INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

IN SILICO ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISM IN BOVINE HSP90AA1 AND HSP90AB1 TRANSCRIPTS

[1]Himangshu Raj, [2]Giasuddin Ahmed & [3]Devojyoti Dutta
[1]Department of Bioengineering and Technology, Institute of Science and Technology, Gauhati University, Guwahati, Assam, India
[2]Department of Biotechnology, Gauhati University, Guwahati, Assam, India
[3]Department of Veterinary Physiology, College of Veterinary Science, Khanapara, Guwahati, Assam, India

ABSTRACT
Heat shock proteins (HSPs) are organized into several families according to their molecular size. HSPs are highly conserved across species, particularly the 90 kDa heat shock protein (HSP90). In eukaryotes there are two major isoforms of HSP90 constituted by gene duplication. The goal of this study was to analyse non synonymous single nucleotide polymorphisms (nsSNPs) in HSP90AA1 and HSP90AB1 using computational methods. Functional effects of nsSNPs were screened with five different *in silico* prediction algorithms and also putative protein structures were modelled for each high risk nsSNPs located in evolutionary conserved regions. Total 54 nsSNPs of HSP90AA1 and 97 nsSNPs of HSP90AB1 were found as high risk nsSNP. Of these13 nsSNPs of HSP90AA1 and 30 nsSNPs of HSP90AB1were important structural residues and 12 nsSNPs of HSP90AA1 and 25 nsSNPs of HSP90AB1 were important functional residues. Wild and mutant residual RMSD for HSP90AA1 ranged from 0.03 to 0.062 and for HSP90AB1 was 0.014 to 0.233. Post translational modifications in the highly conserved residues were influenced by 3 nsSNPs in HSP90AA1 and 8 nsSNPs in HSP90AB1. Our data strongly suggest that the nsSNPs has very high negative impact on structure and/or function of these proteins. Most of these high-risk nsSNPs are located at highly conserved amino acid sites.

KEYWORDS: SNP; HSP90AA1; HSP90AB1; Bovine.

INTRODUCTION

Single nucleotide polymorphism (SNP) is the simplest and most common source of genome variance. Particularly non-synonymous SNPs (nsSNPs) located in protein coding regions are responsible for an amino acid substitution in the corresponding protein product. nsSNPs may affect the protein function by altering protein structure or stability and may affect gene regulation that may not be identified by structure or phylogeny based features (Alshatwi *et al.*, 2012; Dabhi and Mistry, 2014). Agricultural and livestock production has been influenced largely by the effect of global warming which lead to adaptation in new environmental conditions. The study of the molecular basis of traits involved in adaptation in stressful environment has great importance. Heat stress in dairy cattle is one of the main factor which has an important impact in productive and functional traits and gradual adaption in different agro climatic regions (Hansen, 2004; Sanchez *et al.*, 2009; Hoffmann, 2010). The chaperone, HSP90 is one of the most abundant proteins in eukaryotic cells, involved in maintenance of cellular homeostasis, under both physiological and stress conditions (Favatier *et al.*, 1997; Charoensook *et al.*, 2012). HSP90 has two isoforms, HSP90AA1 (the inducible) and HSP90AB1 (the constitutive), which have arisen by gene duplication (Chen *et al.*, 2006; Deb *et al.*, 2014). The expression of HSP90AA1 is heat-inducible and more tissue specific, whereas HSP90AB1 is more or less constitutively and ubiquitously expressed. HSPs are highly conserved across species (Salces-Ortiz *et al.*, 2013, 2015). HSP90AA1 (heat shock protein 90kDa alpha (cytosolic), class A member 1) has 733 amino acids and HSP90AB1 (heat shock protein 90kDa alpha (cytosolic), class B member 1) has 724 amino acids.

