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Health Implications of A1 and A2 Cow Milk Proteins

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Abstract

Milk is an excellent source of high quality proteins, carbohydrates, calcium and other micronutrients. Milk proteins are composed of mainly casein and whey. The casein gene has mutated over time and has resulted into 12 genetic variants. Out of these A1 is the most common one while A2 is the original one. The mutation resulted in the replacement of Proline with histidine at position 67th of the β-casein of A1 variant. A1 β-casein on enzymatic digestion produces beta casomorphine 7. BCM-7 is a seven amino acid long peptide it interacts with the human gastrointestinal tract, internal organs, and brainstem and is associated with the occurrence of type-1 Diabetes, mental disorders and heart diseases. Many auto-immune diseases and sudden infant death syndrome were also known to be potentiated by A1 milk. In a hypothesis, it was suggested that the long time consumption of A1 milk is associated with increased mucous production and is associated with further worsening the respiratory tract diseases particularly asthma. While A1 milk is known to cause heart diseases, A2 milk is known to be athero-protective in nature; its consumption is not associated with any such diseases, because A2 milk produce BCM-9 which have no such ill effects. Indian breeds produced only A2 milk but with the continuous use of the European breeds in selective breeding to increase milk production, gradually A1 allele have propagated through the breeding programme. Now the situation is that most of the cattle population of our country is composed of the crossbred animals which have high milk production but A1 allele in their genome. So It is right time to draw the attention of policy makers, farmers to stop the indiscriminate cross-breeding of our local breeds of cattle with the exotic breeds just to increase the milk production; rather preserve and maintain our own Indian breeds for milk production and use the selection criteria for further enhancement of their production. Initially cross breeding with exotic cattle may appear as a profitable entrepreneurship to do in the present scenario but it will have many serious implications in the future not only from health point of view but also in terms of overall profits in long term of 10-20 years. The germplasm of our indigenous cattle is unique, apart from being a source of A2 milk, which in itself is a great blessing, our cattle breeds have excellent adaptation potential and disease resistance. Besides this, the urine and dung can be utilized for the development of medicinal, cosmetic, organic farming and various other useful products. Hence, strategies should be made in such a way so as to conserve and improve our indigenous breeds. Many western countries are either not aware of these facts or knowingly ignoring the above beneficial effects of Indian zebu cattle and moreover, they were not offered the choice of A1 and A2 milk but fortunately we have it as a matter of chance and blessings of the nature and now it is up to us to decide whether we should use A2 milk having beneficial health promoting potential or regressively destroy our future generations through A1 milk of exotic cows. Also it is pertinent to mention that the conclusions drawn on the basis of the harmful effects of A1cow milk in western countries cannot be applied as such on the people of Indian sub-continent because the A2 milk of Indian Zebu cattle is considered as nectar (Amrit) which is beneficial to heath and makes the body immunologically and mentally strong enough and refractory to most of the infectious diseases, new infections and cancers.

Keywords: Genetic mutations, A1 A2 milk, BCM-7, Opioids, Autism, Autoimmune diseases, Schizophrenia, Leaky Gut disease, Indian Zebu Cattle.

Introduction

Milk from dairy cows has been regarded as nature's perfect food, providing an important source of nutrients including high quality proteins, carbohydrates and selected micronutrients. More than 95% of the cow milk proteins are constituted by caseins and whey proteins. Among the caseins, beta casein is the second most abundant protein and has excellent nutritional balance of amino acids. β-casein protein is coded by the 6th chromosome of bovine genome. Histidine is coded by CAT (cytosine-adenosine-thymine) bases whereas proline is coded by CCT (cytosine-cytosine-thymine); here due to mutation adenosine is replaced by cytosine. This difference of single nucleotide in β-casein gene can be determined by molecular biology methods and individual animals can be identified as whether they carry gene for β-casein A1 or β-casein A2 or both. Different mutations in bovine beta casein gene have led to 12 genetic variants and out of these A1 and A2 are the most common.A2 beta-casein is the beta-casein from cows that have been produced since before they were first domesticated over 10,000 years ago. It has no known negative effects on human health. In the past few thousand years, a natural mutation occurred in Anatolia, Turkey (Ng-Kwai-Hang, 2002). This has resulted in a proportion of cows of European breeds producing a casein variant calledA1 beta-casein. Slowly, these protein variant became dominant in milk which producing A1 milk. The gene encoding beta-casein was changed such that the 67th amino acid in the 209 amino proteins was switched from proline to histidine. This new kind of beta-casein that was created is known as A1 beta-casein which is found in the milk of many crossbred cows such as Holstein, jersey and Friesian. The proline binds very closely to the amino acid next to it in position 66, which is isoleucine, whereas the histidine linkage with isoleucine is easily broken by digestive enzymes. With A2 beta-casein the proline also binds very tightly with the amino acid in position 68. The outcome of all this is that digestion of Al beta-casein can produce a peptide of a string of seven amino acids called beta casomorphin- 7 (BCM7) whereas the evidence is that this does not occur (or at least not to any significant degree) with A2 beta-casein. BCM-7 has been well established as a potent bio-active peptide with opioid activity. It is believed that generation of BCM-7 is the major causative factor associated with A1 milk related health disorders. However, A2 β-casein has not been linked to any of such health issues (Kaminski *et al*., 2007). Milk from dairy cows is providing a high quality source of protein and essential micronutrients like energy, calcium, magnesium and phosphorus to human beings since long time (Bell *et al*., 2006). A significant relationship was observed between bovine milk protein consumption and the incidence of type 1 diabetes and cardiovascular diseases (McLachlan, 2001; Laugesen and Elliott, 2003; Elliott *et al*., 1999; Thorsdottir *et al*., 2000, Virtanen *et al*., 2000; Birgisdottir *et al*., 2002), arteriosclerosis (Tailford *et al*., 2003). Besides, neurological disorders such as schizophrenia and autism (Woodford, 2006), and sudden infant death syndrome were also appeared to be known to be potentiated by milk (Sun *et al*., 1999; Sun and Cade, 1999; Sun *et al*., 2003). Besides, some populations such as the Masai (East African) and Samburu (Northern Kenyan) had virtually no heart disease despite consuming a diet rich in animal milk. But that milk fortunately came from Zebu cattle, which is a breed that carries the A2 allele exclusively (McLachlan, 2001). Western countries, which had similarly high bovine milk consumption from predominantly the Holstein breed, jersey and other breeds had a greater incidence of CVD than nations with low milk consumption. It is so because people of small nations consume fortunately A2 milk. But epidemiological analyses concerning the two alleles of β- casein and the incidence of CVD underscores the apparent relationship between the risk of chronic disease and milk protein variant intake (McLachlan, 2001; Laugesen and Elliott, 2003 Woodford, 2011, Mishra *et al*., 2005). The Food and Agriculture Organisation (FAO) (2012) has reported increase in many chronic diseases arising out of milk Genetic variants in bovine β-casein gene (A1 and B) release a bioactive peptide, β-casomorphin-7 (BCM-7) upon digestion, responsible for many human disorders like Type 1 diabetes, autism, schizophrenia and heart diseases but A2 milk does not cause such type of illnesses (Keith Woodford, 2007; Mishra *et al*., 2009; Sodhi *et al*., 2012). A broad range of studies from American and European investigations has shown reduction in autistic and schizophrenic symptoms with decrease in A1 milk intake.

Basic genetics behind A1 and A2 milk

The A1/A2 status of a cow is determined by a pair of genes present on the sixth chromosome (Rijnkels, 2002). There are two major alleles of the gene that is A1 and A2 beta-casein alleles. A cow carries two copies of the beta-casein gene; she can carry either of A2A2 (homozygous), A1A2(heterozygous) or A1A1 (homozygous) alleles. These alleles are co-dominant i.e., neither is dominant nor recessive and when they are present together they have additive effect. Therefore, a cow with A1A2allele will produce A1 and A2 beta-casein in equal amounts. An A2A2 cow will only produce A2 beta-casein and an A1A1 cow will only produce A1 beta-casein. The Northern European breeds of cows such as the Friesian and Holstein carry the A1 and A2 allele at about equal levels. The Southern European breeds and the Jersey carry the A1 allele at about 35% and 2/3 of A2. Exceptionally, Guernsey breed appears to carry the A1 allele at less than10% and the Scottish Ayrshire breed appears to be well over 50%. In addition, individual herds may carry the allele at levels that are quite different to the average for the breed. If a cow is A2A2 then she is guaranteed to pass on the A2 allele to her progeny. Similarly, an A1 cow is guaranteed to pass on the A1 allele. For an A1A2 cow there is a 50% chance of passing on either of the allele.

