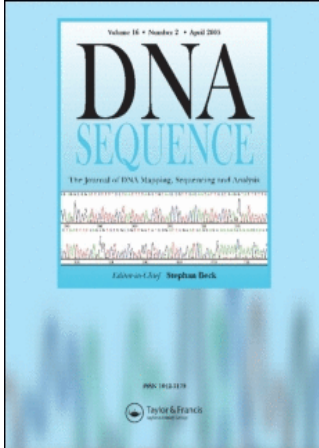


This article was downloaded by:[Hawkins, Gregory]
On: 22 January 2007
Access Details: [subscription number 768583046]
Publisher: Informa Healthcare
Informa Ltd Registered in England and Wales Registered Number: 1072954
Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



DNA Sequence

The Journal of DNA Mapping, Sequencing, and Analysis

Publication details, including instructions for authors and subscription information:
<http://www.informaworld.com/smpp/title-content=t713640135>

Sequence analysis of HSPA1A and HSPA1B in a multi-ethnic study population

To link to this article: DOI: 10.1080/10425170601060707
URL: <http://dx.doi.org/10.1080/10425170601060707>

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article maybe used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

© Taylor and Francis 2007

FULL LENGTH RESEARCH ARTICLE

Sequence analysis of *HSPA1A* and *HSPA1B* in a multi-ethnic study populationRICHARD S. SMITH¹, DEBORAH A. MEYERS¹, STEPHEN P. PETERS¹, WENDY C. MOORE¹, SALLY A. WENZEL², EUGENE R. BLEECKER¹, & GREGORY A. HAWKINS¹¹Center for Human Genomics, Wake Forest University School of Medicine, Winston Salem, NC, USA, and ²Department of Medicine, National Jewish Medical and Research Center, University of Colorado Health Sciences Center, Denver, CO, USA

(Received 23 March 2006)

Abstract

Two copies of the Hsp70 gene, *HSPA1A* and *HSPA1B*, are located on chromosome 6p21. The coding regions of *HSPA1A* and *HSPA1B* are intronless and nearly identical, however, promoter and 3' UTR sequences are different. The coding regions and putative promoter regions of *HSPA1A* and *HSPA1B* were re-sequenced in an affected and unaffected asthma screening panel of US Caucasians, African Americans, and US Hispanics ($n = 72$) to identify polymorphisms. *HSPA1A* and *HSPA1B* were each amplified in two separate whole-gene fragments. The polymorphisms identified were compared to those reported in dbSNP. Nine polymorphisms (one novel) were identified in *HSPA1A*, five of which are coding. Fourteen polymorphisms (five novel) were identified in *HSPA1B*, five of which are coding. One polymorphism (Asp110Glu GAG > GAC) was found in both genes. Two-thirds of the polymorphisms reported in dbSNP were not identified in our screening panel. Although similar in sequence, *HSPA1A* and *HSPA1B* do not share common patterns of polymorphisms.

Keywords: *HSPA1A*, nested PCR, asthma, polymorphism, *HSPA1B***Introduction**

The inducible form of heat shock protein 70 (Hsp70) is an abundant molecular chaperone that plays a key role in the assembly and activation of the glucocorticoid receptor complex. Two single exon genes (*HSPA1A* and *HSPA1B*) encode identical copies of the 641 amino acid protein Hsp70; and are located in tandem along a ~ 15 kb region of chromosome 6p23.1 in the class III region of the major histocompatibility complex (Pratt and Toft 1997; Temple et al. 2004). The coding regions of *HSPA1A* and *HSPA1B* are identical except for six single base substitutions. The promoter and 3' untranslated region (UTR) of the genes, however, have considerable sequence differences suggesting that regulation of *HSPA1A* transcription and translation could be distinct from *HSPA1B*.

