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Research Article

Network analysis of beta casomorphin-7 revealed genes and pathways associated with human diseases

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Abstract

Context: Several epidemiological researches imply a link between exposure to A1 beta casein (BC) containing milk and the incidence of non-communicable diseases in humans. Many breeding policies support BC variants not releasing Beta Casomorphin-7 (BCM-7).

Objective: Insilico network analysis was performed to determine the bioactive targets, pathways and diseases associated with A1 and A2 beta casein and BCM-7.

Results: The analysis revealed 63 bioactive targets for A1 and A2 beta casein with 118 pathways and 30 diseases associated. There were no differences in bioactive targets between A1 and A2 beta casein. For BCM-7, 15 bioactive targets were identified, which were found to be associated with 18 pathways and 4 diseases.

Conclusion: The results showed that the change in amino acid at the 67th position in A1 and A2 beta casein did not affect the bioactive targets. BCM-7 may be held responsible for adverse effects until challenged by clinical trials.

Keywords: Beta Casein; Beta Casomorphin-7; A1 Milk; Human Disease

Introduction

Milk is an essential source of nutrition, including high-quality protein, carbohydrates, and certain minerals. Milk is an oil-in-water emulsion consisting of water (87.7%), protein (3.3%), lactose (4.9%), fat (3.4%), minerals (0.70%), and minor components (3.36%). Milk is primarily composed of two forms of protein: whey protein (14%), and casein (80%) [1]. Casein is made up of multiple components and is the most common protein found in milk and four major subgroups are found. S1 Casein, S2 Casein, Casein, and BC, which are all heterogeneous and contain multiple genetic variations [2]. BC is the second most abundant protein component (25 to 35%) in cow milk and the most common beta casein types are A1 and A2 [3]. Histidine in A1 BC is replaced by proline in A2 BC in the 67th position of the beta-casein chain [1]. Lately, public health concern has grown about the negative consequences of BCM-7, an opioid peptide produced from A1 BC in bovine milk that has been linked to risk factors for noncommunicable illnesses in humans. A1-milk and the BCM-7 peptide may be connected to heart disease, diabetes, autism, newborn mortality, and digestive inflammation [4] while many other researchers contradict the adverse effects of BCM-7 [5]. Hence in this in-silico analysis, we made an attempt to find out bioactive targets, diseases and disease-causing pathways, if any associated with A1 BC, A2 BC and BCM-7, to supplement scientific evidence to resolve conflicting schools of thoughts.

Material and Methods

A1 and A2 variants of Beta Casein protein sequence were downloaded from NCBI Accession number: AAA30431.1 and UTO68600.1 respectively. Signal peptides are removed from both the sequences before subjecting them to downstream analysis. The physicochemical properties of the proteins were assessed using the ProtParam tool of ExPASy database (https://web.expasy.org/protparam/). Sequence alignment was done using Emboss (https://www.ebi.ac.uk/Tools/psa/emboss_water/) to ensure change of amino acid at 67th position in between the two casein variants. Canonical SMILES of the two proteins were generated using ChemAxon webtool (https://datascience.unm.edu/tomcat/biocomp/convert). SMILE file of BCM-7 was downloaded from Pubchem (PCID: 12770774).

Retrieving and analysis of bioactive targets of A1 and A2 beta caseins and BCM-7

The generated SMILES were queried for parsing in BindingDB database with a high similarity search type of 0.85 to reduce FDR to obtain bioactive target of study sequences. KEGG database was used to find bioactive targets associated pathways and diseases in humans (https://www.genome.jp/kegg/). Genes associated with the found bioactive targets and their functions were obtained from the uniprot database (https://www.uniprot.org/). The Panther database is used for annotation of identified genes (http://www.pantherdb.org/). Network construction of identified targets, their associated genes, disease and pathways were constructed using Cytoscape software [6].

Results

The retrieved two proteins were 224 amino acids long. Signal peptide of beta casein protein was identified as the first 15 amino acid residues from the amino terminal end [7]. After removing signal peptides, 209 amino acids were left in each protein. The molecular weights of A1 BC, A2 BC were 23.623 and 23.583 KDa respectively (Table 1). Alignment of two sequences confirmed only one amino acid change in the sequence i.e., at 67th position from the amino terminal end containing histidine (H) in A1 beta casein and proline (P) in A2 beta casein (Figure 1). BCM-7 had a molecular weight of 0.79 KDa, and was devoid of both positive and negative charge bearing amino acids (Table 1).

Properties	A1 Beta casein	A2 Beta casein	BCM-7
Number of amino acids	209	209	7
Molecular weight (KDa)	23.623	23.583	0.790
Asp + Glu (Total number of negatively charged residues)	23	23	0
Arg + Lys (Total number of positively charged residues)	15	15	0
Theoretical Ph	5.24	5.13	5.52
Instability index	98.48 (Unstable)	96.62 (Unstable)	81.23 (Unstable)
Aliphatic index	88.47	88.47	55.71
Grand average of hydropathicity (GRAVY)	-0.362	-0.355	0.114

Table 1: Physiochemical properties of protein sequences used.

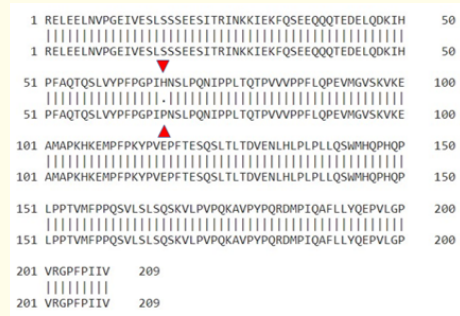

Figure 1: Sequence alignment of A1 and A2 beta caseins displaying one amino acid change at 67th position from amino terminal end.

Network analysis of A1, A2 beta casein and BCM-7

A total of 63 bioactive targets for both A1 BC and A2 BC were identified. KEGG pathway analysis identified 118 pathways related to different molecular functions and disease processes and KEGG figure 2: Network analysis of BCM-7 and its associated genes, diseases and pathways disease database identified 30 associated diseases (Supplementary table 1).

Panther biological process analysis revealed maximum genes (48) were associated with cellular processes (Supplementary figure 1). Same bioactive targets and associated genes, pathways and diseases between A1 BC and A2 BC were identified. The difference of amino acids at the 67th position did not change the target bioactive compounds. For BCM-7, a total of 15 bioactive targets were identified. KEGG pathway analysis revealed 40 pathways related to different molecular functions and disease processes and KEGG disease database revealed 6 associated diseases (Figure 2 and Supplementary table 2). Panther biological process analysis revealed that maximum genes (12) were associated with cellular processes (Figure 3).

Discussion

Amino acid substitution caused by a point mutation in the corresponding DNA sequence alters the physical and chemical characteristics of proteins [8]. But the change in amino acid at 67th position did not lead to change of target bonding bioactive compounds. This finding was in line with the suggestions of Ng and Henikoff [9] that usually around 70% of non synonymous amino acid replacement does not affect protein function. Hence differential action of these two proteins could be due to BCM-7. Digestive enzymes (pepsin, pancreatic elastase, and leucine aminopeptidases) can cut away BCM-7 right close to the histidine at position 67 [10]. Proline at the same position in A2 beta-casein, on the other hand, does not result in enzymatic hydrolysis to generate BCM-7 [11]. Total six diseases were found in network analysis of BCM-7. Out of that, delta-type opioid receptors were found to be involved in eating disorders. Type I diabetes mellitus pathway (KEGG ID: hsa05022) and autoimmune thyroid disease were associated with HLA class I histocompatibility antigen, alpha chain gene. Abnormal fat deposition was noticed in broiler chickens injected with BCM-7 along with differential expression of 39 genes in the liver related to fatty acid production [12]. Excess fat deposition is associated with risk of cardiac diseases [13] and diabetes [14,15]. Proenkephalin-B compound was associated with pathways of neurodegeneration - multiple diseases (KEGG ID: hsa05022). Proteasome subunit beta type-5 compound was associated with Alzheimer disease (hsa05010), Parkinson disease (hsa05012) and Amyotrophic lateral sclerosis (hsa05014) pathways. Kuellenberg., *et al*. [16] in a meta-analysis on laboratory animal model in a quest for intervention of BCM7 found intermediate markers for cardiovascular disease; diabetes including diabetic cardiomyopathy, glucose and insulin concentration, pancreatic oxidative stress and diabetic nephropathy; neurological effects including analgesia and behavioral change. Lucarelli., *et al*. [17] indicated that drinking of cow's milk might worsen behavioral symptoms of autistic children. Reichelt and Knivsberg [18] reported the presence of opioid peptides derived from food proteins in the urine of autistic patients. However, according to the European Food Safety Authority, little was understood about the mechanics of intact peptide transport over the intestinal barrier longer than three amino acids. Even if this occurred, the transmission would be relatively little due to passive diffusion [19] and hence it concluded that no cause and effect relationship could be established between the dietary intake of BCM-7 and various diseases.

