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Comparative Analysis of Mitonuclear Protein Interaction Interfaces and Conformational Differences Between the T3 and I1 Mitochondrial Haplogroups in Mongolian Cattle

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Abstract

Background

Coordination between the mitochondrial and nuclear genomes is essential for the assembly and function of oxidative phosphorylation (OXPHOS) complexes. However, whether structural differences occur at mitonuclear protein interfaces between the T3 and I1 mitochondrial haplogroups in Mongolian cattle remains unclear. We compared mitochondrially encoded protein sequences from 10 Mongolian cattle per haplogroup. We integrated bovine OXPHOS complex structures, AlphaFold 3 predictions, docking with High Ambiguity Driven protein–protein DOCKing (HADDOCK), and Protein–Ligand Interaction Profiler (PLIP) and contact-map analyses to assess associations between amino acid substitutions and local structural differences at mitonuclear interfaces.

Results

Nineteen amino acid substitutions consistently distinguished the haplogroups across eight mitochondrially encoded proteins. Eight occurred at mitonuclear interfaces involving nine nuclear-encoded interaction partners and 11 candidate interaction pairs. Overall backbone conformations were largely conserved between haplogroups, whereas differences were concentrated in local contact networks near substitution sites. Notable haplogroup-associated contact differences occurred at the ND4–NDUFA8 and ND5–NDUFA11 interfaces in complex I and the ATP8–ATP5PD interface in complex V. Because confidence in the ATP8 model was relatively low, the ATP8–ATP5PD results should be interpreted cautiously.

Conclusions

These findings identify three key candidate mitonuclear interfaces for subsequent functional validation and provide structural clues for investigating mitonuclear interaction mechanisms.

Background

Oxidative phosphorylation (OXPHOS) is the primary pathway for adenosine triphosphate (ATP) production in animal mitochondria and is essential for maintaining cellular energy supply and metabolic homeostasis. In beef cattle, the efficiency of mitochondrial energy metabolism is thought to be associated with feed efficiency, production traits, and responses to environmental stress [1, 2]. However, these phenotypic associations are influenced by multiple factors, including nuclear genetic background, tissue status, and environmental conditions. Clarifying the potential relationships between OXPHOS subunit structures and variation in the genes encoding these subunits may provide candidate molecular clues for investigating energy metabolism and environmental adaptation in local cattle breeds. OXPHOS complexes comprise subunits encoded by the mitochondrial genome (mitochondrial DNA, mtDNA) and

the nuclear genome (nuclear DNA, nDNA). Their assembly and function depend on coordinated interactions between mitochondrially encoded and nuclear-encoded gene products [3]. Over evolutionary time, this mitonuclear coordination has been subject to strong functional constraints. Structural and functional compatibility between mitochondrially encoded and nuclear-encoded subunits therefore provides an important foundation for stable mitochondrial function [4].

Bovine mtDNA is maternally inherited, exhibits little recombination, and evolves relatively rapidly. It has therefore long been used to investigate livestock origins, maternal lineages, and population genetic structure [5]. Among bovine maternal lineages, T haplogroups generally represent maternal ancestry from taurine cattle (*Bos taurus*), whereas I haplogroups primarily represent maternal ancestry from indicine cattle (*Bos indicus*) [6]. Taurine and indicine cattle experienced different environmental selection pressures during their evolutionary histories. Nonsynonymous variants potentially associated with adaptation may therefore have accumulated in their mtDNA protein-coding genes. The coexistence of the T3 and I1 mitochondrial haplogroups in Mongolian cattle reflects the retention of both taurine and indicine maternal lineages [7]. Nuclear genomic studies further indicate that the genetic composition of Chinese cattle has been shaped by admixture between taurine and indicine components [8]. Mongolian cattle have long been raised in cold environments under extensive husbandry conditions with limited nutrient availability. Their maternal genetic backgrounds and variants related to mitochondrial function therefore warrant investigation. Previous studies, however, have largely focused on mtDNA sequence diversity, maternal origins, and population genetic structure. They have not directly addressed whether the interfaces between mitochondrially encoded and nuclear-encoded OXPHOS subunits differ structurally across mtDNA backgrounds.

Research on bovine mtDNA has focused largely on maternal lineages and population genetics, whereas the effects of nonsynonymous variants on protein structure, subunit contacts, and mitonuclear coordination have not yet been systematically evaluated. For the T3 and I1 maternal backgrounds in Mongolian cattle in particular, structural evidence remains limited as to whether the relevant amino acid differences occur at direct interfaces between mitochondrially encoded and nuclear-encoded subunits within OXPHOS complexes and whether these differences are accompanied by changes in local contacts, including hydrogen bonds, hydrophobic interactions, and salt bridges. In recent years, tools for protein structure prediction, molecular docking, and interface contact analysis, including AlphaFold 3, High Ambiguity Driven protein–protein DOCKing (HADDOCK), Protein–Ligand Interaction Profiler (PLIP), Protein Interfaces, Surfaces and Assemblies service at PDBe (PDBePISA), and Protein Data Bank in Europe Knowledge Base (PDBe-KB), have provided a feasible means of screening candidate mitonuclear interaction interfaces based on sequence variation [9–13].