A number of single nucleotide polymorphisms (SNPs) have been identified in *HSPA1A* and *HSPA1B*, but due to the high degree of similarity between the coding regions of each gene, many SNPs might have been assigned to both genes. Due to potential miss assignment, an accurate assessment of SNP validity and minor allele frequency (F_{ma}) is not available for either gene. Because of the location of these polymorphisms and the identical sequences flanking each *HSPA1A/HSPA1B* polymorphism, most SNP genotyping methods are incapable of accurately assessing in which gene the polymorphism is located. This in turn limits genotyping of these polymorphisms and thus their use in genetic association studies. In this study, we have re-sequenced *HSPA1A* and *HSPA1B* separately in a multi-ethnic asthma case and control screening panel, and for the first time were able to

Correspondence: G. Hawkins, Section on Pulmonary, Critical Care, Allergy and Immunologic Diseases, Center for Human Genomics, Wake Forest University School of Medicine, Winston Salem, NC 27157, USA. Tel: 1 336 713 7511. Fax: 1 336 713 7566. E-mail: ghawkins@wfbmc.edu

Table I. Primers utilized for sequencing.

Gene	PCR type	Primer name	Sequence
<i>HSPA1A</i>	PCR	Genomic forward	CGCCTCGGTCTCCCTAAG
		Genomic reverse	CTGACCCCAATAAAGGAATAAA
	Nested PCR & sequence	Promoter 1 forward	TCATGGCAGACTGCTTTTTGGACA
		Promoter 1 reverse	GCCCGCCTCGGCCTCTCAA
		Promoter 2 forward	CACCGGCAGTAAATTCAGTCAGG
		Promoter 2 reverse	GGTAAGGGGGAGGCGGGGTAAC
		Promoter 3 forward	TGGGCGACAGAGCGAGACT
		Promoter 3 reverse	AGGGTGGGGGCGTTTGTGTTGA
		Promoter 4 forward	GGGCTCCCTCAATATCAAACCTGC
		Promoter 4 reverse	GGGGCTGGAACGGAACACTGG
		Promoter 5 forward	TGTCCAAGGCTTCCCAGAG
		Promoter 5 reverse	GCCCCGCCCAGGTCAAAGAT
		Coding 1 forward	AGCGCCAGGCCACCAAGGATG
		Coding 1 reverse	GAGCCCCGACCAGGACCGAG
		Coding 2 forward	CTGGGTGGGGAGGACTTTTGACA
		Coding 2 reverse	CCTCTCGCCCTCGTACACCTGGAT
		Coding 3 forward	CAACGGGCGCGACCTGAACAA
		Coding 3 reverse	CTCGCGCTGCACCTCGTCCTC
		Coding 4 forward	AGGGCCATGACGAAAAGACAACAT
		Coding 4 reverse	GGCCCCTAATCTACCTCCTCAATG
		Coding 5 forward	CTTGCCGAGAAGGACGAGT
		Coding 5 reverse	AATTTCAACATTGCAAACACAGGA
	<i>HSPA1B</i>	PCR	Genomic forward
Genomic reverse			GTTTTGCAGCTGCCATCCGACTTT
Nested PCR & sequence		Promoter 1 forward	ACCCCTCACATTTTCTTTTCAGA
		Promoter 1 reverse	TCCCGGTTTTAAGCAATTCCTCTA
		Promoter 2 forward	AAAACAACAGGCGGGAATGGTG
		Promoter 2 reverse	GGAGGCGCTGGTCCGGTAATG
		Promoter 3 forward	AGCTAGGAATGGGGAGGAAATGG
		Promoter 3 reverse	TCGATGTGCTGGGAACTCTGAT
		Promoter 4 forward	GACCAGCGCCTCCCTTCCTC
		Promoter 4 reverse	GACCCGCTTTCCCTTCTG
		Promoter 5 forward	AGAGCGGGCCCTTCCTGTCAA
		Promoter 5 reverse	ATGCCGGTGCCCTGCTCTGT
		Coding 1 forward	GAAGGGGAAAGGCGGGTCTC
		Coding 1 reverse	GCCTCGGCGATCTCCTTCAT
		Coding 2 forward	GGGGATGCGGCCAAGAACCA
		Coding 2 reverse	GAGCCCCGACCAGGACCGAG
		Coding 3 forward	CTGGGTGGGGAGGACTTTTGACA
		Coding 3 reverse	CACCGCCGCCCGTAGG
		Coding 4 forward	CCACGGCCGGGGACACC
		Coding 4 reverse	AGGGCGTTCTTGGCTGACACC
		Coding 5 forward	GCGAGAGGGCCATGACGAAAGA
		Coding 5 reverse	GTCCGCTGATGATGGGGTTACACA
Coding 6 forward		TCACGGCCACGGACAAGAGC	
Coding 6 reverse	AAAGAACAAGGCCCTAATCCAC		
Coding 7 forward	CAGCGAGGCGGACAAGAAGAAGG		
Coding 7 reverse	CTCCCCGCCCCAGTGAACC		

