Whole-Genome comparative analysis reveals genetic mechanisms of disease resistance and heat tolerance of tropical *Bos indicus* cattle breeds

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**Whole-Genome comparative analysis reveals genetic mechanisms of disease resistance
and heat tolerance of tropical *Bos indicus* cattle breeds**

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Abstract

The *Bos indicus* cattle breeds have been naturally selected over thousands of years for disease resistance and thermo-tolerance. However, a genetic mechanism of these specific inherited characteristics needs to be discovered. Hence, in this study, the whole-genome comparative analysis of *Bos indicus* cattle breeds of Kangayam, Tharparkar, Sahiwal, Red Sindhi, and Haryana of the Indian subcontinent was conducted. The genetic variants identification analysis revealed a total of 15,58,51,012 SNPs and 1,00,62,805 InDels in the mapped reads across all *Bos indicus* cattle breeds. The functional annotation of 17,252 genes that comprised both, SNPs and InDels, of high functional impact on proteins, has been carried out. The functional annotation results revealed the pathways that were involved in the innate immune response including toll-like receptors, a retinoic acid-inducible gene I like receptors, NOD-like receptors, Jak-STAT signaling pathways, and the non-synonymous variants in the candidate immune genes. Further, we also identified several pathways involved in heat shock response, hair and skin properties, oxidative stress response, osmotic stress response, thermal sweating, feed intake, metabolism, and the non-synonymous variants in the candidate thermo-tolerant genes. These pathways and genes were directly or indirectly contributing to the disease resistance and thermo-tolerance adaptations of *Bos indicus* cattle breeds.

Keywords: *Bos indicus*, disease resistance, thermo-tolerance, SNPs, InDels

52 Introduction

53 The *Bos indicus* cattle breeds (Zebu cattle) are characterized by the hump at the withers,
54 pendulous skin in the dewlap and navel, draught ability, fertility at the higher temperature,
55 maintenance of production performance in low input system, robustness, adaptability, disease
56 resistance, and thermo-tolerance in tropical climates (Cunningham and Syrstad 1987). Among
57 these characteristics of zebu cattle, thermo-tolerance and disease resistance are being important
58 traits exploited for cross-breeding programs in production commercial dairy and beef cattle
59 production (Cunningham and Syrstad 1987; Madalena et al. 2019; Vijayakumar et al. 2019a).
60 Therefore, the characterization of these two traits in *Bos indicus* cattle at the genome level
61 seems a strategic plan to design sustainable breed selection programs. Further, the
62 identification of genes and genomic variants likely responsible for these fitness traits in indicine
63 cattle breeds will be useful for the selection of disease resistance and heat tolerance animals in
64 the future (Berry et al. 2011; Singh et al. 2014). This may be considered as one mitigation
65 strategies to handle the current global climate-changing scenario (Escarcha et al. 2018; Osei-
66 Amponsah et al. 2019). Thus, tapping the genomic basis of *Bos indicus* cattle breeds requires
67 a timely and effective approach, especially considering the value and importance of these
68 animal genetic resources. The high intensity of artificial selection was applied to *Bos taurus*
69 cattle in comparison with *Bos indicus* cattle to improve economically valuable traits.

70 The innate immune response plays a crucial role in the disease resistance mechanism
71 against pathogen infection. Because its non-specific immune response is activated at the very
72 initial stages in any pathogens/parasitic infection in the animal body system. The potent innate
73 immune system activation is essential for the development of pathogen-specific adaptive
74 immune responses (Mishra et al. 2017). Further, an in-depth and complete understanding of
75 the cattle's natural anti-infectious immunity is essential for the effective management of cattle
76 herds (Ferluga et al. 2020; Vlasova and Saif 2021). The arrival of new high-throughput

sequencing technologies has significantly improved our understanding of the cattle immune system (Meade 2015). It should be pointed out that, the complete knowledge of genes, genetic variants, and molecular pathways responsible for disease resistance traits in indicine cattle breeds that originated in the Indian subcontinent are not identified to date (Meade 2015; Vlasova and Saif 2021).

The thermo-tolerance of cattle breeds is the ability of animals to regulate their body temperature by balancing heat gain and heat loss (Sunil et al. 2011; Paula-Lopes et al. 2013). This adaptive mechanism is achieved through changes in gene expression patterns and the activity of biochemical molecules that regulate cellular functions against heat stress (Bernabucci et al., 2010; Sunil et al. 2011; Paula-Lopes et al. 2013). The previous studies identified several genes contributing to thermo-tolerance in different species (reviewed in Belhadj Slimen et al. 2015; Archana PR et al. 2017). In cattle, well-known and widely studied genes related to heat stress are heat shock proteins (HSPs) and higher expression of these HSPs protects the cell against hyperthermia (Qiu et al. 2006; Collier et al. 2008; Gupta et al. 2013). Further, genes involved in antioxidant production, sweat gland development, and sweating, has also been reported to contribute to the thermo-tolerance mechanism in cattle (Wilke et al. 2007; Akers and Denbow 2008).

