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COMPARATIVE BIOINFORMATIC ANALYSIS OF CYTOCHROME C OXIDASE SUBUNIT1 GENES AND PROTEINS ACROSS SEVERAL MAMMALIAN SPECIES

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Abstract: Cytochrome c oxidase enzyme has an important role in the mitochondrion electron transport chain, plays several main roles in aerobic cellular respiration. In the present study, we have obtained the relation between nucleotide, amino acids and promoter region for the human and mouse Cytochrome c oxidase1 (COI, also COX1). Raw data was retrieved from NCBI and Uniprot websites. Promoter predictions for human and mouse COX1 gene showed that this gene had several common transcription factors such as CREB binding protein, important to histone transcriptional activation, Cation transport regulator homolog 1 (ChaC1), involved in breast and ovarian cancer. Mammalian COI protein sequences were analyzed by the application of bioinformatical tools to predict the protein properties based on biophysical properties, major motifs, conserved domains and transmembrane regions, similar characteristics, secondary and spatial structures. In addition, a relationship among mammalians COI gene was proved by Phylogenetic analysis. Network analysis has indicated that COX1 is closely related to MT-CO2, MT-CO3 and MT-CYB.

Keywords: MT-CO1, comparative analysis, prediction, conserved regions.

INTRODUCTION

Cytochrome c oxidase enzyme (COI or COX1) is a main transmembrane protein complex that is found in the mitochondrion of eukaryotes and several prokaryotes (Stiburek et al. 2006). The length of this gene in vertebrates is about 1545 bp and prior to the start of the cox1 reading frame, a region about 650 bp was known as the 'barcode' region. Barcode researches have been done for a variety of animals (Hajibabaei et al. 2007). Barcode region has been regarded as DNA barcode for phylogenetic analyses (Kim et al. 2007). In addition, one of the COX1 features is known to be conserved so it can be used in phylogenetic studies of the animal or plant species (Robideau et al. 2011). Even among all of the mitochondrial-encoded genes, the MT-CO1 considered to be highly conserved (Castellana et al. 2011). Also COX1 gene is recognized as a marker for finding species-level diversity and biodiversity investigations; it can be validated by polymerase chain reaction (PCR) (Robba et al. 2006). Moreover, COI is the right choice as the marker which can speed up the study of animal metabarcoding (Coissac et al. 2012).

We analyzed several ways to study COI among a group of mammalians via data mining. Cytochrome c oxidase is a protein-phospholipid complex, including 13 unlike protein subunits with molecular weight of 205 KDa for monomeric COX1. Three major subunits (I, II and III) from them are mitochondrial-encoded and contain all the redox-active regions and ten other subunits are nuclear-encoded and surround this central core (Musatov & Robinson 2012). COX1 degradation has major effects on the cellular energy metabolism as an example production of reactive oxygen with a variety of the adverse effects in humans (Sreekanth et al. 2015). Some of the disorders, directly related to mitochondrial encephalomyopathies, are as a consequence of COX1 deficiency, which indicates that mutations in mitochondrial genes encoding structural subunits of the complex lead to nuclear mutations affecting assembly factors (Mick et al. 2011). Structurally, the copper-binding site are conserved in all the known MT-CO1 sequences (Holm et al. 1987).

In this study, bioinformatics and molecular analysis of COI gene will be done in mammalian species to obtain detailed information on COI structure and functions. Analyses of biophysical properties, transcriptional, signal peptide, motifs, domains and protein structure were done to provide insights into the common characters of MT-CO1 genes in mammalians.

MATERIALS AND METHODS

Retrieval of UniGene sequences

Nucleotide and protein sequences were extracted and searched from the NCBI UniGene website (ftp://ftp.ncbi.nih.gov/repository/UniGene/) and EMBL (http://www.ebi.ac.uk/). The data which consisted of 21 nucleotide sequences and 25 protein sequences from various mammals, were related to COX1.