MATERIALS & METHODS

The SNP data of bovine HSP90AA1 (ENSBTAT 00000008225) and HSP90AB1 (ENSBTA T00000001034) genes were retrieved from Ensembl cow (UMD3.1) genome browser (http://asia. ensembl.org/ Bos_taurus/Info/Index). Functional effects of nsSNPs were screened using the following *in silico* algorithms: SIFT (http://sift.jcvi.org/) (Kumar *et al.*, 2009), nsSNP Analyzer (http://snpanalyzer. uthsc.edu/) (Bao *et al.*, 2005), Polyphen-2(http:/ /genetics.bwh. harvard.edu /pph2/ index.shtml) (Adzhubei *et al.*, 2010), PhD-SNP (http://snps. biofold.org/ phd-snp/phd-snp.html) (Capriotti *et al.*, 2006), Panther (http://www. pantherdb.org/) (Mi *et al.*, 2016). nsSNPs predicted to be deleterious or damaging by all five *in silico* algorithms were categorized as high-risk nsSNPs and were selected for further analysis.

Evolutionary conservation of amino acid residues in bovine HSP90AA1 and HSP90AB1 were determined using the ConSurf web server (consurf.tau.ac.il/) (Berezin *et al.*, 2004; Celniker et al., 2013). ConSurf employs an empirical Bayesian method to identify putative structural and functional well as evolutionary conserved residues. Highly conserved residues were predicted to be either structural or functional based on their location relative to the protein surface or protein core. High-risk nsSNPs located at highly conserved sites were selected for further analysis.

I-Mutant suit (http://gpcr2.biocomp.unibo.it/cgi/ predictors/I-Mutant3.0/I-Mutant3.0.cgi), a support vector machine based predictor of protein stability, was used to evaluate nsSNP induced changes in protein stability (Capriotti *et al.*, 2005). High risk and highly conserved nsSNPs were submitted to I-Mutant suit to evaluate any changes in structural stability. For understanding the significance of nsSNPs on protein function, Knowledge about 3D structure of protein is very important. SWISS-MODEL Workspace (http://swissmodel. expasy.org/interactive) was used to generate 3D structural models for wild type HSP90AA1 and HSP90AB1 and each of their high-risk nsSNPs. The SWISS-MODEL Workspace is a web-based integrated service dedicated to protein structure homology modelling. The global and per-residue model quality has been assessed using the QMEAN scoring function (Benkert *et al.*, 2005). Putative phosphorylation sites were predicted using GPS (http://gps.biocuckoo.org) (Xue *et al.*, 2011), NetPhos server (http://www.cbs.dtu.dk/ services/NetPhos/) (Blom *et al.*, 1999), putative sumoylation sites were predicted using the GPS-SUMO 2.0 (http://sumosp. biocuckoo.org /online.php) (Zhao *et al.*, 2014) and putative ubiquitylation sites were predicted using the UbPred (www.ubpred.org) (Radivojac *et al.*, 2010).

RESULTS & DISCUSSION

According to Ensemble databases (Genome assembly: UMD 3.1), the HSP90AA1 gene contains 822 SNPs, 27 insertions and 27 deletions whereas HSP90AB1 genes contains 1241 SNPs, 35 insertions and 21 deletions (Table 1).

TABLE 1: Polymorphism data of HSP90AA1 and HSP90AB1 retrieved from Ensemble genome browser

Variation type	Variation class HSP90AA1				Variation class HSP90AB1			
	SNP	Insertion	Deletion	Total	SNP	Insertion	Deletion	Total
3 prime UTR variant	43	0	2	45	0	0	0	0
5 prime UTR variant	3	0	0	3	11	0	0	11
Downstream gene variant	169	5	5	179	448	16	10	474
Frameshift variant	0	4	1	5	0	5	2	7
Intron variant	212	10	13	235	230	7	5	242
Missense variant	168	0	0	168	245	0	0	245
Missense variant in splice region	4	0	0	4	4	0	0	4
Splice acceptor variant	1	0	0	1	9	1	0	10
Splice donor variant	2	0	0	2	5	0	0	5
Splice region intron variant	11	1	1	13	23	0	0	23
Splice region synonymous variant	3	0	0	3	2	0	0	2
Start lost splice region variant	2	0	0	2	0	0	0	0
Stop gained variant	12	0	0	12	6	0	0	6
Synonymous variant	42	0	0	42	70	0	0	70
Upstream gene variant	150	7	5	162	187	6	4	197
Coding sequence variant	0	0	0	0	1	0	0	1
Total mutations	822	27	27	876	1241	35	21	1297