This study investigated mtDNA coding-region variation in Mongolian cattle belonging to the T3 and I1 maternal lineages. We compared amino acid differences in mitochondrially encoded proteins between the two haplogroups and mapped these sites onto bovine OXPHOS complex structures. We then screened for candidate variants located at interfaces between mitochondrially encoded and nuclear-encoded subunits. By integrating structural modeling, interface contact identification, and contact

network analysis, we assessed potential structural associations between the candidate variants and differences in local subunit interactions. We focused on the ND4–NDUFA8 and ND5–NDUFA11 interfaces in complex I and the ATP8–ATP5PD interface in complex V. This study may provide clues regarding potential OXPHOS structural differences associated with the two mitochondrial genetic backgrounds in Mongolian cattle. It may also provide candidate targets for subsequent validation of mitochondrial function, investigation of mitonuclear interaction mechanisms, and association analyses involving relevant economically important traits.

Methods

Sample Sources and Mitochondrial Haplogroup Selection

Based on previous laboratory research data [14], 10 complete mitochondrial genome sequences from each of the T3 and I1 haplogroups of Mongolian cattle were selected for analysis. The 20 sequences have been deposited in GenBank under accession numbers PZ890225–PZ890244. To illustrate the mitochondrial phylogenetic grouping of the selected samples, a maximum-likelihood phylogenetic tree was constructed using MEGA 12 [15] based on the complete mitochondrial genomes of the 20 Mongolian cattle samples and the Bos taurus mitochondrial reference genome (GenBank: V00654.1). The sequences were aligned using ClustalW, and the tree was constructed using the Tamura–Nei 1993 (TN93) model with 500 bootstrap replicates. Using the Bos taurus mitochondrial reference genome (GenBank: V00654.1), mitochondrial genomes were annotated with MitoFinder v1.4.2 [16], and the sequences of the 13 mtDNA protein-coding genes were extracted and translated into amino acid sequences. After confirming that the T3 and I1 samples formed separate clusters, multiple sequence alignments were performed for each of the 13 mtDNA-encoded proteins across the 20 samples. Because the protein sequences were highly consistent within each haplogroup, 19 consistent inter-haplogroup amino acid substitutions were identified. To exclude potential interference from random individual mutations, the consensus protein sequence of each haplogroup (haplogroup consensus sequence) was used as the input sequence for the mtDNA-encoded subunits in subsequent three-dimensional protein structure prediction and molecular docking analyses. All T3 and I1 models used the same set of bovine nuclear-encoded protein reference sequences. This held the nuclear-encoded subunit sequence background constant and allowed the assessment to focus on the potential effects of amino acid substitutions associated with the two mtDNA haplogroups on the structures of candidate mitonuclear interaction interfaces.

Spatial Mapping and Screening of Candidate Mitonuclear Interaction Interfaces

To determine which of the 19 consistent inter-haplogroup amino acid substitution sites were located at interaction interfaces, we mapped them onto experimentally resolved structures of bovine respiratory chain complexes obtained from the RCSB Protein Data Bank (PDB) (complex I: 5LDW; complex III: 1NTM;

complex IV: 7Y44; complex V: 6ZIQ) [17–20]. Candidate structural interaction partners for each mtDNA-encoded protein containing a substitution site were obtained from the “Interacting Partners” information in PDBe-KB [13]. Entries corresponding to mtDNA-encoded subunits and nonspecific entries such as “Other” were excluded, whereas potential nuclear-encoded interaction partners were retained (Table S1A; Additional file 1). By integrating interface residue information from PDBe-KB and PDBePISA (Table S1B; Additional file 1), we identified the interface residues and spatial contacts between candidate mtDNA-encoded proteins and nuclear-encoded proteins. Only protein pairs in which the substitution sites were clearly located within contact regions were retained (Table 1) for subsequent AlphaFold 3 modeling, HADDOCK docking, and contact network analysis. Although amino acid differences between T3 and I1 were identified in COX3 and CYTB, these sites did not map to contact regions with nuclear-encoded subunits and were therefore excluded from subsequent docking analysis.

Table 1
Candidate mitonuclear protein-protein interaction pairs retained after interface contact screening

OXPHOS complex	Mitochondrially encoded subunit(s)	Nuclear-encoded subunit
Complex I	ND1, ND4	NDUFA8
Complex I	ND1	NDUFA13
Complex I	ND3	NDUFA3
Complex I	ND4	NDUFB11
Complex I	ND4	NDUFB5
Complex I	ND5	NDUFA11
Complex V	ATP6	ATP5MF
Complex V	ATP6, ATP8	ATP5PD
Complex V	ATP6	ATP5MJ

Note: Candidate interaction pairs were retained based on bovine OXPHOS complex structures (PDB: 5LDW, 1NTM, 7Y44, and 6ZIQ) and interface information from PDBe-KB and PDBePISA when amino acid substitution sites distinguishing T3 and I1 were located within mitonuclear contact regions. COX3 and CYTB were not included because their substitution sites did not map to mitonuclear contact interfaces.

Three-Dimensional Structural Modeling and Sequence Preparation for Core Proteins

Based on the within-haplogroup sequence consistency established through preliminary alignment, we extracted the T3 and I1 haplogroup consensus sequences containing the candidate variant sites. AlphaFold 3 (AF3, version 3.0.0) was used to generate monomeric models of the selected core mitochondrially encoded and nuclear-encoded subunits, with the corresponding haplogroup consensus

sequences used as inputs for the mitochondrially encoded subunits. Each amino acid sequence was submitted as a single proteinChain, and the random seed was set to modelSeeds = 1. To model the mature forms of the nuclear-encoded proteins, N-terminal mitochondrial transit peptides (mTPs) were predicted using TargetP 2.0 [21] (Figure S1; Additional file 2). For NDUFB11 and NDUFB5, which were predicted to contain mTPs, the mature peptide regions were determined based on UniProt annotations [22], and the transit peptides were removed before structural prediction. After prediction, model quality was evaluated using the predicted local distance difference test (pLDDT), predicted template modeling score (pTM), and ranking_score metrics output by AF3. The model with the highest ranking_score was selected for subsequent structural comparison and mitonuclear interaction analysis (Table 2). All selected models were visually inspected using PyMOL 3.1.0a0 [23]. Details of nuclear-encoded protein sequence processing and model quality evaluation are provided in Tables S2 and S3 (Additional files 3 and 4). AF3 quality metrics for all 60 mitochondrial-protein model samples are provided in Supplementary Data 1 (Additional file 5).