Bioinformatics analyses

For analyzing COX1 in mammalians several online web services and programs were used. Biophysical properties analysis has been done by ProtParam (available at: http://web.expasy.org/protparam/) in mammalians. Firstly, EST profile results (UniGene) were used for expression analysis and then results were loaded into the R program for designing plot by ggplot2 package. Polypeptide domains were determined by SMART program (www.smart.embl-heidelberg.de), this software could be used for the recognition and study of protein domains in protein

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sequences. Protein analyses were loaded into the MEME program (available at http://meme.nbcr.net) and TMHMM server (available at http://www.cbs.dtu.dk/services/TMHMM). Multiple alignments for 25 sequences of the protein from different species were performed using CLC Genomics software. STRING 10.0 program (available at string905.embl.de) was used for prediction network display by Sequences. The DnaSP software was used for finding codon usage and frequency of amino acids. Recognizing promoter regions in human and mouse was done via Berkeley Drosophila Genome Project software (available at http://fruitfly.org/seq_tools/promoter.html). Signal Peptide finding was carried out by Signal 3.0 program analyses. The secondary structure of COX1 was examined by using the SOPMA (Geourjon & Deleage 1995). The protein 3D structure has performed using the Protein Model Portal (PMP) program. Phylogenetic tree was designed via Molecular Evolutionary Genetic Analysis (MEGA; version 6) software with 500 replications and neighbor joining method.

RESULTS AND DISCUSSION

COI Biophysical properties in mammalians

The *Homo sapiens* COI (AY339402.1) (as a standard sequence) contains 1541 nucleotides (one exon) and is located between 5905 and 7446 nucleotides in the mitochondria genome. Biophysical properties analyses indicated that human COI protein has 513aa with 57.0413KDa mass, Theoretical pI (pH that a specific molecule has got no net electrical charge): 6.19, total number of negatively charged residues (Asp + Glu): 25, total number of positively charged residues (Arg + Lys): 18, Formula: $C_{2699}H_{4022}N_{622}O_{675}S_{33}$, total number of atoms: 8051. Instability index (II) is computed to be 28.97(this classifies the protein as stable), grand average of hydropathicity (GRAVY): 0.682 and aliphatic index: 104.17. Table 1 summarizes the general characteristics of the COX1 sequences in different mammalians. Surprisingly, comparative analysis among protein sequences of mammalians showed that number of amino acids, molecular weight, theoretical pI, total number of negatively and positively charged residues, grand average of hydropathicity were closely related, except in the case of *Mus musculus*. The only aliphatic index factor of *Mus* musculus had little difference with other mammals. In fact, the results of homogeneity information about amino acid sequences among species can be shown that this gene is much more conserved. It is a considerable point in COX1 amino acid sequences; the third codon positions are highly variable. Accordingly, the nucleotide sequence diversity is more than the amino acid sequence diversity, and it may be a good source of information for identification of mammalian species (Hebert et al. 2003; Ward & Holmes 2007).

Tab. 1.

The list of the properties COX1 in different mammalian

Biophysical properties	*Homo sapiens*	*Gorilla gorilla*	*Mus musculus*	*Bos taurus*	*Canis lupus*
Number of amino acids	513	512	433	514	514
Molecular weight	57.041.3	56.9442	48.2776	57.0323	57.0252
Theoretical pI	6.19	6.19	9.62	6.06	6.10
Asp + Glu	25	25	31	25	25
Arg + Lys	18	18	47	17	17
GRAVY	0.682	0.669	0.039	0.685	0.678
Aliphatic index	104.17	103.44	100.48	102.06	101.69

Distribution and expression of COI tissues in some of the mammalian

Mammalian EST analyses showed that COX1 gene had high level expression in heart and brain. The testis and ovary tissues had low level expressing, the main reason was that, the EST profile of these tissues had not been reported in some mammalian (Figure 1).

The study of COX1 gene expression could be effective in tissue abnormalities like ovarian tumor (Bragoszewski et al. 2008). Disorders caused by mutations in cytochrome c oxidase gene are significantly higher in colon cancer. Researchers have identified that cytochrome c oxidase expression level can be as a marker to assess colon cancer risk (Payne et al. 2005).

Tab. 2.