accurately validate and assess the allele frequencies of DNA polymorphisms.

Materials and methods

Polymerase chain reaction and DNA sequencing

The DNA screening panel was composed of 72 subjects: 24 Caucasian, 24 African Americans, and 24 Hispanics. Each ethnic group consisted of 16 individuals with asthma and eight unaffected controls (A genome-wide search for asthma susceptibility loci

in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA) 1997).

PCR primers were designed to independently amplify a ~4.5 kb region of *HSPA1A* and *HSPA1B* (Table I). Each 4.5 kb amplicon contained ~2 kb of promoter and ~500 bp of 3' UTR in addition to the coding region. PCR was performed in 40 µl reactions using 80 ng of genomic DNA, 15 pmol of both the forward and reverse primer, 4–5 U of Taq polymerase (Life Technologies), and IX FailSafe PCR master mix (Epicentre Technologies, Madison, WI, USA). Cycling conditions were: 94°C for 5 min, followed by

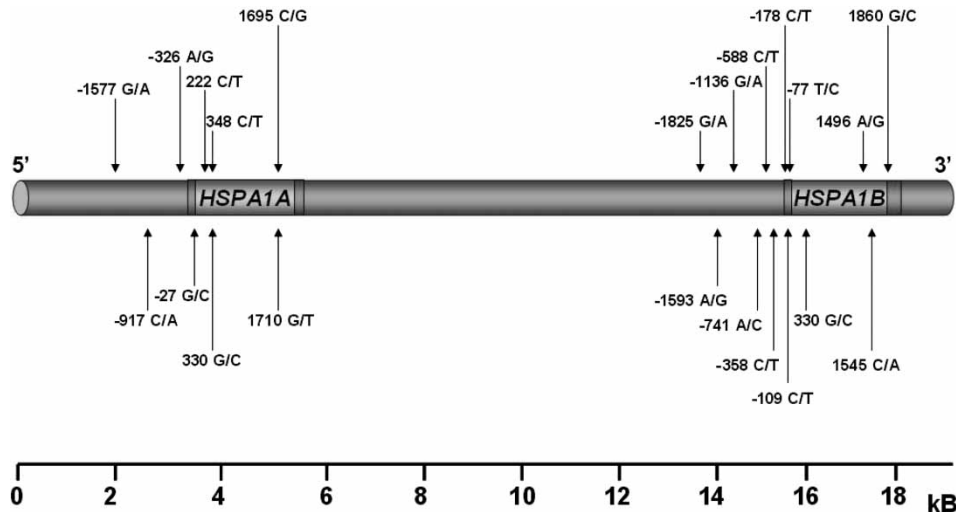


Figure 1. Location of polymorphisms identified in *HSPA1A* and *HSPA1B* genes. Regions marked *HSPA1A* and *HSPA1B* represent coding sequence. The darked regions flanking the coding region represents the 5' and 3' UTR sequences. The *HSPA1A* "Genomic Forward"-primer and "Genomic Reverse"-primer are located between chromosomal positions 31889307 and 31889324, and 31893708 and 31893731 respectively (Table I). The *HSPA1B* "Genomic Forward"-primer and "Genomic Reverse"-primer are located between chromosomal positions 31901566 and 31901589, and 31906221 and 31906244, respectively.

