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Impact of zinc and chromium deficiency on gene expression in type 2 diabetes mellitus

Humma Nayyar, Attya Bhatti✉ & Peter John

The increasing prevalence of type 2 diabetes mellitus (T2DM), largely attributable to poor dietary habits and nutritional imbalances, highlights the need for novel diagnostic and therapeutic approaches. Emerging evidence emphasizes the critical role of trace elements in the pathogenesis and management of T2DM. However, the molecular mechanisms through which zinc and chromium deficiencies contribute to disease progression remain largely unexplored. This study aimed to investigate the role of zinc and chromium in T2DM pathogenesis through a combination of bioinformatics analysis, molecular docking, trace element profiling, and gene expression validation. Relevant genes identified from the literature were analyzed using a bioinformatics pipeline to uncover hub gene networks and regulatory pathways associated with trace element deficiencies. Molecular docking of HUB genes with zinc and chromium enriched compounds were employed to uncover the new therapeutic approach. For experimental validation, serum zinc and chromium levels were measured in fifty T2DM patients and fifteen healthy controls using atomic absorption spectrophotometry. Gene expression profiling of GCK (zinc associated) and GLUT4 (chromium associated) was performed using real time PCR. Bioinformatics analysis revealed that HUB genes affected due to zinc and chromium deficiency were linked to an elevated risk of T2DM. Crucial metabolic pathways was altered due to zinc and chromium deficiency in T2DM.The serum concentration of zinc and chromium were markedly lower in T2DM patients as compared to controls. Level of zinc was significantly lower in T2DM patients with nephropathy and chromium level was significantly were lower in T2DM patients with CVD. Expression of *GCK* and *GLUT4* was reduced by approximately 4–6 folds and 3–4 folds, respectively, in T2DM and its associated complications. The study provide novel insights into the gene-environment interaction driving T2DM and highlight the therapeutic approach of zinc and chromium supplementation in mitigating diseases progression. This study paves the way for future research into personalized nutritional interventions targeting gene expression abnormalities in diabetes management.

Keywords Chromium, Gene expression, Regulatory networks, Trace elements, Therapeutic targets, Zinc

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that has emerged as a one of the most pressing global health challenge of the 21 st century[1]. The prevalence of T2DM is increasing at an alarming pace, currently affecting nearly 2.8% of the global population and expected to exceed 11% by 2026[2].Specifically, South Asia, has experienced a dramatic surge in cases over the past three decades, making the region a critical focus for research and intervention[3].T2DM is characterized by insulin resistance and chronic hyperglycemia, where impaired insulin action across key tissues and subsequent β-cell exhaustion together drive disease progression[4].Despite considerable advances in understanding its etiology, the molecular mechanisms linking lifestyle, nutrition, and gene regulation in T2DM remain incompletely understood. Among nutritional factors, trace elements have gained increasing attention due to their pivotal roles in growth, development, and metabolic regulation. Although trace elements are present at concentrations below 100 µg/g, their imbalance can disrupt essential metabolic pathways and has been increasingly implicated in the pathogenesis of T2DM[5].Their concentrations vary across individuals due to dietary intake, environmental exposures, and genetic factors[6].Altered trace element levels can disrupt cellular homeostasis, leading to changes in gene expression and protein function[7–8]. Understanding these molecular interactions is essential to explain how micronutrient imbalances contribute to the development and progression of T2DM[9,10].

Department of Biomedicine, Atta ur Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad 44000, Pakistan. ✉email: attyabhatti@asab.nust.edu.pk

Zinc is indispensable for pancreatic β-cell function, where it stabilizes insulin in secretory granules[13–12]. Cellular homeostasis is maintained through *zinc transporters*, including *Metallothioneins (MTs)*, *importers (ZiPs/SLC39)*, and exporters *(ZnTs/SLC30)*[16–14]. Dysregulation of these transporters has been linked to altered expression of key genes such as *Glucokinase (GCK)* and *Zinc transporter 8* (*ZnT8)*, both integral to glucose sensing and β-cell activity[20–16]. Clinical evidence also supports zinc's therapeutic potential: randomized controlled trials and meta-analyses have demonstrated that zinc supplementation can improve fasting glucose, HbA1c, and insulin resistance, highlighting its potential as a promising adjunct therapy in T2DM management[17].In addition to zinc, chromium also plays a critical role in regulating carbohydrate, lipid, and protein metabolism[12]. Since the 1950 s, it has been implicated in glucose regulation[18]. Chromium enhances insulin sensitivity by modulating *Insulin receptor substrate 1* (*IRS-1)*, *Akt phosphorylation*, and *PI3-kinase* activity, while also regulating transcription factors such as *Peroxisomes proliferator activated receptor gamma* (*PPARγ)*, which influence adipogenesis and glucose metabolism[25–20].Any deficiency in chromium has been linked to oxidative stress, impaired *Glucose transporter type 4* (*GLUT4)* translocation, and reduced glucose uptake, all of which contribute to insulin resistance[20,21]. A recent population-based study showed that higher dietary intake of chromium and selenium was associated with a greater likelihood of reverting from pre-diabetes to normal glucose regulation, emphasizing its clinical importance[22]. Nevertheless, evidence regarding chromium remains less consistent than zinc, with mixed results across intervention studies, underscoring the need for further investigation.

Despite these insights, most existing studies have focused primarily on circulating levels of zinc and chromium or their general metabolic effects, while their direct influence on gene expression and molecular pathways remains underexplored[23].This gap is particularly critical in regions like South Asia, where high rates of T2DM coincide with widespread micronutrient deficiencies, amplifying disease burden and complicating management. Recent advances in computational biology, high-throughput sequencing, and serum profiling provide powerful opportunities to explore these mechanisms[32–25]. Zinc has consistently demonstrated strong associations with insulin signaling and antioxidant defense, whereas chromium has been suggested to influence glucose metabolism, though evidence remains less conclusive[26,27]. To address this gap, the present study aims to systematically investigate the hypothesis that alterations in zinc and chromium levels can modulate gene expression, thereby contributing to T2DM pathogenesis. We employ a multi-level approach that combines: (1) computational analyses to identify T2DM-associated genes linked to zinc and chromium metabolism, (2) molecular docking to model their interactions with key protein targets, and (3) clinical validation through serum profiling and gene expression analysis in a patient cohort. By integrating nutritional, computational, and molecular approaches, this study seek to uncover novel biomarkers and therapeutic targets that can advance diagnostic precision and inform personalized strategies for T2DM management.

Methods and materials

In-silico analysis

Gene selection criteria

We systematically searched PubMed for peer-reviewed articles published within the past 15 years. Our aim was to identify candidate genes potentially influenced by zinc and chromium status in T2DM.Search terms included combinations such as *"zinc AND gene expression AND diabetes"*, *"chromium AND glucose metabolism"*, *"GCK AND T2DM"*, and *"GLUT4 AND insulin signaling"*. We included original studies in humans or validated animal models that reported gene expression or molecular outcomes relevant to zinc and chromium in T2DM. We excluded reviews, case reports, conference abstracts, and studies not involving zinc/chromium or not reporting gene-level data. The search initially retrieved 256 records; after screening titles and abstracts, 78 articles were reviewed in full text, and 24 studies were finally included for candidate gene retrieval. The detailed study selection process is illustrated in the PRISMA flow diagram (Supplementary data figure S2).

Additionally, we mined gene expression datasets from the NCBI Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/,) to identify differentially expressed genes in T2DM-related tissues, including peripheral blood mononuclear cells, adipose tissue, and liver (accessed 16 July 2024). Where applicable, Ensemble (https://asia.ensembl.org/index., release 112, accessed 16 July 2024) was used to verify gene annotations and transcript information. To provide baseline expression context, we focused on GTEx expression profiles across metabolic tissues (pancreas, liver, adipose, and muscle).Google Scholar was used only as a supplementary source to locate full-text versions of relevant PubMed-indexed studies or to explore additional relevant references.

Data retrieval

Candidate gene-specific information was retrieved from two major biological databases: NCBI Gene (https://www.ncbi.nlm.nih.gov/gene) and UniProt (https://www.uniprot.org). These databases were accessed on (July 20, 2024). From these sources, we collected details on gene functions, chromosomal locations, transcript variants, and reported associations with T2DM.Moreover, information on zinc and chromium-binding domains, functional sites and metal binding was extracted from UniProt for each protein encoded by the selected genes. Protein sequences were downloaded in FASTA format from UniProt for downstream structural and interaction analyses. This integrated approach ensured a comprehensive molecular profile of each gene in the context of trace element imbalance and diabetes pathogenesis.

Identification of HUB genes

The protein–protein interaction (PPI) network obtained from Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) was analyzed and visualized in Cytoscape (version 3.9.1) (https://cytoscape.org/, accessed July 24, 2024) to explore the functional relationships among target proteins. Hub genes were identified using the CytoHubba plugin, which applies topological algorithms to rank nodes in the network. The degree centrality method was primarily used, where the degree value reflects the number of direct connections a protein has in the

network. Proteins with degree values greater than or equal to 5% of total nodes were considered as hub genes. To enhance robustness, results were cross-validated by applying Maximal Clique Centrality (MCC) and closeness centrality algorithms, both of which consistently confirmed the top-ranked hub proteins. These hub genes were subsequently selected as key targets for further functional enrichment and pathway analyses.

KEGG, reactome and enrichment pathways analyses

The identified hub genes were subjected to pathway enrichment analysis to elucidate their involvement in T2DM-related biological processes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.genome.jp/kegg/pathway.html, accessed July 27, 2024) was used to identify key signaling pathways associated with zinc and chromium deficiency. To broaden the scope of analysis, the Reactome Pathway Browser (https://reactome.org/PathwayBrowser/, accessed July 27, 2024) and the EnrichR tool (https://maayanlab.cloud/Enrichr/, accessed July 27, 2024) were also applied. The findings emphasized the role of trace element imbalances in the pathophysiology of T2DM by highlighting the involvement of these genes in a number of metabolic and signaling pathways connected to glucose homeostasis, insulin resistance, oxidative stress, and inflammation.

Protein –protein interaction (PPI) network construction

The STRING database (https://string-db.org/, accessed on August 01, 2024) was used to construct protein–protein interaction (PPI) networks for candidate genes affected by zinc and chromium status in T2DM. STRING integrates direct (physical) and indirect (functional) associations by combining experimental data, computational predictions, and curated resources such as KEGG, UniProt, and Reactome (Szklarczyk et al., 2019).The curated gene list was submitted to the STRING web interface, and networks were generated separately for zinc- and chromium-related datasets using a high-confidence interaction score threshold (≥ 0.7). The resulting interaction maps were examined to identify hub proteins and functional modules relevant to the interplay between trace element imbalances and the pathophysiology of T2DM.

Prediction of 3D structure –swiss model

The SWISS-MODEL service (https://swissmodel.expasy.org/, accessed on August 02, 2024) was used for homology modeling (comparative) modeling to predict the three-dimensional structures of proteins with known sequences but unresolved experimental structures. The modeling process involved aligning the query protein sequences with experimentally determined template structures that showed significant sequence similarity and contained relevant zinc- or chromium-binding ligands. The resulting alignments were used to construct structural models, providing insights into the potential metal-binding sites and structural conformation of the target proteins under trace element-deficient condition.