The percentage identity between COX1 protein sequences (above 92 %) in the multiple alignments of mammalians

Index	Characteristics	Identity (%)
1	Homo sapiens-AEG23663.1 & Pan paniscus-ADA55577.1	99
2	Homo sapiens-AEG23663.1 & Gorilla gorilla-ABV58887.1	98
3	Homo sapiens-AEG23663.1 & Canis lupus familiaris-KF926378.1	93

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4	Homo sapiens-AEG23663.1 & Sus scrofa-NP_008636.1	92
5	Mus musculus-NC_005089.1 & Felis catus-U20753.1	95
6	Mus musculus-NC_005089.1 & Canis lupus familiaris-KF926378.1	95
7	Mus musculus-NC_005089.1 & Capra hircus-KM360063.1	95
8	Mus musculus-NC_005089.1 & Cynomys ludovicianus-YP_009128492.1	95
9	Bos taurus- AF493542.1 & Canis lupus familiaris-KF926378.1	99
10	Bos taurus- AF493542.1 & Odocoileus virginianus-AEP21795.1	99
11	Bos taurus- AF493542.1 & Hydropotes inermis argyropus-YP_006460032.1	99
12	Bos taurus- AF493542.1 & Panthera pardus-EF551002.1	97
13	Sus scrofa- NP_008636.1 & Hydropotes inermis argyropusYP_006460032.1	98
14	Sus scrofa- NP_008636.1 & Bos taurus- AF493542.1	97
15	Sus scrofa- NP_008636.1 & Capra hircus-KM360063.1	97
16	Sus scrofa- NP_008636.1 & Camelus-AP003423.1	96
17	Lepus corsicanus-AHX02685.1 & Felis catus-U20753.1	97
18	Lepus corsicanus-AHX02685.1 & Panthera pardus-EF551002.1	97
19	Lepus corsicanus-AHX02685.1 & Canis lupus familiaris-KF926378.1	97
20	Lepus corsicanus-AHX02685.1 & Bos taurus- AF493542.1	96

COI commonalities in mammalians

Table 2 shows a couple of the percentage of similarity between proteins in the multiple alignment of COX1. The *Homo sapiens* with *Pan paniscus* had the highest homology and *Homo sapiens* with *Sus scrofa* had the lowest homology in COX1 protein sequences of mammalians.

The analysis of TMHMM, indicated that five of selected mammal had twelve transmembrane domains in COX1, equally (Table 3). Among these, the locality of tarnsmembrane amino acids were very similar between *Homo sapiens* and *Gorilla gorilla*. Also the position of tarnsmembrane amino acids were exactly alike among *Mus musculus*, *Bos taurus* and *Canis lupus*.

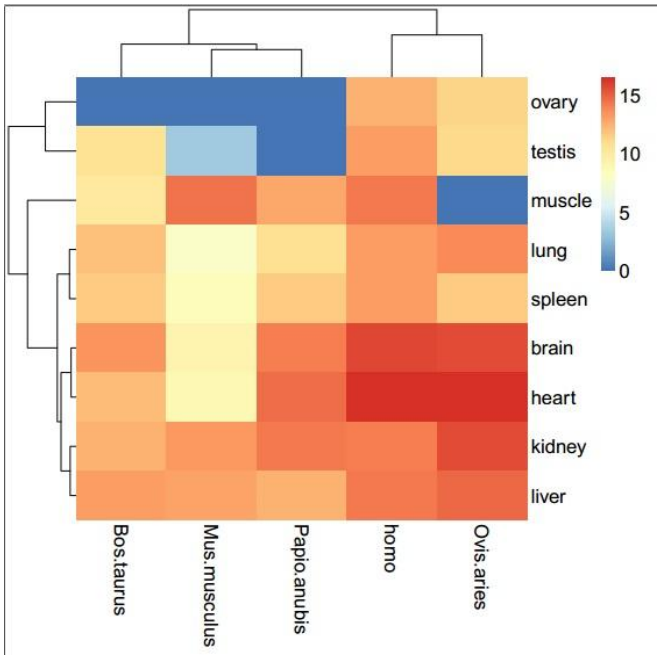

Fig. 1. The frequency distribution and expression pattern of MT-CO1 based on log 2 in tissues.

