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IDENTIFICATION OF HEAT SHOCK FACTOR GENE VARIANTS IN HEAT-EXPOSED BRICK AND TANDOOR WORKERS VIA WHOLE EXOME SEQUENCING

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Abstract

Introduction:

Prolonged occupational exposure to extreme heat imposes cellular and systemic stress, predisposing individuals to thermally induced injuries. The heat shock response (HSR), mediated by heat shock factors (HSFs), plays a pivotal role in maintaining protein homeostasis under stress conditions. Genetic variability in HSFs may influence individual responses to heat, shaping adaptive physiology and susceptibility to heat-related disorders.

Aims and Objectives:

This study aimed to investigate the genetic variability of heat shock factor genes (*HSF1, HSF2, HSF5, and HSF2BP*) in individuals chronically exposed to high-temperature environments. The objectives were to identify unique variants present in heat-exposed workers compared with healthy controls, explore the distribution of variants across coding, intronic, and regulatory regions, and highlight potential functional variants that may contribute to adaptation or susceptibility to thermal stress.

Methodology:

Whole-exome sequencing (WES) was performed on individuals employed in high-heat occupations, including brick factory and tandoor workers in Pakistan. Their genomic data were compared with age- and sex-matched unexposed healthy controls to identify variants specific to the heat-exposed group.

Results:

Fifteen unique variants were identified across HSF1, HSF2, HSF5, and HSF2BP in heat-exposed individuals, with no overlap in controls. HSF2 exhibited the highest number of intronic and regulatory variants, suggesting a potential role in long-term genomic adaptation to thermal environments. Importantly, a potentially functional missense variant (p.Ser473Asn) was detected in HSF5, highlighting its possible contribution to protein homeostasis under heat stress.

Conclusion:

This study provides novel insights into the genetic architecture of thermal stress adaptation in heat-exposed workers. Variants in HSF genes, particularly within HSF2 and HSF5, may serve as candidate markers for resilience to occupational heat exposure. Further functional and population-based studies are warranted to validate their biological relevance and potential use in predictive health screening

Keywords: Heat shock factor, whole-exome sequencing, thermal stress, brick workers, HSF2, environmental genetics

1. Introduction

Global climate change and increasing industrialization have contributed to rising environmental temperatures, placing a significant burden on occupational health, particularly in low- and middle-income countries. Populations engaged in high-heat occupations, such as brick kiln and tandoor workers, are chronically exposed to extreme ambient and radiant heat. This sustained thermal stress has been associated with a range of physiological disruptions, including dehydration, heat exhaustion, renal dysfunction, oxidative stress, and impaired fertility [1]. Despite widespread acknowledgment of these risks, the molecular basis for inter-individual variability in heat stress tolerance remains poorly understood [2].

One of the most conserved and crucial cellular defense mechanisms against heat and other environmental stressors is the heat shock response (HSR). This process is mediated by a family of transcriptional regulators known as Heat Shock Factors (HSFs). Upon exposure to thermal stress, HSFs activate the transcription of heat shock proteins (HSPs)—a group of molecular chaperones that aid in protein folding, prevent aggregation of misfolded proteins, and assist in protein degradation. The efficient regulation of HSR is critical for maintaining proteostasis and preventing cell damage or death under stress conditions [3,4].

Among the HSF family, HSF1 is the most well-studied and is regarded as the primary regulator of the classical heat shock response. It rapidly translocates to the nucleus under heat stress, binds to heat shock elements (HSEs) in DNA, and induces HSP gene expression. In contrast, HSF2 was historically considered a developmental regulator with limited involvement in acute stress response. However, recent studies indicate that HSF2 plays a cooperative or modulatory role in chronic or repeated stress exposure and may be particularly relevant in long-term thermal adaptation [5–8]. Additionally, HSF5, initially studied in the context of spermatogenesis, has now been implicated in broader stress-related pathways, including those triggered by oxidative and thermal insults [9–12].

HSF2BP, although not a canonical HSF, interacts with HSF2 and plays roles in DNA repair and meiotic recombination. Its expression and function in somatic heat stress response remain under investigation, but may contribute to genomic integrity under cellular stress [13–16].