Status of β casein variant in different breeds of Cattle

Researches conducted on indigenous cows (Zebu type),buffaloes and exotic cows (*Taurine* type) have revealed that A1 allele is more frequent in exotic cattle (A1 milk) while Indian native dairy cows and buffaloes have only A2 allele and hence are a source for safe milk i.e. A2 milk (Mishra *et al*., 2009). The A2 allele gene in Indian milk breeds of cows and buffaloes are 100% (Red Sindhi, Sahiwal, Tharparkar, Gir and Rathi), other Indian breeds used for farming, is around 94 per cent (Joshi, 2011) and while in foreign breeds (HF and Jersey), it is around 60 per cent (NBAGR, 2011). A1 β-casein is absent in the milk of pure Asian and African Cattle (Ng-Kwai-Hang and Grosclaude, 2002). So, our indigenous cows and buffaloes produce A2 milk. A study was conducted to sequence characterize β casein gene and identify allelic distribution of A1A2 alleles in native cattle of Ladakh region adapted to high altitude and low oxygen condition. The data showed 2 non-synonymous variations in coding region, while 5'UTR was completely conserved. The 3'UTR showed 2 more variations in Ladakhi samples. Further, the genotyping in 85 Ladakhi cattle for A1A2 alleles revealed that in Ladakhi cattle, A2 allele is predominantly present as reported for some of the other Indian breeds. The frequency of A2 allele was 0.90 and frequency of A2A2 genotype was found to be 0.79 in cattle. The present data strongly indicated that the local cattle breeds of Ladakh have A2A2 genotype and are a source for A2 milk. Systematic efforts should be made for long term conservation and genetic improvement of this invaluable genetic resource of Ladakh (Sharma *et al*., 2018).

Milk protein

Bovine milk proteins are composed approximately of 80% casein and 20% whey (Shah, 2000; Niki *et al*., 1994). But according to some researchers whey proteins constitute about 14% (McLachlan, 2001; Roginski, 2003). It contains four components namely αs1 (CSN1S1, 39–46%), αs2 (CSN1S2, 8–11%), β (CSN2, 25–35%), and κ (CSN3, 8–15%) of total caseins (Eigel *et al*., 1984; Roginski, 2003, Rijnkels, 2002*)* whereas human milk casein is composed of primarily β, and κ *1*. β-caseinis the second most abundant protein and crucial for casein micelle structure. Beta-casein is 30% of the total protein content in cow's milk. The polymorphic status of bovine β-casein is confirmed, and till date 13 allelic variants havebeen identified (Kaminski *et al*., 2007). Amongst these, A1 and A2 variants are reported to be the most common allelic variants of β-casein in dairy cattle (Farrell *et al*., 2004). The polymorphic nature and its association withmilk, fat and protein yield attracted several efforts in evaluating this locus as a potential dairy trait marker (Ikonen *et al*., 1999; Caroli *et al*., 2004; Kucerova *et al*., 2006). Consumption of milk of certain breeds of cow, buffaloes results in the release and possible absorption of bioactive peptides like BCMs. These peptides yielded by the digestion of β-casein have opioid effects similar to morphine, and so named β-casomorphins (β-CMs). The BCMs have unique structural features which is responsible for its affinity with the binding sites of endogenous opioid receptors (Meisel and FitzGerald, 2000).

Digestion of A1 milk

Of the protein variants A1 beta casein yields BCM-7 whereas A2 beta casein does not give rise to BCM-7 upon digestion (Woodford, 2006; Bell *et al*., 2006), but produce BCM-9 (Roginski, 2003; Kostya *et al*., 2004). The full structure of bovine BCM7 is tyrosine-proline-phenylalanine-proline-glycine- proline-isoleucine. In the shorthand of chemistry this is usually written as Tyr-Pro-Phe-Pro-Gly-Pro-Ile. Variants B, C and F all have histidine at position 67 and therefore can be expected to break down just like Al. In contrast, variants A3, D and E all have proline at position 67 and therefore behave the same as A2 in relation to BCM7 release. In one of the *in vitro* study**,** A1 β-casein was digested with combination of enzymes such as pepsin, elastase, leucine aminopeptidase (LAP) and pancreatin (Jinsmaa *et al*., 1999). Along with BCM-7, other degradation products such as BCM-9, BCM-13 and BCM-21 were also produced. BCM-7 is further breakdown to BCM-5 and BCM-3 by dipeptidyl peptidase IV (DPP IV) enzyme present on surface of enterocytes and in blood (Cade *et al.*, 2000). Even if it survives there due to high permeability of intestine specifically in neonates or improper function of DPP (IV) enzymes (autistic patient), blood brain barrier (BBB) is next stopping point for BCM-7. BBB has also tight junction like structure which stops the flow of large peptides. Also, presence of peptidases (in BBB) further inhibits its progression to brain (Egleton *et al*., 1997; Egleton *et al*., 2005). Peptides are generally transported across the intestine by two pathways, transcellular or paracellular. As most of opioid peptides are hydrophobic in nature it is difficult for them to transport by diffusion or paracellular pathway. The PEPT1, H+-coupled transporters, which are found in intestinal epithelium can only transport di to tri-peptides (Ganapathy and Miyauchi, 2005). In 2003 a new transporter was identified in epithelial cell of retinal pigment (RPE) and was called as ARPE-19 (Na+-dependent active transport). This transport system can transport opioid peptides of length of 4 to 13 amino acids (Miyauchi *et al*., 2003). However, its presence in intestine is still unknown. *In vitro* and *in vivo* both studies have demonstrated that BCM-7 can pass through intestinal barrier. Caco-2 cell line is popularly used in *in vitro* study for peptide transportation. This cell line was developed from human colon adenocarcinoma cells (Fogh *et al*., 1977). Caco-2 cells differentiate to enterocytes very quickly. These cells are joined by tight junction, mimicking true intestinal system (Pinto *et al*., 1983). Iwan *et al*., (2008) showed BCM-7 can pass through Caco-2 cell line. In *in vivo* studies, dog pups were used as a model and was found that by feeding bovine casein formula, BCM-7 immunoreactive material (BCMIR) could pass through intestinal barrier in newborns but failed to pass in adults. The length of BCMIR was much higher than BCM-7, consists of 13 amino acids residue, This clearly shows that long peptides can pass through intestine in newborns (Singh *et al*., 1989). BCM-7 production is pH dependent and pH 4.0 is optimum for its production from bovine A1 milk. B allele (A1 like milk, very low frequency of this allele is found in animals) produced maximum amount of BCM-7 at pH 2. However, at pH 4, BCM-7 amount in B type (A1 like milk) was still higher as compare to A1 milk when milk was digested in *in vitro* condition (Noni, 2008). In neonates for few months of their life stomach pH is found in the range of 2 to 5 as compared to 2 in adults (Hamosh, 1996; Berseth *et al*., 2006). Further milk stays longer period of time in neonates, creating more favorable condition for BCM-7 production. The processing of milk does not affect *in vitro* production of BCM-7, but the amount of BCM-7 was found high in case of cheese (Gouda, Cheddar), 15 to 21 mg/kg as compared to UHT or pasteurized treated milk, infant formulas, unprocessed milk and fermented milk which was in the range of 0.04 to 1.16 mg/l during *in vitro* digestion study.