40 cycles of 94°C for 1 min, T_{anneal} (62°C for *HSPA1A*, 58°C for *HSPA1B*) for 1 min, and 72°C for 5 min, and an extension at 72°C for 7 min. The *HSPA1A* amplicon was purified via gel extraction (QIAEXII, Qiagen, Hilden, Germany). Both amplicons were purified in a final step using the Perfectprep PCR Cleanup 96 (Eppendorf, Hamburg, Germany). The purity of each amplicon was verified on a 1% agarose gel.

Nested PCR was performed on individual 4.5 kb amplicons to generate overlapping fragments (Table I). Each of the 20 μl nested PCR reactions contained 40 ng of DNA from the amplicons, 0.5 U Taq polymerase, 15 pmol of both the forward and reverse primer, and IX FailSafe PCR master mix. General cycling conditions were: 94°C for 5 min, followed by 30–35 cycles of 94°C for 1 min, T_{anneal} for 1 min, and 72°C for 1 min, and a final extension at 72°C for 7 min. PCR products were verified on a 1.5% agarose gel and purified using Perfectprep PCR Cleanup 96 (Eppendorf, Hamburg, Germany) prior to DNA sequencing.

DNA sequencing reactions were performed as previously described (Hawkins et al. 2004). Sequencing products were analyzed on an ABI 3730 XL DNA Analyzer (Applied Biosystems, Inc., Foster City, CA, USA). Sequencing reactions were performed on both DNA strands.

Sequence Analysis

Sequence alignment and polymorphism identification were performed using Sequencher 4.2 (Gene Codes, Corp., Ann Arbor, MI, USA). All polymorphisms were validated through observation in two

independent DNA samples or by re-sequencing independently generated PCR products. MatInspector (www.genomatix.de) was used to identify predicted transcription factor binding sites in the promoter region. A search of the region sequenced was performed in dbSNP (www.ncbi.nlm.nih.gov) to record all polymorphisms reported in the region.

Results

The database dbSNP listed 24 and 32 polymorphisms for *HSPA1A* and *HSPA1B*, respectively. We were able to validate only eight polymorphisms in *HSPA1A* and nine polymorphisms in *HSPA1B* (Figure 1, Tables II and III) in our screening panel. Only one novel polymorphism was identified in *HSPA1A* (C/T + 348, hP116P), however, five novel polymorphisms were identified in *HSPA1B* (G/A – 1825, A/G – 1593, C/T – 358, G/C + 330, D110E, and C/A + 1545, I515I). Five coding polymorphisms were identified in *HSPA1A*, only one of which was non-synonymous (G/C + 330, D110E). Five coding polymorphisms were identified in *HSPA1B*, two of which were non-synonymous (G/C + 330, D110E and A/G + 1496, N499S). The non-synonymous coding polymorphism G/C + 330 (D110E) was listed as being present in only *HSPA1A* (rs562047) by dbSNP and thus was considered to be novel to *HSPA1B*.

Only one polymorphism identified in our screening panel occurred in both genes (G/C + 330, D110E). The F_{ma} of this polymorphism was higher in *HSPA1A* than in *HSPA1B*. Five *HSPA1A* polymorphisms (G/A – 1577, A/G – 326, G/C – 27, G/C + 330, D110E, and C/G + 1695, A565A) were present in all three ethnic groups. One *HSPA1A* polymorphism

Table II. *HSPA1A* Polymorphism locations and frequencies.

