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Review Paper

An Insight into the Aetiology of Tropical Chronic Pancreatitis and Fibrocalculous Pancreatic Diabetes

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Abstract

Pancreatitis is a heterogeneous diseases with hereditary and non-hereditary transmission, manifesting itself in acute and chronic forms. Tropical chronic Pancreatitis prevalent in the peri-equitorial tropical regions is a juvenile form of non-alcoholic, idiopathic, calcific pancreatitis with progressive deterioration of the endocrine function leading to diabetes, termed as Fibrocalculous Pancreatic Diabetes (FCPD). This review aims to delineate the genetic insight that traces the relationship of TCP and FCPD as well as causal or modifier role of metabolic stress factors and environmental toxins. The review intends to show a three- hit model as the causal strategy for FCPD. Different studies have thrown light on familial aggregation as the probable basis to consider the genetic predisposition of the disease. Majority of studies done on this aspect favour calling FCPD as the later stage of TCP , although some reports from Bangladesh consider TCP and FCPD as two separate entities. If we go by the view of majority, then FCPD is the logical end-point of TCP. Hence, the suspected genes whose malfunctioning leads to TCP and FCPD are SPINK1 N34S mutation, causing inappropriate activation of trypsinogen to trypsin within pancreatic parenchymal cells and prevents maintenance of integrity of pancreatic acinar cells. CTSB (Cathepsin B) polymorphism (Leu26Val, C595T, T663C, and Ser53Gly) is recorded in good number of TCP patients but its involvement in progression to FCPD is still not clear. CASR (calcium sensing receptor) gene mutation increases risk of TCP and its progress to FCPD.CTRC (Chymotrypsin C) gene mutation is recorded both in TCP and FCPD patients preventing protease and anti-protease balance thereby enhancing supertrypsin activity and auto-digestion of pancreatic parenchymal cells. Some epistatic gene interaction is also predicted between SPINK1 and CTSB genes. Hence, defects in the above mentioned genes are considered to be the first hit for initiating TCP and FCPD. Metabolic stress factors like high carbohydrate and low protein diet along with lifestyles such as excessive smoking, drinking dealing with occupational chemicals causes oxidative stress and decrease in total anti-oxidant capacity considered as the second hit for FCPD. Increased amount of free radical due to oxidative stress has a direct bearing on the immunological functioning of the body. Cell-mediated immunity and auto-immunity due to release of sequestered pancreatic antigen was found in FCPD patients. Hence, immunological malfunction acts as the third hit to enhance progress of TCP (clinically symptomised by severe pain, fibrosis, calcification and progressive endocrine malfunction) to FCPD.

Keywords: Three-hit model, genetic predisposition, SPINK1 N34S, CTSB, CASR, CTRC gene polymorphism, causal or modifier role, metabolic stress factors, altered immune functions.

Introduction

Pancreatitis is a heterogeneous disease with varied etiologies, defined as an inflammatory disease of the pancreas leading to morphologic changes that typically cause pain and/or loss of function[1]. Pancreatitis can be either hereditary or non-hereditary. In the hereditary form vertical transmission of altered gene or set of genes predisposes individual to the risk of pancreatitis, while in case of non-hereditary forms vertical transmission may or may not take place. In both the above mentioned forms (especially non hereditary) of the disease can present itself in acute or chronic state, where complications of recurrent acute pancreatitis leads to Chronic pancreatitis (CP)[2]. Chronic pancreatitis (CP) is a continuing inflammatory disease which eventually leads to morphologic changes characterized by irreversible destruction and fibrosis of the exocrine parenchyma, leading to exocrine pancreatic insufficiency and progressive endocrine failure leading to diabetes[1]. Thus expert "state-of-the-science" reviewers conceded that "chronic pancreatitis remains an enigmatic process of uncertain pathogenesis, unpredictable clinical course, and unclear treatment"[3]. In most developed countries, alcohol causes about 60%-70% of the cases of chronic pancreatitis showing its preponderance in male patients especially termed as alcohol related chronic pancreatitis (ACP), and unknown causes are responsible for 25% of cases, termed as idiopathic chronic pancreatitis (ICP)[4]. Chronic pancreatitis depending upon its geographical prevalence and definitely due to the variation in the symptoms shown by the affected individuals, it can be temperate chronic pancreatitis and Tropical Chronic Pancreatitis (TCP). Tropical chronic pancreatitis differs from temperate zone pancreatitis in its younger age of onset, more accelerated course, higher