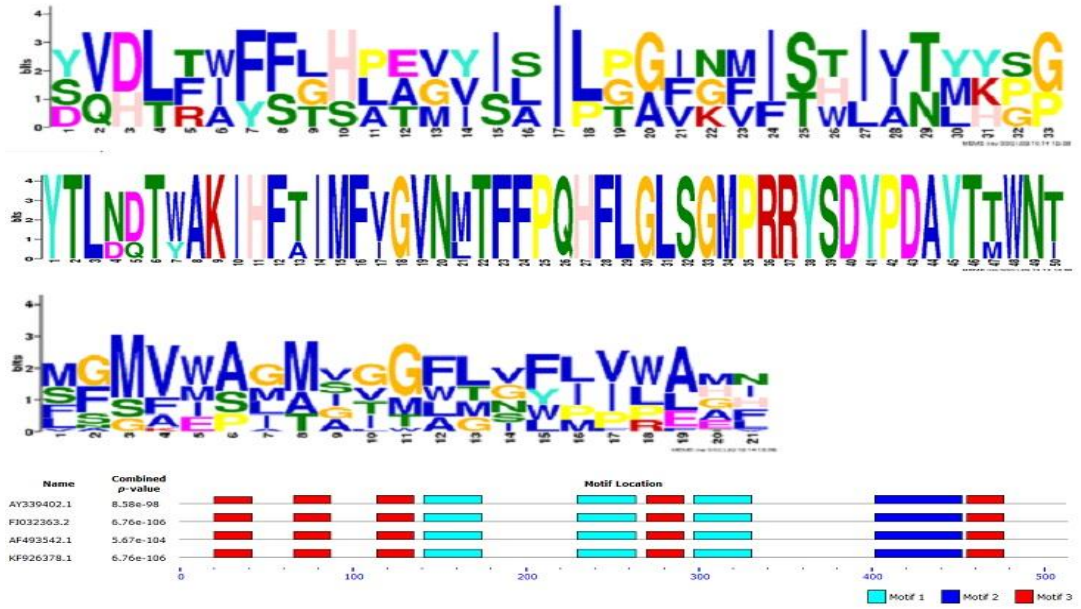

Fig. 2. Motifs for cytochrome c oxidase proteins. The MEME motifs are shown as different colored boxes. Biochemical properties of the various amino acids indicated: Blue; most hydrophobic, Magenta; acidic, Red; positively charged and Green; Polar, non-charged and non-aliphatic residues.

Tab.3.

Prediction of transmembrane helices in COX1 by TMHMM server

	Homo sapiens		Gorilla gorilla		*Mus musculus*		*Bos taurus*		Canis lupus	
	Start	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**
Inside	1	19	1	19	1	14	1	14	1	14
Transmembran	20	42	20	42	15	37	15	37	15	37
Outside	43	56	43	56	38	61	38	61	38	61
Transmembran	57	79	57	79	62	84	62	84	62	84
Inside	80	99	80	99	85	103	85	103	85	103
Transmembran	100	122	100	122	104	126	104	126	104	126
Outside	123	146	123	146	127	146	127	146	127	146
Transmembran	147	169	147	169	147	169	147	169	147	169
Inside	170	181	170	181	170	183	170	183	170	183
Transmembran	182	204	182	204	184	206	184	206	184	206
Outside	205	235	205	235	207	235	207	235	207	235
Transmembran	236	258	236	258	236	258	236	258	236	258
Inside	259	270	259	270	259	267	259	267	259	267
Transmembran	271	293	271	293	268	290	268	290	268	290
Outside	294	302	294	302	291	302	291	302	291	302
Transmembran	303	325	303	325	303	325	303	325	303	325
Inside	326	336	326	336	326	339	326	339	326	339
Transmembran	337	359	337	359	340	362	340	362	340	362
Outside	360	373	360	378	363	374	363	374	363	374
Transmembran	374	396	379	401	375	397	375	397	375	397
Inside	397	408	402	413	398	411	398	411	398	411
Transmembran	409	431	414	436	412	434	412	434	412	434
Outside	432	450	437	450	435	454	435	454	435	454
Transmembran	451	473	451	473	455	477	455	477	455	477
Inside	474	513	474	512	478	514	478	514	478	514

Results of conserve COX1 sequences in some mammalians for evolutionary process showed that VTAHAFVMIFFMVMPIMIGGFGNWLVPLMIGAP DMAFPRMNNMSFWLLPPSFLLLLA and

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ILYQHLFWFFGHPEVYILILPGFGMISHIVTYY SGKKEPFGYMGMVWAMMSIGFLGF sequences are preserved in *Homo sapiens*, *Mus musculus*, *Bos Taurus*, *Canis lupus familiaris* and *Ovis aries* (Supplementary figure 1).

