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Parkinson's disease and lactoferrin: Analysis of dependent protein networks

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ABSTRACT

The etiology of Parkinson's disease (PD) is partially understood. Some have indicated the role of concentration of iron in midbrain of affected individuals. Accordingly, looking into iron-binding proteins and antioxidant activity such as lactoferrin (LTF) may shed some lights into missing puzzles of PD. LTF association with PD-associated protein was investigated in human, chimpanzee, bovine, horse, sheep, chicken, mouse, rat, dog, one and two humped camels. In silico analysis of protein interaction network (PIN) was used to better establish the relationship with the disease. According to STRING facilities in finding interaction between proteins in different species, no pairwise interactions (edges) were found between LTF with PD-associated proteins except of bovine and chicken. SNCA (Alpha-synuclein) was related to bovine LTF and PD signaling pathways. In bovine, more than 85 proteins were associated to PD mostly mitochondrial resident. The network density, network diameter and clustering coefficient of the PIN for PD-associated proteins in bovine were 0.065, 9 and 0.381, respectively. The based on network analysis, we identified hub protein and high-degree protein for NDUFB4 and NDUFAB1, respectively. Those couples of them belonged to ubiquinone. It was concluded that bovine LTF is one of the major proteins in initiation of signaling to PD-associated proteins.

1. Introduction

Parkinson's disease is a neurodegenerative disorder of the central nervous system with tremor, muscle rigidity, and slowness of movement (bradykinesia). The motor symptoms of PD result from the death of dopamine generating cells in the substantia nigra, a region of the mid brain (Rakshit et al., 2014). It has proven, some proteins are affected on the PD as iron binding capability (Berlutti et al., 2011) and direct function as cell protector against toxic stresses and regulator of antioxidant transcriptional (Park et al., 2013). Furthermore, it has been reported that iron has a role in onset of Parkinson's disease (PD) when the concentration rises in substantia nigra. Yet again, it may contribute to the formation of oxygen-derived free radicals and thus to neuronal damage leading to PD (Grau et al., 2001). Availability of reduced iron (ferric or Fe^{3+}) leads to damage lipid membranes, nucleic acids and proteins of pathogens through generation of hydroxyl radicals (Bou-Abdallah, 2012).

Lactoferrin (LTF) is an 80 kDa iron-binding glycoprotein of the transferrin family (García-Montoya et al., 2012). This protein can be found in milk, colostrum and most mucosal secretions including tears, saliva, vaginal fluids, semen (Van Der Strate et al., 2001), nasal and bronchial secretions, bile, gastrointestinal fluids and urine (ÖztaşYeşim and Özgüneş, 2005). LTF has many biological roles such as, anti-carcinogenic, anti-inflammatory and immune modulatory effect against various microorganisms due to its capability to bind to catalytic iron, acting as an inhibitor of free radical formation (Habib et al., 2013). Reports are augmenting that the expression of lactoferrin receptors immune reactivity in micro vessels and neurons of the substantia nigra are increased under Parkinson's and Alzheimer's diseases (Grau et al., 2001; Kawamata et al., 1993; Leveugle et al., 1996).

Based on studies in human, mutations in *SNCA* (Alpha-synuclein), *PARK7* (Parkin7), *LRRK2* (leucine-rich repeat kinase 2), *glucocerebrosidase* (GBA), *PRKN* (protein Parkin), *LRRK2* (leucine-rich repeat kinase 2), *PINK1* (PTEN-induced putative kinase 1) and *ATP13A2* (lysosomal ATPase) genes were reported to conclusively be associated with PD (Farlow et al., 1993–2015; Lesage and Brice, 2009; Davie, 2008). In spite mutations on aforementioned genes associated with either autosomal

Abbreviations: LTF, Lactoferrin; KDa, kiloDalton; PD, Parkinson's disease; PIN, protein interaction network; GO, gene ontology; SNCA, Alpha-synuclein; PARK, protein Parkin; LRRK2, leucine-rich repeat kinase 2; PINK1, PTEN-induced putative kinase1; SNCAIP, Alpha-Synuclein-Interacting Protein; NDUF families, NADH dehydrogenase (ubiquinone) proteins; LOC783686, NADH dehydrogenase (ubiquinone); COX4, Cytochrome *c* oxidase subunit 4.

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Table 1
Identity percent of lactoferrin in different species based on protein homology.

Species	Species										
	M. musculus	*R. norvegicus*	*H. sapiens*	*P. troglodytes*	*E. caballus*	*C. familiaris*	*O. aries*	*B. taurus*	*C. dromedarius*	*C. ferus*	*G. gallus*
M. musculus	100	69.62	70.56	70.75	64.41	67.37	63.98	63.84	66.53	66.39	47.15
R. norvegicus	69.62	100	61.15	61.48	57,67	59.07	57.24	58.65	58.65	58.82	43.00
H. sapiens	70.56	61.15	100	97.61	73.10	73.38	70.85	69.44	73.80	74.05	50.76
P. troglodytes	70.75	61.48	97.61	100	73.56	73.70	70.46	69.76	73.84	74.09	50.90
E. caballus	64.41	57.67	73.10	73.56	100	76.27	72.03	71.33	75.71	75.25	49.51
C. familiaris	67.37	59.07	73.38	73.70	76.27	100	70.34	70.20	75.42	75.53	51.74
O. aries	63.98	57.24	70.85	70.46	72.03	70.34	100	92.09	76.55	76.09	51.04
B. taurus	63.84	58.65	69.44	69.76	71.33	70.20	92.09	100	75.42	75.25	51.60
C. dromedarius	66.53	58.65	73.80	73.84	75.71	75.42	76.55	75.42	100	97.89	49.93
C. ferus	66.39	58.82	74.05	74.09	75.25	75.53	76.09	75.25	97.89	100	49.86
G. gallus	47.15	43.00	50.76	50.90	49.51	51.74	51.04	51.60	49.93	49.86	100