Differences between human and bovine milk

Accordingly, the important differences between human and bovine milk are not related to the overall solids content (which is similar for both) but to their constituents. Human milk is higher in lactose, similar in fat, but much lower in protein than bovine milk. It is also considerably lower in minerals such as calcium, sodium and potassium. In bovine milk about 80% of the proteins are casein proteins whereas in humans the major proteins are whey proteins. Although beta-casein is the most important of the human casein proteins it is different to the beta-casein produced by cows. The human beta-casein is a shorter protein chain and so the analogous positions in relation to the bovine BCM7 are from 51 to 57 instead of 60 to 66. However, all human beta-casein is of the A2 type rather than the Al type (59th proline), which act as major barrier for the production of BCM-7 in humans.

Absorption of BCM-7 and its effects

The next important question is what happens to BCM7 when it is released into the gut. Once again there is no simple answer. In healthy adults it should be difficult for BCM7 to get through the gut wall and into the bloodstream, because the molecule is too large. But it appears there are plenty of exceptions. Almost certainly, it depends on the age, health and genetic makeup of the particular person. Some people suffer from leaky gut syndrome, whereby BCM7 and other peptides pass very easily into the bloodstream. There is also very strong circumstantial evidence that people with stomach ulcers or untreated coeliac disease absorb BCM7 through the gut wall. Babies can absorb BCM-7 in the same way because neonates naturally have increased intestinal permeability for improved nutrient absorption, otherwise they won't be able to absorb colostrum in their mother's milk, and they're at high risk. One of Professor Cade's co-workers, Dr Zhongjie Sun

(1999), has experimentally injected BCM7 into rats and have published evidence that once in the bloodstream, BCM7 can readily pass through the blood brain barrier into the brain where it can bind to opioid receptors. They have also shown that the rats then exhibit behavioural tendencies very similar to those of autism and schizophrenia. They found that the effects could be reversed with administration of naloxone, a well-recognised morphine antagonist. Other scientists have found that BCM7 causes apnoea (breathing dysfunction) in adult rats and newborn rabbits that is analogous to sudden infant death syndrome in humans. The association between these effects and BCM7 was further supported by the ability to reverse the behavioral changes with the opioid antagonist naloxone (Sun *et al*., 1999).

The effects of BCM7 are not restricted to behavioural symptoms. The fact that opioids affect a wide range of immune functions has been known for over a hundred years. This immune effect provides a possible explanation as to why BCM7 appears to be implicated in such a wide range of auto-immune diseases. However, not all of the effects of BCM7 are necessarily due to its opioid characteristics. The tyrosine molecule on the end of the BCM7, combined with the stability of BCM7, gives the milk devil strong oxidant properties. Indeed BCM7 has been shown *in vitro (i.e.* in a test tube) to be a strong oxidant of low-density lipoprotein (LDL, the 'bad' type of cholesterol). Oxidation of LDL is fundamental to the process whereby fatty plaques are laid down in artery walls, leading in turn to heart disease. It seems likely that the effect of BCM7 on heart disease may be two fold, with an opioid-related mechanism (perhaps linked to immune function) and the oxidant properties working like a double-edged sword (Chauhan and Singh, 2013).

A1 milk and heart Diseases

There are three parts to the evidence that Al beta-casein is linked to heart disease. The first is evidence that countries where people have high intakes of Al beta-casein, also have a high incidence of heart disease; this is called epidemiological evidence. The second part is trials involving animals and humans, in particular a trial in which rabbits fed Al beta-casein developed arterial plaque, whereas rabbits fed A2 beta casein did not. The third part is pharmacological evidence showing how the BCM7 that derives from Al beta-casein is linked to oxidation of low-density lipoprotein (LDL), which in turn causes arterial plaque. The first indication that Al beta-casein was related to heart disease came about quite by chance. In 1994 Dr Corran McLachlan was asked by the New Zealand Child Health Research Foundation to review Professor Bob Elliott's work programme. The results were very striking, the correlation between coronary heart disease and Al beta-casein consumption was exceptionally high that is 0.86 in one observation and 0.84 in another. McLachlan also compared the incidence of heart disease in the various states of West Germany. He found that 66% of the variation in deaths from heart disease could be explained by differences in the level of Al beta-casein intake, based on the different breeds of cattle found in each state.

Epidemiological Evidence-MONICA study (A major survey undertaken by the World Health Authority on cardiovascular death rates and the associated risk factors)

The death rates from heart disease in Belfast were three to four times those in Toulouse, despite all the classical risk factors being (McLachlan, 2001) 'virtually identical'. About the only difference of note is that the citizens of Toulouse tend to drink more wine than the citizens of Belfast, who imbibe similar amounts of alcohol, but in drinks other than wine. McLachlan presented data showing that the intake of Al beta-casein was 2.49 times higher in Belfast than in Toulouse. Iceland and Finland provide some more interesting evidence. Ethnically, these Scandinavian peoples are very similar and they have similar diets. However, Finland has one of the highest levels of heart disease in the world, whereas in Iceland the incidence is only about 60% that of Finland. This difference in Al beta-casein intake is because the Norske cows in Iceland have a higher level of A2 beta-casein and a lower level of Al beta-casein in their milk than the Finnish cows. Also Corran McLachlan put forward a suggestion in his *Medical Hypotheses* paper that both the historical increase and subsequent decrease in levels of heart disease worldwide might be linked to the method of pasteurization. He drew together evidence from a range of sources to show that, as pasteurization of milk was introduced in various countries and regions within countries, within a few years there was a marked increase in the level of heart disease. Corran McLachlan (2001) hypothesized that the heat treatment regime used in the Holder method was leading to protein breakdown and providing an increased level of BCM7 from Al beta-casein. He drew together evidence from a range of sources to show that, as pasteurization of milk was introduced in various countries and regions within countries, within a few years there was a marked increase in the level of heart disease. Prior to 1950 the major method of pasteurization was the Holder method (the milk was heated to 63°C for about 30minutes). Subsequently this method fell out of favour, largely because of the distinctive 'cooked' flavour it gave to the milk. In the 1960s there was a move to short-time, high-temperature methods, (about 90°C for 15 seconds) and by 1980s these had become predominant. This change was soon followed by a decline in heart disease levels that cannot be satisfactorily explained in terms of the classic risk factors for heart disease.

Trials -The rabbit story: Al beta-casein is atherogenic

Most important of the animal trials linking Al beta-casein to heart disease was undertaken at the Centre for Research in Vascular Biology at the School of Biomedical Sciences, University of Queensland. It was found that rabbits fed Al beta-casein developed fatty plaque lesions that were both larger and thicker than those of rabbits fed A2 beta-casein. It was concluded that their results demonstrate for the first time that beta-casein A2has a mildly athero-protective effect while beta-casein Al is most definitely atherogenic (Campbell, 2003). Some evidences were also found although only in groups that had no added dietary cholesterol, that the rabbits on A2 diets had lower serum cholesterol levels than those fed Al. The trial used 60 New Zealand white/Lop cross rabbits aged 16-24 weeks, split into 10 groups, each with a different diet. Four of the groups were given Al beta casein in amounts varying up to 20% of the diet, and four were given A2 beta casein. Two groups received whey protein that contained neither Al nor A2 beta casein. Some groups also received additional cholesterol, which was known to induce fatty plaques in the arteries of this breed of rabbits. Each rabbit had its right carotid artery 'balloon de-endothelialised' prior to the trial, to make the rabbits more prone to atherosclerosis. The damage may increase the chance of fatty plaques being laid down in a reasonably short time. As long as all animals in the trial are treated the same way this procedure introduces no bias when comparing one treatment with another. Prior to this trial it had already been widely reported in the scientific literature that casein was linked to atherosclerosis, and Campbell's team referred to previous research that found this with rabbits, monkeys and mice. However, none of these previous studies had looked at which particular component of casein, if any, wasthe problem. What they found was that rabbits fed Al beta-casein developed fatty plaque lesions that were both larger and thicker than those of rabbits fed A2 beta-casein.

