To study the additive predictability of Non-HLA markers in first degree relatives of children with celiac disease

ADPREP STUDY (Additive Predictive Profile)
Study of non HLA markers (Polymorphisms of IL-18 gene & Citrulline) in first degree relatives of patients with celiac Disease
PART I: GENERAL INFORMATION

Name of the Institute/University/Organization submitting the Project Proposal:

- Name: Maulana Azad Medical College
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Status of the Institute: Teaching Tertiary care Medical College

Name and designation of the Executive Authority of the Institute/University forwarding the application:

- i. Name: Dr. Arun Aggarwal
- ii. Sex M/F: Male
- iii. Date of Birth: 2nd March 1963
- iv. Qualification: MBBS, MD
- v. Designation: Professor
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Project Title: To study the additive predictability of Non-HLA markers in first degree relatives of children with celiac disease.

Category of the Project:

Research & Development called for under invited proposals on Celiac disease with a focus on gluten in wheat

- Development, standardization and validation of diagnostic tests and non-invasive biomarkers for celiac disease

Duration: Three Years

Total Cost (Rs.) 51,60,800

Is the project Single Institutional or Multiple-Institutional (S/M)?: Single
**Scope of application indicating anticipated product and processes**

**SCOPE OF APPLICATION:**
Celiac disease, an autoimmune enteropathy that affects the proximal small intestine, is characteristically seen in people who have a genetic susceptibility to gluten sensitivity. Celiac patient’s first-degree relatives are more at risk of acquiring the disease (10%). The objective of the present study is to evaluate the additive predictive value of Non HLA markers like polymorphism of the IL-18 gene and plasma citrulline as genetic and metabolomic signature in predicting celiac disease.

In India where the prevalence of celiac disease is as high as 1 in 100; evaluating first degree relatives may be of vital importance as they may be detecting in the silent phase or as a potential celiac where defining may help stringent follow up and early institution of Gluten free diet.

**ANTICIPATED PRODUCT:**
Project may help create an algorithm for screening first degree relatives of celiac using a build up on the HLA markers utilizing non HLA markers to add to predictive value. Citrulline will also help in following progress and compliance on gluten free diet as it is an important marker of enterocyte damage.

**PROCESS:**

- **Index Case; Complete study**
  - Clinical Variables, Plasma Citrulline, Marsh Score, Ttg Titres, HLA Genotype, IL18 Polymorphisms

- **Recruitment of first degree relatives**
  - Clinical Variables, Plasma Citrulline, Marsh Score, Ttg Titres, HLA Genotype, IL18 Polymorphisms

- **Statistical Analysis**
Project Summary

Summary

Celiac disease is a chronic immune mediated, inflammatory disorder of small intestine triggered by exposure to dietary gluten (Dicke et al. 1950) [1]. It is an important health problem in children of India because of its prevalence range from 1 in 310 (Sood A et al. 2006) [2] to 1 in 100 (Bhattacharya M et al.2009) [3]. Family members of subjects with CD constitute a high risk group and may have atypical and silent CD. Certain HLA haplotype show a strong association with celiac disease, the strongest association signal is HLA class II molecule DQ2 and DQ8.(Tosi et al.1983)[4]. The HLA DQ2 and DQ8 heterodimers are necessary but not sufficient, on its own for the development of celiac disease as they are also common in the healthy population (Polvi et al. 1996) [5]. This indicates that there are other risk genes for celiac disease in the human genome. Several attempts have been made to identify the non-HLA risk genes and genetic variants for celiac disease. In addition to DQ2 and DQ8 coding haplotype, others susceptibility loci for CD are now being evaluated through genome wide linkage and association studies, candidate gene studies, their fine mapping and follow up studies. One of the candidate gene is IL-18 which in synergy with IL12 promotes development of the Th1 lymphocyte response by induction of IFN-γ is a gene likely to be a strong predictor or a strong non HLA marker. Furthermore, increased serum levels of IL18 have been identified in patients with autoimmune diseases. Therefore the additive predictability of Non-HLA marker like IL-18 gene with two polymorphisms at position -607, position -137 in the promoter region might be a useful potential biomarker targeting celiac disease in at risk population. In this study the prevalence of DQ-2 and DQ-8 and IL-18 gene polymorphism in CD patients and in their first degree relatives will be determined.

With the advent of mass spectrometry plasma citrulline has been used as a simple analyte which may also help decision on gut permeability in celiac disease. Other useful amino acids are glutamine and arginine. Citrulline may be a good metabolomic signature suggesting subclinical intestinal damage and may actually help in additive predictive value in detecting potential celiacs amongst first degree relatives of a case. Thus this study aims at

1. Revalidating usefulness of Class II HLA markers in celiac disease
2. Evaluating the utility of non HLA markers as polymorphisms of IL -18 gene involved in pathogenesis of intestine mediated damage
3. Evaluating the utility of citrulline as a metabolomic signature in predictive value and follow up value in celiac disease; suggesting it to be a marker of enterocyte villous damage
PART II: PARTICULARS OF INVESTIGATORS

Principal Investigator:

i. Name  
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Number of research projects being handled at present: FOUR.

