR] = 1.88-1.89 depending on the genetic model; Table 2). The association signals maintained significance under co-dominant and recessive models after multiple testing corrections (P_{corr} = 0.01-0.04; Meff = 2.2767; Table 2).

The results of regression analysis after adjustment for age, sex, smoking status, atopy, and BMI showed a significant association between a similar set of *WDR46* variants (*rs463260*, *rs446735*, *rs455567*, *rs469064*, and *WDR46_ht2*) and decreased FEV1 in asthma patients (P = 0.006-0.03 under co-dominant and dominant models; Table 3), even after multiple comparisons (P_{corr} = 0.01-0.03 in co-dominant and dominant models; Table 3).

Hap.	*rs463260*	*rs446735*	*rs466384*	*rs455567*	*rs469064*	Freq.
WDR46_ht1	G	T	T	C	G	0.687
WDR46_ht2	A	G	T	T	T	0.230
WDR46_ht3	A	G	C	T	T	0.072
Others	.	.	.	.	.	0.011

Figure. Physical map, linkage disequilibrium, and haplotypes of *WDR46*. (A) Schematic gene map and single-nucleotide polymorphisms (SNPs) of *WDR46* on chromosome 6p21.3 (10.11 kbp). Black blocks represent coding exons and white blocks represent 5' and 3' untranslated regions. The first base of translation sites are denoted as nucleotide +1. SNPs in absolute linkage are indicated by brackets (r^2 = 1). (B) LD coefficient (|D'|) among *WDR46* SNPs in a Korean population. (C) Haplotypes of *WDR46*.

Table 2. Association analysis of variation in WDR46 with AERD

SNP/ Haplotype	MAF		HWE*		Co-dominant			Dominant			Recessive			Statistical power
	AERD	ATA	AERD	ATA	OR (95% CI)	$P^{\dagger}$	$P_{corr}^{\ddagger}$	OR (95% CI)	$P^{\dagger}$	$P_{corr}^{\ddagger}$	OR (95% CI)	$P^{\dagger}$	$P_{corr}^{\ddagger}$	
rs463260	0.374	0.250	0.561	0.586	1.88 (1.19-2.99)	**0.007**	**0.010**	1.89 (1.04-3.42)	**0.040**	>0.050	3.71 (1.25-11.00)	**0.020**	**0.040**	33.52
rs446735	0.366	0.255	0.799	0.892	1.74 (1.10-2.75)	**0.020**	**0.030**	1.86 (1.03-3.36)	**0.040**	>0.050	2.58 (0.92-7.22)	0.070	-	35.19
rs466384	0.082	0.068	0.597	0.365	1.21 (0.56-2.58)	0.630	-	1.27 (0.54-2.96)	0.590	-	0.98 (0.06-16.66)	0.990	-	20.86
rs455567	0.368	0.255	0.764	0.892	1.77 (1.12-2.79)	**0.020**	**0.030**	1.89 (1.04-3.42)	**0.040**	>0.050	2.65 (0.94-7.42)	0.060	-	34.97
rs469064	0.361	0.250	0.904	0.586	1.78 (1.12-2.85)	**0.020**	**0.030**	1.85 (1.02-3.36)	**0.040**	>0.050	2.93 (0.97-8.84)	0.060	-	34.95
WDR46_ht2	0.283	0.182	0.687	0.415	1.86 (1.12-3.10)	**0.020**	**0.030**	1.79 (0.98-3.27)	0.060	-	5.42 (1.09-26.93)	**0.040**	>0.050	51.74

Logistic analysis controlling for age, sex, smoking status, atopy, and body mass index.
*P values of Hardy-Weinberg equilibrium (HWE).
†P values at 0.05 levels of significance. ‡P values after multiple testing corrections (Meff=2.2767). Significant values are shown in bold.
WDR46, human WD repeat-containing protein 46; AERD, aspirin-exacerbated respiratory disease; SNP, single-nucleotide polymorphism; MAF, minor allele frequency; ATA, aspirin-tolerant asthma; OR, odds ratio; CI, confidence interval.