Interestingly, and perhaps surprisingly, the biggest differences were in the undamaged aorta (the main artery that exits from the heart) rather than in the damaged carotid artery. The differences in relation to the aorta were statistically significant, some at the $p< 0.05$ level and others at the $p< 0.01$ level, which indicates that the probability of getting these differences by chance is less than 5% and 1%, respectively. The team said that the lesions that were formed 'are termed fatty streaks and closely resemble juvenile fatty streaks that are present in early childhood and are considered the precursors of advanced atherosclerotic plaques'. They concluded that their results 'demonstrate for the first time that beta-casein A2 has a mildly athero-protective effect while beta-casein Al is most definitely atherogenic.

Oxidation of LDL Cholesterol

According to the theory, if we consume lots of antioxidants, which tend to stop these reactions from occurring, and minimise our intake of oxidants, then our chances of getting atherosclerosis and having **a** heart attack are reduced. oxidised LDL is a major cause for Heart diseases. We know that BCM7, with its tyrosine amino acid at one end plus its high resistance to breakdown, will be a powerful oxidant. French scientists Jean Torreilles and Marie-Christian Guerin have shown that BCM7 does indeed oxidise LDL (Torreilles and Guerin, 1995). Also, Czech scientist A. Steinerova and colleagues have published several papers showing that infants exposed to cows' milk have up to ten times more antibodies to oxidised LDL than babies that are breastfed. Steinerova and colleagues have postulated that this is caused by Al beta-casein (Steinerova, 1999; Steinerova 2001). So this is an evolving story, with quite a lot known but still much more to be discovered. According to *Dorland's Medical Dictionary* (online) the Sippy diet is 'a diet formerly used to treat peptic ulcers, consisting of milk, cream and other supposedly bland foods; it was later proved ineffective.' Not only was this diet ineffective: it was subsequently found to cause high rate of death from heart attacks. R.D. Briggs and group (1960) undertook an autopsy study in 15 hospitals in the USA and Great Britain. In Great Britain they found that the death rate from myocardial infarction (heart attacks) amongst ulcer sufferers was nearly 2.5 times as high among those on the Sippy and other high-milk diets compared to those not on a milk diet. In the USA the heart attack death rate of ulcer sufferers on the Sippy diet was six times that of ulcer sufferers not on the Sippy diet (Briggs *et al*., 1960) In both countries the results were highly significant, at $p< 0.01$. When taken together, this means that the likelihood of getting two sets of results like this from a random effect or 'fluke' (i.e. a false association) is less than one in ten thousand. So we can be very confident this is a real effect involving causation linked to milk intake.

A1 Beta Casein and Type 1 Diabetes

The A2 story actually began with diabetes. In late 80's and early 90's the worldwide incidence of Type 1 diabetes kept increasing at about 3% a year. Its incidence varies by over 300-fold across 51 countries (Karvonen *et al*., 2000). About 65,000 children aged up to 14 are newly diagnosed each year and there are almost as many additional new cases each year among young adults. The vast majority are in the developed world. The Scientists also agreed that some human genotypes are more susceptible to Type 1 diabetes than are others. Elliot and Hill (1999) conducted an epidemiological study on the incidence of type I diabetes in children aged 0 to 14 in 10 different countries- Australia, Canada, Denmark, Finland, Germany, Iceland, New Zealand, Norway, Sweden and the USA. The data for the USA came from the city of San Diego. A key feature of these countries is that they are high-income western countries with a European-type lifestyle. This commonality is important because it reduces the likelihood of confounding factors. Elliott and his team concluded that 'Total milk protein consumption did not correlate with diabetes incidence [r2 is equal+0.16] but consumption of the beta-casein Al variant did [r2 = +0.53]. One of the interesting features of the data presented by Elliott and colleagues was that Iceland had the highest milk intake but quite moderate levels of Type 1 diabetes. This seemed to make sense because the Icelandic milk was indeed very low in Al beta-casein (because of the predominance of the Norske breed). However, it seemed important to see whether there was anything else in the Icelandic milk that might be causing the low level of Type 1 diabetes relative to the nearby Nordic countries of Finland, Sweden, Norway and Denmark. McLachlan also pointed out that people such as the Masai and Samburu people of Kenya are 'essentially free' of heart disease despite having very high milk consumption. In both cases the milk they drink is 100% A2. The highest level of heart disease at the time of this analysis was in Finland, where there is a very high intake of Al beta-casein. Birgisdottir and group in 2006 showed that lower consumption of cow milk protein (Al beta-casein) at 2 years of age, rather than among 11-14 year old adolescents, explains the lower incidence of type 1 diabetes in Iceland than in Scandinavia (Birgisdottir *et al*., 2006). (Birgisdottir and Thorsdottir (2002) and Hill and Harris looked at the levels of bovine serum albumin (BSA), immunoglobulin and lactoferrin. BSA was already suspected of causing diabetes and the some others had been being suspecting it to be potentially protective against diabetes. However, the researchers were unable to find any statistical relationship. In fact, for BSA they found that Icelanders had a higher intake than people in the other Nordic countries, so clearly this could not be the predominant cause of diabetes. The diabetes data came from the World Health Organisation Diabetes Monitoring (WHO DiaMond) Project (Karvonen *et al*., 2000), except for the data for Switzerland and Iceland, which were surveyed by the EuroDiab Ace study group. The correlations were remarkable. Considering Al beta-casein from all dairy products except cheese, and excluding the minor variant B which also has histidine at position 67, the relationship had an r2 value of 0.84. In other words, 84% of the variation in diabetes incidence can be explained by variation in Al beta-casein intake. This epidemiological evidence is further supported by research done on animals. In 1997, Bob Elliot and Jeremy Hill conducted a study on NOD (non-obese diabetic) mice that were susceptible to diabetes and found that 47% of the mice fed A1 beta casein developed diabetes compared to none of the mice fed A2 beta casein. Throughout the trial, some of the mice fed Al beta-casein were also given the opioid-reversal drug naloxone in their drinking water. These mice also did not get diabetes. Another similar study, named the Food and Diabetes (FAD) trial, was conducted in 2002, but the conclusion of this study opposes the idea that A1 beta casein poses any health risks. However, this conclusion appears to be very much based on the industry bias of the authors and some technical flaws in the research such as some of the A2 beta casein feed being contaminated with A1. Despite this, certain aspects of the research still showed a statistically significant correlation between A1 beta casein and Type 1 Diabetes. Type 1 Diabetes is often classified as an autoimmune disease and can be caused by the immune system attacking the insulin producing cells of the pancreas. In 1999, scientists in Germany found a strong correlation between type 1 diabetics and high levels of antibodies for A1 beta casein. It's believed that these antibodies are actually based on the amino acid sequence of the problematic opioid BCM7 that's derived from A1 beta casein. Because this sequence has similarities to the protein structures of insulin producing cells in the pancreas, the antibodies attacking pancreas cells along with BCM7 peptides. It is believed that BCM-7 suppresses body's immune system that may enhance the survival of

pathogens such as enteroviruses (Elliot *et al*., 1999; Swinburn, 2004) or bacteria (*Mycobacterium avium*) (Dai *et al*., 2009). These pathogens are ultimately involved in trigger of type 1 diabetes like symptoms. A Second theory says that there is autoantibody generation against beta cells. According to this theory some of the peptide sequences of β-casein which are produced during digestion, mimic the sequence of GLUT-2 transporter (Pozzilli, 1999; Cavallo *et al*., 1996; Inman *et al*., 1993) (involved in glucose transportation in cell). In this response T cells recognize them as an antigen and activate B cells for production of antibodies. Antibodies not only targets the digested segment of β-casein product but also to insulin producing beta cells, causing type 1 diabetes.