prevalence of pancreatic calculi and diabetes, and greater propensity to pancreatic malignancy[5]. Tropical calcific pancreatitis synonymously used term with Tropical Chronic Pancreatitis is a idiopathic, juvenile form of chronic calcific non alcoholic pancreatitis, seen almost exclusively in developing countries of the peri-equitorial tropical world[6,7]. In the most simple of terms, tropical calcific pancreatitis has been described as a disease with "pain in childhood, diabetes in puberty and death at the prime of life"[8]. The diabetic stage of tropical chronic pancreatitis is referred to as Fibrocalculous Pancreatic Diabetes (FCPD). The diabetes is severe and insulin requiring although ketosis resistant although some variation in nature of Diabetes is also reported[5]. In TCP, there is progressive deterioration of endocrine pancreatic function, with development of diabetes in 50% of patients upon follow up, suggesting that FCPD is merely a later stage in the course of TCP[9], although reports from Bangladesh claims TCP and FCPD as two different entities[10,11]. Based on long term follow up of large numbers of patients, it is believed that FCPD is indeed the later diabetic stage or logical end- point of TCP for the following reasons: i. TCP patients are younger than FCPD patients[12], ii. TCP patients are also seen at the impaired glucose tolerance stage[12,10] which is considered to be a prediabetic stage. iii. The presence of SPINK 1 mutations in both TCP and FCPD [11]suggests a common genetic basis.

Regardless of etiology, chronic pancreatitis affects young men and women in the prime of their lives and takes a heavy toll on the quality of life and productivity of the individual.

This review article has the following objectives: i. To develop a genetic insight that traces the relationship between TCP and FCPD, ii. To delineate the causal or modifier role of environmental factors.

Lack of proper, extensive but concentrated epidemiological evidence from the different parts of developing countries along with the lacuna in the genetic analysis to deduce the effect of different regulatory genes leading to FCPD are the major hindrances for which knowledge of FCPD still remains obscure.

Results and Discussion

Classification: *"Same disease with different names or different disease with same name".*

A large no of terminologies have been reported to be used for the disease under consideration (TCP) which are as follows - chronic calcific pancreatitis (CCP)(although the term Chronic calcific pancreatitis is a misnomer as there was no parenchymal calcification in any of the cases studied), fibrocalcific pancreatitis (FCP), chronic calcified pancreatitis, chronic relapsing pancreatitis, chronic progressive pancreatitis, fibrocalculus pancreatitis, tropical pancreatitis. (TP), non-alcoholic-pancreatitis, hereditary pancreatitis, Afro-Asian pancreatitis, idiopathic pancreatitis, chronic calculus pancreatopathy and fibrocalculus pancreatopathy. The only factor common to all these terms is 'chronic' and is the only factor true to this disease[13]. All these terminologies for CCP (as TCP is considered as a type of CCP), came from the imperfect knowledge of the etiopathogenesis of the disease[13]. Global acceptance of the association of malnutrition with diabetes was first expressed by the National Diabetes Data Group (1979)[14] and subsequently corroborated by WHO Expert Committee (1980)[15] Describing "Special types" of diabetes, the technical report acknowledged two sub type with background of malnutrition viz; i. Malnutrition related syndrome of severe non-ketosis diabetes in children in tropics: 'J-type'. ii. Diabetes with fibrosis and calcification of the pancreas and a history of severe childhood malnutrition and also excessive consumption of cyanide especially from cassava. These "special classes" were described under other types of clinical diabetes-subhead miscellaneous[15]. In the final classification by WHO Study Group (1985)[16], the position was altered. Next to the well recognized classes (1) IDDM and (2) NIDDM, Malnutrition-related Diabetes Mellitus (MRDM) was placed No. 3 in the classification table. MRDM was further subtyped as (a) Protein-deficient Pancreatic Diabetes(PDPD) and (b) Fibrocalculous Pancreatic Diabetes (FCPD)[15]. A recent international workshop reviewed the evidence for, and characteristics of diabetes mellitus seen in undernourished populations[17,18]. Whilst it appears that malnutrition may influence the expression of several types of diabetes, the evidence that diabetes can be caused by malnutrition or protein deficiency *per se* is not convincing. Therefore, it is recommended that the class "Malnutrition-related diabetes" (MRDM)[13] be deleted. The former subtype of MRDM, Protein-deficient Pancreatic Diabetes (PDPD or PDDM), may be considered as a malnutrition modulated or modified form of diabetes mellitus for which more studies are needed. The other former subtype of MRDM, Fibrocalculous Pancreatic Diabetes (FCPD), is now classified as a disease of the exocrine pancreas, fibrocalculous pancreatopathy, which may lead to diabetes mellitus.