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Number of research projects being handled at present: Five

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Number of Research projects being handled at present: NONE

Co-Investigator

i. Name  
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Number of Research projects being handled at present: TWO
INTRODUCTION/BACKGROUND (Origin of the proposal)

Celiac disease together with other common immune-mediated diseases, such as type 1 diabetes, rheumatoid arthritis and asthma falls into the category complex diseases because it appears to be causally influenced by multiple risk factors. These factors can be genetic or environmental, or may be comprised of interplay between both. Complex diseases can present with a variety of symptoms and disease manifestations, and they do not conform to the classical Mendelian inheritance patterns, although familial clustering can often be seen. As complex diseases affect a relatively large portion of the human population, and they are a major burden on health care systems, substantial resources are invested in understanding the etiology of these diseases and developing better tools for disease diagnosis and individualized treatment. The challenge to research efforts is that the contribution of each risk factor to the disease is often very slight, and even the healthy population has varying degrees of predisposition in relation to the risk factors. Celiac disease also belongs to a group of inflammatory diseases with autoimmune feature. Its unique feature is that the major environmental risk factor for celiac disease is known: the pathology is triggered by exposure to dietary gluten (Dicke 1950) [1].

Other environmental risk factors for celiac disease also involved. It is well recognized that certain haplotypes within the major histocompatibility complex (MHC) region constitute the main genetic risk factors for celiac disease (Sollid et al. 1989) [6]. Their role in the pathogenesis of the disease has also been confirmed (Molberg et al.1998) [7], making celiac disease a relatively well-characterized disease when compared to several other disorders with complex etiology.

SPECTRUM OF CELIAC DISEASE

Celiac disease is having varied manifestations responsible for wide spectrum of clinical presentations. The varying picture is difficult to understand. Clinical presentations are in form of:

I. Classical celiac disease
The classical or active form is one which manifests with overt clinical picture characterized by the absence of villi (villous atrophy), chronic diarrhea with mal absorption, failure to thrive, weight loss, fatigue and anemia.
2. Silent celiac disease
Silent celiac disease patients are those who are asymptomatic but small intestinal biopsy show villous atrophy. Silent cases are detected by population screening and screening of first degree relatives of celiac disease, 10% of whom are found to have CD.

3. Latent celiac disease
Latent celiac disease patients are those who have a normal jejunal biopsy while on a normal diet but at some other time, before or since, have recovered from a flat jejunal biopsy while on gluten free diet. These patients are in a latent phase of the disease or pre-celiac state that may become symptomatic when exposed to high dose of gluten. They have cellular infiltrate and immunologic response equivalent to active CD.

4. Potential celiac disease
Potent celiac disease patients are those who have a normal jejunal small bowel villous architecture on gluten, but still gluten sensitive. They have the potential to develop celiac disease. In a study, Collin et al. have shown that 30% developed villous atrophy over a period of 7 years. Probably potential celiac disease cases would require a second insult to evolve to the full blown picture of severe mucosal damage. Factors which have been postulated to act as a second insult are, temporary increase in intestinal permeability, an increased gluten intake or adjunct effect of intestinal infections. Potential celiac disease may be suspected in individuals who show positive serological tests (positive EMA, AGA or tTg), a high positive immunological response as seen in active CD and a positive rectal gluten challenge.

5. Refractory celiac disease
Refractory celiac disease is a rare condition which can be defined as persisting or recurring villous atrophy and crypt hyperplasia with increased intraepithelial lymphocytes despite strict gluten-free diet for at least 12 months. Refractory celiac disease can also manifest with severe persisting symptoms and require interventions independent of the duration of gluten-free diet. Refractory celiac disease has two categories. Patients with type I refractory celiac diseases do not have aberrant T cells in their small intestinal epithelium and seem to profit from immunosuppressive treatment. Patients with type II refractory celiac disease demonstrate an aberrant clonal intraepithelial T cell population, and have a high risk of developing overt lymphoma. Type II refractory celiac disease is usually resistant to immunosuppressive therapies: cytotoxic chemotherapy and autologous stem cell transplantation have been suggested for treatment. (Al-Toma et al. 2007) [8]

With the increasing prevalence of celiac disease, so-called atypical symptoms and manifestations of celiac disease have become common and the disease can even be apparently symptom-free. The studies have shown that genetic components play a major role in the induction and manifestation of celiac disease as celiac disease is more common in first degree relatives of the index case. Family studies have also shown that the prevalence of celiac disease is 10% among first-degree relatives of gluten intolerant patients. Till date, studies have evaluated HLA markers but limited studies have evaluated Non-HLA markers and probably none in this ge-ethnic population. Since our understanding of the genetic background and the pathogenesis of celiac disease is incomplete, and is the focus of cutting-edge research.

RATIONALE OF THE STUDY

In view of the rising prevalence of celiac disease to the tune of 1 in 100 (Bhattacharya M et al.2009) [3] in India and considering that first degree relatives would be the most susceptible high risk cohort; screening these potential celiac using non invasive polymorphic and metabolic markers may help quantify the proportion of the cohort which may then be counseled for stringent follow up and corrective measures taken early. This study also stems from the fact that though HLA remains a strong predictor may not be the only molecule conferring susceptibility. ( Polvi et al. 1996) [5]. This study also envisages that citrulline would be a good biomarker which is independent of the nutritional status for susceptibility testing in recognizing subclinical damage, in follow up and adjudging adherence to gluten free diet.
Hypothesis

1. To the date, the HLA locus remains the strongest single risk locus for celiac disease, with the other identified loci having risk effects not quantified in different ethnic groups. IL-18 since is involved in immunopathogenesis of celiac disease; polymorphisms of the IL-18 gene may also help us in additive predictability of the disease in first degree relatives of patients affected by celiac disease.

2. Citrulline (CIT), a non-protein amino acid in circulating blood, is almost exclusively contained in the enterocytes of small bowel mucosa and may represent a reliable marker of functioning enterocyte mass. The aim of this study will be to evaluate the clinical utility of measuring serum citrulline levels in a group of first degree relatives of patients affected by celiac disease. This may help in predicting subclinical damage in potential or latent celiac disease; a high risk cohort being first degree relatives of index patients.

KEY QUESTIONS

1. Do Polymorphisms of the IL-18 gene confer additive predictability over and above evaluation of class II MHC HLA genotype
2. Is plasma citrulline a reliable biomarker which may both predictive value being a marker of enterocyte damage
3. Can we frame a simple algorithm for further utilization of these 2 non HLA markers in the Indian Context?

National status:
As per published data the prevalence of celiac disease in India ranges from 1 in 310 (Sood A et al. 2006) [2] to 1 in 100 (Bhattacharya M et al. 2009) [3]. The disease distribution is slightly higher in urban population. Early diagnosis in urban patients may be because of their direct presentation to tertiary care hospitals while delayed diagnosis in rural population shows that celiac disease is still not being diagnosed at primary health care centers. Celiac disease in India was first spotted in 1966 from New Delhi (Walia S et al. 1972) [9]. In Asian migrant population, celiac disease was described in 1972 (Nelson R et al. 1973) [10]. In India, flexible endoscopic facilities became available to children during second half of 1980's. To take biopsy from jejunum with Crosby capsule was difficult and it was a traumatic experience for the child. Moreover, fluoroscopic help is also needed for this procedure (Sahni A et al. 1989) [11]. But these facilities are not available in all the centers in our country. There is lack of expertise also. In 1980's, the role of antigliadin antibodies, antireticulin antibodies, anti-endomysial antibodies and antitissue transglutaminase was defined and it was shown that these are very sensitive and specific in diagnosing celiac disease. Recently Deamidated Gliadin Peptides, reported to be very sensitive and specific of celiac disease. The main differences in the clinical features of celiac disease as highlighted by the studies from northern India (Meeuw W et al. 1970) [12] as compared to west (Misra R et al. 1966) [13] are the higher incidence of failure to thrive and anemia in India. These two clinical manifestations are features of more severe disease in our country.

With increasing awareness about the varied manifestations of CD and with the availability of reliable non invasive markers (celiac serology) and with the help of genetic testing of HLA marker (Q2 and DQ8) for the disease, it is now being recognized in the atypical form with early diagnosis as compared to earlier studies.
**International status:**

**CLINICAL CHARACTERIZATION**

The clinical picture of celiac disease was first described by Samuel Gee in 1888 (Gee et al. 1888) [14], although the adverse effects of ingested gluten were recognized over 60 years later (Dicke et al.1950) [1]. The various primary symptoms of celiac disease are caused by a flattening of the small intestinal mucosa, known as partial, subtotal or total villous atrophy. Severe mal absorption syndrome has also been common among patients affected with celiac disease. (Mäki and Collin 1997) [15]. The clinical picture of celiac disease has changed over time, and the clinical signs of celiac disease are becoming milder than the symptoms that have previously been defined as classical symptoms. Along with the increasing prevalence of celiac disease, so-called atypical symptoms and manifestations of celiac disease have become common and the disease can even be apparently symptom-free. There are several atypical symptoms associated with celiac disease, such as various neurological symptoms, dental enamel defects, infertility, osteoporosis, aphthosis of the mouth mucosa, joint symptoms and elevated liver-enzyme concentrations and isolated short stature.

**DIAGNOSTIC CRITERIA**

The diagnostic criteria for celiac disease, established by the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN), are small bowel mucosal atrophy with improvement or normalization on a gluten-free diet, and the presence of circulating antibodies specific for celiac disease (Working group of European Society of Pediatric Gastroenterology and Nutrition 1990). Typically, the first step in diagnosis is a serologic test, where celiac disease-specific antibodies (IgA specific for tissue transglutaminase [TGM2] and IgA endomysial antibody [EMA]) are measured from the peripheral blood of the patients.

For IgA-deficient patients a serological test based on immunoglobulin G (IgG) antibodies can be performed. Small intestinal villous patterns from the biopsy specimens are assessed based on Marsh and Oberhuber’s classification of duodenal histological lesions (Oberhuber et al. 1999)[16].Diagnosis of DH is based on the demonstration of granular IgA deposits in sub-epidermal skin (van der Meer 1969)[17]. Traditionally, both positive serology and biopsy results are required for a presumptive diagnosis of celiac disease. However, as the clinical picture of celiac disease has been changing during the past years, gluten intolerance is no longer restricted only to villous atrophy. It is now recognized that individuals with normal mucosal villous structure can have celiac disease, with villous atrophy thought to develop gradually during the disease process (Mäki and Collin 1997) [15]. Genetic testing for HLA DQ2 and DQ8 is very important in diagnosing celiac disease.