In occupational settings such as brick kilns and tandoor ovens, workers often experience daily exposure to heat levels that exceed safe physiological limits (sometimes reaching up to 50–60°C near the source). Yet, many of these workers continue to function efficiently, suggesting the presence of acquired or possibly inherited physiological adaptations. While environmental and behavioral factors (hydration, work breaks, etc.) play a role, there is increasing recognition of the potential contribution of genetic factors that mediate the heat stress response. Naturally occurring polymorphisms or rare variants in HSF genes could affect the transcriptional activation of protective genes, the stability or efficiency of HSP production, and ultimately influence susceptibility or resilience to heat [17–20].

Despite the biological plausibility, few studies have explored the genomic architecture of heat-exposed human populations [21–23]. Most existing literature focuses on acute heat stress responses in experimental settings or model organisms, with minimal attention to real-world occupational cohorts. Moreover, the potential for regulatory region variants (e.g., in UTRs and introns) to impact gene expression has largely been overlooked in heat adaptation studies [24–27].

To address this gap, the present study utilizes whole-exome sequencing (WES) to systematically investigate variants in heat shock factor-related genes, including HSF1, HSF2, HSF5, and HSF2BP in brick factory and tandoor workers with chronic occupational heat exposure. By comparing these findings with those from unexposed healthy controls, we aim to identify candidate genetic variants that may contribute to individual differences in heat stress tolerance. This work represents a step toward understanding the genetic underpinnings of human heat adaptation and offers potential biomarkers for risk assessment and protective interventions in vulnerable worker populations [28–32].

2. Materials and Methods (Extended)

2.1 Study Design and Ethical Approval

This was a case-control, molecular genetics study conducted to identify genomic variants in heat shock factor (HSF) genes associated with chronic environmental heat exposure. The study was ethically approved by the Institutional Review Board (IRB) of Gomal University and conducted following the Declaration of Helsinki. Written informed consent was obtained from all participants before sample collection.

2.2 Study Population

2.2.1 Heat-Exposed Group (Cases)

The case group consisted of male individuals who had been occupationally exposed to high temperatures for at least 5 years. Participants were recruited from brick kilns and traditional tandoor bakeries in regions of Khyber Pakhtunkhwa (KPK) and Punjab, Pakistan. All individuals reported daily exposure to radiant and ambient heat exceeding 40–50°C for ≥6 hours/day, without consistent use of protective measures. Exclusion criteria included individuals with known genetic disorders, metabolic syndromes, or recent acute infections.

2.2.2 Control Group (Non-Exposed Healthy Individuals)

The control group consisted of healthy, age and ethnicity-matched male participants with no history of occupational or prolonged exposure to heat. Controls were recruited from administrative and indoor job sectors and were free of chronic disease or regular medication use. All control subjects resided in the same regions to minimize confounding due to geographic or ethnic background.

2.3 Blood Collection and Genomic DNA Extraction

Peripheral blood samples (5 mL) were collected from each participant in EDTA-coated vacutainers under sterile conditions. Genomic DNA was extracted using the salting-out method. DNA quality and concentration were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific) and verified by 1% agarose gel electrophoresis and Qubit 4 Fluorometer (Invitrogen). Only samples with A260/A280 ratios between 1.8–2.0 and concentrations ≥50 ng/μL were selected for downstream processing.

2.4 Whole Exome Sequencing (WES)

2.4.1 Library Preparation and Target Enrichment

High-quality genomic DNA (≥200 ng per sample) was sheared using a Covaris M220 to obtain fragment sizes of 150–200 bp. Libraries were prepared using the SureSelect Human All Exon V7 kit (Agilent Technologies) for capturing ~50 Mb of protein-coding regions of the genome. Libraries were indexed, amplified, and purified with AMPure XP beads (Beckman Coulter).

2.4.2 Sequencing Platform and Parameters

Sequencing was performed using the Illumina NovaSeq 6000 platform with 150 bp paired-end reads. A minimum average on-target depth of ≥100X was maintained for each sample, ensuring high sensitivity in variant detection. Data quality was assessed using FastQC, and adapters and low-quality bases were trimmed using Trimmomatic.