Further, improving thermo-tolerance capacity among productive cattle breeds through conventional quantitative genetics is difficult because of its negative correlation with the production performance traits (Dikmen et al. 2012; Bernabucci et al. 2014; Carabaño et al. 2014; Nguyen et al. 2016). Hence, understanding the molecular mechanism of thermo-tolerance processes may provide solutions to select heat-tolerant animals with high production potential (Belhadj Slimen et al. 2015; Min et al. 2017; Carabaño, 2016). However, information on molecular pathways and genes responsible for the thermo-tolerance mechanisms of *Bos indicus* cattle that originated in the Indian subcontinent is very scarce (Carabaño et al. 2019).

This study is planned to understand the molecular mechanism and the key genetic variants present in the candidate genes for disease resistance and thermo-tolerance traits in *Bos indicus* cattle breeds in comparison with temperate *Bos taurus* cattle. Our study results were highlighting the unique genome response to disease resistance and thermo-tolerance adaptations of *Bos indicus* breeds of cattle that originated in the Indian subcontinent. Further, the genetic variants identified in this study can be used for the creation of custom-designed SNP genotyping chips, marker-assisted selection, and genomic selection of animals in the future.

Materials and methods

Whole-genome datasets and read mapping

We used whole-genome data of five zebu cattle breeds that originated in the Indian subcontinent including Kangayam (n = 1), Tharparkar (n = 3), Sahiwal (n = 2), Red Sindhi (n = 1) and Hariana (n = 1) breeds available at NCBI public database (Accession no. PRJNA496267 and PRJNA493191) (Table 1). The quality control of raw sequencing reads was performed using the FastQC program (Andrews 2010). The Trimmomatic software was used for quality filtering of the short reads (Bolger et al. 2014). The quality reads were mapped into *Bos taurus* reference genome assembly ARS-UCD1.2 using the Bowtie2 with default parameters (Langmead and Salzberg 2012). The “MarkDuplicates” tool of Picard tools was used to remove potential PCR duplicates from the aligned reads and then the uniquely mapped reads were used for the variant calling step.

Variant calling

Freebayes is a Bayesian genetic variant detector that was used with default parameters to find SNPs (single-nucleotide polymorphisms), indels (insertions and deletions), MNPs (multi-nucleotide polymorphisms), and complex events (composite insertion and substitution

events) in our mapped datasets (Garrison and Marth 2012). After the variant calling step, the 'Snpsift filter' tool was used to remove sequencing and alignment artifacts from the SNPs and Indels with the following parameters '(DP >= 2) | (QUAL >= 20)' (Cingolani et al. 2012). Further, the freebayes output was filtered to include high-quality variants in the candidate genes, i.e., quality (QUAL) > 1, presence of supporting reads on both strands (SAF >0 and SAR > 0) and both sides of the variant (RPL > 1 and RPR > 1), and high quality per alternate observation (QUAL/AO > 4).

Variation annotation

SnEff is a variant annotation and effect prediction tool that was used for functional annotation of identified variants. The identified SNP/Indels were classified as high, moderate, low, and modifier by using the SnEff tool (Cingolani et al. 2012). All analyses were done on the Galaxy server (<https://usegalaxy.org/>) (Afgan et al. 2016). Genes comprising of high and moderate impact were used for pathway analysis in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (Kanehisa and Goto 2000) to relate the biological process and pathways associated with disease resistance and thermo-tolerance of zebu cattle breed adaptability in the tropics (Fig. 1). The Cattle Quantitative Trait Locus database (Cattle QTLdb) (<https://www.animalgenome.org/cgi-bin/QTLdb/BT/index>) contains cattle QTL, candidate genes responsible for 685 traits, and association data curated from 1,049 publications. The candidate genes related to disease resistance and thermo-tolerance were retrieved using 'Search and Analysis Tools' of Cattle QTLdb. In addition, innate immunity related genes were also retrieved from the InnateDB database (<http://www.innatedb.com/annotatedGenes.do?type=innatedb>). The protein-protein interaction (PPI) networks and gene ontology terms analysis were done in the NetworkAnalyst server to gain the functional roles of retrieved candidate genes from Cattle QTLdb and

InnateDB databases in structuring and mediating the biological processes of disease resistance and thermo-tolerance of zebu cattle breeds (Fig. 2 and 3) (Xia et al. 2014).