MEME software results indicated that *Homo sapiens*, *Canis lupus*, *Mus musculus*, *Bos taurus* and *Canis lupus familiaris* cytochrome c oxidases have three major motifs including [SYD][VQ][DH][LT][FTR][IWA][FY][FS][GLT][HS][LPA][AET][GVM][VYI][IS][LSA]I[LP][GPT][GA][FIV][GNK][FMV][IF][ST][HTW][IL][IVA][TN][MYL][KYH][PSG][GP],

YTL[ND][DQ]T[WY]AKIHF[TA]IMF[VI]GVN[ML]TFFPQHFLGLSGMPRRYSDYPDAYT[TM]WN[TI]

and

[MSF][GF][MS][VF][WEIM][ASP][GILM][MAT][AGISV][GITV][GM][FALW][LGMT][GVN][FWY][LIP][VIP][WLP][AEL][AGHM][FHIN] (Figure 2).

Network analysis showed that both Homo sapiens (Figure 3a) and Mus musculus (Figure 3b) MT-CO1 have the maximum similarly interaction with MT-CO2 (mitochondrially encoded cytochrome c oxidase II Gene), MT-CO3 (mitochondrially encoded cytochrome c oxidase III Gene) and MT-CYB (mitochondrially encoded cytochrome b). Results were distinguished based on experiments, co-expression, co-occurrence, gene fusion, neighborhood, databases and textmining factors.

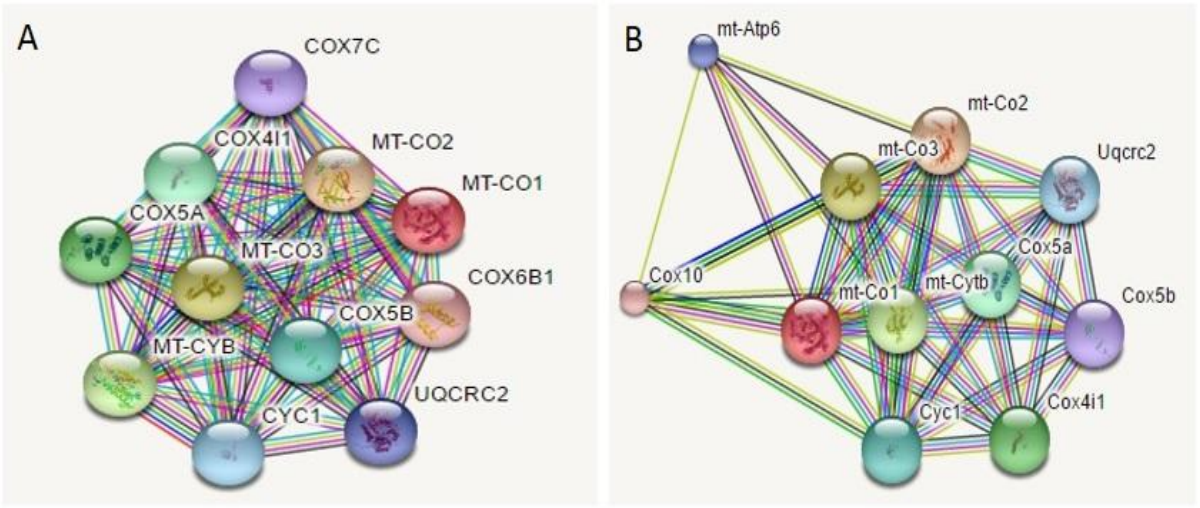
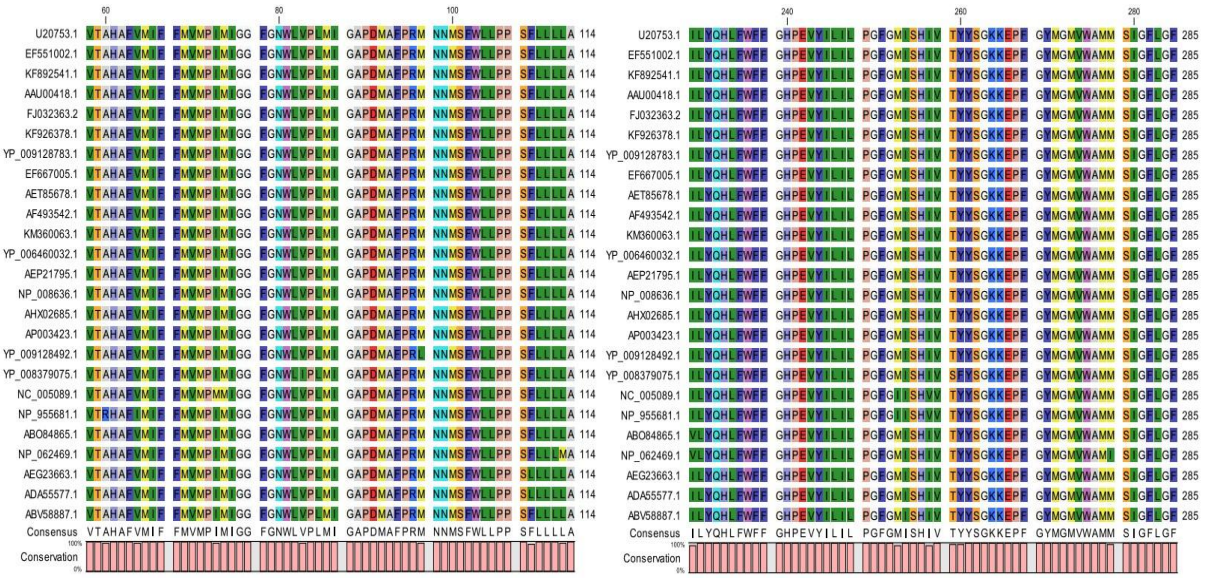