Epidemiology: *Is the world scenario really known to us ?:*
In the middle of the last century, a pattern of peculiar diseases suddenly started appearing in near-epidemic proportions in certain developing regions of the world mainly in the peri-equatorial tropics[6,7]. Grouped as "tropical diseases" these included tropical chronic calculous pancreatopathy (TCCP) also called fibrocalculus pancreatic disease (FCPD) with diabetes, endomyocardial fibrosis, non-atherosclerotic forms of vascular disease called mucoid arteriosclerosis and idiopathic aortoarteriopathy, goitre and idiopathic epidemic neuropathy[19-25]. Initially reported from Uganda, Jamaica, Brazil and South Africa in the 1940's and 1950's, similar conditions were sporadically reported from other places including Brazil, Argentina, Nigeria and in large numbers from Kerala in India in the 1960's, 1970's and 1980's[6,26-28].

The disease was first reported from Indonesia[29], but the largest number of patients has been reported subsequently from south India[30]. It has recently been reported also from China and

Malaysia[29]. The common denominator of the countries afflicted by this malady was their location in the tropics, poverty and poor standards of nutrition. Large segments of population in such countries also regularly consumed cassava (tapioca), a tuber containing starch almost exclusively, with negligible quantities of protein (and amino-acids) as their staple diet. Depending on the features that the reporting authors found most prominent - close association with tropics, poverty and malnutrition earned the disease synonyms such as "Nutritional pancreatitis", "Afro-Asian pancreatitis", "Juvenile pancreatitis", and "Tropical pancreatitis", "Nonalcoholic tropical pancreatitis". At that time, these descriptive terms were useful to segregate such patients from the well recognized entity of "alcoholic pancreatitis"[31,32,33].

With reference to Indian sub-continent, earlier clinic based studies had suggested that the prevalence of FCPD is higher in states like Kerala, Tamil Nadu and Orissa compared to the rest of the country. However, clinic based data are subject to referral bias. In the absence of a national study, it is difficult to estimate the prevalence of FCPD in other parts of the country.

Aetiology: Genetic Basis of the Disease: Familial aggregation: TCP sometimes affects many members of the same family and one study found 17 families with two or more affected members. In a more recent study, familial aggregation was seen in 8% of TCP patients[34]. In some families, there was evidence of vertical transmission of TCP from the parents to the offspring, while in others, there was horizontal distribution of the disease among siblings[35,36]. Familial aggregation suggests, but does not necessarily prove, a hereditary aetiology for TCP, since several family members could be exposed to the same toxic or other environmental factors. There is very little information on the genetic factors associated with Fibrocalculous pancreatic diabetes (FCPD). Ninety-eight first-degree relatives of FCPD patients were subjected to detailed studies, which included glucose tolerance tests, x-ray films of the abdomen, ultrasonography, and studies of exocrine pancreatic function. The study shows that there is a familial aggregation of FCPD with evidence of vertical transmission of the disease from parent to offspring in some families. Routine screening of families of FCPD probands helped to pick up cases in the stage of impaired glucose tolerance. There is heterogeneity in FCPD with respect to familial factors. Some families show marked familial aggregation of FCPD while in others the disease occurs either sporadically or in association with other family members who have abnormal glucose handling[37]. Hence the vertical transmission of the disease cannot be well differentiated.

Suspected genes or in adequate injury protection: Genetic predisposition is a major causal factor for Tropical Chronic Pancreatitis and Fibrocalculous Pancreatic diabetes. The candidate genes taken under consideration for FCPD are as follows:

SPINK1 gene - Serine protease inhibitor Kazal type 1 located in chromosome 5. Phenotypic role – inhibition of immature and inappropriate activation of trypsinogen within the pancreatic parenchymal cells of exocrine pancreas preventing sustained super-trypsin activity. A 6 KDa protein (approx) also termed as pancreatic secretory trypsin inhibitor (PSTI), present in the secretory granules of the acinar cells capable of binding to the active site of trypsin in 1:1 ratio and inhibiting tryptic activities. Other safety mechanisms are the presence of trypsin inhibitors in plasma including α1-antitrypsin and β2-microglobulin, which inhibit the trypsin that leaks into the interstitial space around the pancreas. This gene product is also responsible for maintenance of integrity and regeneration of the acinar cells[38,39].

Mutations- Two distinct recessive type of mutation is identified in SPINK1 gene associated with chronic pancreatitis especially TCP and FCPD. An A>G transition (missense mutation) at 101 nucleotide position in the *SPINK1* gene leading to substitution of asparagine by serine at codon 34 (N34S) has been reported with its highest frequency (approximate 46%) found so far in the Indian population[40]. Similar associations with varying strength have been reported by several studies, establishing *SPINK1* as a strong candidate for contributing to the pathogenesis of TCP[41,42]. It is observed that N34S mutation shows complete linkage disequilibrium with four intronic variants, 56-37T>C, 87+268A>g, 195-604G>A, 195-66_-65insTTTT[43], one of which may be pathogenic .