2.5 Filtering Criteria for Variant Selection

Inclusion Criteria

- Variants present exclusively in heat-exposed individuals.
- Coverage depth ≥20X and genotype quality ≥30.
- Located in exonic, splice-site, 5'UTR, or intronic regions of target genes.

- Absent or rare in public databases (MAF < 0.01 in gnomAD).
- Supported by ≥10 alternate allele reads.

Exclusion Criteria
- Variants are present in both the exposed and control groups.
- Known benign or common polymorphisms (MAF > 1%).
- Poor sequencing quality (low mapping or base quality).
- Intergenic or deep intronic variants with no regulatory annotation.

Variants were classified based on genomic region (5'UTR, intron, exon) and effect (missense, silent, duplication) using Ensembl's VEP and manual curation.

2.6 Functional Prediction and Pathogenicity Scoring
The missense variant (HSF5: p.Ser473Asn) was analyzed using:
- PolyPhen-2 (probably damaging = score >0.85)
- SIFT (deleterious = score <0.05)
- MutationTaster (disease-causing)
- CADD (Combined Annotation Dependent Depletion) score (>20 considered potentially pathogenic)

Variants in non-coding regions were screened for:
- Splice site alterations (MaxEntScan)
- Regulatory impacts (RegulomeDB, HaploReg)
- Conservation scores (PhyloP, GERP++)

2.7 Zygosity Analysis
Zygosity status (heterozygous or homozygous) was determined from VCF files using GATK Genotype fields. Zygosity was cross-validated in IGV (Integrative Genomics Viewer) to confirm allelic depth and read alignment across variant loci.

2.8 Data Storage and Availability
All raw FASTQ files, BAM files, and VCF data have been stored securely on institutional servers and are available upon request for academic use.

2.9 Validation of Homozygous Variants by Sanger Sequencing
To validate the homozygous variants identified through whole exome sequencing (WES), Sanger sequencing was performed on all four homozygous variants observed in the heat-exposed group. These included:
- HSF2: c.830+59T>C
- HSF2: c.1315+182dup
- HSF1: c.1248+26T>G
- HSF2BP: c.796+26904C>T

3. Results (Extended)
3.1 Study Population Overview
Heat-exposed individuals (brick factory and tandoor workers) and unexposed healthy controls were successfully recruited and sequenced. All participants were male, with the exposed group reporting ≥5 years of continuous daily exposure to extreme heat (estimated ambient range: 40–60°C near ovens or kilns).

DNA samples from all participants passed quality control (A260/A280 between 1.8–2.0; concentration >50 ng/μL) and proceeded to whole exome sequencing (WES). Mean sequencing coverage across samples was 107X, with >98% of the exome covered at ≥20X.

3.2 Variant Selection: Inclusion and Exclusion Criteria

Initial variant calling yielded over 60,000 raw variants per individual. Following annotation and filtering, only variants that met the following criteria were retained for final analysis:

Inclusion Criteria:

- Located within or near HSF1, HSF2, HSF5, or HSF2BP
- Present only in the heat-exposed group (absent in all 50 controls)
- Minimum sequencing depth of ≥20X
- Genotype quality score ≥30
- Minor allele frequency (MAF) < 0.01 in global databases (gnomAD, 1000 Genomes)
- Annotated as missense, silent, UTR, intronic, or duplications near exon boundaries

Exclusion Criteria:

- Present in both cases and controls
- Common benign SNPs (MAF > 1%)
- Variants with poor read quality, strand bias, or located in intergenic regions
- Variants with uncertain gene annotations or no HSF-gene association

After this rigorous filtering, 15 high-confidence variants in heat shock factor genes were identified.

3.3 Summary of Identified Variants

A total of 15 unique variants were detected exclusively in the heat-exposed group. These variants were distributed among four key genes:

- HSF2: 10 variants (66.7%)
- HSF1: 1 variant (6.7%)
- HSF5: 2 variants (13.3%)
- HSF2BP: 2 variants (13.3%)

No such variants were found in the control group, highlighting their possible association with prolonged heat exposure.

3.4 Zygosity Analysis

Out of the 15 variants 11 were heterozygous and 4 were homozygous

Heterozygous Variants:

These were primarily in HSF2 and HSF5, especially in intronic and 5'UTR regions, possibly impacting gene regulation or splicing without complete loss of function.