Results and discussion

The *Bos indicus* cattle breeds are known for their unique genetic features like draught ability, disease resistance, and thermo-tolerance in tropical climates. However, a genetic mechanism of these specific inherited characteristics of *Bos indicus* cattle needs to be revealed. Through comparisons of tropical *Bos indicus* cattle breeds genome with temperate *Bos taurus* genome, the genetic variants, biological processes, and signaling pathways responsible for the adaptations of indicine breeds to tropical environments can be identified. To investigate this; a whole-genome analysis study was carried out using the whole-genome sequence of Indian subcontinent originated indicine cattle breeds (Kangayam, Tharparkar, Sahiwal, Red Sindhi, and Hariana) which was previously published and submitted in the public database repository (Table 1). A total of 15,58,51,012 SNPs and 1,00,62,805 InDels were detected in the mapped reads across all indicine cattle breeds, in which Tharparkar cattle exhibiting the highest number of genetic variants followed by Sahiwal cattle (Table 2). SNP quality was assessed by calculating the transition (Ts) versus transversion (Tv) ratio of all detected SNPs. This Ts/Tv ratio is the indicator of potential sequencing error. Ts/Tv ratio in our study ranges from 2.16 to 2.26 (Table 3), which reflects the good quality of the identified SNPs. The observed Ts/Tv ratios were similar to the previous studies in the cattle genome (Choi et al. 2013, 2014; Daetwyler et al. 2014; Iqbal et al. 2019; Zhang et al. 2019). The identified variants were functionally annotated using SnpEff and the variants were classified into locational (e.g., intergenic, intron, and intragenic) and functional (e.g., stop_gained, start_lost and stop lost) categories (Table 4). Among the total identified variants in this study, the highest variants are found in the intron region (Table 4).

The identified SNP/InDels were classified as high, moderate, low, and modifier as per their functionality of the SnpEff program (Cingolani et al. 2012). The ‘high’ impact variants have a disruptive impact on the protein that may cause protein truncation, loss of function, or triggering nonsense-mediated decay. The ‘moderate’ impact variants have a non-disruptive variant that might change protein effectiveness (Cingolani et al. 2012). The functional annotation of high or moderate genetic impact variants may provide a valuable resource for understanding the tropical adaptation and production-related traits of *Bos indicus* cattle breeds (Iqbal et al. 2019). Hence, such functional impact genetic variants identified in all indicine cattle breeds were combined and analyzed to identify the genetic variants present in the candidate genes, biological process, and signaling pathways responsible for the heat tolerance and disease resistance adaptation of indicine cattle breeds. A total of 17,252 genes that comprised both, an SNP and an INDEL, of high or moderate functional impact on proteins, were identified in all indicine cattle breeds.

Pattern-recognition receptors

The innate immune system acts as the first line of defense against microbial infection or tissue damage (Akira et al. 2006; Beutler et al. 2006). Furthermore, host innate immunity is very crucial for the induction and regulation of acquired immunity. Germline-encoded pattern recognition receptors (PRRs) are responsible for recognizing microbial species-specific structures, termed pathogen-associated molecular patterns (PAMPs). The well-known PRRs were identified in this study (Fig. 1), including toll-like receptors (TLRs), a retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and nucleotide oligomerization domain (NOD)-like receptor family pyrin domain containing 3 (NLRP3), which induce the expression of type I interferons (IFNs), pro-inflammatory cytokines, and chemokines (Mishra et al. 2017).

Toll-like receptors

Toll-like receptors are the most extensively studied PRRs and TLRs are recognizing by PAMPs such as lipids, proteins, and nucleic acids (Brubaker et al. 2015). Among the ten TLRs reported in cattle (McGuire et al. 2006), we identified missense variants in TLR3 and TLR4 genes in indicine cattle breeds (Table S1). Cargill and Womack, 2007 reported SNPs and indels for the viral recognition of TLRs in bovine. After PAMP binding into TLRs resulted in the activation of signaling adaptor molecules, including myeloid differentiation factor 88 (MyD88) and TIR domain-containing adaptor inducing interferon (IFN)- β (TRIF), which further introducing downstream enzymatic cascades (Brubaker et al. 2015). MyD88 is very essential adaptor molecule in TLR signaling and most TLRs except TLR3, use MyD88 as the adaptor molecule to activate the canonical NF- κ B pathway for the synthesis of proinflammatory cytokines (Kaisho and Akira 2001; West et al. 2006). The MyD88 associates interleukin-1 receptor-associated kinase (IRAK) 1-IRAK4 to form a complex, it will recruit and activate TNFR-associated factor (TRAF) 6 (Deguine and Barton 2014). We observed non-synonymous SNPs in MyD88 and IRAK4 genes (Table S1). Jann et al. 2009 reported that the MyD88 gene is likely candidate genes underlying QTL regions for resistance to pathogens in cattle. MyD88-knockout (KO) mice experiment results indicates the essential role of MyD88 in the LPS activated innate immune signaling (Kawai et al. 1999). TRIF acts as an essential adaptor in the MyD88-independent pathway of TLR3 signaling (Oshiumi et al. 2003). Further, TRIF-knockout mice show impairment in both TLR3- and TLR4-mediated IRF3 activation and the expression of IFN- and IFN-inducible genes (Yamamoto et al. 2002). This non-synonymous variations in the MyD88 and IRAK4 genes may likely play a role in disease resistance traits observed in the indicine cattle.