SPINK1 mutations in both FCPD patients and TCP patients without diabetes mellitus in comparable frequency was detected. A novel G to T transversion at 215 bp upstream in the SPINK1 promoter region (-215G>T) was also identified in 3 patients, who interestingly also carried an N34S allele, suggesting a compound heterozygote status . Although SPINK1 gene mutation is commonly found in majority of FCPD patients but its mere presence will not induce TCP or FCPD[44].

CFTR gene - Cystic fibrosis transmembrane regulator on chromosome 7. Phenotypic role - CFTR is a ABC transporter-class ion channel that transports chloride and thiocyanate ions across epithelial cell membranes. The molecule that regulates pancreatic duct secretion, regulates the ability to flush activated trypsin into the intestine, especially in the presence of distal duct resistance but further studies are needed for FCPD.

Mutations of the CFTR gene affect functioning of the chloride ion channels in these cell membranes, leading to cystic fibrosis. Based on the very low frequency of CFTR gene mutations in TCP patients, it was concluded that this genetic abnormality had a very small etiologic role, if at all, in patients with TCP[45] although its role in ICP was established[46].

CTSB gene- Cathepsin B on chromosome 8. Phenotypic role - Responsible for localisation within the zymogen granules in acinar cells and activation of pancreatic cationic trypsinogen (PRSS1 gene). There is evidence to suggest that partially purified beef spleen cathepsin B activates trypsinogen to a trypsin-like product. Studies on native and recombinant cationic

trypsinogen assigned a central role of cathepsin B in the development of different forms of pancreatitis [47]. It is also associated with intracellular changes in pH and calcium levels.

Mutation - The coding region of CTSB gene was sequenced to trace polymorphism in TCP patients (Leu26Val, C595T, T663C, and Ser53Gly polymorphisms were analysed)[48] and it was reported that for first time that CTSB polymorphisms are associated with TCP[48]. Still there is scope to determine whether the same polymorphisms are responsible for the progression of TCP to FCPD.

Reg genes (reg 1A) **– codes for lithostathine C** located on chromosome number 2. Phenotypic role - The gene encodes a protein associated with regeneration of pancreatic islets and has a sequence identical to that of pancreatic stone protein**.** Promotes the nucleation of calcite crystals or may prevent pancreatic lithiasis by inhibiting calcite crystal nucleation and growth in the pancreatic juice, and also help in preventing the harmful activation of protease precursors in the pancreatic juice.

Mutation - Restriction length polymorphisms (RFLPs) and possible sequence variants of the reg 1A gene were studied by RFLP analysis, looking for single-stranded conformational polymorphisms (SSCPs) and direct nucleotide sequencing in patients with FCPD and control subjects, no RFLPs were detected using 10 restriction enzymes[49,50]. In patients with FCPD and control subjects, no SSCP variants were detected. Finally, direct nucleotide sequencing of the reg 1A gene from patients with FCPD did not show any differences from the published human reg 1A gene sequence. In conclusion, it seems unlikely that mutations in the coding region of the reg 1A gene are a common cause of FCPD[51].

TCF7L2 gene- transcription factor 7 like protein 2((T-cell specific, HMG-box) also known as TCF4 is a protein acting as a transcription factor on chromosome 10. Phenotypic role- It is a member of the Wnt signaling pathway. Stimulation of the pathway leads to the association of β-catenin with BCL9, translocation to the nucleus, and association with TCF7L2[52] which in turn results in the activation of Wnt target genes, specifically repressing proglucagon synthesis in enteroendocrine cell leading to type 2 diabetes[52].

Mutation– In a majority of TCP patients, progression to diabetes, called fibro-calculous pancreatic diabetes (FCPD) takes place takes place as logical end point, but the nature of the diabetes is controversial. A recent study, hypothesized that investigating a known susceptibility factor for T1D or T2D can help in understanding the type and mechanism of diabetes in FCPD patients. In this study type 2 diabetes (T2D) associated two polymorphisms rs7903146 and rs12255372 in the TCF7L2 gene were analyzed in TCP and FCPD patients. Although no association was found with FCPD independently, data not only suggested that since, TCF7L2 is a major susceptibility gene for T2D, it may be hypothesized that the diabetes in TCP patients may not be similar to T2D. The data also suggested that co-existence of TCF7L2 variants and the SPINK1 and CTSB mutations, can predict susceptibility to exocrine damage, may interact to determine the onset of diabetes in TCP patients [52].