All the ensemble SNP IDs were cross-matched with the NCBI dbSNP database. From these variations only 172 nsSNPs of HSP90AA1 and 245 nsSNPs of HSP90AB1 were selected for analysis. Our analyses included five *in silico* SNP prediction algorithms -SIFT, nsSNP Analyzer, PhD-SNP, Polyphen-2 and Panther. Because each algorithm uses different parameters to evaluate the nsSNPs, so nsSNPs with more positive results are more likely to be truly diseased. Here we considered all damaging, possibly damaging, deleterious mutations as diseased SNP. Here, we classified nsSNPs as high-risk if they were predicted to be deleterious by all five SNP prediction algorithms. We found 54 high-risk nsSNPs of HSP90AA1 and 97 high-risk nsSNPs of HSP90AB1 and were selected for further *in silico* analysis (Table 2). The list of high-risk nsSNPs with their corresponding dbSNP IDs were summerised in Table 3. To further investigate the potential effects of the high-risk nsSNPs, the degree of evolutionary conservation at all amino acid sites were calculated using the ConSurf web server. ConSurf analysis revealed 28 residues of HSP90AA1 and 65 residues of HSP90AB1 in highly conserved (Conservation Score of 8–9) region (Table 4). ConSurf predicted that 13 residues of HSP90AA1 and 30 residues of HSP90AB1were important structural residue (highly conserved and buried) and 12 residues of HSP90AA1 and 25 residues of HSP90AB1 were important functional residues (highly conserved and exposed) (Table 4). Amino acids that are involved in important biological processes, *viz.* located in enzymatic sites or required for protein-protein interactions, tend to be more conserved than other residues. As such, nsSNPs that are located at highly conserved amino acid positions tend to be more deleterious than nsSNPs located at non-conversed sites (Kelly and Barr, 2014). These results indicate that high-risk nsSNPs in conserved regions might induce severe structural changes in the protein. To analyse protein stability change, all the high-risk nsSNPs in conserved regions were submitted in I-mutant suit. Ten variants HSP90AA1 and 25 variants of HSP90AB1 were found to have highly decreased the stability of protein structure (Table 5). All these nsSNPs have free energy change value (DDG) <−1.0 kcal/mole, indicating destabilizing mutations. This may be due to mutant residue which is bigger than the wild type and cannot fit within the available space. Due to carrying the less rigid behaviour, the mutant residue can possibly disturb the original core structure and functional activity of the native protein. A number of studies have shown that decreased protein stability leads to increased protein misfolding, aggregation and degradation. Accordingly, decreased stability typically results in decreased net function (Kelly and Barr, 2014).

TABLE 2: Results of diseased and neutral nsSNPs detected by five algorithms

gene	outcome	SIFT	nsSNP	phdSNP	Polyphen	Panther	all five
HSP90AA1	Diseased	105	89	89	131	74	54
	Neutral	67	83	83	41	98	22
HSP90AB1	Diseased	203	151	175	150	162	97
	Neutral	42	94	70	95	83	25