Genomic variants in the transcription factors play a very essential role in the expression of antiviral type I IFNs and various pro-inflammatory cytokines and chemotactic factors. In TLR-dependent signaling, IRF3/IRF7 primarily functions as transcription factors for antiviral

type I IFN production. The upstream kinase complex TBK1/IKK activates the IRF3 transcription factor, which induces type I IFN expression mediated by TLR3/4 ligands (Fitzgerald et al. 2003). Similarly, activation of the IRF7 transcription factor induces type I IFN production through the MyD88-dependent TLR7/9 signaling pathway (Kawai and Akira 2011.). Further, NF- κ B transcription factor activation is largely responsible for the production of various proinflammatory cytokines and chemotactic factors after TLR ligand engagement (Table S2). In our study, we find missense variants in IRFs (IRF3/IRF7) and NF- κ B transcription factors (Table S1). Among these transcription factors, IRF3 is a likely interesting candidate gene in the context of resistance to viral infections (Pina-Vazquez et al. 2007; Shoemaker et al. 2009; Jann et al. 2009). Similarly, Jann et al. 2009 identified TLR1, MyD88, and IRF3 as the probable candidate genes for the resistance to pathogens in cattle and other livestock species.

Retinoic acid-inducible gene I (RIG-I)-like receptors

The RIG-I-like receptor (RLR) family is comprising of RIG-I, melanoma differentiation-associated gene 5 (MDA5), and LGP2 (Takeuchi and Akira 2010; Mishra et al. 2017). These cytosolic proteins have two N-terminal caspase recruitment domains (CARDs), a central DEAD-box helicase/ATPase domain, and a C-terminal regulatory domain (Takeuchi O & Akira S, 2010). RLRs recognize the genomic RNA of dsRNA viruses and establish the antiviral state of the cell by induction of type I interferon expression (Yoneyama et al. 2004). After dsRNA binding into RIG-I, and consequently, there are conformational changes that expose the CARDs. The CARD domains are ubiquitinated by IFN-inducible E3 ubiquitin ligase, tripartite motif 25 (TRIM25) (Takeuchi and Akira 2010). The CARDs of RIG-I interacting with the N-terminal CARD-containing adaptor mitochondrial antiviral signaling protein (MAVS) (Seth et al. 2005). This modification is required for the activation of RLR signaling. The downstream signaling molecules for the expression of IFN-inducible genes are

shared between the MAVS and TRIF signaling pathways (Takeuchi and Akira S 2010; Xu et al. 2019). MAVS activates signaling cascades leading to the expression of type I IFN genes via activation of IRF3/7 transcription factors (Goubau et al. 2013; Mishra et al. 2017). Further, MAVS signaling induces activation of NF- κ B and the production of pro-inflammatory cytokines and chemokines (Goubau et al. 2013; Xu et al. 2019). We observed non-synonymous variants in RIG-I, TRIM25, and MAVS genes (Table S1). Cargill et al. 2006 reported a single SNP in the RIG-1 gene in bovine.

Nucleotide oligomerization domain (NOD)-like receptor

The NOD-like receptors (NLRs) family consists of a large cytosolic receptor family that responds to bacterial, viral, and fungal infections (Motta et al. 2015). The noninflammasome-forming NOD1 and NOD2 and the inflammasome-forming NALP3, NLRC4, NLRP1, NLRP6, NLRP7, and NLRP12 play vital roles in innate immune responses (Xu et al. 2019). Activation of NOD1 and NOD2 receptor recognize bacteria-derived structures resulted in activation of the canonical NF- κ B and MAPK pathways (Fig. 1), which induce proinflammatory cytokine genes (Chen et al. 2009; Magalhaes et al. 2011). We identified the non-synonymous SNPs in NOD1, and NOD2 genes (Table S1). Pant et al. (2007) identified that a single SNP in the coding region of the NOD2 gene, which was associated with a mastitis-related trait.