Table 2
AF3 model quality for core mtDNA-encoded proteins differing between the T3 and I1 haplogroups

No.	Protein	Haplogroup	Best model	pLDDT	pTM	Ranking score	No. of amino acid substitutions
1	ATP6	T3	seed-1_sample-1	78.52	0.70	0.7310	3
2	ATP6	I1	seed-1_sample-2	84.31	0.80	0.8222	3
3	ATP8	T3	seed-1_sample-2	75.37	0.43	0.9263	2
4	ATP8	I1	seed-1_sample-0	73.47	0.39	0.8862	2
5	ND1	T3	seed-1_sample-4	88.38	0.91	0.9212	1
6	ND1	I1	seed-1_sample-4	88.40	0.92	0.9252	1
7	ND3	T3	seed-1_sample-0	77.73	0.63	0.8707	1
8	ND3	I1	seed-1_sample-4	78.37	0.63	0.8652	1
9	ND4	T3	seed-1_sample-4	90.61	0.94	0.9449	2
10	ND4	I1	seed-1_sample-1	88.46	0.94	0.9352	2
11	ND5	T3	seed-1_sample-1	89.49	0.92	0.9542	7
12	ND5	I1	seed-1_sample-1	88.22	0.91	0.9412	7

Note: Only the six mtDNA-encoded proteins containing substitution sites at mitonuclear interfaces and included in subsequent analyses are shown. COX3 and CYTB were excluded because their substitution sites were not located at mitonuclear contact interfaces; the remaining unlisted proteins showed no consistent inter-haplogroup amino acid substitutions. The best model was selected based on the highest ranking_score. pLDDT and pTM indicate confidence in local structure and overall topology, respectively. The substitution count represents the number of consistent inter-haplogroup amino acid substitution sites between T3 and I1.

Molecular Docking and Local Contact Network Analysis of Mitonuclear Interfaces

To evaluate the potential effects of haplogroup-associated differences on mitonuclear interaction interfaces, we used a three-stage analytical workflow. First, HADDOCK restraint residues were defined, and protein binding was simulated. Interface residues of mitochondrially encoded and nuclear-encoded subunits, verified using PDBe-KB and PDBePISA, were defined as active residues. Haplogroup-associated substitution sites located at the interfaces were also included in the restraint set. The same interface restraint scheme was applied to the T3 and I1 models of each protein pair. The haddock3-restraints module in HADDOCK 3.0 was used to generate ambiguous interaction restraint files for docking 11 mitonuclear protein pairs. A total of 1,000 models were sampled during rigid-body docking, and the top 200 models were selected for semi-flexible refinement.

Second, HADDOCK scores and energy distributions were evaluated. Fraction of common contacts (FCC) clustering was performed using a contact-distance cutoff of 5.0 Å. Based on the HADDOCK ranking, the highest-ranked cluster and its top four representative models were selected. The means and standard deviations of the HADDOCK score, electrostatic energy, van der Waals energy, and desolvation energy were then calculated. For each interface, electrostatic energies were compared between T3 and I1 using a two-sided Welch's t-test. The resulting P values for the 11 interfaces were adjusted using the Benjamini–Hochberg method. One, two, and three asterisks indicate adjusted $P < 0.05$, < 0.01, and < 0.001, respectively. HADDOCK scores, van der Waals energies, and desolvation energies were analyzed descriptively only. The resulting P values were used solely to quantify the separation of electrostatic energies at the docking-model level and did not represent inferential statistical results based on independent biological samples. Details of the restraint residues and residue-numbering mappings are provided in Table S4 (Additional file 6). The PDBe-KB and PDBePISA evidence used for interface selection is presented in Table S1B (Additional file 1).

Third, representative docking conformations were compared, and local contact networks were analyzed. For each candidate interface, the top-ranked model from the highest-ranked cluster was selected as the representative conformation. The T3 and I1 complexes were structurally superimposed, and interfaces showing conformational differences were descriptively identified based on changes in the relative binding orientations of the two subunits. Contact-map analysis was performed on the top four models from the highest-ranked cluster. For each inter-chain residue pair in each model, two complementary geometric criteria were evaluated separately: a Cα–Cα distance of ≤ 9.0 Å and a minimum interatomic distance of ≤ 4.5 Å. A residue pair was retained as a contact-map edge if either criterion was satisfied in at least one of the four models. The occurrence frequency for each criterion was calculated separately across the four models, and the larger of the two values was reported as the summary contact frequency. PLIP 3.0.0 [11] was used to analyze the top-ranked model and identify noncovalent interactions, including hydrogen bonds, hydrophobic interactions, salt bridges, and π-stacking interactions. Key interfaces for detailed analysis were selected by integrating the docking conformations, contact partners near the substitution sites, and changes in noncovalent interactions identified by PLIP. The PLIP results were cross-checked against the contact-map and PDBePISA data and were not used alone to identify key interfaces. Complete contact-map and PLIP data are provided in Additional file 7. The supporting structural and docking model files are provided in Additional file 8.