Fig. 3. Network display predicted for COX1. A: Homo sapiens and B: Mus musculus



Supplementary Fig.1. Multiple alignment of MT-CO1 gene in different species.

Tab. 4.

Promoter predictions for human and mouse cytochrome oxidase gene sequence with score cutoff 0.80

position	Score	Promoter Sequence

Human		
1497-1547	0.97	GTTTGAACATACAAAACCCACCCCATTCCTCCCCACACTCATCGCCCTTA
1817-1867	1.00	CGCCGCCGGGAAAAAAGGCGGGAGAAGCCCCGGCAGGTTTGAAGCTGCTT
1899-1949	0.99	TCGGAGCTGGTAAAAAGAGGCCTAACCCCTGTCTTTAGATTTACAGTCCA
Mouse		
429- 479	0.86	ACATTATTCTAATAAACGCCCTAACAACTATTATCTTCCTAGGACCCCTA
1031-1081	0.93	TACTAATCAACAAAAAAAACCCACGATCAACTGAAGCAGCAACAAAATAC
1202-1252	0.96	TAGCCCTATCCATAAAACTAGGCCTCGCCCCATTCCACTTCTGATTACCA
2156- 2206	0.99	CCGCCGAAAAAAAAAAATGGCGGTAGAAGTCTTAGTAGAGATTTCTCTAC