Table 3. Regression analysis of variation in WDR46 with decrease in FEV1 rate due to aspirin ingestion

SNP	C/C	C/R	R/R	Co-dominant		Dominant		Recessive	
				P^*	$P_{corr}^{\dagger}$	P^*	$P_{corr}^{\dagger}$	P^*	$P_{corr}^{\dagger}$
rs463260	90 (9.11±12.60)	78 (13.90±17.31)	19 (18.03±17.09)	**0.005**	**0.010**	**0.010**	**0.020**	**0.050**	-
rs446735	91 (9.21±12.56)	79 (14.39±17.15)	19 (16.37±17.68)	**0.010**	**0.020**	**0.010**	**0.020**	0.190	-
rs466384	161 (11.44±14.79)	24 (16.23±19.09)	2 (7.70±17.39)	0.370	-	0.260	-	0.680	-
rs455567	90 (9.11±12.60)	78 (14.30±17.25)	19 (16.37±17.68)	**0.010**	**0.020**	**0.010**	**0.020**	0.180	-
rs469064	90 (9.11±12.60)	79 (13.91±17.26)	17 (18.18±17.85)	**0.006**	**0.010**	**0.010**	**0.020**	0.080	-
WDR46_ht2	110 (10.01±13.72)	66 (14.30±17.78)	10 (18.59±14.52)	**0.020**	**0.030**	**0.030**	>0.050	0.110	-

Co-dominant, dominant, and recessive models of regression analysis controlling for age, sex, smoking status, atopy, and body mass index.
C/C, C/R, and R/R refer to major homozygote, heterozygote, and minor homozygote, respectively.
*P values at 0.05 levels of significance. †P values after multiple testing corrections (Meff=2.2767). Significant values are shown in bold.

Functional analyses *in silico*

Functional estimation of SNPs in intronic regions of *WDR46* revealed that none of the analyzed variants act as branch point sites for alternative splicing (data not shown). However, results of another computer simulation analysis showed that the sequence containing the G>A allele of *rs463260* located in the 5' UTR matched a nucleotide sequence within a UTRSite signal of *WDR46*, more specifically, an upstream open reading frame (uORF; Supplementary Table S2).

DISCUSSION

Although ample progress has been made in understanding the pathogenesis of AERD, the observed association between it and genes that were originally thought to play no relevant role in aspirin-induced airway bronchospasm suggests that the exact mechanisms of AERD development remain to be elucidated. The current findings provide important insight into the etiology of aspirin-hypersensitive asthma by reporting, for the first time, a significant association between the *WDR46* variants *rs463260*, *rs446735*, *rs455567*, *rs469064*, as well as the major haplotype *WDR46_ht2*, and the risk of AERD. Furthermore,

with a marginal increase in association signals, similar variants were found to induce significant decreases in FEV1, an important diagnostic marker of respiratory obstruction, among AERD patients compared to ATA controls. The reported association maintained significance under the strict criteria of multiple testing corrections. In particular, the *rs463260* polymorphism exhibited a consistently robust association signal with the risk of AERD (P=0.007, P_{corr}=0.01 in a co-dominant model) and FEV1 (P=0.005, P_{corr}=0.01 in a co-dominant model) decline even after multiple comparisons. The association of *WDR46* with risk of AERD was suggested via co-dominant and dominant modes of genetic inheritance in a Korean population.

Despite the dearth of previous reports, the positioning of the *WDR46* gene in the MHC region is suggested to play a role in AERD development as well as decreased FEV1 due to aspirin ingestion. In further analysis, a uORF was detected in the 5' UTR of *WDR46* and the G>A allele of the *rs463260* polymorphism was found within the start and end positions of the UTRSite signal. The predicted uORF in the 5' UTR is speculated to be among the nine potential ORFs previously identified within *WDR46*.[8] Although the role of uORFs is not yet fully understood, the 5' UTR harbors cis/trans-acting elements that play a crucial role

in the regulation of gene expression by mediating translation efficiency and mRNA stability.[19] Thus, the results of our *in silico* analyses suggest that the relevance of the significant SNP, *rs463260G>A*, in AERD susceptibility may be a manifestation of cis-regulated gene expression.