Results

Mitochondrial Haplogroup Differentiation and Characteristics of Consistent Amino Acid Substitutions in Mongolian Cattle

The maximum-likelihood (ML) phylogenetic tree, constructed from the complete mitochondrial genomes of 20 Mongolian cattle and the *Bos taurus* mitochondrial reference genome (GenBank: V00654.1), showed a clear clustering pattern (Fig. 1a). The 10 T3 sequences and 10 I1 sequences clustered into two distinct branches, with bootstrap support values of 99.6% and 100%, respectively, indicating that the T3 and I1 samples represented two distinct phylogenetic groups in this dataset. Following confirmation of the phylogenetic grouping, amino acid sequence alignment identified 19 consistent inter-haplogroup amino acid substitutions between the T3 (n = 10) and I1 (n = 10) haplogroups (Fig. 1b). These substitutions were distributed across eight mtDNA-encoded proteins: ND1, ND3, ND4, ND5, ATP6, ATP8, COX3, and CYTB. No consistent amino acid substitutions were detected between the two haplogroups in COX1, COX2, ND2, ND4L, or ND6. Because the T3 and I1 samples formed separate clusters in the phylogenetic tree and showed highly consistent amino acid sequences within each haplogroup, these inter-haplogroup substitutions were selected as candidate sites for subsequent structural comparisons. Most were conservative or semi-conservative substitutions between amino acids with similar physicochemical properties. Several representative substitutions, including ND1 T167I, ND3 A82T, ND4 L55V, and ND5 I600M, involved changes in side-chain volume, polarity, or hydrophobicity, providing a basis for subsequent local conformational comparisons and screening of mitonuclear protein interaction interfaces.

Structural Mapping and Screening of Candidate Mitonuclear Interaction Interfaces

The 19 candidate amino acid substitution sites were mapped onto reference structures of bovine respiratory chain complexes and cross-validated using PDBe-KB interface residue annotations and PDBePISA subunit contact analyses. Eight substitution sites were located at direct contact interfaces between mtDNA-encoded and nuclear-encoded proteins (Fig. 2). These sites were distributed across six mtDNA-encoded proteins (ND1, ND3, ND4, ND5, ATP6, and ATP8). Nine directly interacting nuclear-encoded proteins were identified, including NDUFA8, NDUFA13, NDUFA3, NDUFB11, NDUFB5, NDUFA11, ATP5MF, ATP5PD, and ATP5MJ, forming 11 candidate mitonuclear protein interaction pairs. The substitution sites in COX3 (V171I) and CYTB (V356I and I372V) were not located at direct contact interfaces with nuclear-encoded proteins and were therefore excluded from subsequent molecular docking and contact network analyses.

Structural Comparison and Model Evaluation of Core mtDNA-Encoded Proteins between the T3 and I1 Haplogroups

To assess whether amino acid substitutions at mitonuclear interaction interfaces were associated with changes in protein conformation, we used AF3 to predict the monomeric structures of six selected core mtDNA-encoded proteins (ND1, ND3, ND4, ND5, ATP6, and ATP8) and performed whole-protein Cα superposition of the representative T3 and I1 models (Fig. 3). Except for ATP8, the remaining five proteins had root-mean-square deviation (RMSD) values ranging from 0.119 to 1.077 Å (Table 3), indicating that their overall backbone conformations were largely conserved between the two haplogroups and that the consistent inter-haplogroup amino acid substitutions were not associated with pronounced global conformational differences. ATP8 had a higher RMSD (6.071 Å), but it also had the shortest sequence and the lowest mean pLDDT values reported by AF3 among all predicted structures (T3: 75.37; I1: 73.47; Table 2), indicating greater model uncertainty. Therefore, the ATP8 results should be interpreted with particular caution. Overall, the structural differences between T3 and I1 were not primarily characterized by changes in the global fold but appeared to be concentrated in side-chain orientations, local steric effects, or electrostatic surface distributions near the substitution sites. These local differences suggest that haplogroup-associated substitutions may alter local geometric relationships and docking score distributions at candidate interfaces through subtle changes at the side-chain level. Whether these differences affect binding stability and functional output in native complexes requires experimental validation.

Table 3
Whole-protein structural alignment RMSD values for mitochondrially encoded proteins from the T3 and I1 haplogroups

Protein	Amino acid substitution(s)	Mean pLDDT (T3)	Mean pLDDT (I1)	Whole-protein Cα RMSD (Å)	Structural interpretation
ND1	167 T > I	92.1	92.0	0.119	Minor difference in the overall backbone
ND3	82 A > T	82.3	82.9	1.077	Overall backbone largely conserved, with relatively more pronounced local differences
ND4	21 N > S; 55 L > V	94.5	92.4	0.133	Minor difference in the overall backbone
ND5	24 F > L; 90 I > T; 109 Y > H; 272 Y > F; 442 N > S; 507 M > T; 600 I > M	93.6	92.5	0.147	Minor difference in the overall backbone
ATP6	7 T > A; 69 T > A; 154 M > V	82.6	88.3	0.339	Minor difference in the overall backbone
ATP8	28 V > I; 53 T > A	80.7	78.9	6.071	High RMSD; short sequence and low pLDDT; interpret cautiously

Note: RMSD values were obtained by whole-protein Cα-atom superposition of each I1 model onto the corresponding T3 model in PyMOL, with iterative exclusion of atoms showing large deviations. The pLDDT values in Table 3 are the mean values in the B-factor column for Cα atoms in the aligned model PDB files and are used only to assist in evaluating confidence in the regions included in the backbone alignment. By contrast, the pLDDT values in Table 2 are the mean all-atom atom_plddts values from the AF3 output file confidences.json. Because the two calculations use different sets of atoms, the resulting values may differ.