Ligands target identification for molecular docking

To investigate potential therapeutic interactions, we selected ligands that represent the actual forms of zinc and chromium commonly used in supplementation, rather than docking with the free metal ions (Zn^{2+}, Cr^{3+}). Docking with ions alone would not capture the way these elements interact in biological systems, since in vivo they are typically delivered and transported as stable complexes. For zinc, we used zinc gluconate (PubChem CID: 443445), a widely available supplement that releases bioactive zinc ions after ingestion and has been shown to help restore metabolic balance in T2DM[28,29]. For chromium, we considered several trivalent complexes known to improve glucose metabolism and insulin sensitivity, including chromium histidinate, chromium nicotinate, and chromium picolinate. Among these, chromium picolinate (PubChem CID: 22833491) was ultimately chosen as the representative docking ligand due to its established clinical relevance and improves glycemic control[30,31]. Importantly, toxic hexavalent chromium (Cr^{6+}) was not considered in this study. These ligand choices ensured that the docking analysis reflected biologically active and supplement-relevant forms of zinc and chromium, providing greater validity to the investigation of trace element-associated metabolic pathways.

Binding site identification and selection

We identified candidate ligand-binding pockets using a combination of literature evidence and computational prediction. First, previously reported active or binding sites from the literature and experimentally resolved holo structures (PDB entries listed in Supplementary Table S3) were used when available. Second, potential pockets were predicted on apo or holo receptor structures using DoGSite Scorer and CASTp, which detect cavities and estimate pocket volumes and druggability. Pockets were ranked based on druggability score (DoGSite score), pocket volume (> 200 Å^3 considered suitable for small-molecule ligands), and surface exposure.

For proteins with known metal-binding or catalytic functions, we gave preference to pockets enriched with residues commonly involved in metal coordination (His, Cys, Asp, Glu).The docking grid box was centered on the centroid of the selected pocket or at the position of a co-crystallized ligand (where available), with a 6–8 Å margin applied to allow ligand flexibility. For targets with multiple high-scoring pockets, ligands were docked into the top 1–3 sites, and the best-scoring poses were reported. To validate pocket selection, we re-docked co-crystallized ligands (RMSD ≤ 2.0 Å considered acceptable) and performed visual inspection to ensure plausible protein–ligand contacts.

Molecular docking

The crystal structures of target proteins were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org, accessed on August 24, 2024) in PDB format, while the chemical structures of selected zinc- and chromium-based compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on August 24,2024) in SDF format. Protein structures were pre-processed by using Discovery Studio 21.1.0, which involved removal of water molecules and heteroatoms, and addition of hydrogen atoms to optimize the structures for

docking. Docking simulations were performed using PyRx software. The prepared ligands were docked with their respective protein targets, and the binding energy values of the docked complexes were used as the primary criterion to evaluate ligand–target interactions.

Protein structures were pre-processed using Discovery Studio 21.1.0 (assessed on August 26, 2024) which involved the removal of water molecules and heteroatoms, followed by the addition of hydrogen atoms to optimize structures for docking. Ligands were similarly prepared to ensure correct geometry and energy minimization. Docking grid boxes were defined to enclose the active site residues of each protein. Grid center coordinates were set based on reported binding pockets. Different grid parameters were assigned to other proteins according to their respective binding sites. These settings ensured comprehensive coverage of active site residues while allowing flexible ligand access. Post-docking visualization and interaction analysis were performed using Discovery Studio Visualizer. The types of interactions, including hydrogen bonding, π-stacking, and hydrophobic interactions, were examined to provide insights into the structural basis of ligand binding and target modulation.

Trace element analysis and expression profiling of candidate genes

Sample collection

In pilot study, a total of 50 patients with T2DM were recruited from Federal Polyclinic Hospital, Islamabad, along with 15 healthy individuals who underwent routine physical examinations and served as controls. All participants provided written informed consent prior to enrollment. The research protocol was approved by the Research Ethical Committee of Atta-ur-Rehman School of Applied Biosciences, NUST, Islamabad. The study was conducted in accordance with the Declaration of Helsinki and institutional ethical guidelines. From each participant, blood samples (5 mL) was collected in two separate tubes: one tube for the analysis of serum zinc and chromium levels, and the other for RNA extraction and cDNA synthesis to support gene expression profiling. Clinical data, including demographics and complication status, were also recorded. The study included 50 T2DM patients (34 females and 16 males) and 15 healthy controls (8 females and 7 males). Among the diabetic participants, 17 had diabetic nephropathy, 17 had diabetic cardiovascular disease (CVD), and 16 had diabetic retinopathy, based on clinical diagnosis.

Study population and confounder control

Participants were recruited according to predefined inclusion and exclusion criteria to minimize potential confounding factors. Individuals with active infections, inflammatory diseases, or those undergoing dialysis were excluded to reduce bias from comorbid immune or metabolic alterations. Similarly, participants with lifestyle factors known to affect trace element levels (e.g., smoking, alcohol use, or extreme dietary habits) were excluded. Demographic and clinical variables including age, sex, body mass index (BMI), glycated haemoglobin (HbA1c) levels, and diabetes related complications (nephropathy, retinopathy, cardiovascular disease) were recorded for all participants. These variables were considered in the stratification and interpretation of gene expression data. Demographical and biochemical parameters are discussed in (Table 1).

Inclusion and exclusion criteria

A total of 65 participants were enrolled based on availability during the study period. Although the sample size was smaller than originally planned, it was sufficient to provide meaningful insights into the relationship between zinc and chromium levels and complications in type 2 diabetes. Written informed consent was obtained from all participants after they were clearly informed about the study's objectives and procedures. To minimize confounding, individuals with active infections or severe kidney dysfunction (eGFR < 30 mL/min/1.73 m^2 or those on dialysis) were excluded, as such conditions could alter trace element status and inflammatory markers. The inclusion and exclusion criteria applied during participant recruitment are summarized in Fig. 1.

Parameter	T2DM Patients ($n = 50$)	Healthy Controls ($n = 15$)
Gender (n)	16 males, 34 females	7 males, 8 females
Age (years)	52.1 ± 5.17	51.7 ± 6.83
BMI (kg/m^2)	22.7 ± 4.6	21.5 ± 3.14
Fasting blood glucose (mmol/L)	8.1 (6.4–9.5)	4.0 (3.3–4.5)
Random blood glucose (mmol/L)	10.7 (8.7–12.1)	6.3 (5.2–7.1)
HbA1c (%)	> 7.8	< 5.5
Total cholesterol (mmol/L)	4.3 (3.6–5.2)	6.3 (5.1–7.7)
Serum albumin (g/dL)	4.3 ± 0.5	4.1 ± 0.4
Serum creatinine (mg/dL)	0.7 ± 0.3	1.0 ± 0.2
Complications	Diabetic retinopathy, cardiovascular disease, nephropathy	None

Table 1. Demographic and biochemical characteristics of study participants. Values are presented as mean ± standard deviation (SD) for normally distributed variables and median (interquartile range, IQR) for non-normally distributed variables. HbA1c values are expressed as categorical cutoffs. Clinical complications were recorded only in the T2DM group.

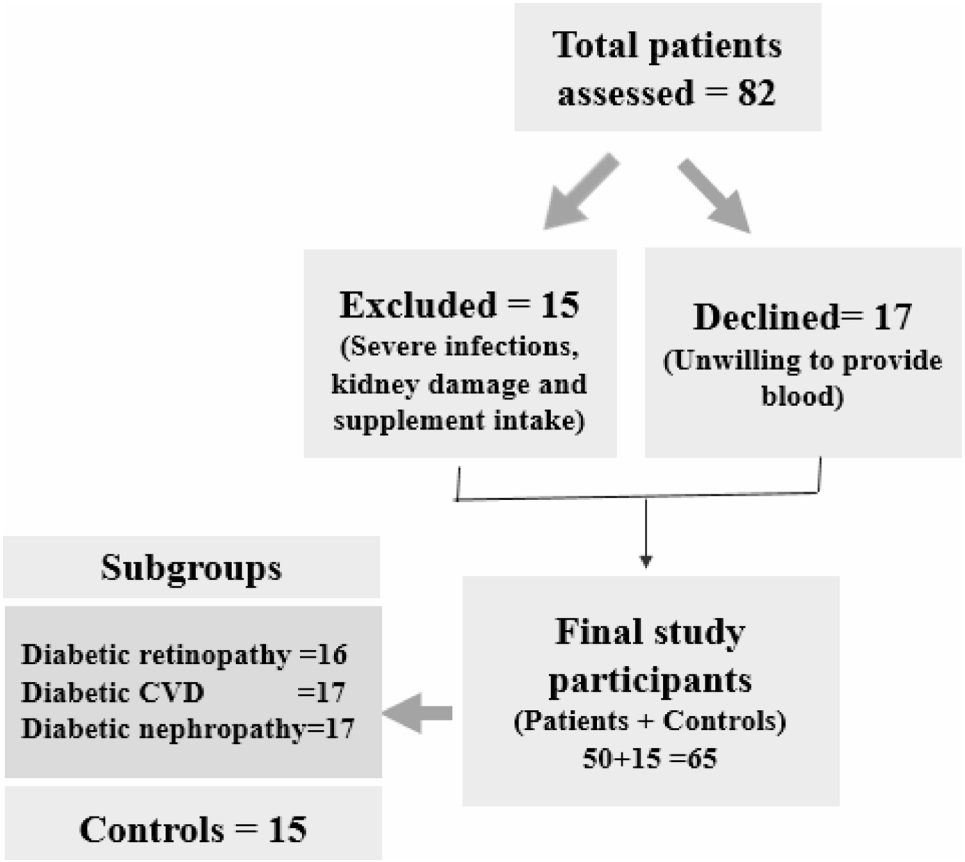

Fig. 1. Participant recruitment, inclusion, and exclusion criteria. A total of 82 patients were initially assessed for eligibility. Of these, 15 were excluded due to severe infections, kidney damage, or prior supplement intake, and 17 declined participation because they were unwilling to provide blood samples. The remaining 50 patients, along with 15 healthy controls, constituted the final study cohort ($n = 65$). Patient subgroups included individuals with diabetic retinopathy ($n = 16$), diabetic cardiovascular disease ($n = 17$), and diabetic nephropathy ($n = 17$). This selection process ensured consistency in the study population and reduced potential confounding factors for serum trace element analysis and gene expression profiling.

Classification of diabetic complications

Diabetic complications were categorized according to their prevalence in the study cohort to maintain sufficient statistical power for subgroup analyses. Patients with multiple complications were classified according to their primary clinical diagnosis, as determined by the treating physician. Sensitivity analyses were conducted to evaluate the influence of overlapping conditions. Participants were recruited during routine diabetes care, screening, or follow-up visits. Complications were defined using standard clinical criteria:

- **Retinopathy**: Funduscopic examination.
- **Cardiovascular disease (CVD)**: History of myocardial infarction, angina, or Electrocardiogram (ECG) confirmed ischemia.
- **Nephropathy**: Estimated glomerulus filtration rate (eGFR) < 60 mL/min/1.73 m^2 or albuminuria > 300 mg/day.