Transcriptional analysis in human and mouse COI promotor region

Our result indicated that existed promoter regions (table 4) were recognized by specific transcription factors such as *Homo sapiens* inhibitor of growth family, member 5 (ING5), CREB binding protein (CREBBP) [is an acetyltransferase acting on histone, that gives an exact tag for transcriptional activation and also acetylates non-histone proteins], Cation transport regulator homolog 1 (ChaC1) [High CHAC1 mRNA expression might be an autonomous index for high risk of cancer relapse in breast and ovarian cancer](Kumar et al. 2012), popeye domain containing 3 (POPDC3) [Decrease expPopeyen of Popdc3 could play a considerable part in the carcinogenesis and progression of gastric cancer](Luo et al. 2012) and EPH receptor A7 (EPHA7) Fibronectin type 3 domain; One of three kinds of interior duplications found in the plasma protein fibronectin, that its tenth fibronectin type III repeat has, an RGD cell identification sequence in a flexible loop among 2 strands. MicroRNA 4484 (MIR4484) was also identified in this promoter region. Also, this MicroRNA was reported in human mitochondria which was corresponded to L-ORF gene (Sripada et al. 2012). In addition 2000 bp upstream of mouse cytochrome c oxidase analyses result indicated that some promoter like *Mus musculus* calpain 11 (Capn11), Calpain, subdomain III exists. Calais are calcium-activated cytoplasmic cysteine proteinases, take part in cytoskeletal remodeling activities, cell differentiation, apoptosis and signal transduction. Collectively, these effects disclose a novel part of CRMP-3in which calpain cleavage of CRMP-3 and the subsequent nuclear translocation of the truncated CRMP-3 evokes neuronal death in retort to excitotoxicity and cerebral ischemia (Hou et al. 2006), interleukin 6 receptor, alpha (Il6ra), exactly this gene on viewpoint Functional Similar with EPHA7 gene in human.

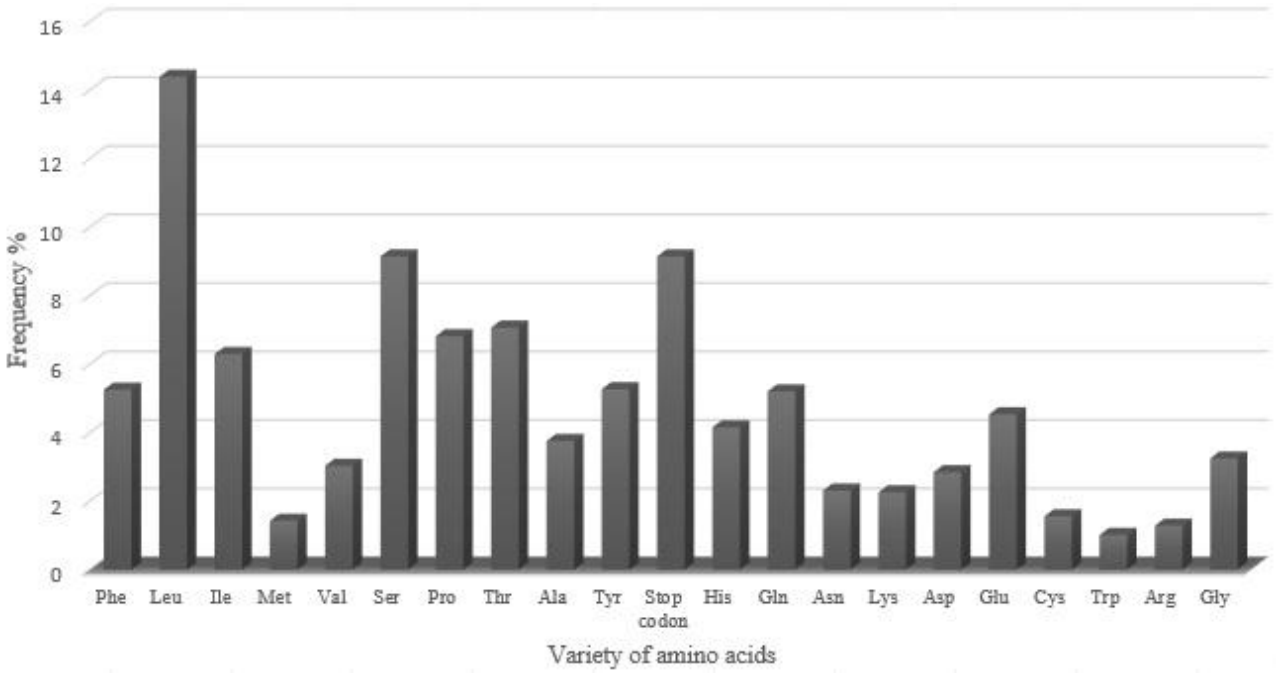


Fig. 4. The frequency of amino acids within sequences containing MT-CO1 domains.