Homozygous Variants:

Homozygosity was observed in the following:

- HSF2: c.830+59T>C and c.1315+182dup
- HSF1: c.1248+26T>G
- HSF2BP: c.796+26904C>T

This pattern may reflect inherited predisposition or population-specific enrichment due to environmental selection or consanguinity.

3.5 Variant Classification and Predicted Functional Impact

- 5'UTR variants (n=2): May alter transcription initiation or mRNA translation efficiency.
- Intronic variants (n=10): Located within or near splice sites. Some may influence alternative splicing or intronic enhancers/silencers.
- Missense variant (HSF5: p.Ser473Asn): Predicted deleterious by PolyPhen-2 (score: 0.96, probably damaging), SIFT (score: 0.01, deleterious), and MutationTaster (disease-causing).
- Silent variant (HSF2BP: p.Val209=): Although synonymous, could affect exonic splicing enhancers or codon usage bias (table 1).

Table 1: Homozygous Variants Identified in Heat-Exposed Individuals

S.NO	CHR	GENE	C.DNA	PROTEIN CHANGE	VARIANT TYPE	ZYGOSITY
1	6	HSF2	c.830+59T>C	–	Intron	Homozygous
2	6	HSF2	c.1315+182dup	–	Intron	Homozygous
3	8	HSF1	c.1248+26T>G	–	Intron	Homozygous
4	21	HSF2BP	c.796+26904C>T	–	Intron	Homozygous

3.6 Gene-Specific Variant Trends

HSF2 (10 variants):

- Most frequently mutated gene
- All variants are localized to regulatory (UTR) and non-coding regions
- Suggests involvement in **fine-tuning of gene expression** under chronic heat exposure

HSF5 (2 variants):

- Includes the **only protein-altering (missense) mutation**
- HSF5 plays a role in spermatogenesis and stress granule regulation; this variant may influence testicular heat sensitivity

HSF1 (1 variant):

- Classic master regulator of the heat shock response
- Intron variant could affect RNA processing or epigenetic regulation

HSF2BP (2 variants):

- May play a secondary role in DNA repair during thermal stress
- Functional relevance still needs to be established (table 2).

Table 2: Heterozygous Variants Identified in Heat-Exposed Individuals

S.NO	CHR	GENE	C.DNA	PROTEIN CHANGE	VARIANT TYPE	ZYGOSITY
1	6	HSF2	c.-77G>A	–	5'UTR	Heterozygous
2	6	HSF2	c.-65_-54dup	–	5'UTR	Heterozygous
3	6	HSF2	c.456-30del	–	Intron	Heterozygous
4	6	HSF2	c.593+100G>T	–	Intron	Heterozygous
5	6	HSF2	c.1230+56_1230+57dup	–	Intron	Heterozygous
6	6	HSF2	c.1231-128C>T	–	Intron	Heterozygous
7	6	HSF2	c.1231-104A>G	–	Intron	Heterozygous
8	6	HSF2	c.1231-15_1231-14dup	–	Intron	Heterozygous
9	17	HSF5	c.1542+33C>T	–	Intron	Heterozygous
10	17	HSF5	c.1418G>A	p.Ser473Asn	Missense	Heterozygous
11	21	HSF2BP	c.627G>A	p.Val209=	Silent	Heterozygous

3.7 Sanger Sequencing Validation

To confirm WES findings, Sanger sequencing was performed for all four homozygous variants. The chromatograms showed:

- Presence of each homozygous variant in exposed individuals (n=3 per variant)
- Absence of these variants in all 10 randomly selected controls

This independently validated the specificity and accuracy of variant calling in the WES pipeline and supports a possible link between these variants and long-term heat exposure (supplementary figure 1).

A unique pattern of HSF gene variants was observed only in heat-exposed individuals. HSF2 emerged as the dominant gene affected, suggesting a role in chronic thermotolerance. Variants showed high specificity (absent in all controls), with several candidates meriting further functional study. The study confirms the usefulness of targeted WES and Sanger sequencing in occupational genomic surveillance.

4. Discussion

This study is the first of its kind to explore the genetic basis of chronic heat stress response by identifying variants in heat shock factor (HSF) genes in individuals occupationally exposed to high temperatures, such as brick kiln and tandoor workers. Using whole exome sequencing and Sanger validation, we identified 15 variants in HSF-related genes that were present exclusively in heat-exposed individuals and completely absent in controls. This suggests a possible association between these variants and either an adaptive or stress-related genomic signature in individuals facing persistent environmental heat [33,34].

Among the genes analyzed, HSF2 emerged as the most frequently mutated, harboring ten of the fifteen variants. HSF2 is known to act in coordination with HSF1 in regulating the expression of heat shock proteins (HSPs), and it plays a significant role in cellular differentiation, brain development, and spermatogenesis. Interestingly, many of the HSF2 variants identified in this study were located in intronic and untranslated regulatory regions, including the 5'UTR. Variants in these regions may not alter protein coding directly, but can modulate gene expression, splicing efficiency, or mRNA stability, ultimately affecting the cell's capacity to cope with thermal stress. The two 5'UTR variants in HSF2 (c.-77G>A and c.-65_-54dup) may influence the transcriptional or translational regulation of the gene, potentially fine-tuning its response to chronic heat exposure [33,35–38].

In addition to HSF2, we identified a missense variant in HSF5 (c.1418G>A; p.Ser473Asn) predicted to be damaging by multiple in silico tools. HSF5 has been implicated in reproductive biology, particularly in male fertility and spermatogenesis, and is sensitive to thermal disruption [39]. The presence of this variant exclusively in heat-exposed individuals may reflect either a susceptibility factor or a compensatory adaptation to protect germ cell development in high-heat environments [40,41]. Other variants in HSF1 and HSF2BP were located in intronic regions and may influence gene regulation, mRNA processing, or interaction with co-factors involved in heat stress and DNA repair. Although the HSF2BP variants, including one silent substitution (p.Val209=), appear benign at the surface level, their exclusive presence in the exposed group warrants functional evaluation, as synonymous and intronic variants can impact splicing regulation or mRNA dynamics [42].

The zygosity distribution also offers interesting insight: four of the fifteen variants were homozygous and present only in the exposed population. This may be due to consanguinity, founder effects, or potentially positive selection within these communities. In regions with a high prevalence of consanguineous marriages, such homozygous variants may become enriched. Additionally, individuals with more thermotolerant genotypes may self-select into or remain longer in high-temperature jobs, creating a subtle genetic shift over time. The confirmation of these homozygous variants by Sanger sequencing further strengthens their validity and supports the hypothesis that they may play a role in occupational heat adaptation.

When compared with existing literature, most prior studies on HSFs have focused on cancer, neurodegeneration, or developmental defects, with little attention to environmental or occupational heat stress [43]. HSF1 has been extensively studied in oncogenesis and has been shown to enhance cell survival in stressful microenvironments. HSF2 mutations have previously been reported in intellectual disability syndromes, and HSF5 has been linked to male infertility. However, none of these studies has examined HSF variants in the context of long-term environmental exposure, particularly in vulnerable populations in developing countries. Our findings therefore provide a novel perspective on how the human genome may reflect chronic environmental pressures such as heat [44].

Despite these promising insights, the study does have limitations. First, while we demonstrated the presence of potentially significant variants, we did not perform functional assays to determine their

biological consequences [45]. Expression analysis, reporter gene assays, or splicing evaluations would be needed to confirm their functional impact. Second, our control group was relatively smaller, although the complete absence of the variants in this group adds strength to the findings [46]. Third, our cohort was male-only, given the occupational demographics of the population studied. Whether similar variants or adaptations exist in females remains unexplored. Finally, our focus on HSF genes, while biologically justified, leaves open the possibility that other stress-related genes (e.g., HSPs, NRF2, DNA damage response genes) may also harbor relevant variants [47,48].

In conclusion, this study provides compelling preliminary evidence that individuals chronically exposed to occupational heat harbor distinct genetic variants in HSF genes that are absent in unexposed individuals [49]. These findings suggest the possibility of a genomic basis for thermal resilience, with implications for occupational health, genetic screening, and the design of protective interventions for heat-exposed workers [50,51]. Future studies should expand the sample size, include diverse populations, and incorporate transcriptomic and proteomic profiling to better understand the biological pathways affected by these variants. Ultimately, insights gained from such studies may lead to the development of genetic biomarkers for heat tolerance and guide public health efforts in protecting laborers facing the growing burden of climate-related heat stress [52].