Examination of expressed sequence tags suggested the presence of alternative splicing within the *WDR46* transcript.[9] Although polymorphisms located in the non-coding intronic regions of the gene were originally thought to have no role in protein function, these intronic variants may also affect alternative splicing, splicing efficiency, or mRNA turnover as has been reported for other disease-causing genes.[20] Thus, an *in silico* analysis was carried out to functionally characterize the intronic *WDR46* polymorphisms. None of the analyzed variants were predicted to be branch point sites for alternative splicing, refuting our hypothesis.

Although the results from power calculations revealed a 35.24% average statistical power to detect effect sizes with the current sample (Table 2), indicating insufficient sample size, the allele frequency of the analyzed variants in our Korean population was similar to that of other Asian ethnic groups (Japanese and Han Chinese; Supplementary Table S1). However, while our findings successfully elucidated the association of *WDR46* variation with aspirin-induced airway inflammation, the lack of an in-depth molecular investigation may be a limitation of this preliminary report. Furthermore, differences in the effects of genetic variation with regard to geographical location and/or ethnicity should also be taken into consideration. The present findings would, therefore, benefit from future studies addressing these limitations. Moreover, the advent of next-generation sequencing (NGS) with one-base ultra-resolution and high-density chip microarrays makes it possible to investigate the association of rare *WDR46* variants with the risk of AERD.

In conclusion, our data provide convincing evidence that *WDR46* variants are associated with the risk for both AERD and a relevant phenotype, FEV1 decline, in a Korean asthma cohort. Because the function of *WDR46* in human diseases remains to be elucidated, our data represent a marked contribution to the knowledge on the role of this gene in airway inflammation.

ACKNOWLEDGMENTS

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Supplementary Table 1. Alleles and genotype distribution of *WDR46* SNPs

SNP	Position	Allele	Genotype			Heterozygosity	MAF				
							Korean	Caucasian[*]	Chinese[*]	Japanese[*]	African[*]
rs463260	5' UTR	G>A	G 90	AG 78	A 19	0.428	0.310	0.500	0.350	0.330	0.714
rs446735	Intron 8	T>G	T 91	GT 79	G 19	0.427	0.310	0.500	0.358	0.332	0.719
rs466384	Exon 10	T>C	T 161	CT 24	C 2	0.139	0.075	-	-	-	-
rs455567	Intron 10	C>T	C 90	CT 78	T 19	0.428	0.310	0.500	0.358	0.329	0.732
rs469064	Intron 10	G>T	G 90	GT 79	T 17	0.423	0.304	0.495	0.354	0.324	0.246

SNP, single-nucleotide polymorphism; MAF, minor allele frequency; UTR, untranslated region.
*Data taken from the International HapMap database (version: release #28; http://www.hapmap.org).

Supplementary Table 2. UTRScan output for *rs463260*

SNP	Input sequence length*	UTRSite signal	Start and end positions of signal	Matched nucleotide sequence
rs463260G>A	361bases	Upstream Open Reading Frame	85-348	ATG GCACCTAGAGAGCTTCATACCTGGTACGCTGCTGAT TGGATGAAGGACAGAGGGCTTCCGGGAGTTTTCAAG CCGACTGTGTGGCAGCTGAGA[G/A]GAGTTTTGCACG TGGATCGCCGTTCGGGTGGGCGAGATGGAGACAGCC CCCAAGCCGGGCAAGGATGTCCCGCCCAAGAAAGA CAAACTTCAGACCAAGAGAAAGGTAGAGGCCTCCCT GGGTGGGAAACGAAGTTTTTAGCTGTGGGGTCGGGG GGCGGGGCG TGA

Nucleotide sequence for the UTRsite signal is predicted using the UTRScan program (http://itbtools.ba.itb.cnr.it/utrscan).
*FASTA sequence taken from National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov).