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In silico identification of genes involved in chronic metabolic acidosis

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ABSTRACT

Aims: Chronic metabolic acidosis (CMA) refers to increased plasma acidity due to disturbed acid-base equilibrium in human body. CMA leads to many dysfunctions including disorders of intestinal metabolism and barrier functions. The human body responds to these intestinal dysfunctions by creating a compensatory mechanism at genomic level in intestinal epithelial cells. This study was to identify the molecular pathways involved in metabolic dysfunction and compensatory adaptations in intestinal epithelium during CMA.

Main methods: In silico approaches were utilized to characterize a set of 88 differentially expressed genes (DEGs) from intestinal cells of rat CMA model. Interaction networks were constructed for DEGs by GeneMANIA and hub genes as well as enriched clusters in the network were screened using GLay. Gene Ontology (GO) was used for enriching functions in each cluster.

Key findings: Four gene hubs, *i.e.*, trefoil factor 1, 5-hydroxytryptamine (serotonin) receptor 5a, solute carrier family 6 (neurotransmitter transporter), member 11, and glutamate receptor, ionotropic, *n*-methyl D-aspartate 2b, exhibiting the highest node degree were predicted. Six biologically related gene clusters were also predicted. Functional enrichment of GO terms predicted neurological processes such as neurological system process regulation and nerve impulse transmission which are related to negative and positive regulation of digestive system processes., intestinal motility and absorption and maintenance of gastrointestinal epithelium.

Significance: The study predicted several important genomic pathways that potentially play significant roles in metabolic disruptions or compensatory adaptations of intestinal epithelium induced by CMA. The results provide a further insight into underlying molecular mechanisms associated with CMA.

1. Introduction

Chronic metabolic acidosis (CMA) is a clinical disorder characterized by an increase in plasma acidity. Under normal health conditions, an equilibrium in the acid-base balance in the body maintains the plasma pH at 7.38–7.42, which is essential for normal functioning of metabolic reactions and physiological processes associated with life [1]. However, sometimes due to abnormal health conditions there is an increase in acid production and the body is not able to get rid of enough acid or does not have enough base to neutralize the normal amount of acid; the resulting disturbance of acid-base balance (pH $<$ 7.35) leads to metabolic acidosis [2]. Chronic metabolic acidosis can last for weeks to years adversely affecting organ functions with serious consequences including coma and death [3].

A human individual normally produces approximately 15,000 mmol of volatile acid (carbon dioxide) and 50 to 100 meq of nonvolatile acid (*e.g.*, sulfuric, phosphoric) every day due to the metabolism of dietary carbohydrates and fats [4]. During the times of intermediary metabolic abnormalities, excess acid production occurs and may lead to CMA. Moreover, buffering mechanisms present naturally in the human body regulate acid-base balance of plasma, of which bicarbonate (HCO_3) system is the major buffering system of the plasma [5]. Deficiency or loss of HCO_3 in the plasma may overwhelm the acid-base homeostasis and lead to CMA. Many risk factors that predispose the body systems for CMA include consumption of processed foods that are low in carbohydrates and high in fats, renal failure, obesity, dehydration, chronic intoxication and diabetes.

In general, CMA has multifactorial underlying causes. CMA is one of the frequently observed clinical complications in patients suffering from chronic kidney disease (CKD) [6]. Of the estimated 10% of the global population affected by CKD, about 2–13% suffer from CMA and about 30–50% CKD patients with estimated glomerular filtration rate of $<$ 30 ml/min have complications of CMA [7]. Under normal conditions, the renal tubules each day reabsorb about 4500 mmols of filtered HCO_3 and also synthesize sufficient new HCO_3 to neutralize the endogenous acid in the body [8]. In CKD, kidneys have a declined ability

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for hydrogen ion secretion and ammonia synthesis [6]. Decreased ammonia production further leads to reduction in acid excretion. Retention of acid and lower HCO_3 reabsorption together with accumulation of sulfates, phosphates and urea in kidney disease lead to CMA [9]. CMA may also result due to chronic laxative abuse, diarrhea, vomiting, tube drainage, fistulas in which hyperchloremic acidosis occurs as a result of loss of HCO_3 [3]. Bicarbonate wasting due to diarrhea concomitantly causes increased chloride and decreased HCO_3 concentrations in plasma. Very recently, suggestions of CMA being associated with the modern western way of food habits have been proposed [10]. Modern food preparation methods render the foods deficient in essential enzymes, vitamins, minerals and bicarbonate and this possibly shifts the internal environment of the body from slight alkaline to acidic causing CMA.

The clinical consequences of CMA manifest in metabolic dysfunction and damage to several body organs including severe muscle wasting, bone demineralization, hypotension, progression of chronic kidney disease, changes in visceral organ blood supply, digestive disorders, *etc*. [6]. CMA is associated with disruption of insulin and insulin-like growth factor signaling leading to skeletal muscle protein catabolism through caspase and ubiquitin-proteasome system resulting in muscle degradation and loss [11–12]. CMA also causes negative calcium balance, bone demineralization, hypercalciuria and exacerbates pre-existing bone disease [13–16]. CMA may advance the progression of CKD by inducing tubulointerstitial injuries through ammonia induced complement activation [17]. CMA is associated with growth retardation in children and sustained treatment with alkali maintained normal stature in children with acidosis [18]. CMA is also associated with negative nitrogen balance and impairment of albumin synthesis, dysfunction of endocrine organs and alteration in secretion and action of many hormones, such as corticosteroids, thyroid hormone and parathyroid hormone, general systemic inflammation, insulin resistance, *etc*. (reviewed in [3–7]).