ACE gene- Angiotensin converting enzyme located on chromosome number 17. Phenotypic role - It is a zinc metallopeptidase which is a key enzyme of the renin-angiotensin system (RAS) and is known to proliferate hepatic stellate cells, has been hypothesized to play a role in pancreatic fibrosis in TCP patients.

Mutation- A polymorphism within intron 16 (g.11417_ 11704del287) of the ACE gene is strongly related to the circulating enzyme levels in a dose dependent manner.ACE insertion/deletion variant does not show any significant association with the pathogenesis, fibrosis and progression of tropical calcific pancreatitis and the fibro-calculous pancreatic diabetes[53].

CTRC gene - chymotrypsin C present on chromosome 1 in humans. Phenotypic role- Chymotrypsin C is a member of the peptidase S1 family. Trypsin-trypsinogen degradation by *CTRC* is an important mechanism for maintaining the physiological protease-antiprotease balance in the pancreas.

Mutation- Rosendahl et al identified chymotrypsin C mutation as a new pancreatitis-associated gene and discovered that loss-of-function alterations in the gene predispose to pancreatitis by diminishing its protective trypsin-degrading activity. The same was shown to be true with TCP patients.Due to CTRC gene mutation it may lead to misfolding of the mutant digestive enzyme in the endoplasmic reticulum (ER), causing ER stress, and killing the cell[54]. Its direct effect on development of FCPD is still obscure.

CASR gene- Calcium sensing receptor located on chromosome number 13. Phenotypic role- Acts as one of the protective mechanism to prevent immature activation and trypsin mediated autodigestion of the acinar cells. This receptor helps to maintain calcium at low level within the acinar cells, as calcium is required for trypsinogen activation.

Mutation- Inappropriate calcium sensing within the acinar cells leads to increased level of calcium within the cells leading to premature trypsinogen activation and autodigestion. 4 novel mutation in CASR gene has been identified and combination of SPINK1 gene mutation with CASR gene, increases the risk of TCP and FCPD[55,56].

Intergenic Interaction: Although the above mentioned mutation of the suspected genes have been documented as the causal factor of TCP but involvement of the genes (apart from SPINK1 gene) in progression of TCP to FCPD is yet to be analysed. As PRSS1 mutation is absent in TCP, but it shows the presence of CTSB polymorphism along with N34S SPINK1

varient mutation, it may hint towards epistatic gene interaction between CTSB and N34S SPINK1[48]. No association of Fibrocalculous pancreatic diabetes was found with restriction fragment length polymorphisms of the insulin receptor gene[57] but an association of Fibrocalculous pancreatic diabetes was found with the hypervariable region in the 5-prime flanking region of the insulin gene[57]. Although no association of fibrocalculous pancreatic diabetes was found with polymorphism of the HLA DR alpha/DQ alpha/DX alpha genes, an association was found with the Taq 1 restriction fragment length polymorphisms of the DQ beta gene which is similar to that found in type 1 but not type 2 diabetes[57]. Primary nature of diabetes in FCPD reveals a type 1 like feature in around 20% case as substantiated by anti-GAD and IA-2 positivty (Hassan et al 2005). FCPD shares common susceptibility genes with both Type 1 and I2 diabetes.

Metabolic and Environmental Stresses: Malnutrition: Protein calorie malnutrition has long been suspected as a likely cause for TCP because of the fact that the disease occurs predominantly in tropical countries where malnutrition is common and because of the reports from some of them including India, Uganda and Nigeria reveal that 80-90% of the subjects with calcific pancreatic come from poor socio-economic strata[58]. Chronic protein under nutrition leads to structural as well as functional alterations in the pancreas. It also makes females more susceptible to pancreato-toxins. A prospective study on north Indian patients revealed that malnutrition was a not a cause but an effect of tropical pancreatitis[59]. FCPD is considered as the secondary diabetic phase of TCP, therefore indirectly it can be predicted that malnutrition has no direct causal bearing on FCPD but malnutrition may have a modifier role by preventing appropriate absorption of protein in FCPD patients.

Environmental toxins: The toxic hypothesis has been centered on consumption of cassava which has cyanogenic glycoside and is used liberally in southern India where TCP is endemic[60]. This theory has also not found wide acceptance because of the following reasons: i. cassava does not feature in the diet of many people who develop TCP; ii. there was no difference in cassava consumption between patients with TCP and those without[61] iii. patients with TCP from northern India do not consume cassava[62] and iv. longterm cassava consumption did not produce diabetes or pancreatitis in a rat model[62]. Hence, cyanogenic toxin consumption cannot be considered as a environmental stress for FCPD development.