The observed distribution of complications reflected their natural prevalence in the study population. While subgroup sizes varied and were relatively small, they were considered adequate for exploratory comparisons within the scope of this pilot study.

Sample preparation and measurement of trace elements

To prevent trace element contamination, blood was collected using certified trace element-free EDTA tubes (BD Vacutainer), and all consumables were acid-washed. Sample processing was done under a laminar flow hood using powder-free gloves. Freshly collected blood was centrifuged at 4000 rpm to separate the serum, which was then undergo through acid digestion using perchloric acid nitric acid, and deionized water. The prepared samples were stored at −4 °C. Zinc concentrations were measured using the AA-7000 Atomic Absorption Spectrophotometer with specific hollow cathode lamps (Zn: 357.9 nm).Chromium concentrations were determined using Graphite Furnace Atomic Absorption Spectrometry (GFAAS; PerkinElmer Analyst 800), with a detection limit of 0.1 ng/mL. Calibration was performed using NIST-traceable standards. (Cr: 410 nm).

Measurement of trace elements

Serum zinc and chromium concentrations were measured using an AA-7000 Atomic Absorption Spectrophotometer (AAS) and Graphite Furnace Atomic Absorption Spectrometry. Digested serum samples were analyzed individually for analyzing Zn and Cr level using element-specific hollow cathode lamps at wavelengths of 357.9 nm (Zn) and 410 nm (Cr).Calibration standards were prepared using the standard addition method to account for matrix effects. Background correction (Zeeman or deuterium) was applied to minimize non-specific absorption. The instrument's control software was used to automate the measurement process and compute concentrations from standard calibration curves, ensuring accurate and reliable trace element quantification. Determination of trace elements using Atomic Absorption Spectrophotometry (AAS) methodology depicted through the Table 2 below:

Pure zinc sulfate and chromium nitrate were initially dissolved in deionized water to prepare stock solutions with a concentration of 1 g/L. These stock solutions were then diluted to produce working standards ranging from 0.1 to 10 mg/L, corresponding to the expected serum concentrations, as well as intermediate standards (e.g., 10 and 100 mg/L). All standards were acid-matched to the digested serum samples by adding identical amounts of 70% perchloric acid and 65% nitric acid, ensuring analytical accuracy and minimizing matrix effects. All measurements were performed using optimized atomic absorption spectrophotometer settings for each element, and a calibration curve was created using at least five calibration points that covered the anticipated concentration range.

Extraction of RNA and cDNA preparation

Total RNA from whole blood was extracted by using TriZol reagent (Thermo Fischer Scientific, Waltham, MA, USA). To prevent deterioration, every reaction was conducted on ice. Purity and concentration of RNA was analyzed by using Nano Drop 2000 (Thermo Fischer Scientific, Waltham, MA, USA).Samples having ration A260/A280 > 1.6 were further used for cDNA preparation. For the synthesis of cDNA, 20 µl of reaction volume was prepared through 100ng of RNA,200 U reverse transcriptase,1.5mM of ddNTPs,100 µM of oligodT, 10 U RNASE inhibitor and DPEC water upto 20 µl. At 42 °C for 60 min the reverse transcription reaction was started and terminated at 69.9 °C for 10 min. The cDNA was stored at −20 °C.

Expression profiling of GCK and GLUT4

Primers for GCK and GLUT4 were designed by the authors using Primer-BLAST (NCBI) (https://www.ncbi.nlm.nih.gov/tools/primer-blast/, accessed on September 25, 2024) with the following criteria: amplicon size 100–200 bp, primer length 18–22 nucleotides, melting temperature 60–66 °C, and GC content 50–60%. Primer sequences were checked to avoid secondary structures, hairpin formation, and primer–dimer interactions. All primers were synthesized by Molecular Biology Products, Pakistan.

Primers used for the expression analysis of GCK forward 5'ACCATTACCTCCCTGAGTCT3', reverse 5'TTCCCATAGATGCCTTCCAC3', GLUT4, forward 5'TTCCTTCTATTTGCCGTCCTC3', reverse 5' CTGTTTTGCCCCTCAGTCATT3'and beta actin (internal control) forward, 5'GGACTTCGAGCAAGAGATGG3', reverse,5'TGTGTTGGCGTACAGGTCTTTG 3'.In each run, no negative controls/template was included for every primer. The reaction mixture consists of 1µL of 200ng of template cDNA, 0.5µM of forward and reverse primer, 10 µL of SYBER Green master mix and nuclease free water to make up 20 µL of final reaction volume. The thermal cycler conditions were: Initial denaturation at 95 °C for 10 min following the 40 cycle of denaturation at 95 °C for 30, annealing for 60 s at 60 °C and extension at 72 °C for 45 s. All the experimental reaction were run in triplicates and their average was used for results analysis. Data was analyzed through comparative cycle threshold method (ΔCt) and normalized as followed by beta actin expression in each sample.

The genes GCK and GLUT4 were chosen for expression validation because they play important roles in T2DM-related pathways that are controlled by zinc and chromium. Zinc status has a significant impact on GCK, a zinc-sensitive gene implicated in glucose phosphorylation and insulin secretion control.GLUT4, a chromium-influenced gene, is a key glucose transporter regulated by insulin signaling, and its impaired translocation is directly linked to insulin resistance. They also appeared as global genes in the PPI network interactions, as well as showing high enrichment in important pathways like glycolysis/gluconeogenesis, insulin signaling, and glucose

Step	Procedure
Sample preparation	Serum samples digested with nitric acid and hydrogen peroxide to release bound elements.
Calibration standards	Certified reference standards matched to serum matrix to ensure validity of measurements.
Instrument calibration	Calibration performed before each batch to maintain instrument precision.
Background correction	Zeeman background correction applied to minimize spectral interference.
Internal quality control	QC samples included in each run; results monitored for consistency.
Replicate measurements	Each sample analyzed in duplicates to confirm repeatability.
Data validation	Measured concentrations cross-checked against standard curves for accuracy.

Table 2. Quality control measures for atomic absorption spectroscopy (AAS)-based trace element analysis in serum samples. Each step ensures accuracy, precision, and reliability of elemental quantification. Zeeman background correction minimizes spectral interference, and calibration procedures guarantee instrument performance.

homeostasis in the KEGG pathway enrichment results. While other genes might participate as well, a balanced approach of network centrality, pathway importance, and responsiveness to trace elements led to the selection of GCK and GLUT4, thus validating the strategy while ensuring relevance through representative reasoning.

Tissue-specific expression from GEO datasets
To further validate the expression of GCK and SLC2A4 in T2DM, we retrieved transcriptomic datasets from the NCBI Gene Expression Omnibus (GEO). The selected datasets met the following inclusion criteria: (i) case–control studies comparing T2DM patients with healthy individuals, (ii) availability of raw or normalized expression data. The details of the included datasets are summarized in supplementary Table 5.These datasets allowed us to examine the expression profiles of GCK and SLC2A4 across relevant tissue.

Statistical analysis

SPSS 21.0 (IBM, Armonk, NY, USA) was used to analyze the results. Normally distributed data were expressed as mean ± standard deviation. Quantitative variables, such as serum zinc and chromium levels, were treated as continuous variables without any transformations. For group comparisons, one-way ANOVA was used for normally distributed data. Data normality was assessed using the Shapiro-Wilk test. For datasets that did not meet the normality assumption ($p < 0.05$), appropriate non-parametric tests such as the Mann-Whitney U test were applied. Effect sizes (Cohen's d for two-group comparisons or η^2 for ANOVA) and 95% confidence intervals (CIs) were calculated and reported alongside p-values to provide a measure of the magnitude and precision of the observed differences. Gene expression data were analyzed by using Graph Pad Prism software (version.10.4.2). A p-value < 0.05 was considered statistically significant.

Results

In-silico results

Acquisition of potential genes affected due to zinc and chromium status in T2DM
To identify genes potentially influenced by zinc and chromium status in type 2 diabetes mellitus (T2DM), a keyword-based search was conducted using the terms "zinc and chromium level affected genes in T2DM" across PubMed and Google Scholar databases. Relevant studies were screened for experimentally validated or reported genes associated with altered expression in the context of trace element imbalance and T2DM. The gene lists from both sources were compiled, and duplicate entries were removed to generate a final set of potential candidate genes for further analysis. This can be depicted through Table 3.

Protein-protein interaction (PPI)
The potential genes that were affected due to zinc and chromium status in T2DM imported into online STRING database (v11.5). Then data from STRING were exported to Cytoscape to reconstruct the protein network and analyze the functional interaction between the potential affected genes. Zinc and chromium level affected genes analysis were performed separately. The STRING database (v11.5) yielded a network of N nodes and M edges, based on combined evidence from experimental and predicted interaction channels (confidence score ≥ 0.7).A PPI interaction of zinc and chromium affected genes were shown in Fig. 2.

HUB genes identification
The PPI interaction for both zinc and chromium affected genes in T2DM were imported into Cytoscape software. The nodes with high degree as CytoHubba were performed were identified as hub genes. Network analyzer degree were used to calculate the average node degree. Hub genes were defined as nodes with a degree ≥ 6, corresponding to the top 5% of highly connected genes within the network. Top 3 hub genes were identified for both zinc and chromium separately as shown in Fig. 3.

Top 3 hub genes were selected for further in-silico analysis to check the effect of zinc deficiency in T2DM (*GCK*, *SLC30A8 and MT3*).Top 3 hub genes were selected for further in silico analysis to check the effect of chromium deficiency in T2DM (*IRS1*, *SLC2A4 and PPARG*).

Functional pathways enrichment analysis
Zinc transporting genes in T2DM *SLC30A8 (ZnT8)* involved in multiple pathways affected by zinc deficiency, including the insulin secretion pathway, glucose homeostasis, and inflammatory signaling pathways. *ZnT8* plays a crucial role in zinc transport within pancreatic β-cells, directly impacting insulin granule formation and

Step	Number of Genes Identified	Description/Notes
Keyword-Based Search	150 genes	Initial list of genes retrieved using relevant keywords in databases and literature searches.
Data Collection	77 genes	Genes remaining after reviewing relevant studies for reported expression changes in T2DM.
Data Consolidation	61 genes	Consolidated list of unique genes combining multiple sources/databases.
Duplicate Removal	28 genes	Genes remaining after removing duplicates across sources.
Final Gene List	16 genes	Final set of candidate genes selected for further functional and pathway analysis.

Table 3. Gene selection procedure. The table summarizes the Stepwise procedure used to identify and refine candidate genes for further analysis. Genes were initially retrieved from keyword-based searches, followed by manual review of relevant studies, consolidation of multiple sources, and removal of duplicates, resulting in a final curated set of 16 genes.