A1 Beta Casein and Nervous Disorders

Because BCM7 is an opioid, it shouldn't be surprising that there is also convincing evidence linking A1 beta casein to mental disorders such as autism and schizophrenia. Opioid receptors are basically found in brain and classified in three groups, μ (MOP), κ (KOP) and δ (DOP) (IUPHAR 2008). MOP or μ receptor is found in brain, immune cells, endocrine glands and to some extent in intestine. The prevalence of autism in particular and its related disorders seems to be increasing at a shocking rate, and although there's a lot of variations in the estimations of what the incidence actually is, some suggest it's as high as 1 in 100 or even higher. The symptoms of autism and schizophrenia can be remarkably similar to those caused by opioids. Incomplete breakdown of certain foods, in particular those containing gluten and casein results in the production of opioids (Reichelt *et al*., 1981). From guinea pig ileum assay (GPI) it was known that bovine milk has opioid effect (Brantl *et al*., 1979; Brantl *et al*., 1981) and it acts most probably through MOP receptor (Barnett *et al*., 2014). The BCM7 derived from A1 beta casein and the gluteomorphin derived from gluten are both opioids that can contribute to these symptoms. This is why so many autistic children have experienced dramatic improvements from following a gluten-free and casein free diet. The connection between autism and opioids is nothing new. In 2000, a team of researchers led by Robert Cade reviewed the existing evidence associating casein and gluten opioids with autism and schizophrenia. They also evaluated new data of their own which was collected from 150 autistic children, 120 schizophrenic adults, 43 normal children, and 76 normal adults. The autistic children and schizophrenic adults consistently showed abnormally high excretion levels of casomorphin and gluteomorphin opioid peptides derived from beta casein and gluten. They were also found to have greatly enhanced antibodies to casein and gluten (Cade *et al*., 2000). Out of the 70 autistic children put on a gluten free and casein free diet, 81% improved significantly within three months, and more than a third of those who didn't improve where still excreting high levels of opioid peptides suggesting that they weren't following the diet. Thus such patients have shown dramatic improvements in their symptoms following gluten free and casein free diet (Cade, 2000; Knivsberg *et al*., 2002; Elder *et al*., 2006). Although the improvement rate for the schizophrenic adults was only 40%, it's believed that many of them didn't stick with the diet long enough to allow of the existing BCM7 molecules to be eliminated from the brain which could potentially take longer than a year. The effects of A1 beta casein on autism and schizophrenia are further supported by animal research. In 1999, Zhongjie Sun and Robert Cade injected the BCM7 opioid derivative of A1 beta casein into rats to determine if it entered the brain. They found that it had attached to a number of different areas of the brain that had been previously shown to be associated with autism or schizophrenia. As a result, it was concluded that BCM7 could pass through the blood brain barrier and affect brain regions similar to those affected by autism and schizophrenia. Bovine BCM-7 has much higher affinity for MOP receptors as compared to human milk BCM-7 (YPFVEPI amino acid sequence of human BCM 7) (Koch *et al*., 1985). Also BCM-7 level in human milk reduces drastically after 2 to 4 months of delivery of new born (Jarmolowska, 2007), reducing overall effect of opioid peptides such as human BCM-7. This kind of reduction in BCM-7 level occurs in bovine milk is still unknown. According to Meisel and FitzGerald (2000) bovine milk proteins also produces opioid antagonist like casoxin A, B and C during its digestion. But overall maximum theoretical yield (mg/g protein) of opioid agonist can be higher as compared to opioid antagonist. BCM-7 has also shown to increase in the expression of genes responsible for producing inflammatory enzymes such as myeloperoxidase (UlHaq *et al*., 2014) that could further increase the expression of MOP receptors in intestine (Pol, 2005). The overall process further enhances the action of this peptide. However information regarding similar type of action is not available for brain. It is also believed that mercury toxicity inactivates cysteine amino acid present in active site of DPP (IV) enzyme which is required for digestion of casein (Bernard *et al*., 2001; Reichelt *et al*., 1981). In neonates high level of mercury toxicity could arise through vaccination, mother's dental amalgams or other environmental factors (Cade *et al*., 2000).

Allergies and Autoimmune diseases

The word 'allergy' was coined in 1906 by an Austrian pediatrician, Clemens von Pirquet, who used it to describe responses in his patients to various agents such as dust, pollen and certain foods. Milk intolerance is different. A person who is intolerant of milk, or one of its constituents, will typically experience bloating and/or diarrhea. Some people may experience constipation before the diarrhea. Several aspects on the effect of nutrients on small intestinal motility are not completely understood today. It may be due to either proteins or other food components. For example, lactose intolerance is caused by an inability to digest lactose (milk sugar) on account of a deficiency of the enzyme lactase. Defilippi and group (1995) analyzed changes of motor activity of the canine small bowel following intragastric administration of casein and soy protein. Casein was followed by a statistically significant decrease of amplitude and frequency of small intestinal contractions, compared to soy protein. Also pretreatment with naloxone suppressed the inhibitory effect of casein, suggesting that stimulation of opioid receptors by BCMs might be responsible for the motility changes (Defilippi *et al*., 1995). According to the American autoimmune Related Diseases Association (AARDA), about 20% of the Americans suffer from auto-immune conditions. In November 2004 there was an article in the Christchurch newspaper, *The Press* about Crohn's disease and ulcerative colitis. A link between MAP and Crohn's, and also ulcerative colitis, has been suspected for about 20 years but has been difficult to pin down. Alongside this article there was the case history of Claire, a lady who suffered from Crohn's disease who was also the president of Colitis Support Group. She described the way it affected both her lifestyle and what she could eat. She explained that among other things she had cut out all dairy products. According to the website of the Crohn's and Colitis Support Society about 35% of Crohn's sufferers and 20% of ulcerative colitis sufferers cannot tolerate milk. The reason is still not clear that why this occurs or which constituent of milk is responsible for it.

It is interesting to consider why some people can digest goat's milk but not cow's milk. Both types are broadly similar in relation to their protein types and lactose content. But goats' milk is A2 milk. There is also evidence from Israel that some people who are allergic to cows' milk can drink camels' milk, which also happens to be A2 (Shabo *et al*., 2005). There has been a widespread belief amongst the

general public, going back at least 100 years that milk consumption can cause excessive production of mucus in the nasal passages and throat (Zoghbi *et al.*, 2006). In the human colon, beta casomorphin-7 (BCM-7), an exorphin derived from the breakdown of A1 milk, stimulates mucus production from gut MUC5AC glands. In the presence of inflammation similar mucus overproduction from respiratory tract MUC5AC glands characterizes many respiratory tract diseases. BCM-7 from the blood stream could stimulate the production and secretion of mucus production from these respiratory glands. Such a hypothesis could be tested *in vitro* using quantitative RT-PCR to show that the addition of BCM-7 into an incubation medium of respiratory goblet cells elicits an increase in MUC5AC mRNA and by identifying BCM-7 in the blood of asthmatic patients. This association may not necessarily be simply cause and effect as the person has to be consuming A1 milk, BCM-7 must pass into the systemic circulation and the tissues have to be actively inflamed. These prerequisites could explain why only a subgroup of the population, who have increased respiratory tract mucus production, find that many of their symptoms, including asthma, improve on a dairy elimination diet (Bartley and McGlashan, 2010). A number of studies have suggested that the exclusion of milk products from the diet may improve asthma symptoms. In the 1950s, Rowe and Rowe suggested that a variety of foods could contribute to asthma and found that in asthma patients, symptoms often improved on an exclusion diet (Rowe and Rowe, 1950; Rowe and Rowe, 1956). Excessive milk consumption has a long association with increased respiratory tract mucus production and asthma.