While BCM-7 may have an effect on the digestive system, it is unknown to what extent BCM-7 is absorbed intact into the blood [20]. BCM-7 was not discovered in the blood of healthy individuals who drank cow's milk, according to studies, however a few studies indicated that BCM-7 may be present in infants [21,22]. The World Health Organization (WHO) proposes that newborns be nursed exclusively for the first six months and that breastfeeding should continue into the second year to support good growth and development [23]. Overall, regulations support breeding for BC variations that do not produce BCM-7, and intake of "A1-like" milk may not be deemed adequate for a healthy and safer lifestyle [24].

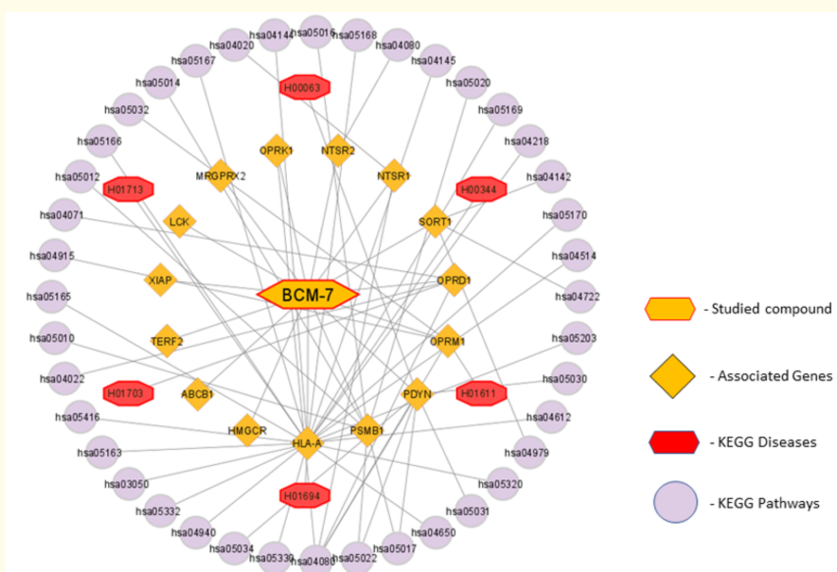

Figure 2: Network analysis of BCM-7 and its associated genes, diseases and pathways disease database identified 30 associated diseases (Supplementary table 1).

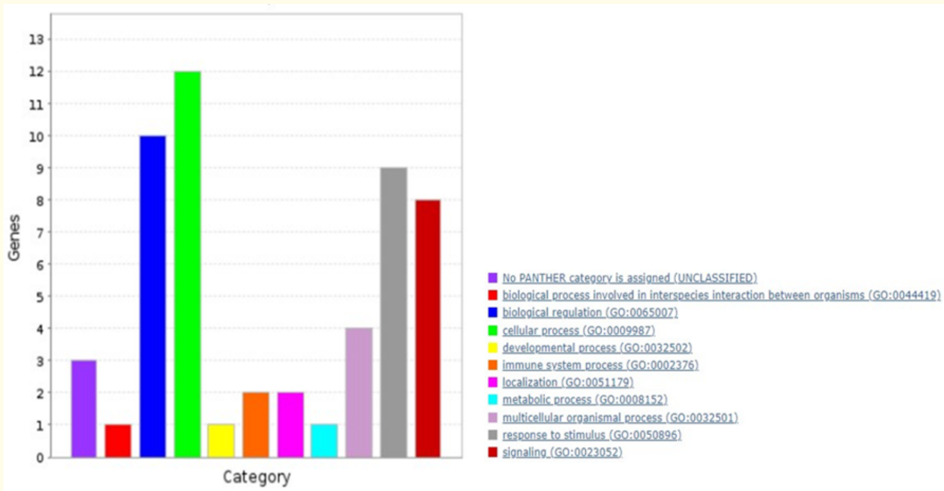

Figure 3: Panther biological process of BCM-7.

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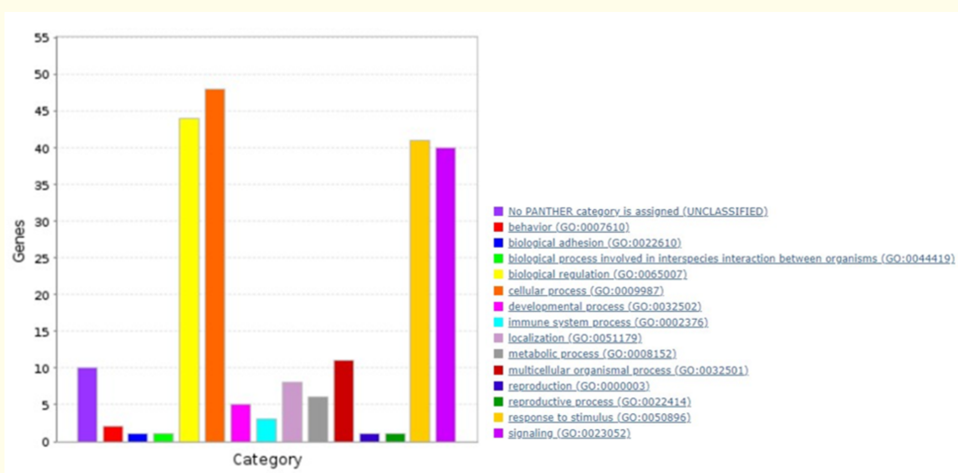

Supplementary Figure 1: Panther biological process of A1 and A2 beta caseins.

Bioactive targets	Uniprot ID	Gene	KEGG Pathways ID	KEGG Pathways	KEGG Disease ID	KEGG Disease
Acetylcholine receptor subunit epsilon	Q04844	CHRNE	hsa04080	Neuroactive ligand-receptor interaction	H00770	Congenital myasthenic syndrome
Angiotensin-converting enzyme 2	Q58DD0	ACE2				
Apelin receptor	P35414	APLNR	hsa04080	Neuroactive ligand-receptor interaction		
			hsa04371	Apelin signaling pathway		
BTB/POZ domain-containing protein KCTD11	Q693B1	KCTD11				
BTB/POZ domain-containing protein KCTD12	Q96CX2	KCTD12	hsa04060	Cytokine-cytokine receptor interaction		
			hsa04061	Viral protein interaction with cytokine and cytokine receptor		
			hsa04144	Endocytosis		
			hsa04151	PI3K-Akt signaling pathway		
			hsa04630	JAK-STAT signaling pathway		
			hsa04658	Th1 and Th2 cell differentiation		
			hsa04659	Th17 cell differentiation		
			hsa05162	Measles		
			hsa05166	Human T-cell leukemia virus 1 infection		
			hsa05200	Pathways in cancer		
			hsa05321	Inflammatory bowel disease		
			hsa05340	Primary immunodeficiency		
Calcitonin gene-related peptide 1	P06881	CALCA				
Calcitonin gene-related peptide type 1 receptor	Q16602	CALCRL				
Cholecystokinin receptor type A	P32238	CCKAR	hsa04020	Calcium signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
			hsa04911	Insulin secretion		
			hsa04972	Pancreatic secretion		
Corticotropin-releasing factor receptor 1	P34998					
Corticotropin-releasing factor receptor 2	Q13324	CRHR2	hsa04024	cAMP signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
			hsa04934	Cushing syndrome		

Bioactive targets	Uniprot ID	Gene	KEGG Pathways ID	KEGG Pathways	KEGG Disease ID	KEGG Disease
Acetylcholine receptor subunit epsilon	Q04844	CHRNE	hsa04080	Neuroactive ligand-receptor interaction	H00770	Congenital myasthenic syndrome
Angiotensin-converting enzyme 2	Q58DD0	ACE2				
Apelin receptor	P35414	APLNR	hsa04080	Neuroactive ligand-receptor interaction		
			hsa04371	Apelin signaling pathway		
BTB/POZ domain-containing protein KCTD11	Q693B1	KCTD11				
BTB/POZ domain-containing protein KCTD12	Q96CX2	KCTD12	hsa04060	Cytokine-cytokine receptor interaction		
			hsa04061	Viral protein interaction with cytokine and cytokine receptor		
			hsa04144	Endocytosis		
			hsa04151	PI3K-Akt signaling pathway		
			hsa04630	JAK-STAT signaling pathway		
			hsa04658	Th1 and Th2 cell differentiation		
			hsa04659	Th17 cell differentiation		
			hsa05162	Measles		
			hsa05166	Human T-cell leukemia virus 1 infection		
			hsa05200	Pathways in cancer		
			hsa05321	Inflammatory bowel disease		
			hsa05340	Primary immunodeficiency		
Calcitonin gene-related peptide 1	P06881	CALCA				
Calcitonin gene-related peptide type 1 receptor	Q16602	CALCRL				
Cholecystokinin receptor type A	P32238	CCKAR	hsa04020	Calcium signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
			hsa04911	Insulin secretion		
			hsa04972	Pancreatic secretion		
Corticotropin-releasing factor receptor 1	P34998					
Corticotropin-releasing factor receptor 2	Q13324	CRHR2	hsa04024	cAMP signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
			hsa04934	Cushing syndrome		

Delta-type opioid receptor	P41143	OPRD1	hsa04022	cGMP-PKG signaling pathway	H01703	Eating Disorders
			hsa04071	Sphingolipid signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
Galanin receptor type 1	P47211	GALR1	hsa04080	Neuroactive ligand-receptor interaction		
Galanin receptor type 2	O43603	GALR2	hsa04080	Neuroactive ligand-receptor interaction		
Galanin receptor type 3	O60755	GALR3	hsa04080	Neuroactive ligand-receptor interaction		
Gamma-aminobutyric acid type B receptor subunit 1/2	Q9UBS5	GABBR1				
Gastric inhibitory polypeptide receptor	P48546	GIPR	hsa04024	cAMP signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
Gastrin/cholecystokinin type B receptor	P32239	CCKBR	hsa04020	Calcium signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
			hsa04971	Gastric acid secretion		
Glucagon receptor	P47871	GCGR				
Glucagon-like peptide 1 receptor	P43220	GLP1R				
Glucagon-like peptide 2 receptor	O95838	GLP2R	hsa04080	Neuroactive ligand-receptor interaction		
Growth hormone secretagogue receptor type 1	Q92847	GHSR	hsa04024	cAMP signaling pathway	H00254	Growth hormone deficiency
			hsa04080	Neuroactive ligand-receptor interaction		
			hsa04935	Growth hormone synthesis, secretion and action		
HLA class I histocompatibility antigen, A alpha chain	P04439	HLA-A	hsa04144	Endocytosis	H00344	Leprosy
			hsa04145	Phagosome	H01694	Stevens-Johnson syndrome
			hsa04218	Cellular senescence	H01713	Diffuse panbronchiolitis
			hsa04514	Cell adhesion molecules		
			hsa04612	Antigen processing and presentation		
			hsa04650	Natural killer cell mediated cytotoxicity		
			hsa04940	Type I diabetes mellitus		
			hsa05163	Human cytomegalovirus infection		