dominant or recessive may be affected on PD, we concentrated on associated proteins to LTF the based on construction of protein interaction networks (PIN) according to LTF characteristics in iron binding capability (Berlutti et al., 2011) and direct function as cell protector against toxic stresses (Park et al., 2013). Due to the fact that some disease including cancers, cardiovascular disease, neurodegenerative disorders, and aging all are associated with increased oxidative stress in tissues (Clements et al., 2006).

2. Materials and methods

2.1. Data collection

To consider lactoferrin (LTF) in different species, at first we decided to focus on the sequences of amino acids to achieve a general comprehension about substitutions rate of amino acid in LTF. The sequences of LTF were obtained from biological database such as Uniprot (http://www.uniprot.org/uniprot/) for *Camelus dromedarius* (*Q9TUM0*), *Bost aurus* (*P24627*), *Homo sapiens* (*E7ER44*), *Equus caballus* (*O77811*), *Ovis aries* (*W5PFP1*), *Mus musculus* (*P08071*), *Rattus norvegicus* (*D3ZAB1*), *Canis familiaris* (*F1PR54*) and *Pan troglodytes* (*H2QMG6*) and also KEGG (http://www.genome.jp/kegg/) for *Camelus ferus* (*102515324*) and *Gallus gallus* (*396241*). It is worth mentioning that the lactoferrin in *Gallus gallus* is a putative uncharacterized protein in egg albumin called TFEW or transferrin (Ibrahim et al., 2006).

2.2. In silico analysis and comparative modeling

Multiple sequence alignments were performed for proteins in each species by MEGA (Tamura et al., 2011). The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al., 1992). The phylogenies' tree with the highest log likelihood was constructed based on each sequences of lactoferrin, as well as the Maximum Likelihood tree for each subunit was built with 1000× bootstrap by CLC Genomics Workbench Version 7.6.4 (www.clcbio.com). To find a relationship between PIN and protein

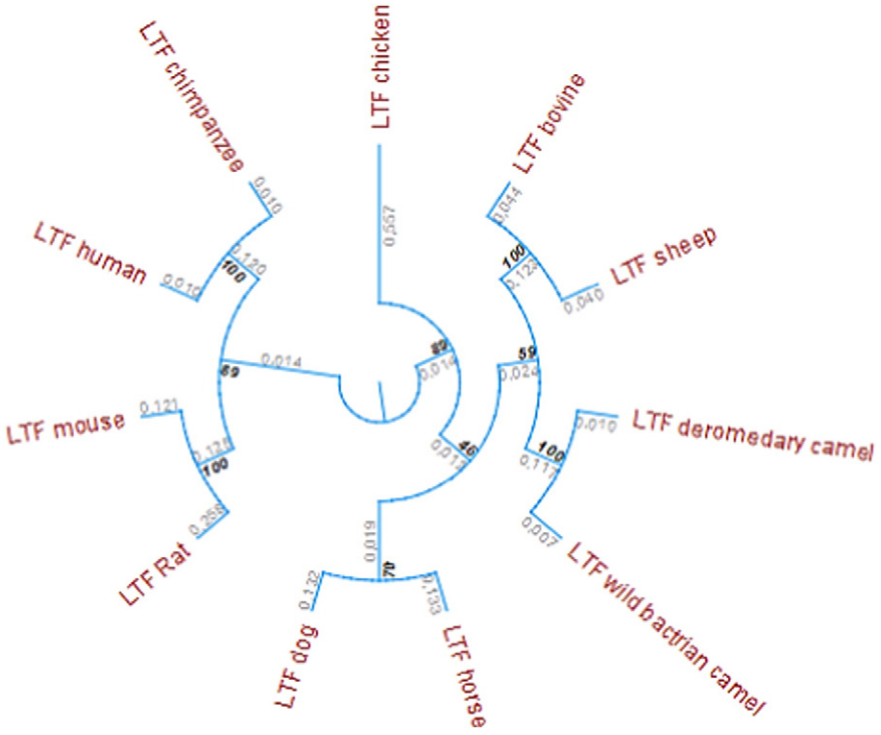

Fig. 1. Molecular phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Initial tree(s) for the heuristic search were obtained automatically as follows. The tree is drawn to scale, with branch lengths (Gray notes) and bootstrap values (Bold and Italic notes) measured in the number of substitutions per site. The analysis involved eleven amino acid sequences.