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Exploring the role of BCHE in the onset of Diabetes, Obesity and Neurological Disorders

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Abstract:
Diabetes, Obesity and Neurological disturbances, most often show co-occurrence. There has been an extensive research in this domain, but the exact mechanism underlying the co-occurrence of the three conditions is still an enigma. The current paper is an approach to establish the role of Butyryl cholinesterase (BCHE) in Diabetes, Obesity and Neurological disorders by performing a comparative analysis with Neuroligin (NLGN2) a protein belonging to the same family. BCHE has its role in glucose regulation, Lipid metabolism and nerve signaling. Emphasis is laid on BCHE's diverse functions whose impediment affects the above mentioned metabolic pathways. Insilco techniques were employed to analyze the sequence, structural and functional similarities of the two proteins. A point mutation is focused which is common to both BCHE and Neuroligin. The mutation occurs at the homologous position in both the proteins making them deficient. This affects the three metabolic pathways leading to the respective disorders. The work describes the pathway that describes the role of BCHE in the onset of obesity mediated diabetes. The pathway further explains the association between Diabetes, Obesity and neurological disturbances.

Keywords: BCHE, Neuroligin, Diabetes, Obesity, Neurological Abnormalities, Insulin Signaling, Signal Transduction.

Background:
Diabetes is one of the most common disorders for the people of all age groups. Vast research is carried out in solving this major problem. There are several proteins that are involved in the onset of diabetes. BCHE (Butyryl cholinesterase) is one such protein having an important role in diabetes whose exact mechanism is yet to be revealed. BCHE has an important role in the regulation of insulin concentration and lipid levels **[1].** It has wide diversity of functions. Being a member of esterase's it has an important role in nerve regulation and signal transduction **[2].** BCHE is reported to have its involvement in adiposity and insulin resistance **[3],** thereby regulating lipid and glucose concentrations respectively. This diverse group of functions makes the protein an important factor in the onset of Diabetes and associates it with Obesity and Nerve disturbances. It has a length of 602 amino acids with three domains which are overlapping **[4],** an abhydrolase, CO esterase and an ACHE which imparts diverse functions to the protein.

Neuroligin (NLGN) is a cell surface membrane protein, involved in synapse formation at the nerve junctions **[5].** It mediates signaling across the synapse and affects the properties of neural networks by specifying synaptic functions. Members of this family may act as splice site-specific ligands for beta-Neurexins and may be involved in the formation and remodeling of central nervous system synapses **[6].** It resides on the P arm of the 17th chromosome in man with a length of 835 amino acids. The domains are similar to BCHE, an Abhydrolase and a CO Esterase that are overlapping.

Neuroligin, Acetyl choline esterase (ACHE) and Butyryl choline esterase (BCHE) belong to a common family of proteins called alpha beta hydrolases **[7]** .Proteins of the alpha/beta-hydrolase fold family share a common structural fold, but perform a diverse set of functions. Neuroligins are a class of proteins that include Neuroligin 1, 2, 3 and 4 with further sub groups. They are cell surface proteins that are involved in the cell to cell adhesion **[8].** Members of the Alpha beta hydrolase fold family are reported to have a common point mutation R to C which causes the respective proteins to get retained within the Endoplasmic reticulum, thereby leading to various multi factorial disorders **[9].**

Methodology:

Identification of proteins sharing sequence similarity to BCHE

Proteins sharing sequence similarity to BCHE were identified using TFASTY. It compares the protein sequences to the translated DNA sequences with frame shifts **[10].**TFASTY uses the same heuristic algorithm as FASTA.

Evaluation of the proteins for sequence similarity

All the sequences that were found to be similar were further validated using BLAST P (pair wise sequence similarity) that can provide the percentage of similarity between the two sequences.

Identification of the homologous sites on the two proteins

The site that is reported to have a mutation at the homologous positions in the two proteins was identified using EMBOSS NEEDLE **[11].** Pair wise Sequence Alignment is used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences.

Analysis of Domains within the proteins

The functional sites that are present on both the proteins have been identified using SMART. The position, classification and function of the total number of domains present in the submitted sequence can be analyzed using SMART **[12].**

Classification study and functional assessment of the protein

To indicate the effect of BCHE in lipid metabolism using its family classification CDD (Conserved Domain Data base) has been used. It gives complete information of various domains and their relation to the families of proteins.

Active and binding site prediction within the proteins

Binding sites that are present in the protein were analyzed using several Insilco tools like CASTP, QSITE FINDER and POCKET FINDER. These tools will locate the preferable sites that are energetically and stearically suitable for ligand binding. These are the most accessible areas on the receptors.