			hsa05165	Human papillomavirus infection		
			hsa05166	Human T-cell leukemia virus 1 infection		
			hsa05167	Kaposi sarcoma-associated herpesvirus infection		
			hsa05168	Herpes simplex virus 1 infection		
			hsa05169	Epstein-Barr virus infection		
			hsa05170	Human immunodeficiency virus 1 infection		
			hsa05203	Viral carcinogenesis		
			hsa05320	Autoimmune thyroid disease		
			hsa05330	Allograft rejection		
			hsa05332	Graft-versus-host disease		
			hsa05416	Viral myocarditis		
IgG receptor FcRn large subunit p51	P55899	FCGRT				
Insulin receptor	P06213	INSR	hsa04010	MAPK signaling pathway	H00719	Lepre-chaunism
			hsa04014	Ras signaling pathway	H00942	Rabson-Mendenhall syndrome
			hsa04015	Rap1 signaling pathway	H01228	Insulin-resis-tant diabetes mellitus with acanthosis nigricans
			hsa04022	cGMP-PKG signaling pathway	H01267	Familial hy-perinsulinemic hypoglycemia
			hsa04066	HIF-1 signaling pathway		
			hsa04068	FoxO signaling pathway		
			hsa04072	Phospholipase D signaling pathway		
			hsa04150	mTOR signaling pathway		
			hsa04151	PI3K-Akt signaling pathway		
			hsa04152	AMPK signaling pathway		
			hsa04211	Longevity regulating pathway		
			hsa04213	Longevity regulating pathway - mul-tiple species		
			hsa04520	Adherens junction		
			hsa04910	Insulin signaling pathway		
			hsa04913	Ovarian steroidogenesis		
			hsa04923	Regulation of lipolysis in adipocytes		
			hsa04930	Type II diabetes mellitus		

			hsa04931	Insulin resistance		
			hsa04932	Non-alcoholic fatty liver disease		
			hsa04960	Aldosterone-regulated sodium reabsorption		
			hsa05010	Alzheimer disease		
			hsa05415	Diabetic cardiomyopathy		
Isoform 1 of Calcitonin receptor (1)	P30988	CALCR	hsa04080	Neuroactive ligand-receptor interaction	H01593	Osteoporosis
			hsa04380	Osteoclast differentiation		
Isoform 4 of Membrane-associated guanylate kinase, WW and PDZ domain-containing protein 3 (4)	Q5TCQ9	MAGI3	hsa04015 Rap1 signaling pathway			
Kappa-type opioid receptor	P41145	OPRK1	hsa04080 Neuroactive ligand-receptor interaction			
Leucyl-cystinyl aminopeptidase	Q9UIQ6	LNPEP	hsa04614 Renin-angiotensin system			
Melanocortin receptor 3	P41968	MC3R				
Melanocortin receptor 4	P32245	MC4R	hsa04080	Neuroactive ligand-receptor interaction	H02106	Genetic obesity
Melanocortin receptor 5	P33032	MC5R				
Melanocyte-stimulating hormone receptor	Q01726	MC1R	hsa04080	Neuroactive ligand-receptor interaction	H00038	Melanoma
			hsa04916	Melanogenesis	H00168	Oculocutaneous albinism
Mu-type opioid receptor	P35372	OPRM1	hsa04080	Neuroactive ligand-receptor interaction	H01611	Alcohol dependence
			hsa04915	Estrogen signaling pathway		
			hsa05032	Morphine addiction		
Neuronal acetylcholine receptor subunit alpha-10/alpha-9	Q9GZZ6	CHRNA10				
Neuropeptide Y receptor type 1	P25929	NPY1R	hsa04024	cAMP signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
			hsa04923	Regulation of lipolysis in adipocytes		
Neuropeptide Y receptor type 2	P49146	NPY2R	hsa04080	Neuroactive ligand-receptor interaction		
Neuropeptide Y receptor type 4	P50391	NPY4R				
Neuropeptide Y receptor type 5	Q15761	NPY5R	hsa04080	Neuroactive ligand-receptor interaction		
Orexin receptor type 2	O43614	HCRTR2	hsa04080	Neuroactive ligand-receptor interaction		