Coeliac disease

Coeliac disease is generally accepted as being an autoimmune disease. For a long time it was believed that people of northern European ethnicity were genetically more prone to this disease than other ethnic groups. However, people from other regions of the world, including the Middle East, northern Africa and India are now being increasingly diagnosed with this disease, and at similar incidence levels to northern Europe (Malekzadeh *et al*., 2005). Once damage occurs, apparently through an autoimmune response, the digestive process is interfered with. Untreated coeliacs patients are also intolerant of milk. This makes sense because the villi are no longer producing the lactase to digest the lactose sugar. But once gluten is removed from the diet, and the intestine wall has had time for self-repair, people with coeliac disease can typically once again digest milk. A recent Danish study published in the *British Medical Journal* found the risk factor for schizophrenia among people with coeliac disease to be 3.2 times higher than in the general population.' Indeed there seems to be general acceptance that coeliac sufferers are considerably more likely than the general population to suffer from neurological conditions. Dr Sun and his colleagues (2003) from University of Florida have found that BCM7 passes through the blood/ brain barrier much more easily and in a different way to the peptides from gluten, and the BCM7 attaches to 45 different parts of the brain. Another piece of evidence that seems to confirm there is something going on in relation to coeliac disease and beta-casein. An Italian group from the University of Rome found that coeliac patients had significantly higher levels of beta-casein antibodies than age-matched controls, and similar levels of these antibodies to people with Type 1 diabetes. Also a 1999 paper by Italian researchers in the journal *Gastroenterology* reporting that the longer coeliac sufferers remain exposed to gluten, the more likely they are to develop another auto-immune disorder. On average, people with coeliac disease had a 14% chance of another auto-immune disease, compared to a 2.8% chance for age-matched controls (Ventura *et al.*, 1999), but among those who were not diagnosed until they were more than 10 years old, 24% had another auto-immune disease as well. They also found similar results for Crohn's disease. They found that Crohn's sufferers were 4.6 times more likely than non-sufferers of Crohn's to also have some other autoimmune diseases (Ventura *et al.*, 2002). Coeliac disease seems to be associated with a great many neurological and developmental conditions (Bushara, 2005).

Crohn's disease and ulcerative colitis

Both diseases are found mainly in northern Europe (particularly Scandinavia and Britain), North America, Australia, New Zealand and South Africa. The incidence of both diseases has increased greatly in the last 60 years. Dr Richard Gearry has been undertaking an extensive survey of IBD sufferers in Christchurch, and identify the risk factors. The results of his survey were that the breastfed babies are less likely to get IBD, breastfeeding has to occur for more than two months to provide the protective effect, and the risk declines the longer that breastfeeding is continued, up to and beyond 12 months (Gearry *et al*., 2010).There is also some evidence from Denmark that Crohn's and ulcerative colitis sufferers have an increased risk of schizophrenia (Eaton *et al.*, 2004). These researchers found an increased risk of 40% for both diseases. There is evidence that these diseases were associated with lesions in the white matter of the brain, as measured by MRI scans (Andus *et al*., 1995).

Sudden infant death syndrome (SIDS)

In developed countries this is the most important cause of death in infants less than one year of age. The link between SIDS and casomorphins that are derived from casein goes back to at least 1988. At that time a paper exploring this link was written by two American scientists from New York University Medical Centre (Ramabadran and Bansinath, 1988). Back then no-one understood that the important casomorphins (BCM7 and its derivatives such as BCM5) were released from A1 beta-casein and not A2 beta-casein. But the possibility that SIDScould be caused by respiratory depression from these opioids was already gaining attention. Since then there have been several trials with young animals showing that injections of BCM7 cause breathing irregularities (Hedner and Hedner, 1987; Taira *et al.*, 1990). Also, it has been shown that young animals absorb BCM7 from the intestine much more readily than do adult animals**.**

Multiple sclerosis

It is a classic autoimmune disease, caused by the body attacking the myelin sheath that surrounds nerves. There are geographical predisposing factor in multiple sclerosis and these may reflect regional dietary differences, and, further, that this factor is directly related to milk production or consumption. A number of biochemical hypotheses are proposed which would predict a resultant weakened blood-brain barrier or immunological defense, or the production of defective myelin, which would then increase susceptibility to the etiological agent, possibly a virus (Agranoff and Goldberg, 1974).Ashton Embry is a Canadian who has played a key role in bringing together the disparate sources of information on the causes of multiple sclerosis. He suggested diet low in dairy products, cereal grains and legumes was the most promising approach “paleolithic approach” (because of its focus on foods that humans ate about 10,000 years ago, prior to the development of agriculture). An epidemiological study by French researchers D. Malosse and colleagues (1992) linked multiple sclerosis to milk

consumption. It showed a strong correlation between milk intake and multiple sclerosis for 27 countries, and that this was statistically significant at $p < 0.001$ (less than 0.1% probability of obtaining such a result by chance). An American study linked multiple sclerosis in the USA to a diet low in fish and high in dairy products (Lauer, 1994). In 2004 a study in the *Lancet* reported that in Sardinia, Italy, people with multiple sclerosis were three to five times more likely than their siblings to have Type 1 diabetes (Marrosu *et al.*, 2004). Also, having relatives with multiple sclerosis increased the risk of being diabetic by a factor of six. However it was not until 2003 that a group of American researchers led by Janice Dorman took some peripheral data they had collected in another study for looking at the clustering of Type 1 diabetes, autoimmune thyroid disease and rheumatoid arthritis. They found a 20-fold increase in the prevalence of multiple sclerosis among their Type 1 diabetic women and concluded that adult women with Type 1 diabetes are at an enormously increased risk of multiple sclerosis (Dorman *et al.*, 2003). But it is fascinating that these two diseases seem to have common genetic risk factors and a common environmental risk factor in relation to milk. This is further supported by the Sardinian study (2004) suggesting that there is indeed a common environmental factor linking to the genetic factor. Michael Dosch (2001) said immunologically Type 1 diabetes and multiple sclerosis are almost the same (Winer *et al.*, 2001). There has been a recent attention to the occurrence of multiple sclerosis and reports finding increased levels of IgA antibodies to gluten, gliadin and casein (Reichelt and Jensen, 2004).

Parkinson's disease

This is a neuro-degenerative disorder which is poorly understood. It causes people to shake and to have difficulty transmitting instructions from the brain to the limbs. It is linked to the loss of dopamine-producing cells in the brain. There are no good data on how the incidence of Parkinson's varies between countries or ethnic groups. Some countries such as China are widely believed to have a very low level and others such as Argentina apparently have a very high level. However, the statistics may not be reliable. The most rigorous analyses of factors linked to Parkinson's disease have been undertaken by a team from the Harvard School of Public Health, led by Dr Alberto Ascherio. The team has numerous publications investigating a wide range of food and lifestyle factors (Chen *et al.*, 2007*).* Their initial key data sources were long-term studies of 50,000 male health professionals and 120,000 nurses, and more recently some 130,000 men and women from the American Cancer Society's long-term Cancer Prevention Study. There was one food item that Chen and colleagues (2007) keep finding associated with Parkinson's disease: milk. And it is the only food type that appears to be a risk factor clearly associated with Parkinson's. A large-scale study of Japanese-American men in Honolulu also found similar results in which dietary intake observed from 1965 to 1968 in 7,504 men ages 45 to 68. Men were followed for 30 years for incident Parkinson's disease (Park *et al.*, 2005). The researchers think it is unlikely to be fat because when they look at total fat in the diet the correlation is less strong. Also, they believe for the same reasons that it is unlikely to be calcium or total protein intake.

Cow Milk Strategy for India

Modern scientific research and Indian belief both confirm that Milk of a cow of Indian breed, fed on greens is indeed nature's best gift to mankind. Grass fed Cow's milk is very rich in EFA' (Essential Fatty Acids) viz. CLA's (Conjugated Linolenic and Linoleic Acids). Such milk has low saturated fatty acid content. (Same is true for beef also). This is universally accepted as a strategy by USDA, and EU Lipgene project. Cows of Indian breeds (*Bos Indicus*) are recognized as producers of A2 type milk, as against cows of Holstein Frisians that belong to *Bos taurus* group and produce A1 type of milk (World Dairy industry led by NZ- the leaders in this field- are reported to be implementing a program to convert all NZ herd to A2 type milk producer by stopping cross breeding with HF A1 semen).