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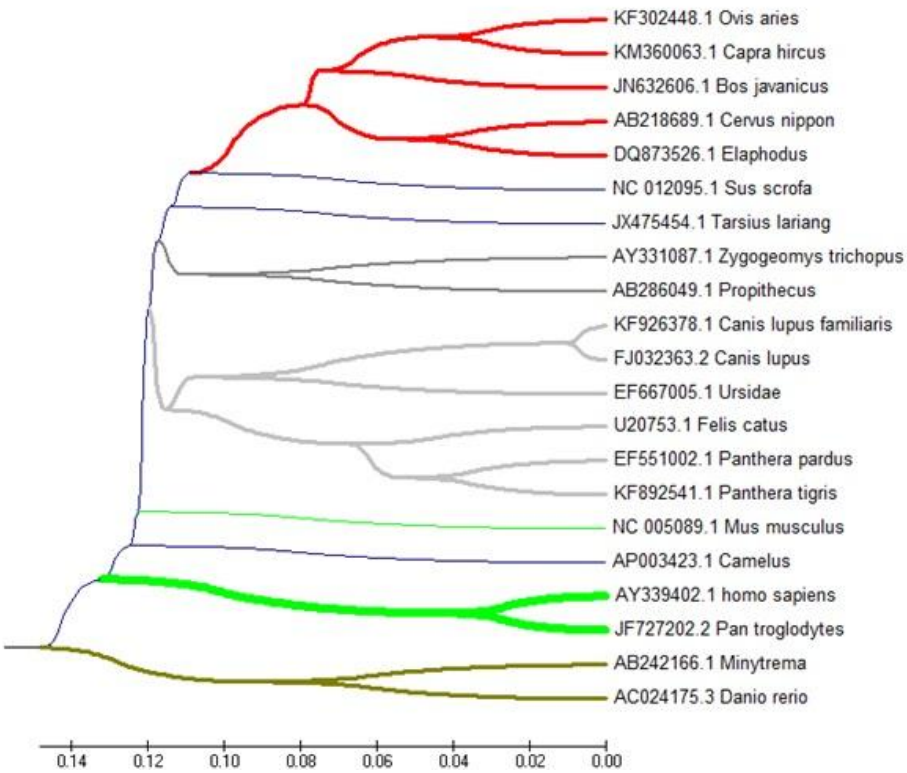
Supplementary Fig.2. The 3D structure of MT-CO1 shows twelve transmembrane domains.



Supplementary Fig.3. The phylogenetic tree of COI from different species by the CLUSTAL-W (MEGA 6) program. The degree of 1,000 bootstrap repeats was given at each node.

Identification of amino acids and protein structure

The study of 22 different selected animals showed that highest frequencies among the terminal codons were TAA and TAG. The results of Tate et al. (1999) about terminal codon have shown that the TAA and TGA codons have highest and lowest efficiency, respectively. In addition, sequences have been analyzed to predict the frequency of amino acids, and the results are shown in (Figure 4). The most abundant amino acid was Leucine and followed by Serine. Generally, the high frequency of these amino acids were similar to previous report in mitochondrial genome (Milbury & Gaffney 2005).

The results of secondary structure revealed that the peptide of human COX1 has 37.23% of alpha helices,

25.73% of extended strands, 11.50% of beta turns and 25.54% of random coils. Thus, alpha helices is dominance of structural elements in this gene. Molecular modeling results showed that the 3D structure of human COX1 had high structure similarities with 3D structures of mouse and cattle (Supplementary figure 2).

Phylogenetic tree analysis

The nucleotide sequences were designed by phylogenetic tree to regulate the evolutionary relationships between different species (Supplementary figure. 3). Phylogenetic tree was separated into two main different branches; one branch is the mammalian and other branch is fishes. One of the main branches was separated to the three major branches such as *Homo sapiens* with accession number AEG23663.1 and *Pan troglodytes* with accession number JF727202.2, ruminants and include Feliformia.

CONCLUSION

To conclude, the rich data set shown in this study will contribute to the better understanding of cytochrome c oxidase in human and other organisms. We analyzed MT-CO1 nucleotides, amino acids and promoter regions for finding their structures, common characteristics and evolutionary relationship in mammalians. Furthermore, the results showed some parts of this gene conserved and maintained in evolution. This study can lead us to understand new things about protein and metabolic engineering in mammalians.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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