Orexin/Hypocretin receptor type 1	O43613	HCRTR1	hsa04080	Neuroactive ligand-receptor interaction		
Pancreatic polypeptide receptor 1	O97505	NPY Y4				
Parathyroid hormone/parathyroid hormone-related peptide receptor	Q03431	PTH1R	hsa04080	Neuroactive ligand-receptor interaction	H00479	Metaphyseal dysplasias
			hsa04928	Parathyroid hormone synthesis, secretion and action	H00495	Eiken dysplasia
			hsa04961	Endocrine and other factor-regulated calcium reabsorption	H00508	Blomstrand syndrome
Peroxisome proliferator-activated receptor alpha	Q07869				H00680	Primary failure of tooth eruption
Peroxisome proliferator-activated receptor gamma	P37231	PPARG	hsa03320	PPAR signaling pathway		
			hsa04152	AMPK signaling pathway		
			hsa04211	Longevity regulating pathway		
			hsa04380	Osteoclast differentiation		
			hsa04714	Thermogenesis		
			hsa04932	Non-alcoholic fatty liver disease		
			hsa05016	Huntington disease		
			hsa05200	Pathways in cancer		
			hsa05202	Transcriptional misregulation in cancer		
			hsa05216	Thyroid cancer		
			hsa05417	Lipid and atherosclerosis		
Potassium voltage-gated channel subfamily A member 1	Q09470	KCNA1			H00749 Episodic ataxias	
Potassium voltage-gated channel subfamily A member 3	P22001	KCNA3				
Pro-neuropeptide Y	P07808	Npy				
Programmed cell death 1 ligand 1	Q9NZQ7	CD274				
Prolyl endopeptidase	P48147	PREP				
Protein ADM2	Q7Z4H4	ADM2	hsa04080	Neuroactive ligand-receptor interaction		
			hsa04270	Vascular smooth muscle contraction		
Prothrombin	P00734					
Proto-oncogene Mas	P04201	MAS1				

Renin	P00797	REN	hsa04614	Renin-angiotensin system	H00541	Autosomal dominant tubulointerstitial kidney disease
			hsa04924	Renin secretion	H00575	Renal tubular dysgenesis
			hsa05415	Diabetic cardiomyopathy	H02011	Familial juvenile hyperuricemic nephropathy
Serine/threonine-protein kinase B-raf	P15056	BRAF	hsa01521	EGFR tyrosine kinase inhibitor resistance		
			hsa01522	Endocrine resistance		
			hsa04010	MAPK signaling pathway		
			hsa04012	ErbB signaling pathway		
			hsa04015	Rap1 signaling pathway		
			hsa04024	cAMP signaling pathway		
			hsa04062	Chemokine signaling pathway		
			hsa04068	FoxO signaling pathway		
			hsa04150	mTOR signaling pathway		
			hsa04270	Vascular smooth muscle contraction		
			hsa04510	Focal adhesion		
			hsa04650	Natural killer cell mediated cytotoxicity		
			hsa04720	Long-term potentiation		
			hsa04722	Neurotrophin signaling pathway		
			hsa04726	Serotonergic synapse		
			hsa04730	Long-term depression		
			hsa04810	Regulation of actin cytoskeleton		
			hsa04910	Insulin signaling pathway		
			hsa04914	Progesterone-mediated oocyte maturation		
			hsa04928	Parathyroid hormone synthesis, secretion and action		
			hsa04934	Cushing syndrome		
			hsa05010	Alzheimer disease		
			hsa05022	Pathways of neurodegeneration - multiple diseases		
			hsa05034	Alcoholism		
			hsa05160	Hepatitis C		
			hsa05161	Hepatitis B		
			hsa05200	Pathways in cancer		

			hsa05205	Proteoglycans in cancer		
			hsa05208	Chemical carcinogenesis - reactive oxygen species		
			hsa05210	Colorectal cancer		
			hsa05211	Renal cell carcinoma		
			hsa05212	Pancreatic cancer		
			hsa05213	Endometrial cancer		
			hsa05214	Glioma		
			hsa05215	Prostate cancer		
			hsa05216	Thyroid cancer		
			hsa05218	Melanoma		
			hsa05219	Bladder cancer		
			hsa05220	Chronic myeloid leukemia		
			hsa05221	Acute myeloid leukemia		
			hsa05223	Non-small cell lung cancer		
			hsa05224	Breast cancer		
			hsa05225	Hepatocellular carcinoma		
			hsa05226	Gastric cancer		
Serine/threonine-protein kinase PLK1	P53350		hsa04068	FoxO signaling pathway		
			hsa04110	Cell cycle		
			hsa04114	Oocyte meiosis		
			hsa04914	Progesterone-mediated oocyte maturation		
Sodium channel protein type 8 subunit alpha	Q9UQD0				H00606	Early infantile epileptic encephalopathy
					H02362	Benign familial infantile seizure
Sodium channel protein type 9 subunit alpha	Q15858	SCN9A	hsa04742 Taste transduction		H00265	Hereditary sensory and autonomic neuropathy
					H00771	Inherited erythromelalgia

Supplementary Table 1: Bioactive targets, associated genes, KEGG pathways and KEGG diseases of A1 and A2 beta casein proteins.