Comparative structural analysis of the two proteins

Secondary and tertiary structural analysis is done to compare the location of the mutational site on the two proteins. SOPMA is a proteomic tool of EXPASY **[13]** which gives the information regarding the secondary structural conformations of the given protein. CPH, HHPRED and PHYRE are employed to analyze the tertiary structures of the protein

Visualization of the 3D structures of the two proteins

To visualize the structure of the protein against X, Y and Z axis RASMOL is employed. The software is basically a command line programme that is executed by commands. The site important in mutation is identified and visualized.

Analyzing the stability of the mutated protein

To check the effect of the mutation on the energy and stability of the protein structure I MUTANT **[14]** is employed. It gives the stability index of the point mutations based on the neural network programme.

Designing a pathway to associate the onset of the three disorders

By aggregating the results from all the above analysis and reports a pathway has been proposed that can considerably justify the association and co occurrence of Diabetes, Obesity and Neurological disturbances with respect to BCHE. The pathway also details the mechanism by which BCHE is involved in causing the three disorders.

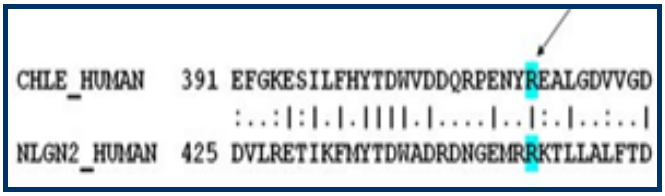

Figure 1: EMBOSS NEEDLE between BCHE and Neuroligin

Discussion:

Table1 (see supplementary material) shows that there are 5 proteins belonging to different classes of neuroligins which share sequence similarity to BCHE. All the proteins showed more than 30% similarity in TFASTY which was further validated using BLAST P.

(Figure 1) shows the alignment of BCHE and Neuroligin. The homologous site that is prone for mutation is highlighted. The sites are 414 and 448 of BCHE and Neuroligin 2 respectively. SMART analysis provided the domain information regarding the two proteins. BCHE has three overlapping domains Abhydrolase (138 – 269), a CO Esterase (9 – 550) and an ACHE (565 – 602). The domains of neuroligin are Abhydrolase (180 - 333) and a CO Esterase (30 – 601). These results indicate that both the proteins share two domains in common a CO Esterase and an Abhydrolase, which represents their functional similarity. **(Figure 3)** depicts that neuroligin has fingerprints that are specific to two classes Cholinesterase and Neuroligin, Which is an indication of its functional similarity to BCHE.

Fingerprint	# of Motifs	SumID	AveID	ProfScore	Ppvalue	Evalue
NEUROLIGIN	4 of 4	380.02	95.00	4349	4.5e-48	3.6e-43
CHOLINESTRASE	2 of 6	81.82	40.91	719	2e-06	0.16

Figure 2: Finger print identification of Neuroligin

This was followed by CDD analysis which showed the presence of a functional region "aes" from 125 to 229 of BCHE, which has a major role in Lipid metabolism. These results indicate that BCHE has a role in Lipid metabolism. Structural

analysis using EXPASY server showed that the site under study is located in the extended strand conformation of both BCHE and Neuroligin. For the determination of the 3D structures of proteins, tools like CPH, HHPRED and Phyre have been employed. The PDB id's 2WIK and 3BE8 are selected as the best one for BCHE and Neuroligin respectively based on the results. I mutant was used to study the stability of the mutated protein.

Position	WT	NEW	Stability	RI	RSA
386	R	C	Decrease	2	11.8

WT: Native amino acid; NEW: New amino acid; RI: Reliability index; RSA: Relative solvent accessible area.

The stability check shows that the mutation causes a decrease in the stability of the protein when compared to the native protein. The 414 site of the sequence would be 386 in structure. Binding and active sites that are present within BCHE were analyzed using tools like CAST P, Pocket finder and Q site finder. The results showed that the mutation under study was falling under one of the pockets which indicate that it causes a major effect on the functional properties of the protein. This emphasizes the importance of the mutation.