Micronutrient Deficiency and Free radical Injury: Chronic pancreatitis in white people has been linked to "heightened oxidative detoxification reactions" induced by cytochrome *P*450-1 within the pancreas and/or liver. It is possible that several factors, including chronic induction of the cytochrome *P*450-1 subfamily of mono-oxygenase by xenobiotics (cigarettes, alcohol, occupational chemicals, dietary corn oil, and so forth) may be involved[63]. Theophylline clearance (a measure of cytochrome *P*450-I activity in vivo) is faster in TCP subjects compared with controls suggesting a role of oxidant stress in causation of TCP[64]. Studies on the antioxidant status of our TCP patients showed low levels of vitamin C and β-carotene and this may well tilt the balance in favour of oxidant stress. Malnutrition induces a state of defective ability to scavenge free radicals, which could enhance the susceptibility for organ damage[65]. Braganza et al have shown that patients with alcoholic pancreatitis as well as other forms of chronic pancreatitis including TP are deficient in antioxidants and hence are more vulnerable to free radical injury[64]. It was also found that patients with TCP do have increased free radical mediated injury as evidenced by high levels of malondialdehyde and decreased anti-oxidant levels[66]. In a recent study on the oxidative stress (OS) and total antioxidant capacity (TAC) conducted on patients with TCP, oxidative stress was measured by lipid peroxidation products (LPO) and superoxide dismutase (SOD), and antioxidant capacity by Ferric reducing ability of plasma and the results showed that patients with tropical chronic pancreatitis had increased oxidative stress and decreased antioxidant capacity[67]. Hence, oxidative stress due to free radicals with decreased antioxidant capacity may act as an important factor for inducing or modifying TCP and FCPD.

Diet and Lifestyle: An experimental evidence showed that monkeys fed with high carbohydrate and low protein intake developed inflammatory and vascular changes in pancreas and heart[68] and the lesions mimicked those of FCPD. Pancreatic calculi was not observed in the study.The experimental diet used was somewhat identical to the diet in the developing countries, but the relevance of this study to FCPD is still not clear.

Geographical and cultural influence: Exploration of gene-environment interaction seems to be the next obvious step to clarify both the nature of pancreatitis and diabetes in TCP and FCPD. A preliminary study in the western half of Bangladesh indicates a north-south gradient in the prevalence of FCPD and this may indicate a possible link with geographical and cultural factors (as the population is genetically homogeneous). However, large scale community based studies, using the genetic, biochemical and epidemiological instruments, are now needed in this area[10].

Altered Immune Response: Occurrence of cell mediated immunity and association of autoimmunity with pancreatic antigen was found in 42.9% of FCPD patients in an independent study conducted[69]. Hence, altered immune function may be an additional domain to induce FCPD.

Relation between TCP and FCPD

The relationship between tropical calcific pancreatitis (TCP) and fibrocalculus pancreatic diabetes (FCPD) is still unclear. Comparison of the age of onset shows that FCPD patients had a late age of onset with a difference of more than a decade (21 yrs vs. 35 yrs). It has also been shown that the patients with TCP

are younger than the FCPD patients and majority of them have an abnormal Glucose tolerance test. This strongly suggests that FCPD may be gradually evolving diabetes in the background of TCP[70]. N34S variant of SPINK 1 is a susceptible gene for FCPD Bhatia et al also found a strong association with SPINK 1 trypsin inhibitor mutations and a high prevalence of N34S in FCPD and TCP without diabetes suggest that both entities have similar genetic predisposition[71]. However, different genetic/environmental factors may be involved to account for phenotypic variability in TCP patients with FCPD[68]. Fibrocalculus pancreatic diabetes patients present with a significantly lower BMI compared with TCP patients. Analysis of the family history reveals that some kind of environmental factors seem to play a predominant role in the development of diabetes in FCPD patients, although these factors remain to be identified. Both TCP and FCPD patients predominantly come from a rural background[68] Measurements of early renal haemodynamic and microvascular changes (glomerular filtration rate, kidney size, microalbuminuria and microtransferrinuria) indicate an early renal involvement in FCPD patients[72]. Tropical calcific pancreatitis subjects have approximately twice as high fasting C-peptide values compared with FCPD patients. Findings of single stranded DNA measurements suggest the involvement of oxidative damage in FCPD patients. Ketosis resistance is the most conspicuous clinical feature in the FCPD group and this relative absence of ketosis is probably due to a defect in the ketone body synthesis pathway and/or in the regulation of counterbalancing hormones[68]. Hence it can be predicted that FCPD should not be considered only as a form of secondary diabetes consequent to generalized pancreatic damage in TCP. FCPD is found to be extremely heterogenous with respect to the diabetic symptoms shown by the patients, some require only oral drug treatment while others with ketosis require insulin for survival.

