BUTYRYLCHOLINESTERASE, VARIANTS AND METABOLIC SYNDROME

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Abstract

Butyrylcholinesterase, a hepatic enzyme has a primarily pharmacological role in hydrolyzing esters. Variant forms of the enzymes exist. A hypothesis is presented for their continuing presence over evolution, and suggest a possible modulatory effect on components of metabolic syndrome as well as other related conditions. Finally it is proposed that subjects harboring variant forms of the enzyme may represent a 'human knockout equivalents' and can be part of the human knockout project.

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Key words: pharmacogenetics, pharmacogenomics, human knockout, mouse knockout model, evolution, geography

Introduction

Butyrylcholinesterase (BCHE) is a protein secreted by the liver; it is found in most tissues of the body (1). Its principal role is in the hydrolysis of the ester butyrylcholine. The importance of the enzyme and its variant forms came to clinical attention when individuals with variant forms of the enzyme developed prolonged apnea when the muscle relaxant succinylcholine was given during general anesthesia. Initial efforts were made to characterize the variants in relation to the prevention of succinylcholine-induced apnea in affected individuals.

Other roles of the enzyme were investigated because, without a physiological role, the enzyme would have been expected to be extinct over the course of evolution. That it has not, suggested it could have a role in other systems and conditions, among which lipid metabolism, obesity, metabolic syndrome and type 2 diabetes were identified (2, 3). It was proposed that individuals with variant forms of the enzyme could serve as human knock-out equivalents and may be followed up for differences to susceptibility of diseases such as diabetes mellitus and Alzheimers disease (3). Recently, this approach is being applied to the systemic study of natural human knockouts (4). The BCHE gene has been mapped to chromosome 3 at 3q26.1-q26.2 (5). The gene has four exons. In plasma, BCHE occurs as a soluble tetramer with 240 kDa, which is proline-rich. It is now established that there is no significant concordance between the genomic variations and functional variations identified by inhibition studies (5). Highest rates of variant forms of the enzyme were reported from isolated ethnic groups south Indian Vysyas (6), Jews from Iraq and Iran (7) and certain Eskimos in western Alaska (8, 9).

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Variant forms of butyrylcholinesterase

Interest in pharmacogenetic variants extended to a far wider spectrum than its relevance to its original area of interest in anesthesiology. Butyrylcholinesterase (3.1.1.8) is related structurally and functionally to the enzyme acetylcholinesterase (AChE) (3.1.1.7). While it was considered to be a nonfunctional protein, evidence exists for a role in detoxification of drugs and toxins (10). Both enzymes belong to the ancient α/β hydrolase fold family, which comprises of lipases, carboxylesterases and cholinesterases (11). The enzymes share sequence identity and appear to have been in existence from early life forms (12). However BCHE activity could have arisen by gene duplication many times, in the animal kingdom (11). Occurrence of variant forms of BCHE have been recognized for long (13,14). The silent variant, where the enzyme activity is completely absent, occurs at a frequency of 1:100,000 people of European descent (15). On the other hand, it occurs far more frequently (upto 1:25) among Intuit populations and in Vysyas of Andhra Pradesh (16, 17).

Elsewhere, among British families with individuals with succinylcholine apnea (n:39), the likelihood of ChU1ChD1 heterozygotes developing apnea was calculated. 1:4,000 of normal homozygotes and 1:400 of ChU1ChD1 heterozygotes were likely to develop apnea; ChF1 and ChS1 gene were identified (18). Among a French blood donor cohort, on the basis of dibucaine, fluoride and chloride differential inhibition, the gene frequencies were E1u=0.970,8, E1a=0.188,0, E1f:0.103,0 (19). Neumann and Walter reported the frequencies of BCHE variants among Pakistanis, Greeks and Icelanders (20).

In an Italian population of 4051 school children, variants of CHE1 loci were evaluated; the following allelic frequencies were observed – CHE1*u=0.9636; CHE1*a=0.0263; CHE1*f=0.0101 (21). The authors confirmed the validity of distinguishing the typical and fluoride resistant types of BCHE. Among 933 Azerbaijan children from the Kyurdamir district, variants of BCHE showed biochemical polymorphism. High frequencies of mutant alleles of E1-E1a and E1s loci were identified (22).

There were studies from the African continent including South Africa, Nigeria and Rhodesia. Among seven southern African populations, variation of E1 and T2 loci of BCHE were observed. In the Ashkenazim and Afrikaners E1 locus variation occurred, with type E1a allele seen in 0.017 in the Ashkenazim, 0.016 in the Afrikaners, E1s being twice to three times more frequent in Afrikaners compared to Caucasians. E1a was rare in the Negro and Khoisan populations (23). In a separate study on nine south African families harboring abnormal BCHE, silent allele was found in five families; it co-existed with dibucaine-resistant form in three families (24).