**Genetics of celiac disease**

The mode of inheritance of celiac disease is not known, although there is strong evidence for genes playing an important role in the disease susceptibility. The proband-wise concordance for celiac disease is 75-86% between monozygotic twins (Nistico et al. 2006) [18], whereas the proband-wise concordance of celiac disease between dizygotic twins has been estimated to be 16.7-20% (Nistico et al. 2006) [18]. The difference in concordances between monozygotic twins and dizygotic twins provides an estimate of the size of the genetic component in celiac disease, which appears higher than in many other complex immunological disorders. According to (Nistico et al. 2006) [18], the heritability of celiac disease is up to 87%. Family and twin studies can be used in estimating the proportion of genetic and environmental risk factors in the disease prevalence because celiac disease is more common in first degree relatives of the index case. The studies have shown that genetic components play a major role in the induction and manifestation of celiac disease. **Family studies have also shown that the prevalence of celiac disease is 10% among first-degree relatives of gluten intolerant patients (Dube et al. 2005) [19].**
**HLA genes**

The MHC region on human chromosome 6p21.3 is the most polymorphic region in the entire human genome. The allelic diversity of certain genes is extremely high, e.g. the HLA-B gene has more than 1100 known alleles and the HLA-DRB1 gene has more than 600 known alleles (IMGT/HLA Database: http://www.ebi.ac.uk/imgt/hla/allele.html - 12.02.2009) (Robinson et al. 2003) [20]. The celiac disease-associated DQB1 and DQA1 genes have 96 and 35 alleles, respectively. The MHC region harbours several genes that have crucial roles in controlling and regulating immune responses. The HLA genes can be divided into three classes according to their main functions: HLA class I and II genes are responsible for presenting antigens; HLA class III genes comprise a more heterogenic group of various, mostly immune related genes. HLA genes are associated with several autoimmune and inflammatory disorders (Revelle et al. 2006) [21] such as ankylosing spondylitis (with B27), narcolepsy (with DQB1*0602), type 1 diabetes (with DRB1*0301-DQB1*0201 and DRB1*04-DQB1*0302), rheumatoid arthritis (with DRB1*0401/0404/0405/0101), systemic lupus erythematosus (with DRB1*0301/*1501/*1503*/08), Siögren’s syndrome (with DRB1*0301), systemic sclerosis (with DRB1*11/*0301/*1502), and autoimmune thyroid disease (Graves’ disease a autoimmune thyroiditis) (with DRB1*0301-DQA1*0501-DQB1*0201 haplotype). Some of these associations are relatively weak, whereas for other diseases the particular HLA haplotype is always required for disease onset.

Certain HLA haplotypes show a particularly strong association with celiac disease, which was demonstrated as early as 1972. The strongest association signal was subsequently refined to the HLA-DRB1 gene which has 606 known alleles. The celiac disease-associated DQB1 and DQA1 genes have 96 and 35 alleles, respectively. The MHC region harbours several genes that have crucial roles in controlling and regulating immune responses. The HLA genes can be divided into three classes according to their main functions. HLA class I and II genes are responsible for presenting antigens; HLA class III genes comprise a more heterogenic group of various, mostly immune related genes. HLA genes are associated with several autoimmune and inflammatory disorders (Revelle et al. 2006) [21] such as ankylosing spondylitis (with B27), narcolepsy (with DQB1*0602), type 1 diabetes (with DRB1*0301-DQB1*0201 and DRB1*04-DQB1*0302), rheumatoid arthritis (with DRB1*0401/0404/0405/0101), systemic lupus erythematosus (with DRB1*0301/*1501/*1503*/08), Sjögren’s syndrome (with DRB1*0301), systemic sclerosis (with DRB1*11/*0301/*1502), and autoimmune thyroid disease (Graves’ disease a autoimmune thyroiditis) (with DRB1*0301-DQA1*0501-DQB1*0201 haplotype). Some of these associations are relatively weak, whereas for other diseases the particular HLA haplotype is always required for disease onset.

The HLA DQ2 and DQ8 heterodimers are necessary but not sufficient, on their own, for the development of celiac disease, as they are common also in the healthy population (Polvi et al. 1996) [5]. This indicates that there are other risk genes for celiac disease in the human genome. Several attempts have been made to identify the non-HLA risk genes and genetic variants for celiac disease. Along with increasing knowledge about variation in the human genome, as well as innovations to genotyping methodology, our understanding of the genetic factors predisposing to celiac disease is growing.