Bioactive targets	Uniprot ID	Gene	KEGG Pathways ID	KEGG Pathways	KEGG Disease ID	KEGG Disease
3-hydroxy-3-methylglutaryl-coenzyme A reductase	P04035	HMGCR				
ATP-dependent translocase ABCB1	P08183	ABCB1				
Delta-type opioid receptor	P41143	OPRD1	hsa04022	cGMP-PKG signaling pathway	H01703	Eating Disorders
			hsa04071	Sphingolipid signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
E3 ubiquitin-protein ligase XIAP	P98170	XIAP				
HLA class I histocompatibility antigen, A alpha chain	P04439	HLA-A	hsa04144	Endocytosis	H00344	Leprosy
			hsa04145	Phagosome	H01694	Stevens-Johnson syndrome
			hsa04218	Cellular senescence	H01713	Diffuse panbronchi-olitis
			hsa04514	Cell adhesion molecules		
			hsa04612	Antigen processing and pre-sentation		
			hsa04650	Natural killer cell mediated cytotoxicity		
			hsa04940	Type I diabetes mellitus		
			hsa05163	Human cytomegalovirus infec-tion		
			hsa05165	Human papillomavirus infec-tion		
			hsa05166	Human T-cell leukemia virus 1 infection		
			hsa05167	Kaposi sarcoma-associated herpesvirus infection		
			hsa05168	Herpes simplex virus 1 infec-tion		
			hsa05169	Epstein-Barr virus infection		

			hsa05170	Human immunodeficiency virus 1 infection		
			hsa05203	Viral carcinogenesis		
			hsa05320	Autoimmune thyroid disease		
			hsa05330	Allograft rejection		
			hsa05332	Graft-versus-host disease		
			hsa05416	Viral myocarditis		
Kappa-type opioid receptor	P41145	OPRK1	hsa04080	Neuroactive ligand-receptor interaction		
Mas-related G-protein coupled receptor member X2	Q96LB1	MRG-PRX2				
Mu-type opioid receptor	P35372	OPRM1	hsa04080	Neuroactive ligand-receptor interaction	H01611	Alcohol dependence
			hsa04915	Estrogen signaling pathway		
			hsa05032	Morphine addiction		
Neurotensin receptor type 1	P30989	NTSR1	hsa04020	Calcium signaling pathway		
			hsa04080	Neuroactive ligand-receptor interaction		
Neurotensin receptor type 1/2	O95665	NTSR2	hsa04080 Neuro-active ligand-receptor interaction			
Proenkephalin-B	P01213	PDYN	hsa04080	Neuroactive ligand-receptor interaction	H00063	Spinocerebellar ataxia (SCA)
			hsa05017	Spinocerebellar ataxia		
			hsa05022	Pathways of neurodegeneration - multiple diseases		
			hsa05030	Cocaine addiction		
			hsa05031	Amphetamine addiction		
			hsa05034	Alcoholism		
Proteasome subunit beta type-5	P20618	PSMB1	hsa03050	Proteasome		
			hsa05010	Alzheimer disease		
			hsa05012	Parkinson disease		
			hsa05014	Amyotrophic lateral sclerosis		

			hsa05016	Huntington disease		
			hsa05017	Spinocerebellar ataxia		
			hsa05020	Prion disease		
			hsa05022	Pathways of neurodegeneration - multiple diseases		
Sortilin	Q99523	SORT1	hsa04142	Lysosome		
			hsa04722	Neurotrophin signaling pathway		
			hsa04979	Cholesterol metabolism		
Telomeric repeat-binding factor 2	Q15554	TERF2				
Tyrosine-protein kinase Lck	P06239	LCK				

Supplementary Table 2: Bioactive targets, associated genes, KEGG pathways and KEGG diseases of BCM-7