Comparison of Docking Scores and Energy Components at Candidate Mitonuclear Interaction Interfaces

HADDOCK 3.0 docking results showed that differences in HADDOCK scores between the T3 and I1 haplogroups across the 11 candidate mitonuclear protein interaction interfaces were complex-specific and interface-dependent. Neither haplogroup consistently yielded lower scores across all interfaces. Overall, T3 models yielded lower HADDOCK scores at most interfaces in complex I, whereas I1 models yielded lower scores at most interfaces in complex V. Based on subsequent integrated analyses of representative docking conformations and local contact networks, the ND4–NDUFA8, ND5–NDUFA11, and ATP8–ATP5PD interfaces were selected for detailed analysis, while the remaining interfaces were retained for comparison.

Among the seven candidate interfaces in complex I, T3 models yielded lower HADDOCK scores than I1 models at five interfaces: ND1–NDUFA8, ND4–NDUFA8, ND1–NDUFA13, ND4–NDUFB5, and ND5–NDUFA11. The ND4–NDUFA8 interface showed a relatively pronounced score difference, with the mean score for T3 being 13.21 points lower than that for I1 (T3: −78.87 ± 2.07; I1: −65.66 ± 11.51). Its energy components were characterized mainly by lower electrostatic and van der Waals energies in T3 (electrostatic energy: T3, − 78.38 ± 4.81 kcal·mol⁻¹; I1, − 63.93 ± 32.56 kcal·mol⁻¹; van der Waals energy: T3, − 39.29 ± 2.27 kcal·mol⁻¹; I1, − 24.08 ± 10.33 kcal·mol⁻¹). At the ND5–NDUFA11 interface, the mean HADDOCK score for T3 was 5.01 points lower than that for I1 (T3: −111.11 ± 3.79; I1: −106.10 ± 4.78). The energy components showed different directional patterns. T3 had lower van der Waals and desolvation energies (van der Waals energy: T3, − 65.24 ± 4.68 kcal·mol⁻¹; I1, − 50.16 ± 3.60 kcal·mol⁻¹; desolvation energy: T3, − 46.95 ± 4.27 kcal·mol⁻¹; I1, − 11.43 ± 6.61 kcal·mol⁻¹), whereas I1 had lower electrostatic energy (I1: −265.25 ± 49.02 kcal·mol⁻¹; T3: −139.40 ± 51.61 kcal·mol⁻¹) (Fig. 4).

The four candidate interfaces in complex V showed a scoring pattern different from that observed in complex I. I1 models yielded lower HADDOCK scores than T3 models at the ATP8–ATP5PD, ATP6–ATP5PD, and ATP6–ATP5MJ interfaces, whereas T3 yielded a lower score only at the ATP6–ATP5MF interface. The ATP8–ATP5PD interface showed a relatively pronounced score difference, with the mean score for I1 being 7.64 points lower than that for T3 (I1: −119.77 ± 1.98; T3: −112.13 ± 4.83). Its energy components were characterized mainly by lower electrostatic energy in I1 (I1: −264.75 ± 19.52 kcal·mol⁻¹; T3: −75.74 ± 20.90 kcal·mol⁻¹) (Fig. 4).

Together, these results showed that shifts in HADDOCK score distributions between the T3 and I1 haplogroups at mitonuclear protein interaction interfaces varied by complex and interface. T3 scores were lower at most interfaces in complex I, whereas I1 scores were lower at most interfaces in complex V. Representative docking conformations, contact maps, and PLIP-based analyses of noncovalent interactions were subsequently integrated to assess whether these differences in score and energy distributions were accompanied by changes in specific residue contact networks.

Conformational and Contact Network Characteristics of Key Mitonuclear Interaction Interfaces

To evaluate the relationship between T3- and I1-associated amino acid substitutions and local contact features at mitonuclear interaction interfaces, we first compared representative binding conformations at 11 candidate interfaces between the two haplogroups. Five interfaces showed relatively clear conformational differences: ND4–NDUFA8, ND5–NDUFA11, ATP6–ATP5MF, ATP8–ATP5PD, and ATP6–ATP5MJ. We then analyzed the local contact networks of all candidate interfaces using contact-map analysis and PLIP-based identification of noncovalent interactions. The substitution sites and adjacent regions at the ND4–NDUFA8, ND5–NDUFA11, and ATP8–ATP5PD interfaces showed relatively pronounced local contact rearrangements (Fig. 5).

At the ND4–NDUFA8 interface, ND4 Leu55 in the T3 background primarily formed a hydrophobic contact with the nuclear-encoded subunit NDUFA8 Phe169. Following the L55V substitution in the I1 haplogroup,

the primary contact partner of ND4 Val55 shifted to NDUFA8 Leu167. The replacement of Leu with Val altered side-chain volume and hydrophobicity and was accompanied by a change in the local hydrophobic contact partner between ND4 and NDUFA8. ND4 is a core mitochondrially encoded subunit of the complex I membrane arm, whereas NDUFA8 is a nuclear-encoded accessory subunit involved in maintaining membrane-arm stability. These local contact changes provide candidate structural clues for understanding complex I assembly or structural fine-tuning between the two mitochondrial genetic backgrounds.