**IL-18 genes**

Candidate gene *IL18* (112.0 Mb) is also known as interferon-gamma-inducing factor. Though this gene, located at chromosomal position 11q22.2-22.3, is some distance from the major linkage peaks in the region, it may still fall within the region of linkage as it is located between a marker shown to be linked to disease (D11S4111) at position 115.8 MB and the next tested marker (D11S898) at 101.0 MB which was not linked to disease (Holopainen et al.2001)[24]. *IL18* is a pro inflammatory cytokine which, in synergy with IL12, promotes development of the Th1 lymphocyte response by induction of γ interferon (IFN-γ). The latter is highly produced in Celiac Disease lesions, and is known to play an important role in inflammatory and infectious diseases (McInnes IB et al.2000) [26]. Furthermore, increased serum levels of *IL18* have been identified in patients with autoimmune diseases such as RA (Gracie JA et al.1999) [26] and acute asthma (Tanaka H et al.2001) [27]. Two polymorphisms in the promoter region of the gene have shown evidence of altering *IL18* protein expression. One polymorphism located at position -607 has been found to disrupt a potential cAMP response element protein-binding site, while the other at position -137 alters a consensus H4TF-1 nuclear factor binding site. Multiple sclerosis patients homozygous for the -607C and -137G alleles have higher levels of *IL18* mRNA compared to other diplotypes, suggesting that these polymorphisms do indeed regulate activity of the gene (Giedraitis V et al. 2001) [28]. More recent results point to a haplotypic effect based on other polymorphisms (Barboux et al. 2007) [29]. Numerous genetic association studies have been carried out on these SNPs and others in the gene, to investigate if any association exists with various autoimmune diseases. Significant association has been shown between *IL18* and type 1diabetes, Crohn’s disease, inflammatory bowel disease, R.A and asthma.
**Citrulline in celiac disease**

In human, citrulline (plasma concentration about 40 micromol/L) is an amino acid involved in intermediary metabolism and that is not incorporated in proteins. Circulating citrulline is mainly produced by enterocytes of the small bowel. For this reason plasma or serum citrulline concentration has been proposed as a biomarker of remnant small bowel mass and function (Crenn P et al. 2008) [30]. This pilot study evaluated more than 500 patients and observed that decreased level of plasma citrulline correlated with the reduced enterocyte mass independently of nutritional and inflammatory status. A close correlation between small bowel remnant length and citrullinemia was found. In addition, diagnosis of intestinal failure was assessed through plasma citrulline levels in severe small bowel diseases in which there is a marked enterocyte mass reduction. Thus in India where protein energy malnutrition is commonplace citrulline may still serve as a reliable indicator. However Miceli E evaluated fifty healthy volunteers, 21 patients with untreated coeliac disease and 6 patients with refractory coeliac disease Serum citrulline levels and duodenal lesions were evaluated at the time of diagnosis, and after at least 24 months of gluten-free diet. They concluded that, serum citrulline levels turned out to be low in coeliac disease, the clinical utility of their measurement is, at least, questionable in this condition (Miceli E et al. 2008) [31]. Hence the utility of measuring needs further exploration.

**The relevance of the proposed study:**

Till date, studies have evaluated HLA markers but limited studies have evaluated Non- HLA markers and probably none in this geo- ethnic population. Considering this to be a disease of significant impact, if the first degree relatives of index case are detected early, this may help in early prediction of the disease and more stringent follow up of screen positive first degree relatives. This may improve our understanding of the genetic background and if plasma citrulline proves to be a good biomarker, it may help in prediction, diagnosis and prognosis of potential and latent celiac disease.

**OUTCOME & SIGNIFICANCE OF THE STUDY**

1. Help in understanding non HLA markers involved in pathogenesis of celiac disease in susceptible high risk group.
2. Understand utility of citrulline as a biomarker in celiac disease.

**Other implications of the study in first degree relatives**

With the increasing prevalence of celiac disease, so-called atypical symptoms and manifestations of celiac disease have become common and the disease can even be apparently symptom-free. Of potentially even greater significance are reports on relatives without celiac disease at risk for other diseases, often associated with celiac disease. For example, a serological survey study of first-degree relatives of children with documented celiac disease suggested that autoimmune diseases may be increased in addition to biopsy-defined, but “sub-clinical” celiac disease. In addition, first-degree relatives may also develop the closely linked dermatological disorder, Dermatitis Herpetiformis (Reunala T et al. 1996 )[32]. Moreover, patients with type-1 diabetes have an increased risk of celiac disease and biopsy-defined, but essentially asymptomatic and unrecognized celiac disease (Marchese A et al.2012) [33]. Finally, although lymphoma risk seems to have fallen in the past 4 decades, individuals with first degree relatives affected with celiac disease have an increased lymphoma risk(Hervonen K et al. 2005) [34]. These studies, then, have important implications for risk of familial forms of celiac disease in relatives, particularly first-degree, but also for a host of other genetically-related clinical disorders, even in the absence of celiac disease. In future, expression of different celiac disease phenotypes and their individual specific risks of different diseases may be more readily defined with precise genetic markers or more precise genetic signatures.
Preliminary work done by the Investigator on this problem

The laboratory is diagnosing celiac disease since 2007 and has 350 patients diagnosed till date. A pediatric gastroenterology clinic runs every Friday and caters to the need of these patients including issues of compliance. The other co-investigators Dr A.S. Puri is Head Department of Gastroenterology which caters to adult celiac which has 100 patients on follow up. Dr Praveen and his team cater to a different geo-ethnic area and have 690 patients on follow up.

Plasma citrulline has been evaluated in 30 patients with celiac disease and found to be low. This has been done by tandem mass spectroscopy using ABI LCMSMS and later confirmed by amino acid analysis using post column derivitization using ninhydrin.
AIMS & OBJECTIVES

- To study the prevalence of HLA types DQ2 and DQ8 in first degree relatives of an index case with celiac disease. This would be evaluated by the % of first degree relatives testing positive for DQ2 & DQ8 as the verifiable indicator.