Interferon stimulated genes (ISGs) signaling pathway

Activation of the various PRRs resulted in the production of IFNs, pro-inflammatory cytokines, and chemokines (Table S2) (Takeuchi and Akira 2010). Three IFN types have been identified, such as type I IFN (IFNA and IFNB), type II (IFNG), and type III (IFNL) (Chelbi-Alix MK and Wietzerbin J, 2007). The type I and II IFNs genes bind to IFNAR1/IFNAR2 and IFNGR1/IFNGR2 corresponding receptors of the same cell or neighboring cells, which activate the Jak-STAT signaling (Fig. 1). Activation of Jak-STAT signaling leads to the

phosphorylation and nuclear translocation of IFN-stimulated gene factor 3 (ISGF3), a complex comprising of signal transducer and activator of transcription 1 (STAT1), STAT2, and IRF9. ISGF3 induces the expression of IFN-inducible antiviral genes such as MX1, OASL, IFITM5, IFITM3, IFIT5, PKR, TGFB1, GBP 1, GBP 7, and RSAD2 among others (Mishra et al. 2017). We identified non-synonymous variants in IFNAG, IFNB, OASL, IFITM5, IFITM3, IFIH1, and PKR genes in the zebu cattle genome (Table S1). Polymorphisms in some of these genes may likely be associated with resistance to viral infections in mice and humans have been reported (Sadler and Williams 2008). However, very limited information is only available for bovine. The type I interferons of bovine are polymorphic and associated with BHV-1 infections (Ryan et al. 1993; Muller-Doblies et al. 2002). The MX genes were expressed in response to BHV-1, BRV, and single-stranded RNA paramyxoviridae virus infection in cattle (Nakatsu et al. 2004; LeRoy et al. 2005). The OASL protein degrades the viral RNA in the cytosol and polymorphisms in the OASL gene have been associated with flavivirus resistance in mice (Perelygin et al. 2005, 2006a). PKR protein effectively reducing viral replication by decreases viral protein synthesis (Chinsangaram et al. 2001, Gil et al. 2006). Previous literature reported the evidence for polymorphisms in some of these ISGs in cattle (Ryan and Womack 1993; Nakatsu et al. 2004), however, no associations with disease resistance have been defined in cattle (Glass et al. 2010). Collectively, our results indicate that activation of various innate immune pathways and the presence of nonsynonymous variations in TLRs, RLRs, NLRs, signaling adaptor molecules, transcription factors, and IFN-inducible genes may likely enhance disease resistance immune response against infectious diseases in indicine cattle breeds.

Molecular mechanism of heat shock protein (HSP) synthesis and function related to thermo-tolerance

Heat shock proteins (HSPs) genes are very extensively studied genes that are associated with thermo-tolerance in cattle (Archana et al. 2017). The expression of the HSPs is mainly

controlled at the transcription level by a family of heat shock transcription factors (HSF) (Morimoto 1993). Under heat stress or hyperthermia conditions, the HSF1 dissociate from HSP to form trimers of the HSF1 complex (Pirkkala et al. 2001). In the nucleus, the homotrimeric HSF1 binds with the heat shock elements (HSE) present in the promoter region of the HSPs genes resulted in enhanced HSP gene transcription (Guertin and Lis 2010). The following heat shock factors such as HSF1, HSF2, HSF2BP, HSF4, and HSF5 were expressed in cattle. Among these HSFs, HSF1 is the major regulator of HSP70 transcription in eukaryotes (Trinklein et al. 2004). Further, HSF1 has been associated with carbohydrate metabolism, transport, cytoskeleton, and ubiquitination during heat stress conditions in cattle (Page et al. 2006).

HSP increases the thermo-tolerance of animals through their functioning as molecular chaperones (Gupta et al. 2013; Belhadj Slimen et al. 2015). The HSP maintains protein homeostasis through facilitating the assembly and translocation of newly synthesized proteins, prevention of inappropriate protein aggregation, and repairing and refolding of aggregated proteins during cellular response to heat stress (Table S3) (Hightower 1991; Moseley 1997; Feder and Hofmann, 1999). In the cattle genome, 26 genes encoding molecular chaperones that may contribute heat tolerance to zebu cattle have been identified. These included members of HSP70 family (HSPA4L, HSPA8, HSPA1A, HSPA2, HSPA1L, HSPA6, HSPA4, HSPA5, HSPA13, and HSPA9), HSP60 (HSPD1), HSP90 (HSP90AA1, HSP90AB1, HSP90B1), HSP10 (HSPE1), HSP110 (HSPH1) and HSP40 (DNAJA1, DNAJB1, DNAJC1, DNAJC8, DNAJC3, DNAJC17, etc.). We identified non-synonymous variants in HSF1, HSP70 family (HSPA4L, HSPA1A, HSPA1L, HSPA6, HSPA4, HSPA5, HSPA13, and HSPA9) and HSP90 family (HSP90B1) genes in indicine cattle breeds (Table S1).