Opportunity & Challenge

This presents for India a great opportunity to build on the tremendous strength of our nation, being already the largest producer of Milk in the world. Indian breeds of cows represent the world's largest A2 milk producing herd. Traditionally Indians were raised on A2 Milk, which accounted for excellent health of Indians. Milk of Indian breeds of Cows enjoys excellent USP in India, in preference over cross bred cow's milk or buffalo milk. This presents tremendous commercial opportunity. Per capita consumption and availability of liquid milk in India is about the lowest in the world and has a fantastic Demand Potential. This is where a tremendous challenge to Animal Science experts of India, and the greatest economic development opportunity is being visualized. Milk of only Indian breeds of Cows, (Confirmed by laboratory type testing as being free from BCM 7(Beta Caso Morphine 7), or a herd DNA tested for A2 milk producing cows) should be supplied as a separate product. In this category, States such as Gujarat that produce large quantities of natural A2 Milk from its Gir cows fits excellently well to take a lead. Pure milk of Indian breeds of Cows commands very good premium prices in India. Low fat A2 milk as the premium grade A2 milk, can command very high premium prices not only in India but around the world. EU under Lipgene project is conducting research simultaneously in 21 laboratories in Europe to produce designer's natural milk that has low total fat and high EFA content. India has the unique privilege of having an unbroken Cow Milk tradition going back to thousands of years. Stall feeding of cattle and concentrated prepared feed, is a rather recent development. Cows raised in Pastures as reported in Kautilya's Arth Shastra had total fat content of less than 1%. This is when Cow's Milk was truly Amrit- Nature's Nectar- a preventive and cure for all self-degenerating diseases of human body. Taking guidance from our ancient cow management practices in Vedas and other Sanskrit texts, it is not difficult for us in India to produce within foreseeable future, a 'Designer's Milk' with low total fat content. In the process of reducing fat content, the milk yield also goes up (Chauhan and Singh, 2013). Indians over thousands of years had enjoyed low fat high EFA, A2 type Milk of Indian cows. The high Omega 3 content of this milk explains the secret of the well-recognized highly developed capacity of Indian brains in the world. Low in total Fat & high EFA Milk of Indian breeds of Cows will be the most highly prized A2 milk for commercial considerations.

Typing of animals for A1 and A2 gene

Allele specific PCR (AS-PCR): Keating and group (2008) described this concept of allele specific PCR (AS-PCR). It uses DNA from bovine blood and finds single nucleotide polymorphisms (SNPs) and mutations. Then selective amplification is achieved by designing a primer such that its 3' end will match or mismatch with base of allele. Primer strand will extend only when its 3' end is a perfect complement to the allele present in DNA. Thus, if a single base polymorphism occurs, the genotyping results can be observed by simply

observing presence and absence of PCR products on the gel. Allele specific PCR is carried out by using a common forward primer (Bwtp3; 5'GCCCAGATGAGA GAAGTGAGG-3') and reverse primers with either T or G at the 3' end (5'-GATGTTTTGTGG GAGGCTGTTAT/G-3') to amplify an 854 base pair fragment. This method can easily detect that whether the bovine will produce A1A1, A2A2 or A1A2 type of milk.

Amplification created restriction site PCR (ACRS-PCR): Raies and group (2009) have described the method for typing of animals using DNA from bovine blood. This method uses the concept of amplification created restriction site (ACRS-PCR). It is quite similar to allele specific PCR. 121 base pair length of DNA is amplified. Forward primer sequence is made of identical sequence, but reverse primers are made such that out of 18 bases, one base penultimate to the 3' end is not able to form hydrogen bond with base present at complementary strand of amplifying DNA. Because of one base difference between A1 and A2 beta-casein, a restriction site is created in A2 but not in A1 gene which is recognized by a endonuclease enzyme; it cuts the DNA of A2A2 genotype into two parts, which appears as two bands on the gel, but appears as one band in case of A1A1 genotype. Three bands are observed in A1A2 genotype.

Single strand conformation polymorphism PCR (SSCP-PCR): Barroso and group (1999) have described a method for typing of animals using DNA from bovine blood. There is electrophoretic separation of single-strand nucleic acid based on subtle differences in sequence which results in a different secondary structure and a measurable difference in mobility through the gel. It detects whether the bovine will produce A1A1, A2A2 or A1A2 type of milk. This method only tells that a mutation exists. However DNA sequencing has to be performed to know the nature of the mutation that caused an electrophoretic mobility shift in a given sample. Also, highly standardized electrophoretic conditions are required for this method.

Taq Man method: Manga and Dvorak (2010) gave this method of typing of animals for A1 and A2 gene. First DNA is isolated from somatic cells of bovine milk then ssDNA probe (18 bases long) is made specific for A1 and A2 beta-casein gene that contains at 5' end fluorescent agent and at 3' end a quencher. Quencher inhibits the activation of fluorescent agent in native form of probe. During PCR, Taq Man probe (ssDNA labeled with fluorescent and quenching agent) binds to A1 or A2 beta-casein gene at their sense strand along with forward primer and reverse primer. DNA polymerase breaks the probe during amplification process, activating fluorescent agent present on it at specific wavelength. This method is highly sensitive.

Detection method of BCM-7 in urine / blood: RIA/ELISA methods are described for assaying BCM-7 in urine or plasma (Kost *et al*., 2009; Sokolov *et al*., 2014). Sokolov and group (2014) have detected BCM-7 in picogram level in urine of normal as well autistic children with the help of ELISA method. The concentration of BCM-7 was much higher in urine of autistic children as compared to normal ones. Commercially available kit such as S-1334 (ELISA based kit from Bachem company) can detect BCM-7 in beta- casein digest at nanogram level.

Typing of animals on the basis of presence or absence of BCM-7: This method first requires isolation of β-casein from milk followed by *in vitro* digestion by gastrointestinal enzymes and separation of generated peptides by HPLC-MS method. The sequence and mass of peptide helps in identification of BCM-7 as well as in typing of milk (Noni, 2008).

A2 Milk Based Infant food

A2 milk is considered most important for baby food and milk formulae. This presents India with an excellent commercial opportunity to become a world leader in Infant milk food supplies. According to Hindu mythology as well as the Indian traditional medical practices (both the classical systems like Ayurveda and Siddha and the oral practices of the rural villagers) cow milk has rejuvenatory, health protecting and health promoting properties and hence has been said as the best one among vitalizers. The cow milk is a healthy food because of low calorie, low cholesterol and high micro-nutrients/vitamins. Compared to buffalo milk it has high moisture, carotene, thiamine, riboflavin, vitamin C, sodium potassium; and on other hand is low in protein, energy (kilocalories), calcium, phosphorous, fat and cholesterol. These properties serve unique purposes. Buffalo milk may be richer but it is the cow milk that sharpens intellect, gives swiftness of body, stability of emotions and a serene nature to the one who drinks it. Cow milk is an integral part of balanced diet. Cow's milk contains substances like carotenes, vitamin A, vitamins of B complex group and vitamin C. It also contains substances like flavones, sterols and phenols. All these chemical agents delay the processes involved in aging. It is known that the fatty acids and amino acids present in the fat of cow milk are different than those in buffalo milk. These components render cow milk very nutritious and growth promoting for infants and children. The use of milk proteins as nutritional supplements to enhance dietary protein quality is very feasible as they can provide lysine and tryptophan, the limiting amino acids of wheat and maize proteins. A 250 ml serving of cow milk contains riboflavin equivalent to 50% of the daily requirement of a pre-school child. Milk plays an important role in meeting the requirements of many essential nutrients, and hence milk is considered as a protective food. The proteins of milk are of a high biological value. The digestibility of milk proteins is rated higher (96%) then that of plant proteins (74-78%). It is easily digestible, the amino acids composition makes its protein easily digestible, and reaching the nerves of the brain it acts as a brain tonic and is good for kidney. Lactose favors the absorption of calcium and phosphorous and the synthesis of some B complex vitamins in the small intestine. The lactic acid bacilli present in milk are important. Lactose, the principal milk sugar, promotes the growth of lactic acid producing bacteria in intestine thus creates a desirable condition that inhibits the growth of proteolytic and putrefying bacteria in the intestine and may increase the solubilization and absorption of calcium. Lactose also promotes the utilization of magnesium and phosphorus present in the milk.