TABLE 3: NCBI dbSNP ID of high risk nsSNPs detected by all five algorithms

HSP90AA1 (54 no.s)				HSP90AB1 (97 no.s)					
dbSNP_ID	SNP	dbSNP_ID	SNP	dbSNP_ID	SNP	dbSNP_ID	SNP	dbSNP_ID	SNP
rs448808905	D157G	rs457080512	D527Y	rs467576297	I28T	rs476146966	F208I	rs441461852	L533P
rs480688641	Q159P	rs471495972	V531G	rs434674471	N30D	rs476146966	F208V	rs477589392	K551N
rs458313136	W162C	rs435851406	E536G	rs466916233	E42G	rs472491124	I209R	rs452962738	L563R
rs478434151	W162L	rs450212828	F537I	rs481934012	D61E	rs472491124	I209T	rs435607410	R583W
rs478434151	W162S	rs450212828	F537V	rs432261085	T89P	rs441085645	G210C	rs455803986	S587P
rs479151805	V172A	rs458149592	G539R	rs474678867	G92S	rs470954293	Y211D	rs472693540	C590S
rs463629514	D175G	rs471641348	T546P	rs437206991	L98I	rs448653296	I213L	rs434921762	I591N
rs472236324	E234G	rs451284009	K547E	rs457251500	V99A	rs462333746	I213N	rs434921762	I591S
rs482496258	N292H	rs433359682	E558V	rs474141038	S108P	rs462333746	I213T	rs451793946	V592G
rs474629407	L342F	rs447781901	K559T	rs436520569	G109R	rs432760549	L215F	rs471847721	S594R
rs467637937	L364V	rs483094634	L572P	rs453395271	D122H	rs449604817	L215P	rs445006481	T599N
rs448282125	V366G	rs443552719	C573W	rs473616751	I126S	rs436293173	E218G	rs480615391	N601T
rs477116173	C375R	rs440825071	M576K	rs476135429	F129I	rs457378189	E224G	rs449209761	M602I
rs452372633	E393A	rs472117700	K577E	rs444018549	G130R	rs474267855	I225N	rs466054434	R604G
rs432321927	P396S	rs476285477	V584G	rs460948680	V131A	rs453569706	N283S	rs465376851	I605N
rs453141164	S400A	rs441573316	V584L	rs474660206	F133V	rs458529674	P287L	rs465376851	I605S
rs436038368	S400Y	rs522401604	C598R	rs460250088	Y137S	rs481248313	I296T	rs465376851	I605T
rs450525898	Q405P	rs446039721	S603I	rs477219583	V139L	rs450878970	F304C	rs457608487	K607E
rs464967293	V412G	rs447535061	Y605N	rs445827953	E141G	rs467876089	Y305H	rs442361898	A608D
rs445523692	I413S	rs462489377	V664F	rs478963604	V201E	rs436555419	K306Q	rs473030067	Q609H
rs442999290	E427A	rs460831928	L679Q	rs478963604	V201G	rs467243743	L316F	rs452842345	Q609R
rs438640994	F442L	rs442757887	I699M	rs460513544	V202G	rs208189445	F334L	rs481998444	D613G
rs450117948	F508I	rs451134399	E721G	rs469506187	K204E	rs446286665	D385G	rs449227536	E627D
rs478358085	L512R	rs451134399	E721V	rs483198403	H205D	rs477579644	D385H	rs466000298	P630R
rs441215622	E518Q	rs482595169	D723Y	rs483198403	H205N	rs438328017	S391C	rs452192138	L658Q
rs459626889	V519G	rs444285615	E730D	rs448714857	H205P	rs456176684	F433L	rs475520905	F659V
rs472616844	V519M	rs475729502	D733H	rs468842579	H205Q	rs476271798	S434Y	rs461112565	T661P
				rs434281858	S206A	rs441884600	N436K	rs440274379	G667A
				rs447194038	S206C	rs442224835	S462P	rs440274379	G667V
				rs434281858	S206P	rs479359832	E471A	rs445224871	H676N
				rs434281858	S206T	rs463979053	R475L	rs458834968	H676P
				rs447194038	S206Y	rs469741685	E478G		
				rs432815006	Q207P	rs435343753	Y484S		

Theoretical structural models were generated for each high risk conserved nsSNPs using Swiss Model portal, which is a fully automated protein structure homology-modelling server. Swiss Model uses QMEAN (Qualitative Model Energy ANalysis) score for model quality estimation. All the models for each high risk conserved nsSNP were then compared using SwissPdb Viewer. Root mean square deviation (RMSD) for each mutant residues were determined (Table 5). A higher RMSD typically indicates greater deviation between wild type and mutant structures. Residual RMSD for HSP90AA1 ranged from 0.03 to 0.062 and for HSP90AB1 0.014 to 0.233 (Table 3).

TABLE 4: Degree of evolutionary conservation score of the high-risk nsSNPs, detected by CONSURF neural-network algorithm

HSP90AA1		HSP90AB1			
nsSNPs	Residue type, Score	nsSNPs	Residue type, Score	nsSNPs	Residue type, Score
W162C	BS,9	I28T	BS,9	E224G	EF,8
W162S	BS,9	N30D	BS,9	N283S	EF,9
W162L	BS,9	E42G	EF,9	P287L	EF,8
N292H	EF,9	G92S	BS,9	F304C	BS,9
L342F	BS,9	L98I	B,8	Y305H	B,8
L364V	BS,9	S108P	EF,9	K306Q	EF,8
V366G	BS,9	G109R	EF,9	D385H	EF,9
C375R	B,8	D122H	EF,9	D385G	EF,9
E393A	EF,8	I126S	BS,9	S391C	BS,9