Among the HSPs, the HSP70 and HSP90 genes are mainly associated with thermo-tolerance development in livestock species (Feder et al. 1999; Hue et al. 2013; Belhadj Slimen

et al. 2015). HSP70 family in bovines, HSP70-1 (HSPA1) and HSP70-2 (HSPA1L) proteins are highly conserved, and most abundant proteins (Hansen 2004; Basiricò et al. 2011; Salces-Ortiz et al. 2013; Kishore et al. 2014). The upregulation of HSP70, HSP90, HSP32, HSP40, HSP60, and HSP110 proteins were observed and playing a crucial role in thermal adaptation in cattle, buffalo, broilers, sheep, and goats (Mazzi et al. 2003; Yu et al. 2008; Mishra et al. 2011; Gupta et al. 2013; Banerjee et al. 2014; Mehla et al. 2014; Pawar et al. 2014; Shaji et al. 2015).

The overexpression of HSP70 in the intestine significantly improves digestion and absorption function in the broiler during heat stress (Table S3) (Hao et al. 2012). Further, the ATPase activity of HSP70 is regulated by HSP40 through interacting with the J domain of the HSP70 proteins (De Maio 1999). HSP90 proteins assist in protein folding and protein stabilization (Table S3) (Panaretou et al. 2002). The HSP protein in extracellular fluids is involved in immuno-modulation through the signaling of innate and acquired immune pathways (Table S3) (Mishra et al. 2011). Further, the HSP70 along with HSP27 and HSP90 proteins is involved in the inhibiting of apoptosis in mammalian cells (Table S1) (Garrido et al. 2001). The previous study suggesting the role of HSP70 in the induction of antitumor immunity through the production of chemokines from tumor cells via the TLR4 signaling pathway in mice (Chen et al. 2009). Chen et al. (2009) reported that HSP70 (including HSP70i and HSC70) is the major HSP responsible for the autocrine induction of chemokines from tumor cells. In *Bos indicus* breeds, the reproductive performance difference observed in these breeds has been associated with polymorphism in HSP70 (Table S3) (Rosenkrans et al. 2010; Cushman 2013). Variations in the HSP70 family genes and other HSP genes are a potential strategy adopted by different cattle breeds to different seasons. Hence, the HSP genes serving as the suitable genetic markers for the selection of thermal tolerance cattle (Kumar et al. 2015). The previous studies reported that polymorphisms in HSP genes associated with heat-tolerant

such as HSP90AB1, in Thai native cattle, the 5'-UTR region of HSP70 in Holstein cows (Basirico et al. 2011), the HSF1 gene, and HSP70A1A gene in Chinese Holstein cattle (Li et al. 2011; Deb et al. 2014). Our results were in close agreement with the previously published studies. In summary, our functional annotation results and non-synonymous variations in HSP genes would explain part of tropical cattle adaptability of zebu cattle breeds.

The prolactin-releasing hormone (PRLH) stimulates prolactin release and prolactin has an influence on hair morphology and thermo-tolerance in the taurine Senepol cattle (Fig. 1) (Littlejohn et al. 2014; Vijayakumar et al. 2019b). Further, the increased plasma prolactin concentration is to be connected with the altered metabolic state of heat-stressed animals (Sunil et al. 2011; Gupta et al. 2013). In indicine cattle breeds, we identified missense variants in the PRLH gene (Table S1). These results together suggest that the PRLH mutation possibly confer a selective advantage to the thermo-tolerance mechanism of indicine cattle. Heat stress rises the production of a large number of free radicals and reactive oxygen species (ROS) which cause cytotoxicity (Sunil et al. 2011; Belhadj Slimen et al. 2015). In this condition, the host activates the oxidative stress response through increased activities of antioxidant enzymes like SOD1, GPX7, GSTM2, GSTM4, SLC23A1, and SLC23A1 to destroy free radicals and maintain hemostasis in the body (Table S3) (Belhadj Slimen et al. 2015; Davidson et al. 1996; Sunil et al. 2011; Gupta et al. 2013; Chen et al. 2016). Kim et al. 2014 suggested that variations in antioxidant enzymes like the SOD1 gene possibly play an important role in heat tolerance traits observed in African cattle. Similarly, we observed missense variants in the SOD1 gene in indicine cattle breeds (Table S1).