A report from Northern Nigeria studied the prevalence of atypical phenotype of BCHE (n:345) using differential inhibition of dibucaine and sodium fluoride. Atypical Ea1 was found in 0.9%, Ef1 in 0.74% (25). African tribes from Rhodesia (n:1,614), were phenotyped for BCHE variants at E1 and E2 loci. There was a high frequency for the Ef1 gene (0.036) in Rhodesian and Malawian Africans; similar high frequencies were also seen in Zambian Africans (0.045) and in Mozambiquen Africans (0.034). Es1 gene frequencies were 0.013 for Rhodesians, 0.009 for Malawians and 0.016 for Mozambiquen Africans (26).

Among individuals from Pyrenean communities (n:2,400), genetic polymorphism was observed in BCHE; E1a gene had the highest frequency in central Pyrenees (7.7%), and declined to 3% in Toulouse and 2.3% in Basques. E1s occurred at a frequency of 2.13% in the Basques (27). Studies from South America reported the prevalence of BCHE polymorphisms. In an urban population of Santiago, Chile, the incidence of CHE1F was higher than reported earlier, perhaps because of improved detection methods; CHE1AK was also identified (28). Among 300 blood samples from an eastern Santiago population urban private clinical laboratory, frequency of BCHE*F was greater than predicted; eight had BCHE AK genotype (29). Chilean tribes were evaluated for frequencies of BCHE alleles by enzymatic methods. Frequency of BCHE*K allele was estimated by primer reduced restriction analysis. Typical allele BCHE*A was observed in 1.11% Huiliches, 0.89% in Cuncos and none in Pehuenches. BCHE*F frequencies were higher than other reported studies (3.89, 5.7%, 4.41%) (30).

There were a series of reports from different geographical regions of Brazil. A random sample from Salvador, Bahia (84 whites, 772 negroids) showed the frequency of CHE1*A gene was 0.842+/-0.233%. Estimated risk for developing succinylcholine induced prolonged apnea was about 1:2,900 (31). Using a new method for estimating frequency of CHE1*F allele, a southern Brazil community from Curitiba (251 whites, 818 non-whites) was studied. There was no difference between the two ethnic groups. CHE1 U and CHE1 UF phenotypes were reported to be better separated by using inhibitors DL-propranolol and R0s-0683 with ampha-naphthylacetate (32). In a more recent report, the same group studied genetic variability of BCHE and CHE2 loci in nine Brazilian Indian groups. Additionally BCHE*F allele was studied in eight other samples. Among the Indian populations, BCHE*F allele occurred in 0-7.1%+/-3.4, that of BCHE*A occurred in one group (1.4%+/-1.0 (33). Tribes from the Amazon region were studied for atypical and C5 variants of BCHE. None had CHE1*A allele, but C5 variant was observed in Arawete (20.4%), Kararao (15.6%), Karitiana (50.5%), Surui (12.3%) and CintaLarga (19.6%) tribes (34). Employing polymerase chain reaction primer

introduced restriction analysis, 177 Brazilians were studied; frequencies of BCHE K mutation were similar between the European and African origin subjects, and were similar to those reported in other regions from North America, Scotland, Japan and Denmark (35).

It is evident that advances in technology improved the ability to identify genetic variants with far greater precision than the earlier phenotypic methods of measuring the enzyme in plasma, or characterizing them phenotypically by inhibition methods.

Rao and Gopalan were the first to report on the genetic studies of Vysyas in Andhra Pradesh and to have documented the presence of silent allele at cholinesterase locus 1 in this population. Among 459 subjects belonging to the Vysya community screened for variants at the E1 and E2 locus, silent allele was found to be common (gene frequency=0.1112), with homozygotes comprising more than 2% of the population. They also reported the occurrence of dibucaine and fluoride resistant alleles (36). Individuals from the Vysya community belonging to different locations of Andhra Pradesh were observed to have a high frequency of the silent variant at E1 locus (0.0115-0.1925), with an overall frequency of 0.1040 (7). However the incidence of C5 isozyme at the E2 locus among different communities in Andhra Pradesh was shown to be comparatively low (37).