At the ND5–NDUFA11 interface, ND5 Ile600 in the T3 background formed specific spatial contacts with residues including NDUFA11 Ala2. Following the I600M substitution in the I1 haplogroup, changes in side-chain volume and hydrophobicity at this site were accompanied by a shift in the local contact pattern with NDUFA11. The C-terminal transmembrane helix of ND5 directly contacts NDUFA11 and contributes to the stability of complex I and its supercomplexes. Therefore, local changes at this interface may affect higher-order complex assembly between the two mitochondrial genetic backgrounds.

In complex V, the ATP8–ATP5PD interface showed relatively pronounced differences in contact patterns. In the T3 background, ATP8 Val28 formed additional geometric contacts with ATP5PD Phe140 and Glu142. PLIP identified the interaction between Val28 and Phe140 as a hydrophobic interaction. Following the V28I substitution in the I1 background, Ile28 primarily formed geometric contacts with ATP5PD Thr143 and Leu161 and formed a new hydrogen bond with Arg156. The current models indicated that the V28I substitution was accompanied by a local shift from hydrophobic contact to hydrogen-bond formation, suggesting a change in local geometric contacts near this site. However, ATP8 has a relatively short sequence, and confidence in its AF3 models was comparatively low (mean pLDDT: T3, 75.37; I1, 73.47). Structural differences at this interface should therefore be interpreted cautiously, and their biological effects require experimental validation.

In contrast to the relatively pronounced rearrangements at the ND4–NDUFA8, ND5–NDUFA11, and ATP8–ATP5PD interfaces, the remaining candidate mitonuclear interfaces showed relatively conserved contact features between the two haplogroups (Figure S2; Additional file 9). For example, at the ND3–NDUFA3 interface in complex I, despite the ND3 A82T substitution, both haplogroups retained the core geometric contact and noncovalent interaction between ND3 residue 82 and NDUFA3 Arg47. Similarly, at the ATP6–ATP5PD interface in complex V, the contact patterns between ATP6 residue 69 and ATP5PD Tyr152, Trp153, and Pro154 were largely consistent between the T3 and I1 backgrounds. PLIP did not identify direct noncovalent interactions involving ATP6 residue 69 in either haplogroup. Although the other interfaces showed some differences in HADDOCK scores or local geometric contacts, no combined evidence from contact-map and PLIP analyses directly linked these differences to the substitution sites. Overall, the potential effects of haplogroup-associated substitutions on mitonuclear interaction interfaces were interface-specific and concentrated at a limited number of interaction sites. Contact-map and PLIP results for all candidate interfaces are provided in Table S5 (Additional file 10).

The complete model-level values and statistical results underlying Fig. 4 are provided in Source Data 1 (Additional file 11).

Discussion

Maternal mitochondrial lineages in cattle populations reflect their history of domestication, dispersal, and hybridization. The T lineage is mainly associated with maternal ancestry from taurine cattle (*Bos taurus*), whereas the I lineage is mainly associated with maternal ancestry from indicine cattle (*Bos indicus*) [24, 25]. Combinations of different mtDNA haplogroups and nuclear genomic backgrounds may affect OXPHOS function depending on their compatibility, thereby influencing energy metabolism and related phenotypes. OXPHOS complexes are encoded by both mitochondrial and nuclear genomes. Their normal assembly and function depend on coordinated matching and co-evolution between mitochondrially encoded and nuclear-encoded subunits [26]. A study of African admixed cattle (*Bos taurus* × *B. indicus*) identified selection on nuclear-encoded genes related to mitochondrial function. The study also suggested that mtDNA copy number and regulation of related gene expression may contribute to maintaining mitonuclear compatibility [27]. Furthermore, direct contacts between mitochondrially encoded and nuclear-encoded subunits in complex I can result in mitonuclear incompatibility under specific genetic combinations [28]. A study using bovine inter- and intra-species cybrids also showed that different mtDNA backgrounds may affect oxygen consumption rate, ATP turnover, and the expression of metabolism-related genes [29]. Together, these findings suggest that the potential effects of mtDNA background may be influenced by the compatibility between mitonuclear protein partners. In this study, structural differences between the T3 and I1 haplogroups in Mongolian cattle were concentrated mainly in local contact networks at specific mitonuclear interfaces rather than in overall protein backbones. Mapping these differences to specific mitonuclear interaction interfaces provides candidate structural clues for understanding differences between mtDNA haplogroups in Mongolian cattle.

Electrostatic interactions at protein interfaces can influence the recognition of protein-binding regions and local binding modes [30]. Among the 11 candidate mitonuclear interaction pairs, the ND4–NDUFA8, ND5–NDUFA11, and ATP8–ATP5PD interfaces showed relatively pronounced differences in binding conformation and contact patterns. At the ND4–NDUFA8 interface, the calculated electrostatic energy was lower for T3 than for I1. In contrast, at the ND5–NDUFA11 and ATP8–ATP5PD interfaces, it was lower for I1 than for T3. Thus, the direction of the electrostatic energy difference varied among interfaces. These differences may be associated with changes in side-chain volume, steric effects, and local packing caused by amino acid substitutions. Such changes may alter interfacial geometric complementarity and the distances and orientations of neighboring polar residues. At the ATP8–ATP5PD interface, the lower electrostatic energy for I1 was accompanied by a hydrogen bond between the guanidino group of ATP5PD Arg156 and the backbone carbonyl group of ATP8 Ile28, consistent with a change in the local polar interaction network. At the ND4–NDUFA8 interface, changes in contact partners may have altered the spatial arrangement of neighboring residues and indirectly affected electrostatic interactions. At the ND5–NDUFA11 interface, although the electrostatic energy was lower

for I1, the van der Waals energy, desolvation energy, and HADDOCK score were lower for T3, suggesting possible compensation among different energy components. Overall, the ATP8–ATP5PD, ND4–NDUFA8, and ND5–NDUFA11 interfaces were characterized, respectively, by polar-network rearrangement, altered spatial organization, and possible compensation among energy components. These interface-specific patterns indicate that the structural effects of T3- and I1-associated substitutions differ among interaction interfaces.