P396S	EF,9	F129I	BS,9	F433L	B,8
S400Y	BS,9	G130R	BS,9	N436K	EF,8
S400A	BS,9	V131A	BS,9	R475L	EF,9
Q405P	EF,9	F133V	BS,9	L533P	B,8
V412G	BS,9	V139L	BS,9	R583W	EF,9
I413S	BS,9	K204E	EF,8	S587P	B,8
F508I	BS,9	H205Q	EF,9	I591S	B,8
E518Q	EF,9	H205N	EF,9	I591N	B,8
V519M	BS,9	H205D	EF,9	V592G	B,8
V519G	BS,9	H205P	EF,9	T599N	B,8
D527Y	EF,9	S206T	BS,9	N601T	EF,9
T546P	EF,8	S206Y	BS,9	M602I	BS,9
K547E	EF,9	S206C	BS,9	R604G	EF,8
K559T	EF,8	S206A	BS,9	I605N	BS,9
K577E	EF,8	S206P	BS,9	I605S	BS,9
V584G	B,8	Q207P	EF,8	I605T	BS,9
V584L	B,8	F208I	BS,9	Q609R	EF,9
E730D	EF,8	F208V	BS,9	Q609H	EF,9
D733H	EF,9	I209T	BS,9	D613G	EF,9
		I209R	BS,9	E627D	EF,9
		Y211D	B,8	T661P	BS,9
		I213T	BS,9	G667A	BS,9
		I213N	BS,9	G667V	BS,9
		I213L	BS,9		

B: Buried residue; E: Exposed residue; S: Structural residue; F: Functional residue

Highest deviations were observed in L342F (HSP90AA1) and V131A (HSP90AB1) mutants. To investigate how nsSNPs may influence the post-translational modification of HSP90, we used a variety of *in silico* prediction tools (GPS 2.1 and NetPhos 2.0, UbPred server) to identify putative PTM sites. There were 43 serine-specific, 41 threonine-specific and 23 tyrosine-specific sites In HSP90AA1 and 55 serine-specific, 38 threonine-specific and 23 tyrosine-specific sites in the HSP90AB1 protein. Of these 1 serine 1 threonine 1 sumoylations were in high risk conserved nsSNP sites of HSP90AA1 and 4 serine 2 threonine 2 tyrosine in high risk sites of HSP90AB1 (Table 6).

Polymorphisms within HSP90AB1 were not causative for the physiological responses; however, the results propose that this gene is an attractive candidate for heat tolerance and should at least be used as a genetic marker to select appropriate breeds for hot climates (Charoensook *et al.*, 2012). Pattern of differentiation at nonsynonymous SNPs potentially subject to natural selection and synonymous or silent SNPs that are presumably closer to selective neutrality, but otherwise subject to similar effects from demographic effects and nearby selective sweeps.

TABLE 5: Protein destabilizing nsSNPs and RMSD values of high risk nsSNP located in highly conserved region

HSP90AA1			HSP90AB1		
nsSNPs	Stability (DDG)	RMSD	nsSNPs	Stability (DDG)	RMSD
W162C	LD (-1.29)	0.04	I28T	LD (-2.19)	0.028
W162S	LD (-1.45)	0.04	E42G	LD (-1.30)	0.047
L342F	LD (-1.12)	0.062	G92S	LD (-1.31)	0.023
L364V	LD (-1.86)	0.049	L98I	LD (-1.04)	0.037
V366G	LD (-2.06)	0.039	I126S	LD (-2.05)	0.024
P396S	LD (-1.59)	0.03	V131A	LD (-1.86)	0.233
V412G	LD (-1.43)	0.027	F133V	LD (-1.40)	0.031
I413S	LD (-1.53)	0.02	V139L	LI (-1.15)	0.039
V519G	LD (-2.04)	0.044	F208V	LD (-1.53)	0.073
V584G	LD (-2.02)	0.026	I209T	LD (-2.27)	0.033
			I209R	LD (-1.65)	0.043
			I213T	LD (-2.13)	0.034
			I213N	LD (-1.91)	0.036
			F304C	LD (-1.36)	0.014
			Y305H	LD (-1.03)	0.015
			D385H	LD (-1.19)	0.039
			D385G	LD (-1.65)	0.053
			F433L	LD (-1.22)	0.036
			L533P	LD (-1.39)	0.064
			I591S	LD (-2.33)	0.029
			I591N	LD (-2.10)	0.042
			V592G	LD (-2.67)	0.029
			R604G	LD (-1.10)	0.068