Position*	dbSNP accession	Map coordinates	Alleles [†]	Minor allele [‡]	Minor allele frequency			
					Caucasian American	African American	Hispanic	dbSNP
- 1577	rs12213612	31889936	G/A	A	0.083	0.114	0.083	NA
- 1248	rs11414738	31890265	A/-	-	-	-	-	NA
- 1234	rs6933097	31890279	A/T	T	-	-	-	NA
- 1233	rs9279425	31890280	A/-	-	-	-	-	NA
- 1083	rs12526722	31890430	A/C	C	-	-	-	NA
- 917	rs16867582	31890596	C/A	A	0.071	0.045	0	0.014
- 591	rs4713489	31890922	G/A	A	-	-	-	NA
- 340	rs11576011	31891173	G/A	A	-	-	-	NA
- 326	rs1008438	31891187	A/G	G	0.429	0.682	0.521	0.404
- 97	rs11557922	31891416	T/C	C	-	-	-	NA
- 64	rs11576009	31891449	G/A	A	-	-	-	NA
- 27	rs1043618	31891486	G/C	C	0.522	0.705	0.375	0.464
- 7	rs2242667	31891506	A/C	C	-	-	-	0.002
79	rs11557923	31891591	G/T (E27-)	T	-	-	-	NA
222	rs1043620	31891734	C/T (1741)	T	0.104	0	0.042	NA
330	rs562047	31891842	G/C(D110E)	C	0.261	0.357	0.167	0.038
348		31891860	C/T(P116P)	T	0.109	0	0	-
635	rs11557924	31892147	T/C (I212T)	C	-	-	-	NA
637	rs11557921	31892149	G/T (D213Y)	T	-	-	-	NA
1053	rs1061581	31892565	G/A (Q351Q)	A	-	-	-	0.333
1695	rs506770	31893207	C/G (A565A)	G	0.063	0.455	0.104	0.983
1710	rs541340	31893222	G/T (V570V)	T	0	0.023	0	0.969
1905	rs508603	31893417	C/T (P635P)	T	-	-	-	NA
1920	rs508633	31893432	A/G	G	-	-	-	0.000[¶]

Bold, identified only in dbSNP (www.ncbi.nlm.nih.gov); NA, not Available; * Position relative to the ATG start codon, where the first base in the start codon is + 1; [†] In parentheses is amino acid change and position; [‡] Based on allele frequency in all three populations; [¶] Frequency is too low to be reported.

Table III. *HSPA1B* Polymorphism locations and frequencies.

Position*	dbSNP accession	Map coordinates	Alleles†	Minor allele‡	Minor allele frequency			
					Caucasian American	African American	Hispanic	dbSNP
- 1825		31901882	G/A	A	0	0.021	0.021	-
- 1593		31902114	A/G	G	0	0.021	0	-
- 1136	rs2763979	31902571	C/T	T	0.417	0.625	0.438	0.478
- 741	rs7771177	31902966	A/C	C	0	0.063	0	NA
- 646	rs5026931	31903061	G/T	T	-	-	-	NA
- 588	rs13217108	31903119	C/T	T	0.042	0.043	0	NA
- 453	rs11576017	31903254	C/T	T	-	-	-	NA
- 429	rs11576016	31903278	C/T	T	-	-	-	NA
- 383	rs11576015	31903224	G/A	A	-	-	-	NA
- 358		31903349	C/T	T	0	0.063	0	-
- 338	rs11576011	31903369	G/A	A	-	-	-	NA
- 192	rs11576014	31903515	G/A	A	-	-	-	NA
- 178	rs6457452	31903529	C/T	T	0.146	0.167	0.083	0.068
- 167	rs11576013	31903540	G/C	C	-	-	-	NA
- 135	rs2607020	31903572	G/A	A	-	-	-	NA
- 109	rs11576012	31903598	C/T	T	0.042	0	0	NA
- 87	rs2607019	31903620	T/C	C	-	-	-	NA
- 77	rs2607018	31903630	T/C	C	0	0.021	0	NA
- 68	rs2607017	31903639	G/C	C	-	-	-	NA
79	rs11557923	31903785	G/T (E27-)	T	-	-	-	NA
330		31904036	G/C(D110E)	C	0.063	0.104	0.031	-
379	rs12190359	31904085	A/G (M127V)	G	-	-	-	NA
635	rs11557924	31904341	T/C (I212T)	C	-	-	-	NA
637	rs11557921	31904343	G/T (D213Y)	T	-	-	-	NA
1053	rs1061581	31904759	G/A(Q351Q)	A	0.542	0.292	0.438	0.333
1496	rs483638	31905202	A/G (N499S)	G	0.045	0.095	0	NA
1545		31905251	C/A(I515I)	A	0	0.094	0	-
1860	rs539689	31905566	G/C (G620G)	C	0.368	0.475	0.219	0.460
118	rs3036297	31905750	- /AGTTA	AGTTA	-	-	-	NA
121	rs9281590	31905753	- /AAGTT	AAGTT	-	-	-	NA
249	rs1061623	31905881	A/C	C	-	-	-	0.020
266	rs9267570	31905898	G/T	T	-	-	-	NA