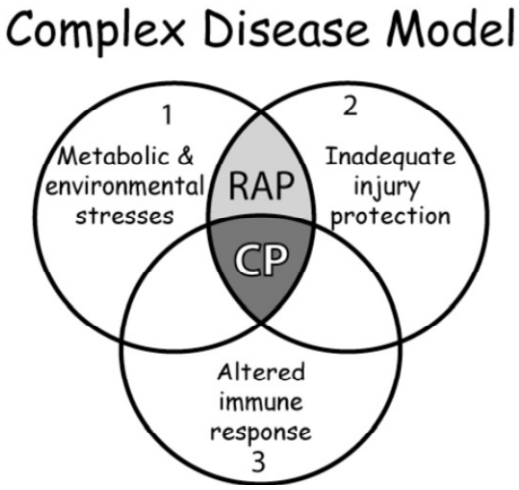
Complex Disease Model

This model tend to differentiate between TCP and FCPD, as both present with more or less similar genetic basis- SPINK gene mutation[68] although having different clinical profile.

Chronic pancreatitis is modelled as a complex trait in which one or more factors must be present in each of at least three domains before chronic pancreatitis develops. The three major genes with mutations that increase susceptibility to chronic pancreatitis (PRSS1, SPINK1 and CFTR) are all in the domain of "inadequate injury protection" and lead to recurrent acute pancreatitis (RAP) in the presence of a sufficiently strong metabolic or environmental stressor. Only the subset of patients with an altered immune response favoring fibrosis develop chronic pancreatitis (CP), but this response requires RAP to direct it to the pancreas rather than other organs[68].

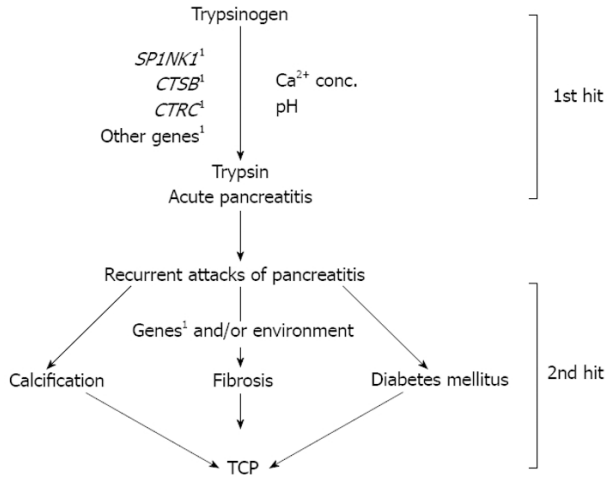
In patients with recurrent acute pancreatitis: – Normal response = healing, Factor "A" Anti-inflammatory immune response = fibrosis, Factor "B" = B-type - severe, continuous pain, Factor "C" = Calcifications, Factor "D" = Diabetes mellitus.

Types of "tropical pancreatitis": RAP + A+B+C = tropical calcific pancreatitis (TCP), RAP + A+C+D = fibrocalulous pancreatic diabetes (FCPD).



Relationship between TCP and FCPD according to Complex Disease Model

Since TCP is a complex disease, in addition to candidate gene analysis which has undoubtedly been influential, there is a necessity for a more comprehensive and holistic approach to understand its etiopathogenesis, to help early detection and discover possible treatment. The role of environmental factors as disease modifiers cannot be undermined. An in-depth study of the contribution of dietary- and lifestyle-related factors, and their association with genetic variants would yield interesting leads.



Two hit model for the pathogenesis of TCP. First hit contributing to the pathogenesis of TCP is likely to be loss of balance between activation events and degradation of active trypsin leading to presence of persistent "super-trypsin after Swapna Mahurkar, D Nageshwar Reddy, G Venkat Rao, and Giriraj Ratan Chandak as given in "Genetic mechanisms underlying the pathogenesis of tropical calcific pancreatitis".