A genetic variation identified in the BHMT2, PLCB4, CFTR, ITPR2, SLC9A4, and MC5R genes may likely play important role in the thermotolerant mechanism of zebu cattle (Table S1). The BHMT2 gene is involved in the regulation of homocysteine metabolism with beneficial effects in heat-stressed animals through its activity against osmotic stress and

protection from protein denaturation (Cottrell et al. 2015; Del Vesco et al. 2015). The PLCB1 and PLCB4 are enzymes involved in the energy metabolism process and positively selected in bovines in response to adaptation to dry arid environments (Kim et al. 2015; Li et al. 2019). The zebu cattle lose incredible amounts of heat through the sweating mechanism (Akers and Denbow 2008). Taye et al. (2017) reported that the genes such as ITPR2, ITPRIP, CFTR, SCNN1D, and SLC9A4 were involved in the thermal sweating of African cattle. Further, Wnt signaling and calcium signaling pathways likely responsible for thermal sweating were identified in the KEGG pathways analysis (Fig. 1) (Cui et al. 2014; Cui and Schlessinger 2015; Taye et al. 2017). MC5R regulates exocrine glands and controls the secretion of sebum (Zhang et al. 2006). Sebum lubricates skin and hair with the function of hydrophobic protection against overwetting and for thermal insulation in cattle (Zouboulis 2004; Akers and Denbow 2008).

Mammalian skin hair and coat color provide physical protection and thermoregulation functions in cattle (Jian et al. 2014). Cattle have a thermo-tolerant mechanism through the enhanced conductive and convective heat loss and reduce the absorption of solar radiation (Hansen 2004; Lenis Sanin et al. 2016). Previous studies reported the non-synonymous variation in genes such as SLC45A2, MLPH, RAB17, and ATRN involved in the regulation of melanogenesis processes is very important for the differences in cattle breeds in pigmentary traits (Sturm 2009). Similarly, we identified non-synonymous variations in the above said gene list in the zebu cattle genome (Table S1). In summary, we identified various non-synonymous variations in the majority of candidate genes and the biological processes may likely be related to thermo-tolerance in indicine cattle breeds. Our results were in close agreement with the previous studies involved in different cattle breeds (Bolormaa et al. 2011; Fortes et al. 2012; Lee et al. 2013; Gurgul et al. 2016; Taye et al. 2017a; 2017b; Dias et al. 2017; Nayeri et al. 2017; Edea et al. 2018; Alshawi et al. 2019). However, the limitation of the present study is

there is a possibility of obtaining false-positive results as common in this kind of study. Hence, validation with GWAS, candidate gene approach, and gene expression analysis are suggested.

Conclusion

This study provides a comprehensive overview of the molecular mechanism of disease resistance and thermo-tolerance of Indian subcontinent originated cattle breeds. So far, the available literature information on disease resistance and thermo-tolerance of *Bos indicus* cattle breeds mostly belong to African zebu cattle breeds. Only very few studies were available for Indian subcontinent originated cattle breeds. Hence, our study results may likely provide the molecular pathways putatively responsible for these traits of the cattle breeds that originated in the Indian subcontinent. Further, this molecular mechanism of specific climate-resilient adaptive animals to Indian subcontinent tropical environments is currently required to combat climate change-related adverse effects and to sustain livestock production systems in Indian subcontinent countries. The identified genetic variants provide a useful resource for further studies to phenotypically differentiate *Bos taurus* and *Bos indicus* cattle breeds.

Competing Interests

The authors have declared that no competing interests exist.

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Data Availability

The datasets were used for this analysis are from previously submitted datasets in NCBI which have been cited.

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832 **Table 1.** Details of the whole genome dataset are included in the study.

S. No	Project accession	Run accession	Description	Submitted by	URL	Reference
1	PRJNA496267	SRR8156151	Sahiwal	Big Data Center	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8156151	Iqbal et al. 2019
		SRR8156141	Sahiwal	Big Data Center	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8156141	
		SRR8156150	Red Sindhi	Big Data Center	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8156150	
		SRR8156142	Tharparkar	Big Data Center	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8156142	
		SRR8156149	Tharparkar	Big Data Center	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8156149	
		SRR8156146	Hariana	Big Data Center	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8156146	
2	PRJNA493191	SRR8053288	Kangayam	AgriGenome Labs Pvt. Ltd., Kerala, India	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8053288	-
		SRR8053289	Tharparkar	AgriGenome Labs Pvt. Ltd., Kerala, India	https://www.ncbi.nlm.nih.gov/sra/?term=SRR8053289	

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837 **Table 2.** The number of variants identified in different *Bos indicus* cattle breeds.