	I605N	LD (-1.66)	0.027
	I605S	LD (-2.15)	0.027
	I605T	LD (-2.02)	0.034

DDG: free energy change value; LD: Large Decrease; LI: Large Increase; RMSD: Root Mean Square Deviation

TABLE 6: *in silico* prediction of post-translational modification of HSP90AA1 and HSP90AB1

	GPS			NetPhos			SUMO	UbPred
	Serine	Threonine	Tyrosine	Serine	Threonine	Tyrosine		
HSP90AA1	43	41	23	21	9	10	17	10
high risk	1	1	0	0	0	0	1	0
conserved	(S400)	(T546)					(K547)	
region of								
HSP90AA1								
HSP90AB1	55	38	23	22	9	12	16	9
high risk	4	2	2	1	0	1	0	0
conserved	(S108, S206,	(T599,	(Y211,	(S108,		(Y305)		
region of	S391, S587)	T661)	Y305)	S587)				
HSP90AB1								

CONCLUSION

Our data strongly suggest that the 10 nsSNPs of HSP90AA1 and 25 nsSNPs of HSP90AB1 has very high negative impact on structure and/or function of these proteins. Most of these high-risk nsSNPs are located at highly conserved amino acid sites. In addition to these findings, we also identify few sites that may undergo post-translational modification, including sites that coincide with the location of high-risk nsSNPs. This study is the first systematic and extensive *in silico* analysis of functional SNPs in the bovine HSP90AA1 and HSP90AB1 transcripts. From our study, we can roughly estimate the proportion of nsSNPs subjected to differential positive or negative selection. The *in silico* profile of nonsynonymous variants might indicate that natural selection has a significant influence on the diversity and evolution of HSP90 isoforms that many of these changes may reflect structural and functional variants deserving of follow-up population level as well as phenotypic study. Future functional *in vitro* studies, can contribute to elucidate the polymorphisms that involved in expression variation and protein stability as response to environmental conditions. This prediction can be further tested through larger population-based studies.

REFERENCES

Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S. and Sunyaev, S.R. (2010) A method and server for predicting damaging missense mutations. Nat Methods 7: 248–249.

Alshatwi, A.A., Hasan, T.N., Syed, N.A., Shafi, G. and Grace, B.L. (2012) Identification of Functional SNPs in BARD1 Gene and In Silico Analysis of Damaging SNPs: Based on Data Procured from dbSNP Database. PLoS ONE 7(10): e43939.

Bao, L., Zhou, M. and Cui, Y. (2005) nsSNPAnalyzer: identifying disease-associated nonsynonymous single nucleotide polymorphisms. Nucleic Acids Res. 33: W480–2.

Benkert, P., Tosatto, S.C.E. and Schomburg, D. (2008) QMEAN: A comprehensive scoring function for model quality assessment. Proteins 71:261–277

Berezin, C., Glaser, F., Rosenberg, J., Paz, I., Pupko, T., Fariselli, P., Casadio, R. and Ben-Tal, N. (2004) ConSeq: the identification of functionally and structurally important residues in protein sequences. Bioinformatics 20: 1322–1324.

Blom, N., Gammeltoft, S. and Brunak, S. (1999) NetPhos 2.0 Server: Sequence- and structure-based prediction of eukaryotic protein phosphorylation sites. Journal of Molecular Biology 294(5): 1351-1362.

Capriotti, E., Fariselli, P. and Casadio, R. (2005) I-Mutant 2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res. 33: W306–10.

Capriotti, E., Calabrese, R. and Casadio, R. (2006) Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. Bioinformatics 22: 2729–2734.

Celniker, G., Nimrod, G., Ashkenazy, H., Glaser, F., Martz, E., Mayrose, I., Pupko, T. and Ben-Tal, N. (2013) ConSurf: Using Evolutionary Data to Raise Testable Hypotheses about Protein Function. Isr. J. Chem. 3-4:199-206.