- To study the polymorphisms of the IL-18 gene in patients with Celiac disease and their first degree relatives. The polymorphisms at position & would be evaluated in the index cases and their first degree relatives with equal number of non-related controls of the same ethnicity. This will answer whether polymorphisms of the IL-18 gene are more prevalent in case versus control population as an indicator to disease susceptibility in our index cases. Further the number or proportion of these being positive in first degree relatives of index case would confer the predictability of this marker in first degree relatives. Both would be verifiable indicators.

- To study the utility of plasma citrulline as a marker for subclinical presentation and enterovillus loss in first degree relatives of patients with celiac disease. Citrulline concentrations in index cases and their first degree relatives would be evaluated. In index cases Plasma citrulline levels would be compared with degree of villous atrophy adjudged using Marsh score. In first degree relatives of index case and in matched healthy controls citrulline estimation would be done to evaluate this potential biomarker for predictive use as a verifiable indicator

- To assess the additive value of both as susceptibility markers in patients with celiac disease and in their first degree relatives above values generated from HLA status would be computed.

PLAN OF WORK (METHODOLOGY)

Inclusion criteria for subjects:

Confirmed cases of celiac disease up to 18 years and their first degree relatives.

Exclusion criteria for subjects:

Cases of celiac with renal dysfunction

Criteria for Controls:

Age and sex matched healthy subjects who are not relatives or siblings of the index cases.

Sample Size:

Total 1000 samples (first degree relatives)

Variables:

Study group would be the index case for which the parameters noted would be:

1. Presentation- typical/atypical
2. Presence of co-morbidities
3. Marsh score
4. TTG/DgP titres
5. HLA status
6. Polymorphisms of the IL-18 gene
7. Plasma citrulline levels
Variables to be evaluated in the siblings

1. Clinical presentation
2. Co-morbidities
3. TTG & Deamidated gliadin protein
4. HLA status
5. Polymorphisms of the IL-18 gene
6. Plasma citrulline levels

Methodology for the study of IL-18 polymorphism

Amplification of IL-18 gene promoter region

The -607 and -137 polymorphisms will be detected using sequence-specific PCR as described by Giedraitis et al., (2001) [28]. A common reverse primer 5’- TAACTCATCAGGACTTCC-3’ and two forward primers 5’- TTGCAGAAGTGTAAATTATTAC-3’ and 5’- TTGCAGAAAGTGAAAAATTATTAA-3’ is used for position -607 specific PCR. An amplification product of 196-bp will be detected. A control forward primer 5’- CTTGGCTATCATCAGGAGAA-3’ will be used to amplify a 301-bp fragment encompassing the polymorphic site to serve as an internal positive amplification control. PCR reactions will be performed in a final volume of 20 μl consisting of 50 mM KCl, 10 mM Tris–HCl pH 8.3, 2.0 mM MgCl2, 0.20 mM dNTP, 50 ng genomic DNA and 0.5 U Taq polymerase. One sequence specific primer (for C allele or A allele) and the common reverse primer will be included in every reaction mixture at a concentration of 0.4 μM. In addition, the internal positive control primer will be added to reaction mixtures at a concentration of 0.4 μM. Two PCR reactions will be performed for every individual DNA sample. Initially, denaturation will be performed at 94°C for 2 min. This will be followed by seven cycles of 94°C for 20 s, 64°C for 40 s and 72°C for 40 s and 25 cycles of 94°C for 20 s, 57°C for 40 s, 72°C for 40 s and a final extension will be done at 72°C for 5min. Amplified products will be visualized on 2% ethidium bromide-stained agarose gel following electrophoresis. For position -137 specific PCR, a common reverse primer 5’ AGGAGGGCAAAAATGCAGTGG-3’ and two forward primers 5’- CCCCAACTTTTACGGAAAGAAAC-3’ and 5’- CCCCAACTTTTACGGAAAGAAAC-3’ will be used. An amplification product of 261-bp will be detected. A control forward primer 5’- CCAATAGGACTGATTATTCCGCA-3’ will be used to amplify a 446-bp fragment encompassing the polymorphic site to serve as an internal positive amplification control. PCR reactions will be performed as mentioned for position -607 except that the primer concentrations were 0.4μM for internal control and 0.5μM for the reverse and specific forward primers used.