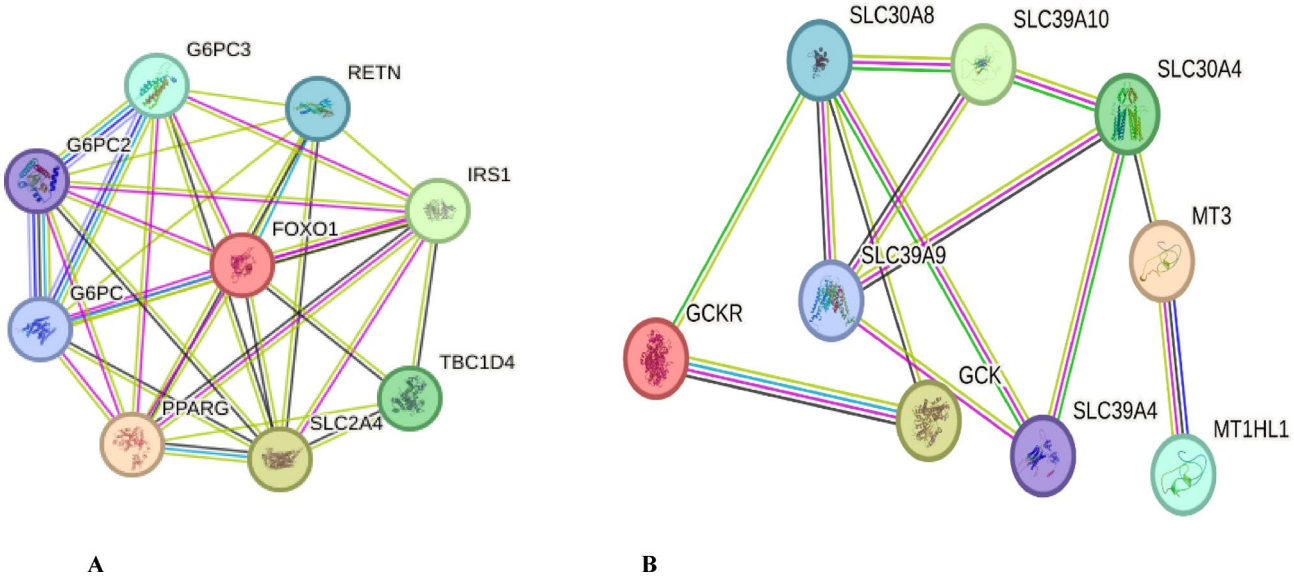

Fig. 2. Protein–protein interaction (PPI) networks of genes affected by serum zinc and chromium levels in T2DM.(**A**) PPI network of 9 genes influenced by zinc status in T2DM, including *G6PC* (glucose-6-phosphatase), *FOXO1* (forkhead box protein O1), and *IRS1* (insulin receptor substrate 1). The network illustrates predicted and experimentally validated interactions, with nodes representing proteins and edges representing interactions.(**B**) PPI network of 9 genes influenced by chromium status in T2DM, including *SLC30A* (solute carrier family 30 member A), *GCK* (Glucokinase) and *GCKR* (Glucokinase regulatory protein).The networks were constructed using STRING (v11.5) with a confidence score ≥ 0.7 and visualized in Cytoscape. Each node represents a gene/protein, and edges indicate functional or physical interactions. Separate networks highlight zinc- and chromium-affected genes, facilitating comparison of their roles in T2DM pathophysiology.

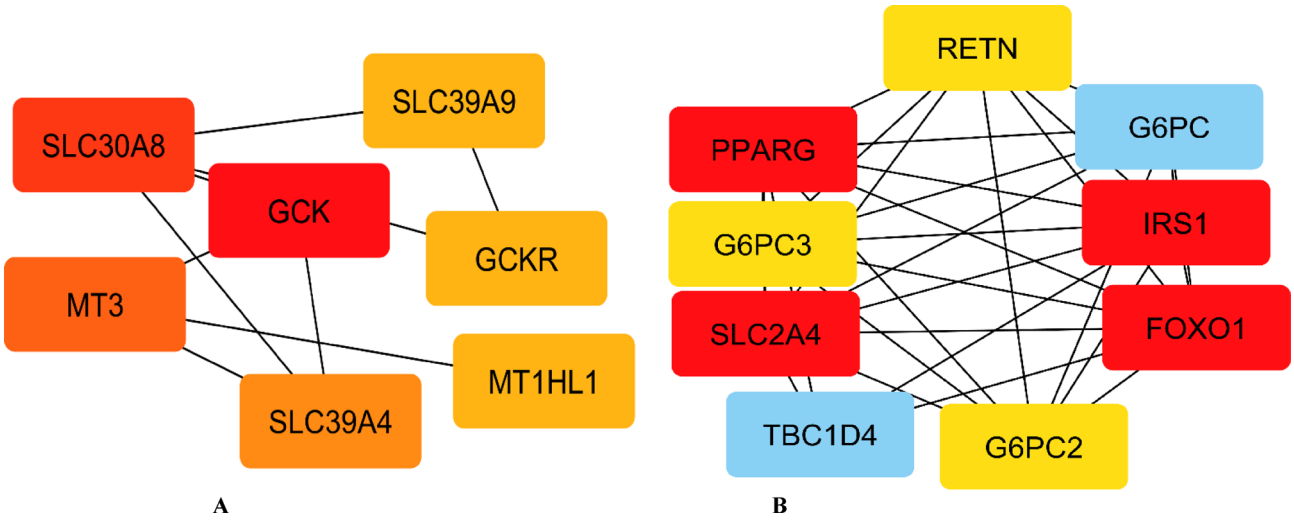

Fig. 3. Hub genes affected by serum zinc and chromium levels in T2DM. **(A)** Hub genes identified from the zinc-affected gene network in T2DM. **(B)** Hub genes identified from the chromium-affected gene network in T2DM. Genes are color-coded according to their level of differential expression: **Highly affected**: Red and dark orange, **moderately affected**: Orange and yellow and **Less affected**: Blue. Hub genes were determined using the CytoHubba plugin in Cytoscape, based on nodes with degree ≥ 6, representing the top 5% of highly connected genes in each network. The networks illustrate key genes potentially regulating T2DM pathophysiology under altered zinc or chromium levels.

release.*MT3 (Metallothionein 3)* can affected by zinc deficiency through its regulatory role in insulin signaling, *FOXO* signaling, and the *MAPK signaling pathway*. *MT3* is critical for maintaining cellular zinc homeostasis and mitigating oxidative stress. *GCK (Glucokinase)* can implicated in glycolysis/gluconeogenesis, insulin secretion, and the mTOR signaling pathway. Zinc deficiency can impair *GCK* activity, thereby disrupting glucose metabolism and energy balance in β-cells and hepatocytes as shown in Fig. 4.

Chromium transporting genes in T2DM Chromium deficiency can dysregulate insulin signaling, AMPK signaling, and the mTOR signaling pathway. *IRS1* is a central mediator in the insulin cascade, and impaired function may contribute to insulin resistance.*SLC2A4 (GLUT4)* are involved in insulin signaling, diabetic cardiomyopathy, and AMPK signaling pathways. Chromium deficiency may reduce *GLUT4* translocation, impairing glucose uptake in muscle and adipose tissues. *PPARG (Peroxisome Proliferator-Activated Receptor Gamma)* is a key regulator affected by chromium deficiency, influencing the *PPAR* signaling pathway, adipocytokine signaling, and lipid and atherosclerosis pathways. Disruption in *PPARG* function can worsen insulin sensitivity and metabolic control. It can be depicted by Fig. 4.

PPI interaction of zinc level affected proteins-ZnT8 (SLC30A8), Metallothionein and GCK
Protein- protein interaction of *ZnT8*, *MT* and *GCK* with other interlinked proteins that paly crucial role in T2DM. Alteration in the level of zinc can affect zinc transporter 8, metallothionein, Glucose kinase protein which can further interact with other proteins. (Fig. 5A, B, C). The protein–protein interactions *of ZnT8*, *MT* (metallothionein), and *GCK* (glucokinase) with other interlinked proteins play a crucial role in T2DM. Alterations in zinc levels can impact the expression or activity of *ZnT8*, *MT*, and GCK, which in turn interact with multiple proteins involved in glucose metabolism, insulin signaling, and overall T2DM pathophysiology. The protein-protein interaction of these key proteins are presented below (Fig. 5A–C).

PPI interaction of chromium level affected proteins
Protein- protein interaction of IRS1, GLUT4 and PPARG with other interlinked proteins that play crucial role in T2DM. Any alteration in the chromium level can affect the functional proteins can affect IRS1, GLUT4 and PPARG which further interlinked to other protein that can directly or indirectly affect other interlinked proteins. As Fig. 5D, E,F illustrates this in better way.

3D structure prediction of zinc affected proteins through swiss model
Homology modeling was performed to predict the 3D structures of proteins affected by zinc status using Swiss-Model. Details of the templates, including PDB ID, sequence identity, coverage, and resolution, are provided in Supplementary Table S4. The resulting 3D structures were subsequently used for molecular docking analyses, as shown in Fig. 6A–C.

3D structure prediction of chromium affected genes through swiss –Model
Homology modeling was performed to predict the 3D structures of proteins affected by chromium status using Swiss-Model. Details of the templates, including PDB ID, sequence identity, coverage, and resolution, are

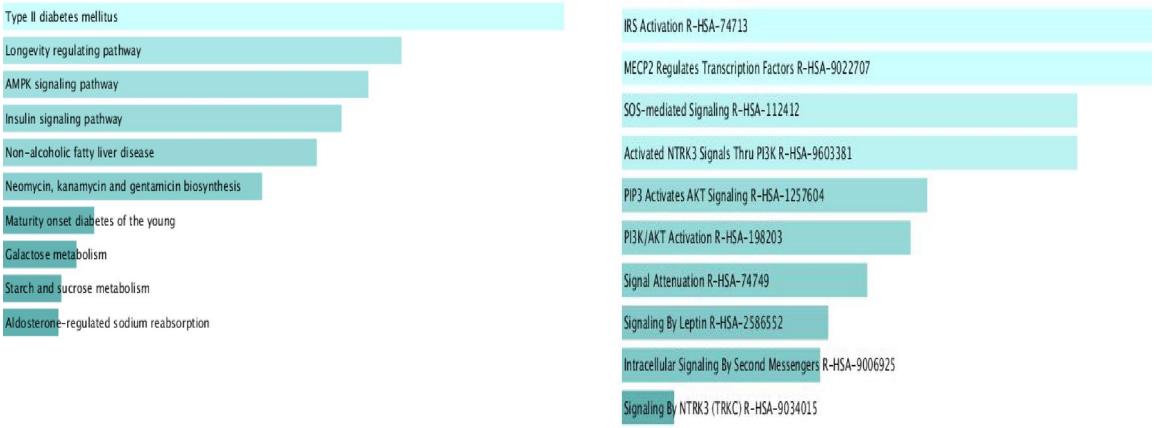

Fig. 4. Functional and pathway enrichment analysis **(A)** KEGG pathway enrichment analysis for genes affected by serum zinc *(ZnT8*, *MT*, *GCK)* and chromium *(IRS1*, *GLUT4*, *PPARG)* levels, highlighting their involvement in key T2DM-related pathways. Pathways are ranked from most to least significant.**(B)** Reactome pathway enrichment analysis for the same set of zinc- and chromium-affected genes, showing their roles in biological processes and signaling pathways relevant to T2DM.Pathway enrichment analyses were performed to identify biological functions and molecular pathways influenced by altered zinc and chromium levels. The figure illustrates the top significant pathways, providing insights into the mechanistic roles of these trace elements in T2DM pathophysiology. KEGG pathway information was obtained from the KEGG database (Kanehisa and Goto, 2000; Kanehisa et al., 2024). Source: Kyoto Encyclopedia of Genes and Genomes (KEGG), Kanehisa Laboratories https://www.kegg.jp/.