The ND4–NDUFA8 interface is located within the membrane arm of complex I, and local structural coordination at this interface may influence conformational coupling and energy transduction. PLIP contact analysis showed that the hydrophobic contact between ND4 Leu55 and NDUFA8 Phe169 was relatively prominent in the T3 background, whereas ND4 Val55 mainly contacted residues such as NDUFA8 Leu167 in the I1 background. ND4 is a core mtDNA-encoded subunit of the complex I membrane arm and participates in proton translocation and long-range conformational coupling [31, 32]. NDUFA8 contributes to the assembly, stability, and local structural organization of complex I [33–35]. Biallelic NDUFA8 variants can reduce complex I subunit levels and lead to assembly defects and decreased enzymatic activity. Complementation with wild-type NDUFA8 can ameliorate these abnormalities [36, 37]. Fu et al. further reported that NDUFA8 increased complex I activity, reduced mitochondrial reactive oxygen species (ROS), and upregulated uncoupling protein 1 (UCP1), thereby promoting the browning of white adipose tissue [38]. These findings suggest that NDUFA8 may participate in processes related to mitochondrial energy metabolism and thermogenesis. At the cellular level, Lapshin et al. found that ectopic expression of an optimized wild-type ND4 in cells harboring the MT-ND4 m.11778G > A variant reduced ROS and mitochondrial calcium levels and improved mitochondrial membrane potential [39]. At the animal level, Kim et al. generated mice carrying the mt-Nd4 m.11185G > A variant as a model of the human MT-ND4 m.11778G > A variant associated with Leber hereditary optic neuropathy (LHON). These mutant mice showed reduced retinal ganglion cell numbers and impaired vision, and reducing the mutant load improved the visual phenotype [40]. Together, these studies provide evidence for the important roles of ND4 and NDUFA8 in complex I function. Based on the local contact differences observed in this study, we speculate that hydrophobic contact rearrangement associated with the ND4 L55V substitution may fine-tune conformational coupling and energy transduction within the complex I membrane arm. This structural effect could influence mitochondrial thermogenesis and potentially contribute to haplogroup-associated variation in thermoregulation between T3 and I1.

The ND5–NDUFA11 interface is located in the membrane arm of complex I, in a region adjacent to complex III. Local structural coordination at this interface may influence the stability of complex I and respiratory-chain supercomplexes. We found that the ND5 I600M substitution between the T3 and I1 haplogroups was accompanied by a change in the contact pattern with NDUFA11, suggesting that this site may influence the local spatial organization of the interface. ND5 is the largest mtDNA-encoded membrane subunit of complex I, and its C-terminal transmembrane helix directly contacts NDUFA11 [17]. NDUFA11 contributes to maintaining the stability of complex I and its supercomplexes. Human NDUFA11 splice-site variants can cause severe complex I deficiency [41]. Knockout of *nduf-11*, the *Caenorhabditis*

elegans homolog of NDUFA11, also destabilizes complex I and its supercomplexes and causes impaired respiratory function, abnormal mitochondrial cristae morphology, and developmental defects [42]. These findings further support the importance of NDUFA11 in maintaining the structural stability of complex I and its supercomplexes. Evidence from human studies and cellular models indicates that the MT-ND5 m.13513G > A variant is associated with clinical phenotypes including Leigh syndrome, MELAS, and LHON, as well as reduced mitochondrial membrane potential [43]. At the animal level, Kim et al. generated mice carrying the heteroplasmic MT-ND5 m.12336C > T nonsense mutation and observed reduced levels of complex I-related proteins, decreased ATP levels in brain tissue, mitochondrial cristae damage, and neurobehavioral and metabolic abnormalities [44]. Trucchi et al. found that the ND5 G527E substitution in the endangered Apennine brown bear was accompanied by reduced mitochondrial membrane potential and oxygen consumption, increased ROS levels, and altered mitochondrial turnover [45]. Together, these studies provide evidence for the importance of ND5 and NDUFA11 in maintaining complex I, respiratory-chain supercomplexes, and mitochondrial energy metabolism. Based on the local contact differences observed in this study, we speculate that contact rearrangements associated with the ND5 I600M substitution may alter structural complementarity at the ND5–NDUFA11 interface and the stability of respiratory-chain supercomplexes. These changes could, in turn, affect the capacity of Mongolian cattle to maintain mitochondrial energy homeostasis under cold or nutrient-limited conditions.