Conclusion

Inapproprite activation of trypsinogen (mainly cationic trypsinogen) to trypsin wi thin the pancreatic acinar cells is mainly due to inadequate and inappropriate serine protease inhibitors (coded by SPINK1 gene) cathepsin B for the untimely activation of cationic trypsinogen within the zymogen granules inside the acinar cells along with loss of chymotyrpsin C function (which in turn would lead to degragation of actve trypsin). These mutation if supplemented by loss of calcium sensing genes within acinar cells lead to increase level of intracellular calcium and pH alteration which stimulates premature activation of trypsinogen. So even without reported PRSS1 and2 mutation that encodes trypsinogen itself, FCPD patients suffer from severe exocrine pancreatic insufficiency. Occurrence of these events along with environmental stress factors like diet, lifestyle, oxidative stress and free radical injury leads to acute pancreatitis and recurrent bouts of acute pancreatitis are actually manifestation of chronic pancreatitis found typically in tropical belt. Hence irreversible exocrine pancreatic damage with fibrosis, calcification and insufficient endocrine function autoimmune dysfuctioning and excessive inflammatory reaction leads TCP to progress to FCPD. Although TCP and FCPD has common genetic predisposition but varied environmental and immunologic factors may play the causal or modifier role for progression of TCP to FCPD.

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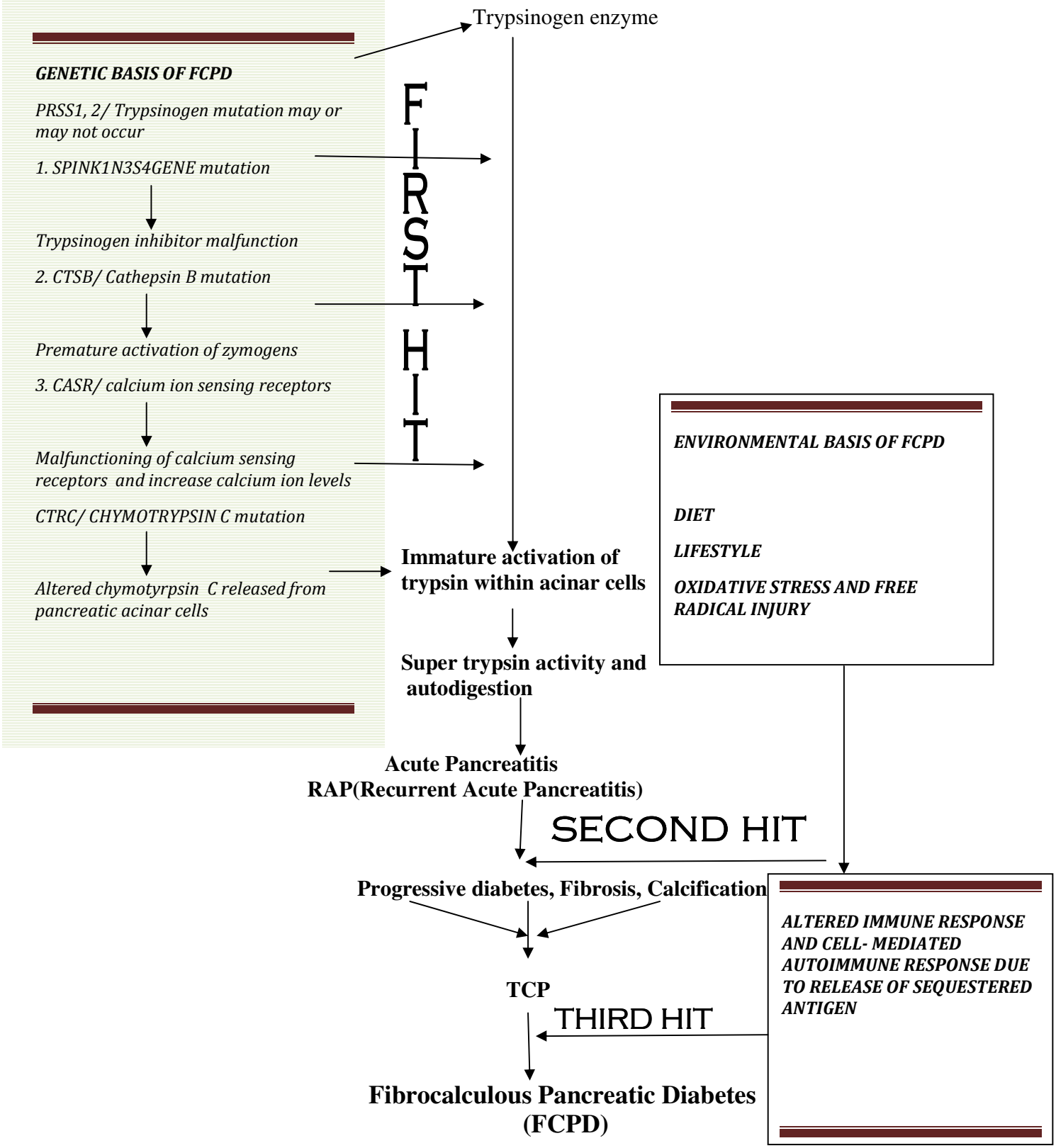

Figure-
Three Hit Model to Trace the Aetiology of FCPD