Bold, identified only in dbSNP (www.ncbi.nlm.nih.gov); NA, not available; * Position relative to the ATG start codon, where the first base in the start codon is + 1; † In parentheses is amino acid change and position; ‡ Based on allele frequency in all three populations.

(G/T + 1710, V570V) occurred only in African Americans, while one *HSPA1A* polymorphism (C/T + 348, P116P) occurred only in Caucasians. Five *HSPA1B* polymorphisms (G/A - 1136, C/T - 178, G/C + 330, D110E, G/A + 1053, Q351Q, and G/C + 1860, G620G) were present in all three ethnic groups. Five polymorphisms (A/G - 1593, A/C - 741, C/T - 358, T/C - 77, and C/A + 1545, I515I) occurred only in African Americans, while one polymorphism (C/T - 109) occurred only in Caucasians. No polymorphisms from either gene were exclusive to Hispanics.

Several significant differences were noted between our data and that of dbSNP. First, the polymorphism rs1061581 (G/A, Q351Q) was listed as being in both *HSPA1A* and *HSPA1B*, whereas this polymorphism only occurred in *HSPA1B* in our screening panel. Second, rs506770 (C/G, A565A) is listed in dbSNP as having C as the minor allele, whereas we identified G as the minor allele. Finally, rs541340 (G/T, V570V) is listed in dbSNP as having G as the minor allele, whereas we identified T as the minor allele.

The promoter sequences (2000 bp 5' of the start codon) of *HSPA1A* and *HSPA1B* were evaluated by MatInspector. No *HSPA1A* polymorphisms altered predicted transcription factor binding sites. Two *HSPA1B* polymorphisms (-741 A/C and -588 C/T) altered predicted transcription factor binding sites. The minor allele of -741 A/C converts a predicted NMP4 (nuclear matrix protein 4)/CIZ (Cas-interacting zinc finger protein) site (ggAAAaaacat) to a predicted Fkh-domain factor FKHRL1 (FOXO) binding site (aagacggaACAacatc). The minor allele of -588 C/T converts the predicted myelin transcription factor 1-like neuronal C2HC zinc finger factor 1 binding site (cgaaAGTTacctt) into a predicted HMGI(Y) high-mobility-group protein I (Y), architectural transcription factor organizing the framework of a nuclear protein-DNA transcriptional complex (ggtAATTtctggg).

Discussion

This is the first detailed re-sequencing study defining genetic variations in *HSPA1A* and *HSPA1B*. While most polymorphisms identified in this study had similar F_{ma} to that reported in dbSNP, two polymorphisms (rs506770 and rs541340) were found to have significantly different F_{ma} in our screening panel, possibly due to reporting errors in dbSNP. Because past genomic builds identified only one gene encoding both proteins, any assignment of polymorphisms to the gene during these previous genomic builds would have been incorrect. A comparison of polymorphisms listed in Tables II and III with the dbSNP database indicates that approximately 66% of the *HSPA1A* and *HSPA1B* polymorphisms listed were not identified in our screening panel. There are several possible

explanations for this observation. First, many of the dbSNP polymorphisms could have arisen from errors in initial sequencing of genomic DNA or errors in data interpretation. Second, many of polymorphisms in dbSNP were derived from expressed sequences, and thus could have been the result of post-transcriptional events such as cloning errors and errors in RT-PCR. In addition, polymorphisms could also be introduced by post-transcriptional events such as RNA editing. Our study also was designed to detect only the most common polymorphisms based on the number of chromosomes screened in each ethnic group. Based on the equation:

$$P = 1 - (1.0 - F_{ma})^{2N}$$

where P is the statistical power of detection, F_{ma} is the threshold minor allele frequency, and N is the number of subjects sequenced; our power to detect polymorphisms with a F_{ma} 0.05 in 24 subjects was approximately 92%, and thus we did not detect some rare polymorphisms identified in dbSNP. However, we believe that our study is comprehensive in identifying the most common variants ($F_{ma} > 0.05$) in these two genes within our study population.