Charoensook, R., Gatphayak, K., Sharifi, A.R., Chaisongkram, C., Brenig, B., and Knorr, C. (2012) Polymorphisms in the bovine HSP90AB1 gene are associated with heat tolerance in Thai indigenous cattle. Tropical Animal Health and Production 44: 921–928.

Chen, B., Zhong, D. and Monteiro, A. (2006) Comparative genomics and evolution of the HSP90 family of genes across all kingdoms of organisms. BMC Genomics 7: 156.

Dabhi, B. and Mistry, K.N. (2014) In silico analysis of single nucleotide polymorphism (SNP) in human TNF- α gene. Meta Gene 2: 586–595.

Deb, R., Sajjanar, B., Singh, U., Kumar, S., Singh, R., Sengar, G. and Sharma, A. (2014) Effect of heat stress on the expression profi le of Hsp90 among Sahiwal (*Bos*

indicus) and Frieswal (*Bos indicus* × *Bos taurus*) breed of cattle: A comparative study. Gene 536: 435–440.

Favatier, F., Bornman, L., Hightower, L.E., Gunther, E. and Polla, B.S. (1997) Variation in hsp gene expression and Hsp polymorphism: Do they contribute to differential disease susceptibility and stress tolerance? Cell Stress & Chaperones 2: 141–155.

Hansen, P.J. (2004) Physiological and cellular adaptations of zebu cattle to thermal stress. Anim. Reprod. Sci. 82: 349–360.

Hoffmann, I. (2010) Climate change and the characterization, breeding and conservation of animal genetic resources. Anim Genet. 41: 32–46.

Kelly, J.N. and Barr, S.D. (2014) In Silico Analysis of Functional Single Nucleotide Polymorphisms in the Human TRIM22 Gene. PLoS ONE 9(7): e101436.

Kumar, P., Henikoff, S. and Ng, P.C. (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 4(7): 1073-1081.

Mi, H., Poudel, S., Muruganujan, A., Casagrande, J.T. and Thomas, P.D. (2016) PANTHER version 10: expanded protein families and functions, and analysis tools. Nucleic Acids Res. 44(D1):D336-42.

Radivojac, P., Vacic, V., Haynes, C., Cocklin, R.R., Mohan, A., Heyen, J.W., Goebl, M.G. and Iakoucheva, L.M. (2010) Identification, Analysis and Prediction of Protein Ubiquitination Sites. Proteins: Structure, Function, and Bioinformatics 78(2): 365-380.

Salces-Ortiz, J., González, C., Moreno-Sanchez, N., Calvo, J.H., Pérez-Guzmán, M.D. and Serrano M.M. (2013) Ovine HSP90AA1 Expression Rate Is Affected by Several SNPs at the Promoter under Both Basal and Heat Stress Conditions. PLoS ONE 8(6): e66641.

Salces-Ortiz, J., Ramón, M., González, C., Pérez-Guzmán, M.D., Garde, J.J., García-Álvarez, O., Maroto-Morales A., Calvo, J.H., and Serrano M.M. (2015) Differences in the Ovine HSP90AA1 Gene Expression Rates Caused by Two Linked Polymorphisms at Its Promoter Affect Rams Sperm DNA Fragmentation under Environmental Heat Stress Conditions. PLoS One 10(2): e0116360.

Sanchez, J.P., Misztal, I., Aguilar, I., Zumbach, B. and Rekaya, R. (2009) Genetic determination of the onset of heat stress on daily milk production in the US Holstein cattle. Journal of Dairy Science 92: 4035–4045.

Xue, Y., Liu, Z., Cao, J., Ma, Q., Gao, X., *et al.* (2011) GPS 2.1: enhanced prediction of kinase-specific phosphorylation sites with an algorithm of motif length selection. Protein Eng Des Sel. 24: 255–260.

Zhao, Q., Xie, Y., Zheng, Y Jiang, S., Liu, W., Mu, W., Liu, Z., Zhao, Y., Xue,Y. and Ren, J. (2014) GPS-SUMO: a tool for the prediction of sumoylation sites and SUMO-interaction motifs. Nucleic Acids Research 42(W1):W325-W330.