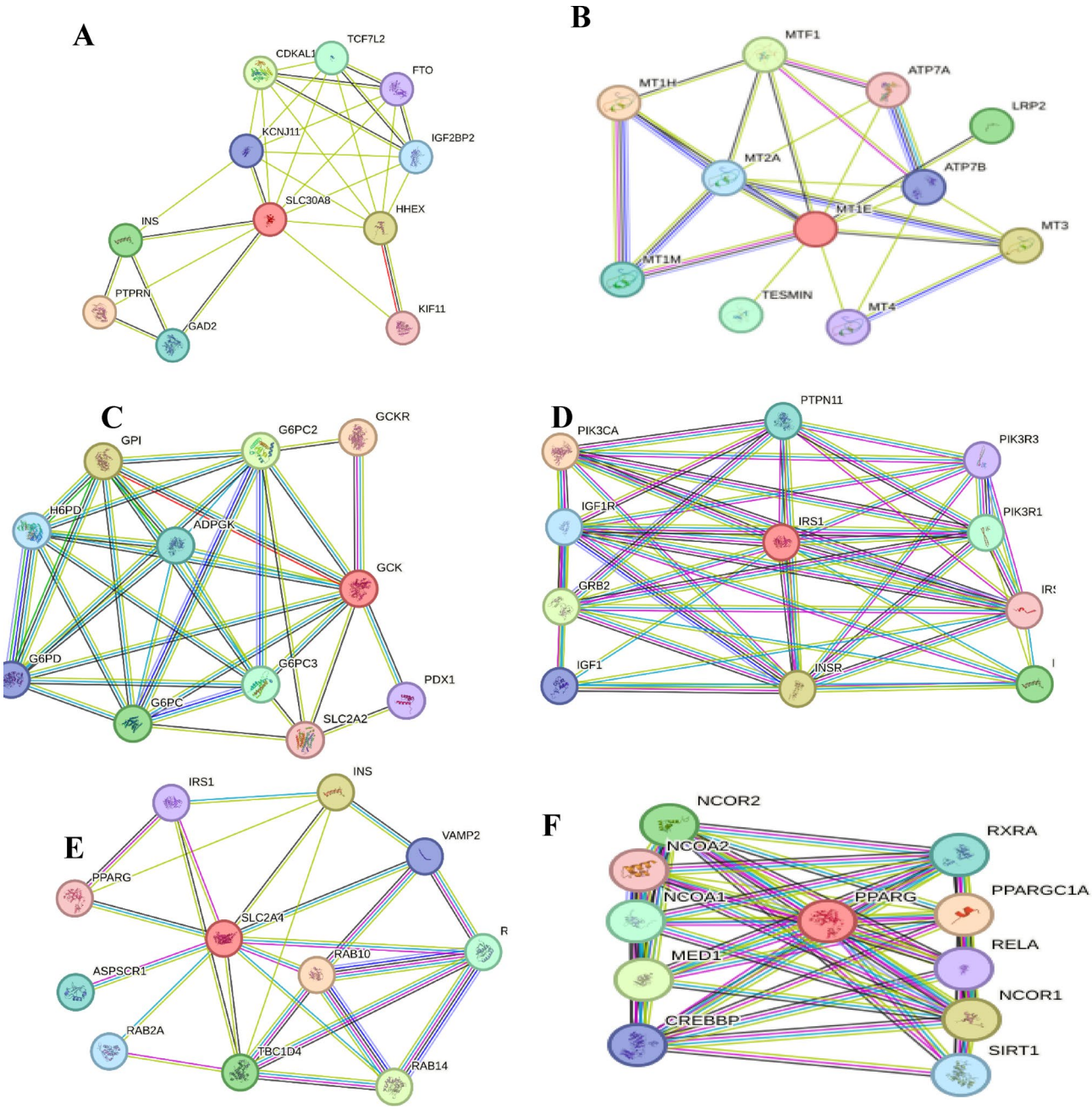

Fig. 5. Protein–Protein Interaction (PPI) Networks of Zinc- and Chromium-Responsive Hub Genes in T2DM. (**A**) represent PPI networks for zinc level–associated hub genes SLC30A8 (ZnT8)High Confidence value (o.700),(**B**) depicts the PPI network for zinc level associated hub gene MT3, and (**C**) represent the PPI network of zinc level associated hub gene GCK, respectively.(**D**)represent PPI networks for chromium level–associated hub genes: IRS1(**E**) depicts the PPI network for zinc level associated hub gene SLC2A4 (GLUT4), and(**F**) represent the PPI network for zinc level associated hub gene PPARG, respectively. Nodes are color-coded based on the magnitude of gene expression changes: Red/Dark Orange: Highly affected, Green/Orange/Yellow: Moderately affected and Blue: Less affected. All these PPI interaction were retrieved having high confidence value (0.70).

provided in Supplementary Table S4. The resulting 3D structures were subsequently used for molecular docking analyses, as shown in Fig. 6D–F.

Molecular docking

Zinc and chromium enriched compounds for docking *GCK*, *ZnT8* and *MT* were docked with zinc gluconate and *Glut4* and *PPARG* were docked with chromium picolinate.3-dimenstional structures of *GCK*, *MT*, *ZnT8*. *PPARG* and *Glut4* were downloaded from Protein Data Bank (PDB).3D structures of compounds were down-

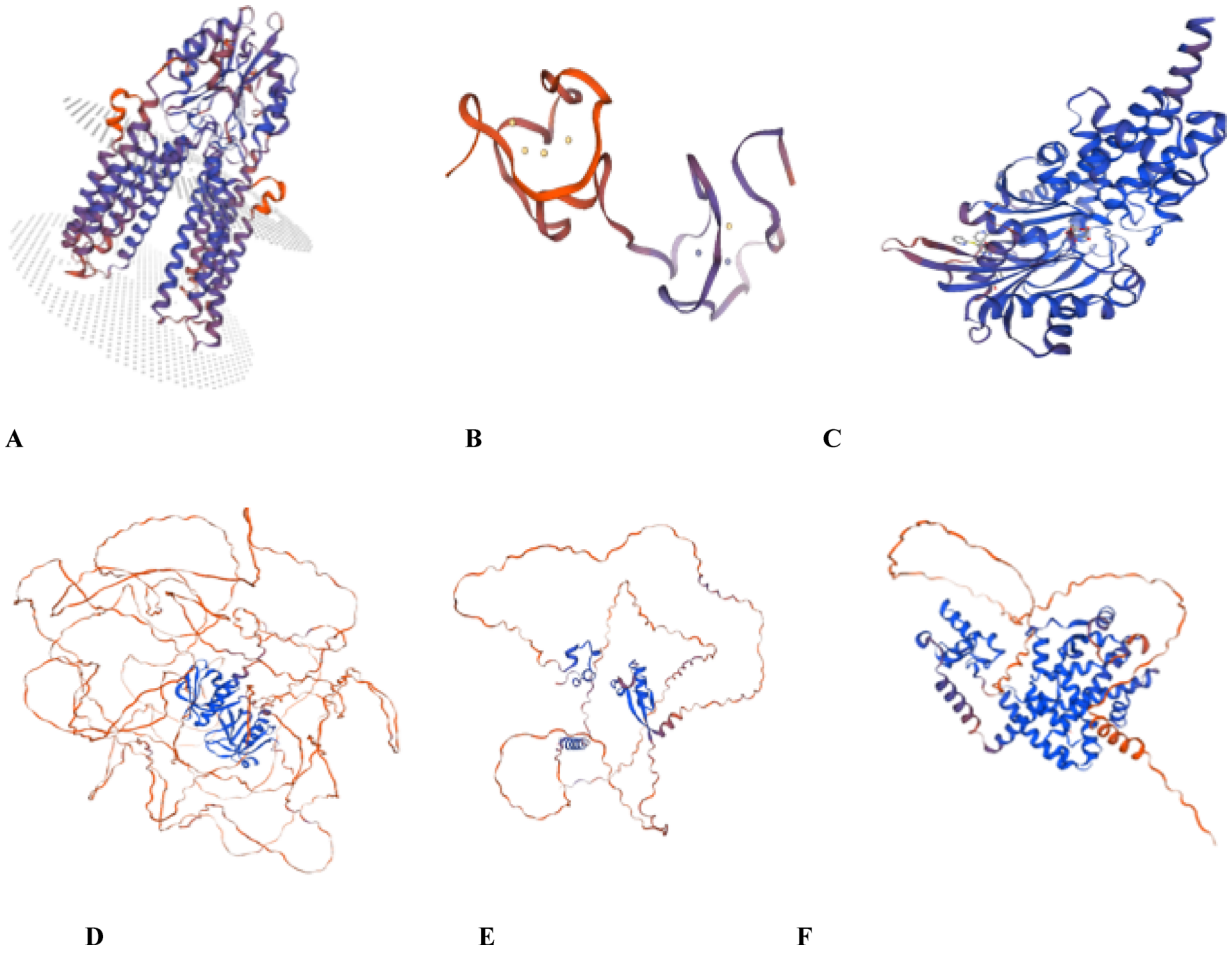

Fig. 6. 3D structure through Swiss model. Structure prediction of zinc level affected proteins (**A**) *Znt8 (**B**) MT* (**C**) *GCK*. Chromium level affected proteins (**D**) *IRS1 (E) GLUT4 (F) PPARG*. The 3D structures were predicted using Swiss-Model based on high-confidence templates. These models were used for subsequent molecular docking analyses to explore interactions of zinc- and chromium-responsive proteins in T2DM.

loaded from PubChem. The molecular weight of zinc gluconate were 455.685 g/mol and chromium picolinate molecular weight were 418.3 g/mol. These structures were then cleaned to remove water molecules and ions and energies were minimized using Discovery studio and PyRx softwares. The molecular docking of genes are shown in Fig. 7.

Although molecular docking simulation showed that binding of the target proteins with the zinc/chromium compounds might have strong binding affinities, we also realize that binding affinity on its own cannot validate the biological importance. The docking results give structural information concerning possible interactions, but lacking are functional results such as alterations in gene expression or activity within the protein. Hence, there are additional validation studies required in vivo and in vitro to prove the importance of these assumed interactions in real biological systems.