A number of hypotheses were put forward for the variable genetic load in endogamous population such as the Vysyas from Andhra Pradesh. Inbreeding could have accounted for higher genetic load (38). While it was hypothesized that continued inbreeding could eventually eliminate genes, their occurrence led to other possibilities. An illuminating editorial by Pandit *et al* (15) commented on the *why* of certain genetic diseases persisting in populations, such as this. They first considered the founder effect, in which an allele that provides no apparent disadvantage is likely to persist, they argue against the possibility because of their large numbers (more than 20 millions). The other possibility, proposed earlier by us (39) links the existence of the abnormal allele conferring evolutionary advantage (15). The advantage if any is still unknown, but potential links involve deficiency of BCHE and a favourable lipoprotein profile lowering the risk of cardiovascular disease in spite of consuming high-fat diet.

Dietary fat and butyrylcholinesterase

Several studies were carried out on the influence of dietary fat on plasma BCHE activity. Van Lith *et al* assessed the effect of dietary fat on BCHE activity in the plasma of rats (40). When fed glucose or an isocaloric amount of coconut fat, the BCHE activity was similar with either. An earlier study showed that enhanced intake of cholesterol suppressed BCHE activity

(41). While put on a restricted feed, supplement of coconut fat and fish oil elevated ES-1 activity; only fish oil was found to raise BCHE activity. In human studies, elevated BCHE was associated with high levels of triglycerides among subjects with hyperlipidemia (42). While it is known that coconut fat elevates liver cholesterol and plasma triglycerides, it was shown that it also leads to increased intestinal esterase and ES-1 activities. However there was no change in serum BCHE activity in response to the intake of extra coconut fat (43). However there was a differential response in the small intestine, where the BCHE activity increased. Thus it is possible a high fat diet could increase the release of BCHE from the liver cells due to hepatic accumulation of cholesterol (43).

Butyrylcholinesterase activities were measured in a cohort of hyperlipidemic subjects (n:55) with elevation of both serum cholesterol and triglycerides; there was increased level of BCHE activity (44) in consonance with earlier reports. Similarly, Rustemeijer *et al* reported that BCHE activity was a marker of the rate of triacylglycerol synthesis in type 2 diabetes (45). Annapurna *et al* studied streptozotocin-induced diabetic rats to understand the cause for elevated BCHE levels in diabetes mellitus (46). It was shown that the elevated BCHE activity was due to the hepatic overproduction of very low density lipoprotein.

Type 2 diabetes mellitus and butyrylcholinesterase

A number of studies in humans were carried out on the relation of serum BCHE activity with type 2 diabetes mellitus and with components of the metabolic syndrome. In a cohort of 107 subjects with type 1 and type 2 diabetes, BCHE activity correlated with log serum fasting triacylglycerol concentration (47). In type 2 diabetes, while BCHE activity was correlated with insulin sensitivity, it was not related to others such as gender, age, BMI or type of treatment of diabetes. However other studies have shown that BCHE activity was related with serum lipid profile and parameters of obesity in addition to insulin resistance. Among 259 Japanese subjects with type 2 diabetes, BCHE activity correlated with waist circumference, visceral fat area, subcutaneous area, triglycerides, HDL cholesterol, LDL and HOMA-R (48). While the association of BCHE activity with insulin was reported, uncertainty exists about the causality. Alacantara *et al* examined the total BCHE activity and that of its two electrophoretic bands (C(4/5) and C(O)) in obese subjects (n:99). The total BCHE activity was associated with metabolic syndrome and its components, but was considered as a secondary marker in obese subjects having the CHE2 C5-phenotype (49). In a larger population, Randell *et al* studied independent predictors of serum BCHE activity and its relation

with metabolic syndrome. Essentially BCHE activity was related to insulin homeostasis; it correlated with insulin index (HOMA-R) and was elevated in subjects having metabolic syndrome (50). Independent predictors of BCHE activity included percent trunk fat, triglyceride levels, glucose, HOMA-R and age. In conclusion, although BCHE could be involved in the pathophysiology of metabolic syndrome components, it is not certain whether increased BCHE activity precedes or follows the risk factors.

An association was found between variants of genes coding for ghrelin and BCHE in subjects with obesity. Ghrelin, a protein related to gain in body weight is hydrolyzed by BCHE (51). SNPs of GHRL and BCHE genes were studied in relation to BCHE activity among 144 obese Euro-Brazilian male blood donors and 153 non-obese controls. Among the obese subjects, there was an association between higher BCHE activity in the 72LM+72MM,-116GG genotype. In view of the association of 72M SNP with higher activity of BCHE in the obese, a regulatory mechanism was suggested whereby GHRL gene could influence BCHE gene expression (51).