Methodology for the study of HLA typing

HLA-DQ2 heterodimer is coded by the DQA1*05 and DQB1*02 alleles. The DQ2 heterodimer can also be encoded in trans configuration by the DQ2.2 (DR7-DQ2) and DQ7 (DR5/6-DQ7) haplotypes, with the DQA1*05 allele deriving from DRB1*11, *12 or *13 haplotypes (DRB1*11/12/13, DQA1*0505, DQB1*0301) and the DQB1*02 allele deriving from a DRB1*07 haplotype (DRB1*07, DQA1*02, DQB1*0202). The DQ8 heterodimer encoded by the DR4-DQ8 haplotype (DRB1*04, DQA1*03, DQB1*0302). It is common in celiac patients who do not carry the DQ2 heterodimer. Both DQ2 and DQ8 heterodimers have a central role in the pathogenesis of celiac disease. HLA DQ2 & DQ8 typing will be carried out in index case and all first degree relatives by PCR amplification with above listed primers as described by O.Olerup et al 1993. [35]
<table>
<thead>
<tr>
<th>HLA (DQ 2.5) DQA1*0501</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5'-ACGGGTCCCTCTGGCCAGTA-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-AGTTGGAGCGTTTAATCAGAC-3'</td>
</tr>
<tr>
<td>HLA DQB1*0201</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5'-GTGCCTCTTGAGCAGAAG-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GCAAGGTCGTGGAGCT-3'</td>
</tr>
<tr>
<td>HLA (DQ 2.2) DQA1*0201</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5'-ACGGGTCCCTCTGGCCAGT-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-CAGGATGTTCAAGTTATGTTT-3'</td>
</tr>
<tr>
<td>HLA (DQ 8) DQA1*0301</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5'-TTCACTCGTCAGTGACCAT-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-CAAATTGCGGGTCAAATCTT-3'</td>
</tr>
<tr>
<td>DQB1*0302</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5'-GACGGAGCCGTCGTCGTTA-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-AGTACTCGGTCAGGCC-3'</td>
</tr>
</tbody>
</table>

Plasma Citrulline estimation:

**Mass Spectrometry**

Measurement of Citrulline will be done by extraction of dried blood spot (3.5 mm dried blood spot equivalent to 7 ul of blood) with a solution containing stable isotope labeled Internal Standards supplied by Neo-Base Non-Derivatized MS-MS kit(Perkin Elmer,Finland). Experimental design includes reconstitution of amino acid internal standard and 1:110 dilution with extraction solution. Blood spots then punched (Sample + Controls) and 100 ul of internal standard will be added with multichannel pipette and incubation took place for 45 min. at 45°C while shaking. Extract is then transferred to a second V-bottomed heat resistant microtitre plate. Samples will analyzed in tandem mass spectrometer (Applied Biosystem) equipped with a liquid chromatography autosampler, where extracted sample is delivered to the ion source of mass spec. Triple-quadrupole mass spectrometer having magnetic field around the rods which is controlled by varying an applied radio frequency potential, is used to separate and quantitates ions based on their mass to charge ratio (m/z) through a process called CAD (Collisionally Activated Dissociation). Only specific product ion is allowed to reach the detector where non-specific ions and parent ions will filter out and send to collision cell where CAD takes place.

The mass spectrometer would use deuterated citrulline standard and software analysis would be done by Analyst Launcher

**High Performance Liquid Chromatography**

HPLC with post column derivitization using ninhydrin will be used for final computation of citrulline levels. The internal standard used will be procured from sigma and run on Agilent HPLC model number 1200 series using FLD detector. Analysis of Area under the curve will be done by Chemstation Software. Age and sex matched controls will be evaluated using the same technique.
STATISTICAL ANALYSIS

Simple correlation coefficient will be estimated to quantify the relationship between clinical variable and status of HLA type and IL18 SNPs and plasma citrulline. The mean ± SD will be calculated for different groups. Two-way analysis of variance will be employed to test for the difference in mean values between the groups; student t-test will be also employed to compare the mean difference wherever appropriate. Statistical significance will be determined by Chi-square test with Yates' correction (wherever needed) or by 2-sided Fisher exact test, Mann Whitney U test and student’s t-test wherever applicable, using SPSS software. The p values of less than 0.05 will be considered significant.

ALGORITHM / PLAN OF STUDY

TTG, DGP and Biopsy proven confirmed Celiac Cases

→ First Degree Relatives

→ Clinical Evaluation

→ HLA typing for DQ2 & DQ8

Polymorphic Analysis for Non-HLA marker: IL-18 & plasma citrulline analysis

→ Evaluation & Characterization into type if positive

Follow up of screen positive patients for 3 years for symptomatology TTG titers for manifest disease. Follow up in index cases for adherence using citrulline @ 6 monthly intervals.
Alternate strategies (if the proposed experimental design or method does not work what is the alternate strategy)

If our proposed plan of work does not work we will plan for genotyping of MYO9B gene, CTLA 4 and ICOS gene.

Genome-wide association studies may be making candidate gene studies a less popular approach to study disease-gene correlations, but there is still a niche for well designed and well-powered candidate gene studies. The first genome-wide linkage scan in celiac disease was published in 1996 (Zhong et al.1996). [36] Since then, 12 other whole-genome linkage scans have been performed. With the exception of the MHC locus, the results of the linkage scans have been somewhat contradictory. However, a number of chromosomal regions have been repeatedly highlighted, e.g. 5q31-33 (CELIAC2) (Liu et al. 2002) [37], 2q32 (CELIAC3) ( Rioux et al. 2004) [38].