Binding energies of docked compounds Binding energies of all docked compounds like zinc gluconate with *ZnT8*, *MT* and *GCK*. The most efficient binding was shown with *GCK* gene that is −5.8 kcal/mol. Binding of chromium picolinate with GLUT4 and *PPARG* gene were performed. The efficient binding was shown with *PPARG* gene that is −5.0 kcal/mol. Binding energy of zinc level affected proteins *ZnT8* were − 5.2 kcal/mol, *MT* were − 4.2 and *GCK* were − 5.8 kcal/mol. For chromium level affected protein for *GLUT4* −4.9 kcal/mol and *PPARG* were − 5.0 kcal/mol.

Validation through wet lab analysis

Zinc and chromium level in T2DM patients

About fifty T2DM patients serum zinc and chromium level were compared with fifteen healthy controls. The zinc and chromium level were measured in milligram per liter. The average zinc level in T2DM patients were 1.258 ± 0.375 while in controls 2.56 ± 1.735. A significant difference in blood zinc levels was observed between the control and zinc-deficient groups ($p = 0.0087$). The mean difference was 0.9 mg/L, with a 95% confidence interval of (0.33, 1.47). The effect size, calculated using Cohen's *d*, was 1.38, indicating a large effect and a substantial reduction in zinc levels among deficient individuals. *P value* is < 0.05 which shows that the results

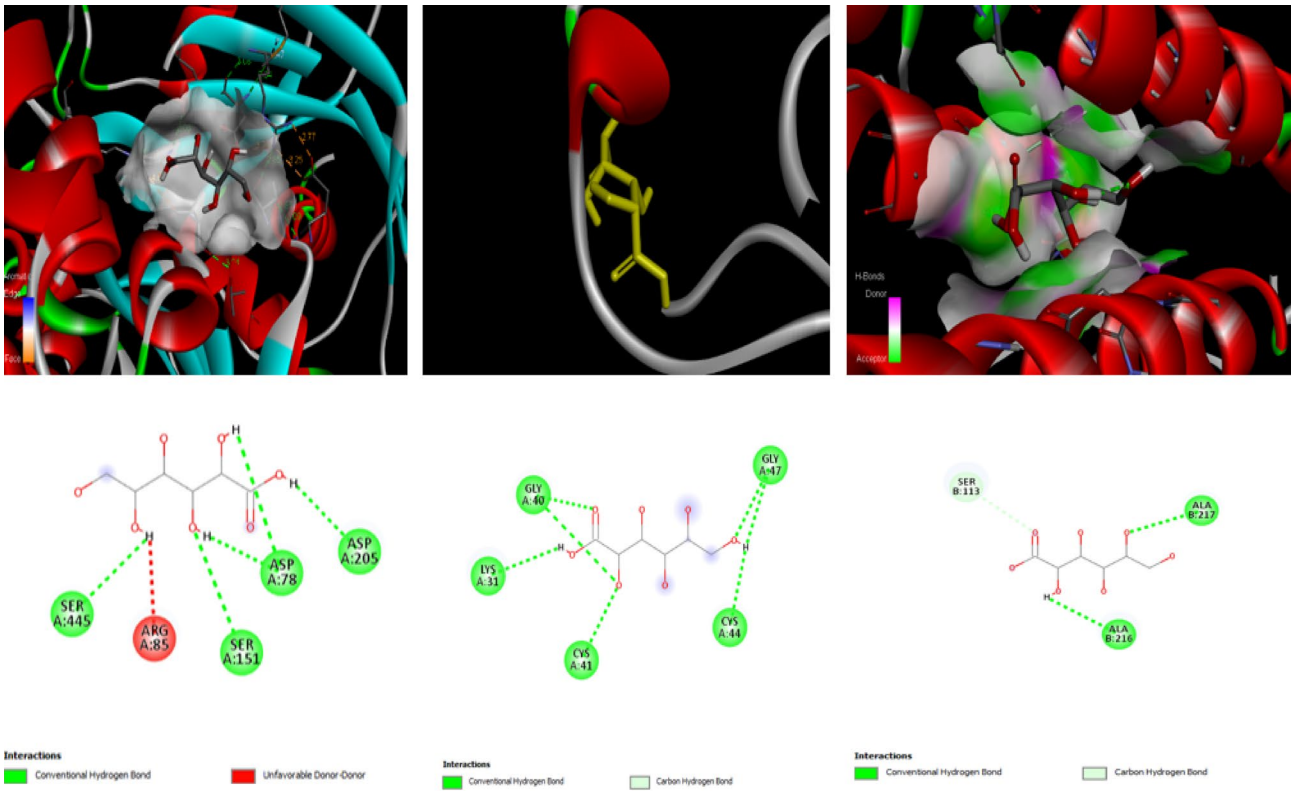

Fig. 7. Molecular docking of zinc- and chromium-affected proteins associated with T2DM.Docking simulations were performed using the homology-modeled 3D structures of zinc-responsive proteins (GCK (**A**), MT (**B**), ZnT8 (**C**)) and chromium-responsive proteins (GLUT4 (**D**), PPARG (**E**)). The ligand–protein binding poses are shown with key interacting residues highlighted. Zinc-responsive proteins demonstrate binding conformations relevant to glucose sensing and metal ion regulation, while chromium-responsive proteins show interactions associated with glucose transport and transcriptional regulation.

are significant statistically. A significant reduction in blood chromium levels was observed in T2DM patients (0.029 ± 0.007) compared to healthy controls (0.18 ± 0.095), with $p = 0.0018$. The mean difference was 0.15, and the 95% confidence interval ranged from 0.09 to 0.21. The effect size (Cohen's *d*) was 2.21, indicating a very large effect and highlighting a substantial chromium deficiency in T2DM patients. As presented in Table 4.

Out of 50 T2DM patients, 17 had diabetic nephropathy, 17 had diabetic cardiovascular disease (CVD), and 16 had diabetic retinopathy. The mean zinc level in patients with nephropathy was 0.22 ± 0.11 mg/L, which was significantly lower than in those with CVD (0.62 ± 0.14 mg/L) and retinopathy (0.88 ± 0.13) ($p = 0.01$). Similarly, the chromium level in patients with CVD was 0.021 ± 0.009 mg/L, significantly lower than in nephropathy (0.032 ± 0.011 mg/L) and retinopathy (0.034 ± 0.012) subgroups ($p = 0.03$).

Comparison to reference values

Serum zinc levels in the control group (2.56 ± 1.74 mg/L) were within the lower boundary of the commonly accepted reference range (2.5–7.5 mg/L)[32,33]. In contrast, T2DM patients (1.26 ± 0.38 mg/L) and all diabetic complication subgroups exhibited values well below the clinical reference threshold, indicating a state of zinc deficiency. Similarly, the control group chromium levels (0.18 ± 0.095 mg/L) were consistent with reported reference values (0.12–0.24 mg/L)[34]. However, T2DM patients and all complication groups had chromium concentrations markedly below this range (0.021–0.034 mg/L), suggesting a clinically relevant chromium insufficiency. These findings reinforce the pathological significance of trace element imbalance in T2DM progression and its complications.

Expression profiling of *GCK* and *GLUT4* in blood of T2DM (zinc and chromium deficient patients)

There was a low expression of *GCK* observed in peripheral blood of T2DM patients (0.3585 ± 0.4057), patients as compared to healthy controls (2.3806 ± 0.2236). In general, there was 4–6 fold decrease in expression of *GCK* in all the studied T2DM patients. There was also low expression of *GLUT4* observed in the blood of T2DM (0.4791 ± 0.51245) patients compared to healthy controls (1.9845 ± 1.4544) validated with Cohen's d and FDR-adjusted p-values. Hence there was almost 3–4 fold decrease in expression of *GLUT4* in T2DM patients as compared to healthy controls.

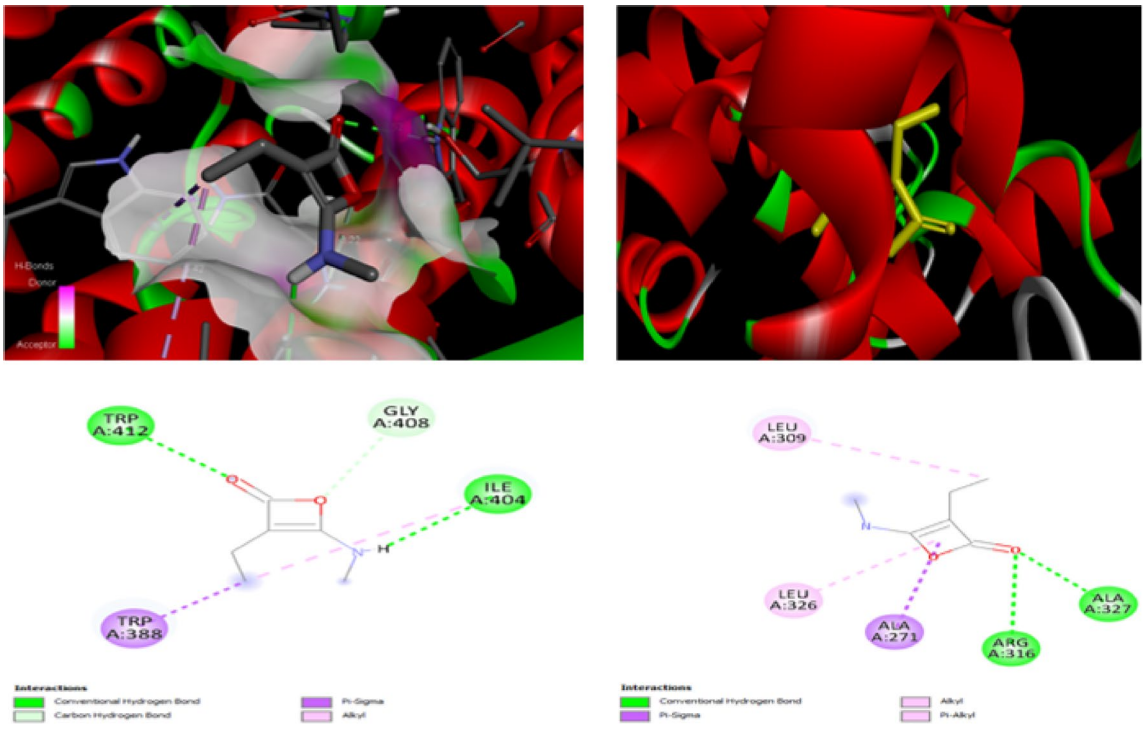

Fig. 7. (continued)

Group	Zinc (mg/L) Mean ± SD	95% CI (Zinc)	Cohen's d (vs. Control)	Chromium (mg/L) Mean ± SD	95% CI (Chromium)	Cohen's d (vs. Control)
Control	2.56 ± 1.74	2.11–3.01	–	0.18 ± 0.095	0.15–0.21	–
T2DM	1.26 ± 0.38 *	1.12–1.39	0.89	0.029 ± 0.017 *	0.023–0.034	1.77
Diabetic Nephropathy	0.22 ± 0.11 *	0.18–0.26	1.74	0.032 ± 0.011 *	0.028–0.036	1.57
Diabetic CVD	0.62 ± 0.14 *	0.56–0.68	1.39	0.021 ± 0.009 *	0.018–0.024	1.91
Diabetic Retinopathy	0.88 ± 0.18 *	0.81–0.95	1.02	0.034 ± 0.012 *	0.030–0.038	1.47

Table 4. Serum zinc and chromium levels in T2DM patients and its complications compared with healthy controls. Values are presented as mean ± standard deviation (SD) along with 95% confidence intervals (CI). Effect sizes are expressed as cohen's d relative to healthy controls. Zinc (mg/L) and chromium (mg/L) levels were significantly reduced in T2DM patients and further declined in complication groups, including diabetic nephropathy, cardiovascular disease (CVD), and retinopathy (*$p < 0.01$ compared to controls). The largest effect sizes were observed for zinc deficiency in nephropathy (d = 1.74) and chromium deficiency in CVD (d = 1.91), highlighting the substantial trace element disturbances associated with disease progression.