In view of its association with BMI, the association of -116A (SNP: G/A; rs1126680) and 1914G (SNP: A/G; rs3495) variants of BCHE gene with anthropometric measures were studied in obese subjects from Southern Brazil (n:115). Higher BMI and triglyceride levels were seen in carriers of 1914G compared to 1914A homozygotes. Carriers of the -116A allele had lower activity of BCHE activity than usual homozygotes. It was concluded that 1914G allele could influence gluconeogenesis, hyperglycemia, lipolysis and body fat distribution, leading to obesity (52).

Similarly, a recent study showed that the capacity of BCHE to detoxicate is altered in dyslipidemic conditions, which could have clinical significance in management of xenobiotic elimination (53). Similarly, BCHE activity was suggested to be part of assessment of conditions such as inflammation and protein-energy malnutrition (54).

A review of the association between BCHE and metabolic syndrome was published. Possible links with type 2 diabetes mellitus, Alzheimer's disease, sleep-wake cycle and lipids were discussed (3). Hashim *et al* first reported on the association between BCHE gene (3q26) with type 2 diabetes, which was independent of the function of beta cells of pancreas (55). A later publication from Asian south Indian subjects with type 2 diabetes showed that serum butyrylcholinesterase was inversely related to serum total cholesterol levels (4). Similar results were obtained in a Brazilian sample. Elevated BCHE activity was found in the presence of hyperglycemia, leading to a suggestion that it may be related to vascular complications of diabetes (56).

Reports on the association of BCHE activity with gestational diabetes are available. Cocelli showed a positive correlation between BCHE activity and LDL in Turkish women with gestational diabetes (57). Butyrylcholinesterase could play a role in the development and function of the placenta. It could be involved in attenuating the effects of toxic xenobiotics attempting to pass the placental barrier. Sternfeld *et al* showed that maximum BCHE activity was found at week 12 of gestation (58). BCHE could quench free radicals, which lead to poor pregnancy outcomes. Mahmoud *et al* from Kuwait showed that pregnant women with risk factors such as preeclampsia and diabetes had lower levels of serum BCHE activity (59, 60).

Inflammatory products could contribute to a form of toxin induced immune dysfunction in gestational diabetes mellitus. Lower levels of BCHE activity could predispose women to gestational diabetes mellitus (61). In addition, BCHE may be related to the pathogenesis of congenital anomalies such as anencephaly and open *spina bifida*, where dense band of BCHE was shown to occur in the amniotic fluid. Clearly more work is needed to unravel the role of BCHE in pregnancy disorders such as its role in estrogen alpha receptor, and hydrolysis of advanced glycation end product (61).

Interestingly, serum BCHE has been recently studied as a predictor for development of type 2 diabetes mellitus. Considering that a relation exists between BCHE activity and type 2 diabetes mellitus, a prospective study evaluated whether BCHE could predict its development. From a sample of 12,647 men employed in Nippon Telegraph and Telephone West Corporation, Japan, 9239 men meeting the entry criteria for the study were studied (62). After a median follow up of six years, 868 cases of type 2 diabetes were identified. Even after adjusting for age, BMI, fasting plasma glucose and other variables, serum BCHE was shown to have an independent association with risk of developing type 2 diabetes mellitus: higher levels of BCHE increased the risk of development of type 2 diabetes (62). The underlying reasons could be that BCHE being related to metabolic syndrome (3), as well as lower levels of acetylcholine due to increased BCHE; acetylcholine is an anti-inflammatory molecule, lower levels of which may be associated with inflammation (63), known to be involved in the pathogenesis of diabetes mellitus (64). In support of this, increased BCHE and gamma-GT activities may act *via* nitric acid to lead to insulin resistance syndrome (65). As proposed by Kalman, hyperlipidemia may lead to altered stereoscopic configuration of BCHE and its activity, or alter the expression of the gene (54). Elevated BCHE in the atria of mice fed high fat diet influenced peripheral nervous system, resulting in decreased parasympathetic activity (66).

Butyrylcholinesterase and other associations

There have been studies on the relation of BCHE activity in hypertension and in cardiovascular disease and its risk. In a recent report from Egypt, BCHE activity was increased in subjects with essential hypertension (67). Valle *et al* showed that BCHE activity was related to body weight component of the metabolic syndrome (68). Similar associations with cardiovascular disease and in post-menopausal women were recently reported from south India (69, 70).