The ATP8–ATP5PD interface connects the F□ membrane domain of complex V to the peripheral stalk, and local structural coordination at this interface may influence complex assembly and ATP synthesis. PLIP analysis showed that ATP8 Val28 formed a hydrophobic contact with ATP5PD Phe140 in the T3 background, whereas ATP8 Ile28 formed a hydrogen bond with ATP5PD Arg156 in the I1 background. This difference was accompanied by changes in the neighboring contact network. ATP8 is a mitochondrially encoded subunit of the complex V F□ membrane domain and contributes to the structural organization of the membrane domain and the stability of the complex [20]. The ATP5PD gene encodes subunit d of the ATP synthase peripheral stalk and contributes to the formation of the peripheral stalk and assembly intermediates involving ATP6 and ATP8 [46]. A functional study reported that reduced expression of ATP5H (now ATP5PD) decreased ATP production and oxygen consumption and induced mitochondrial metabolic reprogramming [47]. Jonckheere et al. reported that the MT-ATP8 m.8529G > A (p.Trp55Ter) nonsense variant can cause abnormal complex V assembly and reduced enzymatic activity and is associated with apical hypertrophic cardiomyopathy and neuropathy [48]. These findings indicate that both impaired ATP5PD function and structural disruption of ATP8 may interfere with complex V-dependent ATP synthesis and energy metabolism. At the level of animal phenotypes, genetic diversity of ATP6 and ATP8 in Tibetan yaks has been associated with high-altitude adaptation [49]. Different mitochondrial genotypes have also been associated with differences in energy metabolism in North American yak satellite cells and milk production traits in Holstein cattle in Croatia [50, 51]. These observations suggest that mtDNA background may be associated with environmental adaptation, energy metabolism, and production traits. Furthermore, Shen et al. identified the ATP8 V28I substitution in two independent lineages of deep-sea fishes [52]. This is the same amino acid

substitution observed in the present study, suggesting that ATP8 residue 28 may have evolutionary and functional relevance. Considering this evidence together with the local contact changes observed in this study, we speculate that contact rearrangements associated with the ATP8 V28I substitution may affect structural coordination between the membrane domain and the peripheral stalk of complex V and may influence ATP synthesis. These potential effects could contribute to haplogroup-associated variation in energy utilization and production traits between T3 and I1.

In contrast, although the T3 model had a lower HADDOCK score at the ND1–NDUFA8 interface, PLIP analysis did not detect a clear rearrangement in the local contact network. ND1 is located near the junction between the peripheral and membrane arms of complex I and contributes to the structural environment associated with ubiquinone binding and reduction, as well as to conformational coupling in the proximal membrane arm [31, 32, 53]. Local contact differences of varying degrees were also observed at interfaces such as ND1–NDUFA13 and ND4–NDUFB11, whereas the core contacts at the ND3–NDUFA3 interface were relatively conserved. These observations indicate that the effects of T3- and I1-associated substitutions vary among the different mitonuclear interaction interfaces of complex I.

Mongolian cattle have long been raised under production conditions characterized by cold climates, extensive management, and relatively limited nutrient availability. Their mitochondrial genetic backgrounds and variants related to mitochondrial energy metabolism therefore warrant investigation. The three key candidate mitonuclear interaction interfaces identified in this study, ND4–NDUFA8, ND5–NDUFA11, and ATP8–ATP5PD, may provide targets for future studies of the mechanisms underlying differences in mitochondrial function, environmental adaptation, and related production traits across different mtDNA backgrounds. However, because the candidate sites were screened mainly on the basis of mitochondrial genomic differences, protein structure prediction, molecular docking, and interface contact analysis, the results represent predictive evidence at the structural bioinformatics level. Because these findings have not been experimentally validated using measurements of mitochondrial respiration, ATP production, respiratory-complex enzymatic activity, ROS levels, animal production performance, or environmental adaptation phenotypes, we cannot directly infer that either the T3 or I1 haplogroup confers a functional advantage in the efficiency of energy metabolism, production performance, or stress resilience. Future studies should incorporate nuclear genomic background data from larger populations, mitochondrial functional assays, and association analyses of production traits to validate the actual biological effects of these candidate interfaces.

Conclusions

This study identified 19 consistent inter-haplogroup amino acid substitutions between the T3 and I1 mitochondrial haplogroups in Mongolian cattle and mapped eight of them to candidate mitonuclear interaction interfaces. Structural modeling and contact-network analysis highlighted three key candidate interfaces: ND4–NDUFA8, ND5–NDUFA11, and ATP8–ATP5PD. These findings support only model-level associations with local structural differences and do not permit inferences about functional advantages or causal mechanisms between the haplogroups. Nevertheless, these interfaces provide candidate

targets for future studies integrating nuclear genomic data, mitochondrial functional assays, and association analyses of production traits.

Abbreviations

AF3
AlphaFold 3
ATP
adenosine triphosphate
Cα
alpha carbon
FCC
fraction of common contacts
HADDOCK
High Ambiguity Driven protein–protein DOCKing
LHON
Leber hereditary optic neuropathy
ML
maximum likelihood
mTP
mitochondrial transit peptide
mtDNA
mitochondrial DNA
nDNA
nuclear DNA
OXPHOS
oxidative phosphorylation
PDB
Protein Data Bank
PDBe-KB
Protein Data Bank in Europe Knowledge Base
PDBePISA
Protein Interfaces,Surfaces and Assemblies service at PDBe
PLIP
Protein–Ligand Interaction Profiler
pLDDT
predicted local distance difference test
pTM
predicted template modeling score
RMSD

root-mean-square deviation
ROS
reactive oxygen species
SD
standard deviation
TN93
Tamura–Nei 1993 model
UCP1
uncoupling protein 1.

Declarations

- **Ethics approval and consent to participate**

Not applicable. This study analyzed existing complete mitochondrial genome sequence data generated in previous laboratory work and involved no new animal sampling or animal experimentation.

- **Consent for publication**

Not applicable. This manuscript contains no data from any individual person.

- **Availability of data and materials**

The complete mitochondrial genome sequences analyzed in this study have been deposited in GenBank under accession numbers PZ890225–PZ890244. The processed structural-analysis data, Figure 4 statistical results, contact-map data, PLIP results, and representative structural and docking files supporting the findings are provided in Additional files 1–11.

- **Competing interests**

The authors declare that they have no competing interests.

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- **Authors' contributions**

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