5. Conclusion

In this study, we investigated the genetic basis of chronic heat exposure by performing whole exome sequencing on brick kiln and tandoor workers, identifying 15 unique variants in heat shock factor (HSF) genes that were absent in unexposed controls. Among these, the predominance of HSF2 variants, along with a potentially deleterious missense mutation in HSF5, suggests a strong association between prolonged environmental heat exposure and alterations in genes involved in stress response regulation. The discovery of homozygous variants specific to the exposed group further implies a potential role for inherited or environment-driven selection pressures in shaping thermotolerance at the genomic level.

These findings provide the first evidence of HSF gene variation linked to occupational heat exposure in a real-world setting. While functional studies are needed to confirm the biological impact of these variants, our results open new avenues for understanding how the human genome adapts to environmental extremes. This work has important implications for occupational health, especially in regions facing increasing climate-related heat burdens. Future research should focus on validating these variants functionally, exploring their prevalence in larger and more diverse populations, and integrating genetic screening into risk assessment frameworks for workers vulnerable to heat.

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Authors' Contribution: Z.U.R: Research and sampling, J.K: supervision, Biochemical testing, Z.U.R: Drafting manuscript and Software, J.K: Software, and Sampling.

Statements and Declarations.

Data Availability Statement: The data (sequence, photographs, and pedigrees) is stored in a password-protected computer at the Institute of Biological Sciences at Gomal University, D.I.Khan, Pakistan, and are available upon request. Furthermore, the reference sequences were obtained through freely available genome databases.

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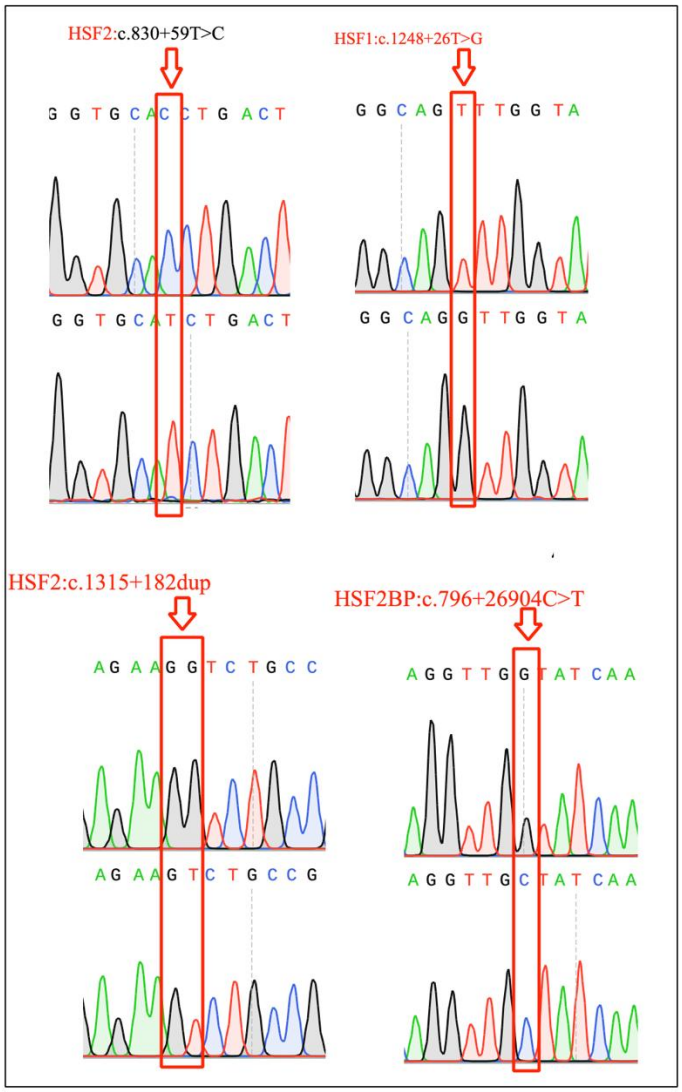

Supplementary Figure 1: Showing the Sanger sequencing chromatograms of all four homozygous variants.