Correlating the expression of GCK and GLUT4 in T2DM associated complications

Gene expression of *GCK* and *GLUT4* were compared among T2DM patients with nephropathy, retinopathy and CVD. There was a low expression of GCK in T2DM with nephropathy (0.2215 ± 0.4532) than *GLUT4* (0.5103 ± 0.4901)0.2–4 folds decreased expression of *GCK* than *GLUT4* were observed in diabetic nephropathy patients. In diabetic CVD patients, the expression of *GLUT4* (0.2121 ± 0.4783) were lower than *GCK* (0.4870 ± 0.4343)0.2–4 fold decreased expression of *GLUT4* than *GCK*. In diabetic retinopathy patients, the expression of *GCK* (0.3911 ± 0.4083) were lower than *GLUT4* (0.4570 ± 0.4884). There was a significant difference as patients with diabetic nephropathy have lower *GCK* expression than others groups and diabetic CVD patients have lower *GLUT4* expression than other groups. The p value can be calculated to show the significant results statistically and depicts that results are highly significant ($P < 0.05$).The relative gene expression of *GCK* and *GLUT4* in T2DM patients without and with complication are shown in (Fig. 8).

GCK and *GLUT4* gene expression in 50 T2DM patients with zinc and chromium deficiencies and 15 healthy controls were performed. Significant downregulation of both genes ($p < 0.05$) was observed in T2DM patients compared to controls. *GCK* expression was more suppressed in diabetic nephropathy patients ($p = 0.0001$), while *GLUT4* showed greater downregulation in diabetic CVD patients ($p = 0.0001$). GLUT4 expression decreased most severely in diabetic CVD (3.2-fold, p value < 0.01), followed by retinopathy (2.4-fold, p-value = 0.02) and nephropathy (1.9-fold, p-value = 0.03), aligning with known insulin resistance in vascular complications. For diabetic retinopathy, *GCK* expression was significantly reduced ($p = 0.0021$) as compared to *GLUT4*. Zinc and

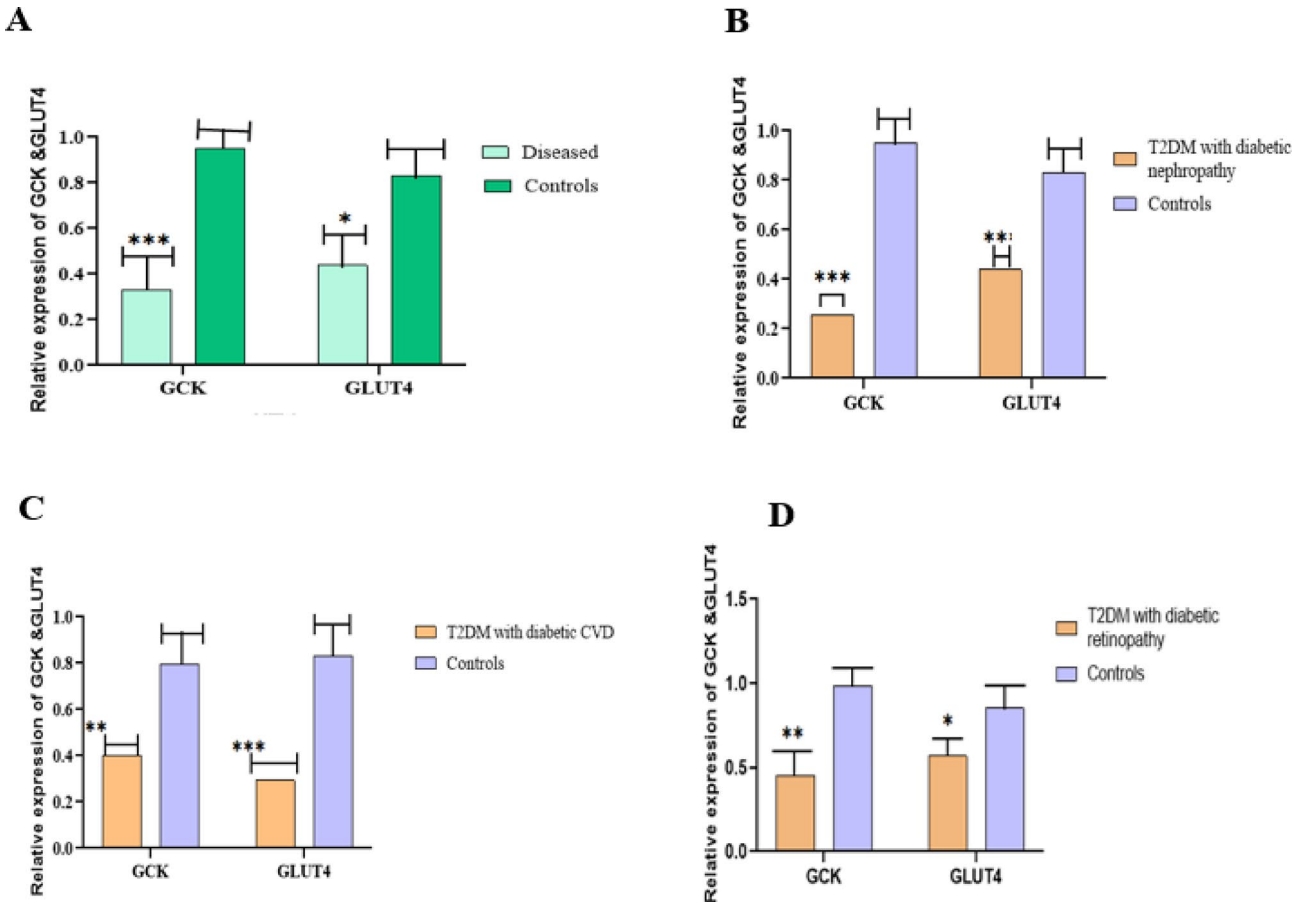

Fig. 8. Relative gene expression of *GCK* and *GLUT4* among T2DM and its associated complications. Expression of *GCK* and *GLUT4*. **(A)** The relative expression of GCK gene and GLUT4 were measured among T2DM patients. Zinc and chromium deficient patients ($n = 50$) were compared with healthy controls ($n = 15$).The downregulation of GCK and GLUT4 gene were shown in T2DM patients with different zinc and chromium level. The zinc and chromium level in T2DM patients were deficient as ($p < 0.05$) as compared to controls. **(B)** Diabetic nephropathy patients with zinc and chromium deficient ($n = 17$).GCK expression were more downregulated with significant value ($p = 0.0001$) **(C)** Diabetic CVD patients with zinc and chromium deficient ($n = 17$). The significant downregulation of GCK and GLUT4 patients were observed in T2DM patients with Zn and Cr level deficient. GLUT4 expression were more downregulated with significant value ($p = 0.0001$). (**D**) Diabetic retinopathy patients with zinc and chromium deficient ($n = 16$).GCK expression were more downregulated with significant value ($p = 0.0021$). Representative data were presented as mean ± SEM of triplicates experiments.

chromium levels were consistently lower in T2DM patients compared to controls. GCK expression decreased 2.1-fold in retinopathy ($p = 0.02$), 4–6-fold in nephropathy ($p = 0.0001$), and 3.0-fold in CVD ($p = 0.01$) compared to controls.

Tissue-level expression of GCK and SLC2A4 from GTEx database To assess the tissue-specific relevance of our target genes, we analyzed publicly available transcriptomic data from the GTEx Portal. GCK expression was highest in the liver and pancreas, two central metabolic organs responsible for glucose regulation and insulin production. Conversely, SLC2A4 (GLUT4) expression was strongly enriched in skeletal muscle and adipose tissues, which are the primary sites of insulin-mediated glucose uptake. These expression patterns confirm the biological roles of both genes and support their dysregulation in T2DM. The GTEx profiles also provide additional support for the validity of blood-based gene expression as a surrogate for systemic metabolic disturbances in T2DM. (See Supplementary Figure S1 and S2 showing GTEx expression profiles of GCK and SLC2A4 across different tissues.).For more details see supplementary Table 5.

Multiple testing correction

Multiple hypothesis testing was conducted across the key comparisons using the Benjamini-Hochberg False Discovery Rate (FDR) correction. Table 2 (supplementary data) presents the raw and FDR-adjusted p-values for the primary variables, along with corresponding fold changes and statistical significance. The results showed that zinc levels were significantly reduced in T2DM patients, and this association remained robust after multiple

testing correction. Zinc also demonstrated significant associations with altered gene expression. In contrast, while serum chromium levels were lower in patients compared to controls and initially suggested a trend toward association, this difference did not remain statistically significant after FDR adjustment and should therefore be interpreted with caution.

Discussion

Trace elements are present in small quantities in the human body, their significance to health cannot be overstated. Previous studies have demonstrated that proper trace element homeostasis is essential for regulating blood glucose and mitigating tissue damage in T2DM patients[35,36]. Consistent with this evidence, our study combined computational analysis, molecular docking, and patient-based validation to investigate how zinc and chromium deficiencies may alter gene expression and contribute to T2DM pathogenesis. Among the two elements investigated, zinc demonstrated greater consistency and stronger statistical significance, highlighting its potential role in the pathogenesis of T2DM. This finding is in agreement with earlier reports that zinc is indispensable for insulin synthesis, storage, and secretion in pancreatic β-cells[37,38]. In addition, zinc has antioxidative, anti-apoptotic, and membrane-stabilizing properties that protect vascular endothelial integrity, thereby reducing the risk of diabetic complications[39,40]. Our analysis identified *GCK*, *ZnT8*, and *MT* as zinc-related hub genes, all of which are known to be critical for glucose sensing and β-cell function. Prior studies have similarly linked zinc deficiency with altered expression of these genes, further supporting their importance in the progression of T2DM[41,42].Chromium, another essential trace element, is primarily involved in the regulation of carbohydrate, lipid, and protein metabolism[43]. In our study, chromium showed associations with *PPARG*, *GLUT4*, and *IRS1*, genes that are central to insulin signaling and glucose uptake. These findings are consistent with earlier evidence that chromium supplementation improves insulin sensitivity and enhances *GLUT4* translocation and *IRS1* activity[44,45].However, it is noteworthy that chromium-related associations did not retain statistical significance after false discovery rate (FDR) correction. This suggests that chromium's effects may be more subtle or context-dependent compared to zinc, and should be interpreted with caution. Larger cohort studies and functional experiments are required to clarify the precise role of chromium in T2DM.