Regarding digestive disorders, CMA may affect the alkaline pH of bile and pancreatic juice, which can change the biochemical properties of these secretions and lead to serious digestive problems [10]. For example, acidification may reduce the antimicrobial activity of pancreatic juice leading to intestinal dysbiosis, decrease in pH may prematurely activate the pancreatic juice in the pancreas and cause pancreatitis, and acidification of bile may result in precipitation of bile salts causing dysfunction of biliary system and formation of biliary calculi. CMA also leads to metabolic and barrier dysfunction of intestines [19]. In an *in vitro* study [20], defective epithelial barrier was seen when rat intestinal epithelial cells were exposed to lipopolysaccharides. The condition was suggested to be due to cytoplasmic acidification resulting from extracellular acidosis. Cellular processes like apoptosis, proliferation, lipid peroxidation, *etc*. which affect the survival of intestinal epithelial cells are also sensitive to CMA [21–22].

Using a CMA rat model [23], CMA was shown to induce genomic changes in the duodenal epithelial cells with up-regulation or down-regulation of 684 genes. These genomic changes likely resulted in duodenal cell dysfunction and also were potential genomic compensatory adaptations in response to defective intestinal epithelial cells to avoid or minimize the effect of disturbed function [23–25]. The genomic adaptations have been thought to result in alterations in trans-epithelial transport of water, amino acids, inorganic phosphate, nutrients like Na, Cl, K, HCO_3, Ca^{2+} and major elements [25–29].

The objective of the present study was to characterize and understand the molecular pathways and functional inter-relationships of CMA induced genomic changes in intestinal epithelial cells by *in silico* approaches using a set of 88 differentially expressed genes (DEG) from the reported previous study [23]. For identification of the genes and pathways, interaction network of DEGs was constructed and hub genes as well as enriched clusters in the network were screened. Gene Ontology (GO) was used for functional interactions among DEGs and over represented biological functions were identified by functional enrichment analysis of each cluster.

2. Materials and methods

2.1. Datasets

Computational analyses were done on 88 DEGs from an earlier experimental study in rats on gene expression profile of duodenal epithelial cells in response to CMA [23]. These DEGs represented the Illumina's microarray featuring high-performance Bead Array technology that was carried out on mRNA samples from duodenal epithelial cells exposed to long-standing acidemia. The dataset of 88 DEGs consisted of 49 up-regulated and 39 down-regulated genes representing the mRNAs whose expression levels altered by > 10-fold in response to CMA.

2.2. Network analyses

GeneMANIA webserver (www.genemania.org/) was used for studying interaction among DEGs and other related genes in the network [30]. Using GeneMANIA, functional interaction among DEGs was determined on the basis of GO term “biological process” and *R. norvegicus* (rat) as reference species. Predicted correlation among network genes incorporated various parameters which include co-expression, biological pathways, similarity in protein domains, co-localization, physical and genetic interactions, and predicted interactions.

2.3. Identification of hub genes

Analysis of interaction networks inferred from GeneMANIA was carried out using Cytoscape 2.8.2 [31]. Scale free property is shown by biological networks [32] where hubs represent nodes having multiple connections in the network. Hubs were determined by computing node degree distribution values using Network Analyzer plugin of Cytoscape and the genes with highest value of node degree distribution were considered as hubs.

2.4. Community analysis

Greedy community-structure detection algorithm *via* GLay (http://brainarray.mbni.med.umich.edu/sugang/glay) plugin in Cytoscape was applied in determining modules with functional property [33]. In each cluster, over represented biological functions were identified by subjecting clusters to a functional enrichment analysis. Only those communities were focused for functional enrichment analysis which had at least 10 nodes. DAVID functional analysis tool was used for carrying out functional enrichment analysis.

3. Results

3.1. Network construction and identification of hub genes

Of the total 88 DEGs, only 86 genes were identified by GeneMANIA. Network analysis of 86 DEGs using GeneMANIA led to enrichment of neurological processes like neurological system process regulation and nerve impulse transmission (Table 1). Other GO term “biological processes” which were over-represented included regulation of synaptic transmission, exocytosis, neurotransmitter transport and axon part (Table 1).

Initial network which consisted of 180 genes and 2771 nodes was reduced to 180 genes and 2461 edges after filtering by eliminating edges and self-loops which appeared in duplicate. Upregulated genes, downregulated genes and additional related genes predicted by GeneMANIA are shown (Fig. 1) with Network genes represented by circles and interactions between these genes represented by edges.

The genes with highest value of node degree distribution were considered as hubs and the analyses indicated three hubs. Of the three