S. No	Type of variants	Kangayam	Tharparkar	Sahiwal	Red Sindhi	Tharparkar_Pakistan	Haryana
1	SNP	1,95,40,715	1,84,47,104	3,02,72,761	1,77,67,764	3,36,33,054	2,12,02,095
2	MNP	19,51,318	15,88,122	13,72,995	10,97,380	16,24,353	8,20,756
3	INS	8,49,551	7,99,245	6,99,406	5,89,231	8,72,434	6,20,425
4	DEL	9,00,655	8,24,621	6,84,992	6,84,044	8,81,898	6,18,835
5	MIXED	4,51,688	4,91,832	2,12,097	1,95,019	2,79,742	1,52,790
	Total	2,36,93,927	2,21,50,924	3,32,42,251	2,03,33,438	3,72,91,481	2,34,14,901

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843 **Table 3.** The number of effects by impact identified in different Bos indicus cattle breeds.

S. No	Type of variants	Kangayam	Tharparkar	Sahiwal	Red Sindhi	Tharparkar_Pakistan	Haryana
1	HIGH	17,078	23,017	51,104	8,700	31,282	18,013
2	LOW	3,03,074	3,07,052	18,62,740	3,05,130	5,22,767	3,96,765
3	MODERATE	1,83,068	1,83,087	12,65,155	1,61,896	6,06,921	4,24,566
4	MODIFIER	9,46,39,357	9,06,99,322	15,49,96,738	8,62,15,209	9,38,13,969	6,04,77,873
5	Ts/Tv ratio	2.166	2.1931	2.1662	2.2634	2.24	2.1901

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855 **Table 4.** The number of effects by type in different *Bos indicus* cattle breeds.

S.No	Type of variants	Kangayam	Tharparkar	Sahiwal	Red Sindhi	Tharparkar_Pakistan	Haryana
1	3_prime_UTR_variant	3,96,922	3,85,074	11,16,812	3,78,124	7,88,568	5,77,996
2	5_prime_UTR_premature_start_codon_gain_variant	12,622	13,230	29,891	13,452	22,908	16,657
3	5_prime_UTR_variant	91,253	97,645	3,12,672	99,839	2,00,295	1,50,644
4	Conservative_inframe_deletion	754	776	695	662	604	367
5	Conservative_inframe_insertion	1,529	2,777	678	469	533	337
6	Disruptive_inframe_deletion	502	579	549	377	329	222
7	Disruptive_inframe_insertion	836	1,388	442	232	307	216
8	Downstream_gene_variant	52,72,628	50,99,841	97,81,382	50,22,053	51,88,496	34,55,491
9	Frameshift_variant	8,056	12,925	3,652	2,863	2,853	1,972
10	Gene_fusion	20	270	0	0	0	0
11	Initiator_codon_variant	30	37	411	33	238	157
12	Intergenic_region	1,47,22,123	1,35,51,397	1,84,58,394	1,21,35,884	2,10,34,988	1,29,02,426
13	Intragenic_variant	7,89,552	7,55,667	12,09,242	7,13,634	3,37,797	2,09,957
14	Intron_variant	3,42,25,372	3,29,94,472	5,59,39,360	3,15,28,499	6,09,69,728	3,96,85,948
15	Missense_variant	1,83,959	1,86,543	12,64,066	1,60,785	6,05,986	4,23,836
16	Non_coding_transcript_exon_variant	1,84,061	1,81,420	4,34,306	1,78,583	2,77,263	2,01,288
17	Non_coding_transcript_variant	3,38,45,164	3,26,89,713	5,84,19,394	3,12,53,278	324	220
18	Splice_acceptor_variant	2,703	2,772	11,056	2,150	7,497	3,742
19	Splice_donor_variant	2,119	2,562	16,799	1,451	6,655	5,989
20	Splice_region_variant	61,035	62,285	2,85,941	57,579	1,38,723	91,239
21	Start_lost	390	381	1,867	338	766	638
22	Stop_gained	4,873	6,909	15,614	1,812	11,925	4,849
23	Stop_lost	391	376	2,564	326	1,933	1,019
24	Stop_retained_variant	158	193	721	157	316	253
25	Synonymous_variant	2,40,578	2,43,219	16,10,092	2,43,662	3,91,382	3,07,235
26	Upstream_gene_variant	51,65,082	49,98,275	95,69,089	49,54,954	51,34,209	33,73,050

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858 **Figure Legends**

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860 Fig. 1. KEGG pathway analysis of genes comprising of high and moderate impact.

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862 Fig. 2. A protein-protein interaction network was constructed based on candidate genes involved in the disease resistance mechanism.

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864 Fig. 3. A protein-protein interaction network was constructed based on candidate genes involved in the thermotolerance mechanism.

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