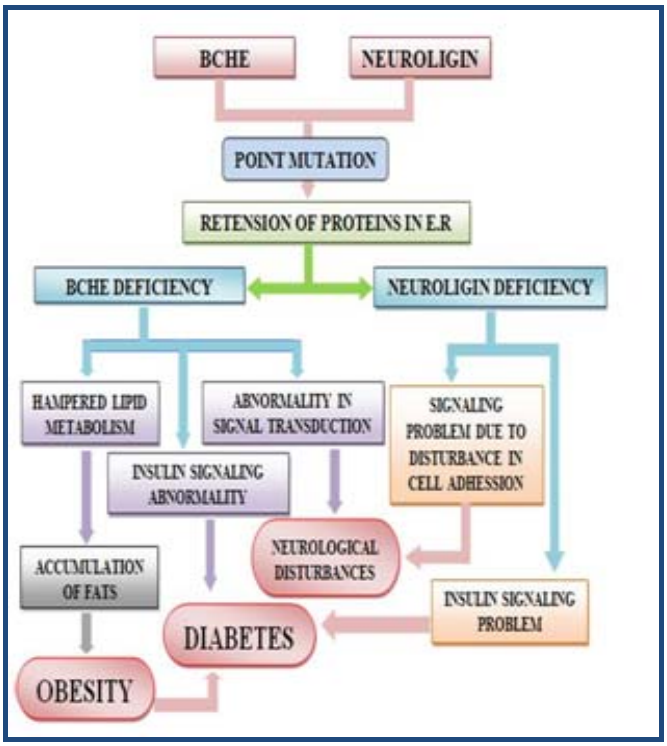

Figure 3: Pathway that associates Diabetes, Obesity and Neurological disturbances.

The pathway explains the mechanism of co occurrence of Diabetes, obesity and Neurological disturbances. It describes the role of BCHE in all the three disorders **(Figure 3).** The work emphasizes the role of BCHE in causing Diabetes, Obesity and Neurological disturbances with focus on its multi functional ability. The importance of BCHE in Nerve regulation is shown by considering its Sequence, structural and functional similarity to Neuroligin. BCHE, ACHE and Neuroligin structurally belong to a family of alpha beta hydrolase fold as reported, which can also be inferred from the similar structural patterns shown by FINGERPRINT SCAN. The presence of common domains by SMART and CDD analysis depicts the probable role of these proteins in insulin signaling.

A point mutation R to C is known which is specific to alpha beta hydrolase fold family of proteins. Reports show that the mutation occurs in the homologous sites in all the three proteins which was further proved by EMBOSS. This mutation leads to the retention of the respective proteins in Endoplasmic Reticulum thereby causing their deficiency. Neuroligin and BCHE both are involved in regulation of insulin production by different mechanisms **[15].** Neuroligin-2 is expressed on the β cell surface and binds with high affinity to another β-cell surface protein. Extracellular interactions involving NL-2 increase insulin expression and secretion by β cells. These results are consistent with the hypothesis that NL-2 promotes the development and maintenance of the insulin secretory machinery in β cells through trans-cellular interactions **[15]**.

BCHE is a member of Esterase Lipase super family. Through CDD analysis, it was found that BCHE contains a region Aes which is involved in lipid metabolism thus justifying the role of this protein in regulation of lipids. The role of BCHE in glucose metabolism could be through insulin signaling. Any alteration to this protein leads to a disturbance in its function. The point mutation R414C is known that causes the retention of the protein within the Endoplasmic reticulum in turn leads to deficiency. This condition causes a disturbance in the insulin signaling thus hinders the glucose metabolism. This leads to the onset of diabetes. BCHE being a member of Ester lipase super family has a role in Nerve signal transduction. The deficiency of BCHE alters the signal transduction of neurons, a potential cause of Neurological disturbances.

Further the mutation causes alteration in the lipid metabolism leading to the accumulation of fats and provoking the onset of Obesity. This suggests the probable role of BCHE in Obesity mediated Diabetes. Reports show that there are two transcription factors FOXA 2 and HNF1A that become non-functional due to the accumulation of fat in obese condition **[16].** These factors are required for the activity of GnT-4a glycosyl transferase which regulates the glucose metabolism by affecting glucose transporters **[16]** Altered transcription factors cause inactivation of GnT-4a glycosyl transferase leading to the disturbance in glucose metabolism causing Diabetes.

The pathway proposes two possible mechanisms by which BCHE is involved in Diabetes, Direct role through insulin signaling abnormality and an indirect role through Obesity mediated Diabetes. The pathway demonstrates the association of BCHE to Diabetes, Obesity and Neurological disturbances. This mechanism can further support the hypothesis that Obesity, Diabetes and Neurological abnormalities are associated to each other in their onset.

Conclusion:

The role of BCHE in Diabetes, Obesity and Neurological Disorders has been analyzed through the above Insilico approach. Association of BCHE in the onset of the three

disorders and the co occurrence of the three conditions has been proposed by means of a pathway.

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Supplementary material:

Table 1: Proteins that are found to have sequence similarity to BCHE as shown by TFASTY and validated using BLASTP

S. no	Name	Accession No	% Identity using TFASTY	% Identity using BLAST
1	Neuroligin 4, X-L	31317255	34.526%	34%
2	Neurlogin 4, Y	256222770	34.165%	34%
3	Neuroligin 3	262359973	34.549%	34%
4	Neuroligin 2	31317254	33.509%	34%
5	Neuroligin 1	31317253	33.836%	33%