Because of the nearly identical coding regions, the *HSPA1A* and *HSPA1B* loci may have been derived by a duplication event. Despite this possibility, only one polymorphism, (G/C + 330 D110E), was common to both *HSPA1A* and *HSPA1B*. This mutation, which produces the conservative amino acid change aspartate to glutamate in the IB domain of lobe I, lies in a region not critical to secondary structure (Zhang and Zuiderweg, 2004). Since this mutation occurs in both genes, the mutational event that introduced the polymorphism probably occurred before gene duplication, however, we can not exclude that the coding change originated independently in both genes. It is not known which gene predominantly expresses the Hsp70 protein; however, the fact that most of the coding polymorphisms are synonymous in both genes indicates that there could be selective pressure to conserve the Hsp70 protein sequence. Unlike the coding regions, however, the putative 5' promoters and 3' untranslated regions are dissimilar, indicating that regulation of *HSPA1A* and *HSPA1B* gene expression may be significantly different. Sequence variations in the promoter regions of *HSPA1A* and *HSPA1B* make this possibility more likely. This is supported by a recent study where the C allele of the polymorphism -178 T/C *HSPA1B* was associated with lower levels of *HSPA1B* expression (Temple et al., 2004). Our analysis of promoter sequences of *HSPA1A* and *HSPA1B* identified only two polymorphisms (*HSPA1B* -588 C/T and -741 A/C) in the promoter of either gene that alter predicted transcription factor binding sites. Reporter assays, electromobility shifts assays (EMSA), or a combination of reverse

transcription and real time PCR will need to be utilized to determine the effects of these promoter polymorphisms on gene expression/transcription.

In summary, we have identified the most common polymorphisms in *HSPA1A* and *HSPA1B* in a screening panel of asthmatics and normal controls from three ethnic groups. Our study indicates that most of the *HSPA1A* and *HSPA1B* polymorphisms identified in dbSNP are rare ($F_{\text{ma}} < 0.05$) or were derived during post transcriptional events, and probably do not exist in the genomic sequence. Our study also has revealed additional sequence variation in the 5' and 3' regions of each gene, which may cause each gene to have unique patterns of gene expression regulation. Therefore, the functional effects of *HSPA1A* and *HSPA1B* polymorphisms are most likely involved in regulation of mRNA and protein levels rather than altering the structure and function of the Hsp70. While our study does have value in the selection of polymorphisms for association analysis, genotyping polymorphisms in the coding regions of *HSPA1A* or *HSPA1B* will be difficult using standard genotyping techniques due to the shared sequence identity.

Acknowledgements

We want to thank Catherine S. Brewer and Abdoulaye F. Diallo for their assistance. This work was supported by NIH 1R21HL077916 to GAH and NIH 1R01HL/69197 to ERB.

References

- Temple SE, Cheong KY, Ardlie KG, Sayer D, Waterer GW. 2004. The septic shock associated HSPA1B1267 polymorphism influences production of HSPA1A and HSPA1B. *Intensive Care Med* 30:1761–1767.
- Pratt WB, Toft DO. 1997. Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev* 18:306–360.
1997. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). *Nat Genet* 15:389–392.
- Hawkins GA, et al. 2004. Identification of polymorphisms in the human glucocorticoid receptor gene (NR3C1) in a multi-racial asthma case and control screening. *DNA Seq* 15:167–173.
- Zhang Y, Zuiderweg ER. 2004. The 70-kDa heat shock protein chaperone nucleotide-binding domain in solution unveiled as a molecular machine that can reorient its functional subdomains. *Proc Natl Acad Sci USA* 101:10272–10277.