The milk proteins are useful in the diet of patients suffering from liver and gall bladder diseases, hyperlipidemia and diabetes. The easy digestibility of milk fat makes it a valuable dietary constituent in diseases of stomach, intestine, liver, gall bladder, kidney and disorders of fat digestion. Milk fat is reported to have antibacterial and fungicidal activity against gram-negative bacteria and certain molds. Milk fat has a protective effect against human tooth decay ascribed in part to adsorption of milk fat onto the enamel surface and in part to antimicrobial effect of milk fatty acids. The low content of protein in cow's milk from buffalo milk makes it ideal for infants and people with renal disorders. Patients with impaired kidney functions rely on protein with high biological value for relieving strain on the excretory function of the kidney.

It is a rich source of vitamins like B2, B3 and vitamin A which help increasing immunity. It is a good source of zinc, which is required for synthesis of insulin by the pancreas and for immunity function. The substances in milk which have an antimicrobial effect are immunoglobulins, lactoferrin, lysozyme, lactoperoxidase and vitamin B12-binding protein. The immunoglobulins, mainly IgA are not broken down by the digestive enzymes. Thus, they not only act against the microorganisms in the intestine but also prevent the absorption of foreign proteins. Lactoferrin is an iron binding glycoprotein that occurs in cow milk at a level of 0.2 mg/ml. A number of milk enzymes viz. lactoperoxidase, xanthin oxidase and lysozyme are involved in antibacterial mechanisms. Several peptides with opium like (sleep inducing) activity have been extracted from the degradation products of milk proteins viz. ß-casomorphins (from ß-casein), exorphin (from µS1 casein), ß-lactostensin (from lactoglobulin) and serorphin (from serum albumin) which can prolong gastrointestinal transit time exerting anti-diarrhoeal effect. Certain peptides from casein stimulate the production of immunoglobulins. Immune-stimulatory peptides from milk can stimulate the phagocytic activities of murine and human macrophages and enhance resistance against certain bacteria. Its yellow substance "Carotene" (Vitamin A) increases the visual strength. Vitamin A deficiency is a major cause of widespread blindness among children in India. A 250 ml serving of cow milk contain vitamin A sufficient to meet 75% daily vitamin A requirement of pre-school child (Chauhan, 2017).

It helps in reducing acidity, a common problem today and thus reduces chances of peptic ulcer. Cow milk fat component is potential anti-carcinogenic agent, which help in reducing chances of colon, breast and skin cancer. A specific fatty acid (a cis-trans isomer of linoleic acid) has been identified in milk fat, which appears to be an inhibitor of cancerous growth. Conjugated linoleic acid (CLA) in cow milk prevents the uncontrolled spread of cancer-affected cells. CLA in mouse models has been shown to protect against the induction and proliferation of chemically-induced skin, stomach, colon, prostate and mammary tumors. It has drawn a great deal of attention among dairy, medical and nutrition scientists. Other components of milk fat also have biological effects with anti-cancer properties. Among these, sphingomeyelin, a component of the milk fat globule membrane, and thus particularly rich in buttermilk, contains the biologically-active components ceramide and sphingosine. CLA fed before puberty may impart long-lasting protection against induction and proliferation of tumors. Research suggests that CLA can decrease the amount of fat in the blood the amount stored in the body. These effects could help in the fight against two other major killers, heart disease and obesity.

It contains practically half the fat content of buffalo milk thus reduces the risk of coronary heart diseases. It is specifically beneficial to heart patients by reducing formation of serum cholesterol. It has been suggested that the regular intake of milk keeps blood vessels healthy. Low fat content helps one keep fit and to check obesity. Unique physical, chemical and biological properties of milk fat contribute to the ease of digestibility compared with other fats. Milk turns down the tendency of the fat cells to store the day's calories, and increases the amount frittered away as heat. Calcium is a critical signaling agent, helping all sorts of cells figure out what they need to do. Apparently, the type of cell which listens when calcium talks are the fat cell. When there's plenty of calcium in the blood, fat cells get the message to quit storing fat and start burning it. Calcium pills aren't a perfect substitute for the complex package of nutrients found in milk. It is one of the best natural anti-oxidants and thus neutralizes the oxidative stress produced in body through action on free radicals. A skin care cream containing cream or ghee from cow's milk is said to render the skin fair and smooth. Less phosphorous content of cow's milk allows better absorption of calcium. Calcium and Phosphorus in cow milk help in balanced absorption of nutrients and are good for healthy growth especially for children. Potassium helps in development of healthy mind. Studies show that milk and products may decrease the risk of osteoporosis through their effects on growth. It not only helps against diabetes but also has good sugar for diabetic patients. Milk consumption, therefore, enables the diabetic person to obtain the biologically highly valuable milk proteins without running the risk of rise in blood glucose levels.

A distinct advantage of cow's milk over human milk is that it's a better source of vitamin K which prevents hemorrhagic disease of newborn. Folic acid prevents anemia and neural tube defects in newborn. It is best for infant feeding after mother's milk and a good supplementary food for adults. It is a fine blend of all the nutrients necessary for growth and development of young once. There is general agreement that breast milk of the mother is the best food for infant. Human colostrum is rich in immunologic factors, which prevent infection in the newborn babies. However, changing social and cultural patterns have led to change from breast to formula feed especially among urban elites and working mothers. Cow milk is the best alternative option in such a situation. Also, milk is the first food a child takes to survive and is the best and most complete of all foods. It has more minerals and vitamin K except iron and copper, than human milk to meet the nutritional requirements of young infants. Cow milk contains tonic, is energetic and conducive to heart and brain, and advances age and potency. Dietary pattern varies widely in different parts of world depending upon socio-economic status, customs, taboos etc. but milk surpasses all these barriers, as it is the only food widely available and acceptable. In Mahabharta, Yaksa asked Yudhisthira "What is nectar?" Yudhisthira replied "Cow's milk is nectar." In India, boiled whole cow's milk is routinely used (Chauhan, 2004). The milk of cow can be used in various forms as other milk. Skimmed milk powder supplemented with vitamin A, D and pyridoxine, forms a comparatively cheap food of high nutritive value. It is useful for the treatment of malnutrition, the nephritic syndrome and the cirrhosis of liver. Toned milk is a useful source of proteins for malnourished children and pregnant women. When cow milk is heated (as in pasteurization), homogenized or acidified to produce a softer curd/dahi, the protein is used by infants as efficiently as is the protein of human milk. Other products are Khoa (Mava) Chhana (cottage cheese), Yoghurt, Lassi (Butter milk) and Ghee (Clarifled butter: Butter-fat). Dairy products can supercharge almost any diet. It has been observed that dieters who got dairy products lost 70% more weight than those who avoided it.

Therefore, it is right time to draw the attention of policy makers, farmers to stop the indiscriminate cross-breeding of our local breeds of cattle with the exotic breeds just to increase the milk production; rather preserve and maintain our own Indian breeds for milk production and use the selection criteria for further enhancement of their production. Initially cross breeding with exotic cattle may appear as a profitable entrepreneurship to do in the present scenario but it will have many serious implications in the future not only from health point

of view but also in terms of overall profits in long term of 10-20 years. The germplasm of our indigenous cattle is unique, apart from being a source of A2 milk, which in itself is a great blessing, our cattle breeds have excellent adaptation potential and disease resistance. Besides this, urine and dung can be utilized for the development of medicinal, cosmetic, organic farming and various other useful products. Hence, strategies should be made in such a way so as to conserve and improve our indigenous breeds. Many western countries are either not aware of these facts or knowingly ignoring the above beneficial effects of Indian zebu cattle and moreover, they weren't offered the choice of A1 and A2 milk but fortunately we have it as a matter of chance and blessings of the nature and now it is up to us to decide whether we should use A2 milk having beneficial health promoting potential or regressively destroy our future generations through A1 milk of exotic cows. Also it is pertinent to mention that the conclusions drawn on the basis of the harmful effects of A1cow milk in western countries cannot be applied as such on the people of Indian sub-continent because the A2 milk of Indian Zebu cattle is considered as nectar (Amrit) which is beneficial to heath and makes the body immunologically and mentally strong enough and refractory to most of the infectious diseases, new infections and cancers.

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