Computational analysis of zinc and chromium-associated genes using bioinformatics tools revealed that these genes participate in multiple pathways directly linked to the pathogenesis of T2DM. Protein–protein interaction (PPI) analysis further highlighted the hub proteins and their interconnections, underscoring their central role in maintaining glucose homeostasis. 3D structure of proteins was further used for docking. To explore therapeutic relevance, the three-dimensional structures of these proteins were subjected to molecular docking with zinc and chromium compounds. Modeling free Zn^{2+} and Cr^{3+} ions would require specialized metal-aware docking protocols or quantum mechanics/molecular mechanics (QM/MM) approaches to capture coordination chemistry, which was beyond the scope of the present study. Bioavailability of zinc and chromium varies by compound. We used gluconate and picolinate due to their safety and established use, but future studies should compare alternative formulations to optimize efficacy. Docking results revealed that zinc gluconate and chromium picolinate display favorable binding affinities with key proteins, offering structural insights into how supplementation may help restore molecular functions disrupted by trace element deficiencies.Although *ZnT8* and *GCK* have been linked to insulin production in earlier research, our docking and expression results confirm that these proteins are functionally impaired in trace element deficiencies. Therefore, our results support the increasing amount of research that points to these genes as possible targets for treatment. To validate the in-silico results, trace element analysis in T2DM patients reveals considerably lower serum zinc levels than healthy controls, suggesting a strong link between zinc deficiency and T2DM[46]. This observation aligns with previous studies reporting a negative relationship between serum zinc concentrations and diabetic complications[18,19]. Zinc deficiency has been implicated in oxidative stress, impaired insulin secretion, and heightened susceptibility to complications, which is consistent with our finding of particularly reduced zinc levels in patients with nephropathy[47,48]. Similarly, serum chromium concentrations were also significantly reduced in T2DM patients compared with controls ($p < 0.05$). Chromium plays an important role in glycemic regulation, and its deficiency has been linked to alterations in lipid metabolism and an increased risk of cardiovascular disease (CVD)[49]. Our subgroup analysis revealed that T2DM patients with CVD exhibited lower chromium levels, reinforcing earlier reports that chromium can enhance insulin sensitivity[50]. Unlike some studies that did not differentiate between diabetic complications, our data highlight subgroup-specific associations, adding novel insight into the relationship between trace element deficiencies and T2DM. It is worth noting, however, that the role of chromium in improving insulin receptor activity and glycemic control may be less consistent or population-dependent than traditionally assumed. While computational pathway analysis identified chromium-linked targets within insulin signaling cascades, the modest serum-level differences suggest that chromium deficiency may exert subtle or context-specific effects not fully captured in cross-sectional measurements. From a therapeutic perspective, it is important to emphasize that only trivalent chromium compounds are safe and widely used as dietary supplements. Higher oxidation states, such as hexavalent chromium [Cr(VI)], are toxic and were not considered in our study. Our recommendations are therefore limited to nutritionally relevant doses of trivalent chromium[51].

Subgroup analyses revealed lower zinc and chromium levels in T2DM patients with nephropathy, CVD, and retinopathy compared to healthy controls. However, each subgroup included only 16–17 participants, limiting statistical power and increasing the risk of type II error. Therefore, these findings should be interpreted as preliminary and hypothesis-generating rather than confirmatory. To verify the computational predictions, gene expression analysis was performed to evaluate the potential effects of zinc and chromium deficiencies on key genes involved in glucose metabolism and insulin signaling. Genes were selected based on protein–protein interaction (PPI) networks and high enrichment in critical pathways such as glycolysis/gluconeogenesis, insulin signaling, and glucose homeostasis, as identified by KEGG pathway enrichment. Expression profiling of *GCK* and *SLC2A4* (*GLUT4*) was conducted using qPCR, and results were analyzed via CT values.In T2DM

patients, both *GCK* and *GLUT4* were downregulated. Subgroup analyses showed distinct patterns: in diabetic CVD patients, both genes were downregulated, with *GLUT4* exhibiting a more pronounced reduction and statistically highly significant results. In diabetic nephropathy patients, both genes were also downregulated, but *GCK* expression showed the greatest reduction compared to other groups, with significant results. In diabetic retinopathy patients, downregulation of *GCK* and *GLUT4* was observed, but the differences were not statistically significant relative to other groups. Finally, transcriptomic analysis further supported our serum findings. GEO datasets and GTEx expression profiles consistently showed enrichment of *GCK* in pancreatic and hepatic tissues and *SLC2A4* in muscle and adipose tissues. This cross-validation across independent resources strengthens the reliability of our research. These findings suggest that zinc and chromium deficiencies likely contribute to the downregulation of *GCK* and *GLUT4*.Chronic hyperglycemia and systemic inflammation associated with prolonged illness can induce transcriptional alterations independently of trace element status. Thus, a complex interplay of metabolic, dietary, and disease-related variables may underlie the observed gene downregulation. Future studies will extend gene expression analyses to additional zinc- and chromium-sensitive genes to identify potential therapeutic targets. Overall, integrating computational docking with gene expression analysis provides compelling evidence that zinc and chromium are critical for maintaining the expression and function of genes essential for glucose metabolism and insulin action. It is important to note that docking analysis predicts potential molecular interactions but does not establish causality or direct regulatory effect.

Although this investigation employed peripheral blood for gene expression profiling in place of tissue samples from the pancreas, liver, or muscle the organs most prominently involved in T2DM earlier research has validated the employment of blood as a good surrogate for systemic disease status. Peripheral blood is not just readily available and least invasive but also mirrors dynamic physiological responses to metabolic and inflammatory cues. For T2DM, previous studies provided evidence that blood transcriptomic signatures are associated with insulin resistance, beta-cell dysfunction, and inflammation. Moreover, the systemic influence of zinc and chromium on pathways such as oxidative stress, inflammation, and insulin signaling justifies the examination of blood-derived transcriptomes[52,53].Our findings highlight zinc deficiency as a robust feature of T2DM, consistent with prior studies linking zinc to insulin signaling, β-cell function, and oxidative stress regulation. Although chromium initially showed an association with T2DM, this did not remain significant after multiple testing correction, indicating that its role in disease pathology should be interpreted with caution. While chromium may still contribute to glucose metabolism, larger studies and more sensitive methods are required to clarify its involvement. Markedly reduced zinc and chromium levels in T2DM and its complications not only support their role in disease pathophysiology but also carry important clinical implications. Serum zinc and chromium measurements may serve as potential diagnostic or prognostic biomarkers, particularly for identifying patients at risk of complications such as nephropathy, cardiovascular disease (CVD), and retinopathy[46, 54]. Integrating trace element assessment into routine clinical evaluations could enhance early detection of metabolic imbalance. From a therapeutic perspective, the observed deficiencies highlight the potential value of targeted supplementation strategies, which should be evaluated in randomized controlled trials (RCTs).Tailored micronutrient interventions, alongside standard antidiabetic regimens, may therefore provide an adjunctive approach for managing T2DM and delaying complication onset. Future clinical trials should investigate the efficacy and safety of zinc and chromium supplementation in well-characterized T2DM subgroups, with attention to dosage, bioavailability, and long-term outcomes. Such strategies could help translate trace element research into practical clinical interventions. While zinc deficiency was robustly associated with altered gene expression, chromium findings were attenuated after correction for multiple testing. Therefore, chromium's role in this cohort should be considered tentative. Nonetheless, prior evidence linking chromium to glucose metabolism suggests a potential contribution that warrants validation in larger, adequately powered studies. In our analyses, chromium showed suggestive but not definitive associations with T2DM, whereas zinc remained the primary robust finding across both unadjusted and adjusted analyses.

Limitations and future directions

This study has several limitations. First, the relatively small sample size, particularly in subgroup analyses, may have reduced statistical power, making the findings exploratory rather than definitive. Second, the cross-sectional design prevents causal inference; longitudinal or interventional studies are needed to determine whether correcting trace element deficiencies directly improves outcomes. Third, gene expression was measured in peripheral blood rather than metabolic tissues, which may not fully capture tissue-specific regulation. Fourth, only messenger RNA (mRNA) expression was analyzed, without protein-level or functional validation, limiting mechanistic conclusions. Fifth, trace element levels were assessed using atomic absorption spectrophotometry without cross-validation by more sensitive techniques (e.g., ICP-MS), and dietary intake, supplement use, and comorbidities were not fully controlled, leaving room for confounding. Finally, molecular docking results provide predictive but not functional evidence, requiring in vitro or in vivo confirmation. Confounders such as diet, supplement use, diabetes duration, and comorbidities were not fully adjusted, and future studies with multivariate analysis are needed to clarify their impact.

Despite these limitations, our findings provide preliminary insights into the role of zinc and chromium deficiencies in T2DM, offering a foundation for future mechanistic studies and clinical trials aimed at validating their utility as biomarkers and therapeutic targets.

Conclusion

This study highlights the roles of zinc and chromium in insulin signaling and glucose metabolism, providing evidence of their involvement in T2DM pathogenesis. Zinc deficiency emerged as a robust and statistically significant feature. In contrast, chromium showed a suggestive but non-significant association after correction,

warranting further investigation. Monitoring these trace elements may provide diagnostic and prognostic value. Targeted supplementation should be evaluated in randomized controlled trials (RCTs) to establish its efficacy and safety as a cost-effective adjunct to conventional therapy, with the potential to improve glycemic control and reduce complications. However, causal relationships remain unconfirmed. This study also did not account for individual variability, such as genetics, diet, and supplementation response. Future large-scale longitudinal studies and randomized controlled trials are needed to determine efficacy, safety, and optimal supplementation protocols, as well as to clarify chromium's role. Collectively, these findings support zinc and chromium as promising biomarkers and adjunctive therapeutic targets in T2DM management.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author, Dr. Attya Bhatti, upon reasonable request. Some data presented in this manuscript are derived from an unpublished study. In accordance with ethical guidelines, any overlap with previous data has been clearly indicated. No duplicate publication or data misuse has occurred. The findings reported here are original and focus specifically on the associations between trace elements and type 2 diabetes mellitus (T2DM), thereby contributing new insights to the field.

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Author contributions
Conceptualization: Humma Nayyar, Attya Bhatti, Peter John.Data curation: Humma Nayyar.Formal analysis: Humma Nayyar, Attya Bhatti, Peter John.Investigation: Humma Nayyar, Attya Bhatti, Peter John.Methodology: Humma Nayyar, Attya Bhatti, Peter John.Project administration: Humma Nayyar, Attya Bhatti, Peter John. Resources: Humma Nayyar, Attya Bhatti, Peter John.Software: Humma Nayyar.Supervision: Attya Bhatti, Peter John.Validation: Humma Nayyar, Attya Bhatti, Peter John.Visualization: Humma Nayyar, Attya Bhatti, Peter John.Writing – original draft: Humma Nayyar.Writing – review & editing: Humma Nayyar, Attya Bhatti, Peter John.

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Additional information

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Correspondence and requests for materials should be addressed to A.B.

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