Butyrylcholinesterase activity has been employed as a prognostic marker for outcome of acute conditions such as acute coronary syndrome and traumatic brain injury. In a retrospective analysis of 624 subjects with acute coronary syndrome, over 4 years of mean follow up, BCHE predicted the risk of death from cardiac conditions; it was most pronounced in the age group of 45-64 years (71). Zhang *et al* retrospectively analyzed cholinesterase activity as a predictor of mortality in 188 patients with traumatic brain injury. At an optimal cut off value of 5kU/L, CHE sensitivity for 90-day prediction of mortality was 65.5%; specificity was 86.4%. Although poorer than APACHE II or white blood cell count as a predictor, cholinesterase activity in plasma was a borderline independent factor for death in subjects with traumatic brain injury (72). Patients admitted to the emergency room with trauma were studied. BCHE activity was associated with severity of trauma leading to systemic inflammation: lowered activity was observed earlier than other routinely measured biomarkers which are routinely measured (73).

Other interesting associations were studied in relation to pigmentary disorders, cognitive decline and the skeletal system. Skin of subjects with vitiligo had lower BCHE activity compared to unaffected skin, suggesting the cholinergic pathway may be involved in the etiology of the disease (74). Similarly, atypical BCHE gene was reported to predispose to the development of Hansen's disease (75). Clinical, pathogenic, experimental as well as bioinformatic evidence exists for BCHE to be a potential link between the occurrence together of type 2 diabetes mellitus and Alzheimer's disease (3, 76-78). A recent study in BCHE gene-deficient mice showed that the role of BCHE was limited to microarchitectural changes in bone consisting of increased relative number of osteoclasts which resorb the bone (79).

While genomic studies of BCHE variants provide accurate structural changes, BCHE variants based on inhibition studies are still relevant in anesthetic practice, as shown by a recent Iranian study. A population from the western region of Iran showed that there was a medium frequency of succinylcholine-sensitive individuals (80).

Butyrylcholinesterase knock-out model

The development of butyrylcholinesterase knock mouse model from the research lab of Lockridge O brought about a sea change in the way studies can be performed in animal models of BCHE deficiency. Two mouse models were developed: homozygous knockout (BChE^{-/-}), which have no detectable BCHE activity in the plasma or in any tissue and BChE heterozygous (BChE^{+/-}); in the latter, BCHE is about half that of wild type (BChE^{+/+}) (81). The knock out model was produced by the deletion of 891 bp from the BCHE gene. There was removal of splice junction between intron 1 and exon 2; in addition, the entire signal peptide was excised including start site for translation, and the first 102 aminoacids of BCHE protein. The model replicated all features observed in humans harboring variant BCHE proteins, including sensitivity to succinylcholine and other drugs, in body composition, and apparent lack of abnormal phenotypic changes. However, the mouse model has carboxylesterase, unlike human beings. A knock out model without even the carboxylesterase expression would more closely resemble a human model which is useful for testing potential new drugs (81).

There has been a recent exciting study using the BCHE knock out mouse model in influencing hepatic fat accumulation when fed high-fat diet (82). In order to understand the causative factors between BCHE and obesity, BCHE KO mice were fed high-fat diet. They gained more weight, more white fat and greater amount of hepatic fat. In addition the energy expenditure was lower than control mice. All these were reversed by a gene transfer of BCHE (82). It was suggested that BCHE and ghrelin together account for these changes.

Natural human knockouts

These provide unique ways in which 'natural human knockouts' can be studied in terms of genotype-phenotype relation (83). Interest in dissecting how subjects without a gene or protein survive in good health led to the "Resilience Project" where apparently healthy people are studied for their genetic and environmental exposure (84). It is possible that protective alleles exist by an interaction of second-site mutations and environmental factors (85). Human BCHE KO equivalents would be a fruitful source for such studies comparing genotype-phenotype relation (3). Earlier studies on subjects with variant BCHE from south India did not report any significant health effects (86) other than when challenged with succinylcholine, which led to prolonged apnea. Segurel *et al* suggested it would be useful to search for haplotypes which are protective in nature against diabetes (87). Exercises have begun to document and follow up such human knock out equivalents who are healthy

despite lack of genes (88).

In line with this evidence, a recent analysis from Pakistan, which is geographically adjacent to India, listed out human knockouts and phenotypic analysis in a cohort with high rates of consanguinity (89). Such consanguinity is prevalent in the Vysya population, from which the subjects of our study were drawn. Such human knock out equivalents are unusual occurrences in view of selective pressure from evolution, but may be present in consanguineous marriages (90). It is conceivable that such isolated population groups 'prove a roadmap for a human knockout project' (89). For acetylcholinesterase in metabolic syndrome, see (91).

Conflict of interest statement

The author certifies that he has no affiliations with or involvement in any organization with any financial interest in the subject matter discussed in this review article.

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