The chromosomal region 19p13 (CELIAC4) has shown evidence for genetic linkage with celiac disease, with the strongest reported linkage signal coming from the Dutch population. A subsequent fine-mapping study revealed association of five SNPs in the 3’ end of the myosin IXB gene (MYO9B) with celiac disease (Monsuur et al. 2005). [39] The function of MYO9B is relatively poorly understood. It is a single-headed processive motor that contains a Rho-GTPase-activating protein domain similar to genes involved in tight junction functions ( Bruewer et al. 2004). [40] Thus, it is possible that variations in MYO9B play a role in the impairment of epithelial permeability of the small intestine (Matter and Balda 2003). [41] leading to increasing amounts of gluten entering the lamina propria. The MYO9B association with celiac disease has also been replicated in the Spanish population (Sanchez et al. 2007) [42], as well as in other pathologies, such as inflammatory bowel disease (Cooney et al. 2009) [43], refractory celiac disease (Wolters et al. 2007) [44], rheumatoid arthritis (Sanchez et al. 2007) [42] and type 1 diabetes (Santiago et al. 2008). [45]

The CELIAC3 locus on chromosome 2q32 has shown linkage with celiac disease and several other autoimmune diseases (Gough et al. 2005) [46]. This region harbours the CTLA4, ICOS and CD28 genes, all of which belong to the CD28 family of T cell co-stimulatory receptors expressed on T cells following activation. CD28 is required for the activation of T cells (Yamada et al. 2002) [47]. In contrast, CTLA4 is a negative regulator of T cell responses and maybe required for the induction of tolerance (Yamada et al. 2002) [47]. It mediates the effects of regulatory T cells known to be important in suppressing immune responses. ICOS is necessary for the maintenance of humoral immunity, as it is involved in antibody isotype-switching and also induces the secretion of Th2 cytokines.

A number of studies have suggested that CTLA4 and ICOS are the strongest candidate genes in the region. Despite several fine-mapping attempts, the risk-conferring genetic factor still remains unknown. Association of the CELIAC4 region and celiac disease has been reported in several populations (Brophy et al. 2006) [48]. However, ICOS appears to give the strongest linkage and association signals in the Finnish population ( Haimila et al. 2009) [49]. The linkage disequilibrium (LD) pattern in the region is complex, consisting of two main haplotype blocks: one covering CTLA4 and the first exon of ICOS, and the other spanning the rest of ICOS. This makes it challenging to pinpoint the risk gene using genetic approaches. Population heterogeneity in the region may also explain the inconsistent results.
BIBLIOGRAPHY


35- O.Olerup A. Aldener, A. Fogdell. HLA-DQB1 and –DQA1 typing by PCR amplification with sequence specific primers (PCR-SSP) in 2 hours. Tissue Antigen1993:41:119-134.Munksgaard, 1993


**Timelines: (Please provide quantifiable outputs)**

<table>
<thead>
<tr>
<th>Period of study</th>
<th>Achievable targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Months</td>
<td>Sample collection &amp; study of clinical pattern of patients</td>
</tr>
<tr>
<td>12 Months</td>
<td></td>
</tr>
<tr>
<td>18 Months</td>
<td>Molecular Study for DQ-2/8 and IL-18 and plasma citrulline analysis</td>
</tr>
<tr>
<td>24 Months</td>
<td>Molecular Study for DQ-2/8 and IL-18 and plasma citrulline analysis</td>
</tr>
<tr>
<td>30 Months</td>
<td>Molecular Study for DQ-2/8 and IL-18 and plasma citrulline analysis</td>
</tr>
<tr>
<td>36 Months</td>
<td>Interpretation of results</td>
</tr>
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</table>

**Name and address of 5 experts in the field**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name</th>
<th>Designation</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dr. Neerja Gupta</td>
<td>Clinical Geneticist</td>
<td>Genetics Unit, Dept. of Pediatrics, AIIMS, New Delhi-29</td>
</tr>
<tr>
<td>2.</td>
<td>Dr. Gurjit Kaur</td>
<td>Associate Professor</td>
<td>Govt. Medical College &amp; Hospital, Sector-32, Chandigarh -160 031, Email:<a href="mailto:gurjitkaur123@rediffmail.com">gurjitkaur123@rediffmail.com</a></td>
</tr>
<tr>
<td>3.</td>
<td>Dr. Savita Attri</td>
<td>Associate Professor</td>
<td>Dept. of Pediatrics, PGIMER, Chandigarh</td>
</tr>
<tr>
<td>4.</td>
<td>Dr. Ujjal Poddar</td>
<td>Professor</td>
<td>Department of Pediatric Gastroenterology, Sanjuy Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India. Email: <a href="mailto:ujjal@sgpgi.ac.in">ujjal@sgpgi.ac.in</a></td>
</tr>
<tr>
<td>5.</td>
<td>Dr. AK Patwari</td>
<td>Retired Professor</td>
<td>Lady Hardinge Medical College Campus, New Delhi 110 001, India.</td>
</tr>
</tbody>
</table>
## PART IV: BUDGET PARTICULARS

**Budget (In Rupees)**

### A. Non-Recurring (e.g. equipments, accessories, etc.)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Item</th>
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<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>1.</td>
<td>Gel Documentation System with thermal printer</td>
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<tr>
<td>2.</td>
<td>Electrophoretic Unit</td>
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<tr>
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<td>Minifuge</td>
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</tr>
<tr>
<td>4.</td>
<td>Weighing Machine (0.01 mg)</td>
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<td>5.</td>
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<td>50,000</td>
</tr>
<tr>
<td>6.</td>
<td>Electronic Pipettes</td>
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<td></td>
<td>1,80,000</td>
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<td>(i)</td>
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<td>(ii)</td>
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<td>(iii)</td>
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<td>(iv)</td>
<td>100 µl - 1000 µl</td>
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Sub-Total (A) = 12,80,000

### B. Recurring

**B.1 Manpower (See guidelines at Annexure-III)**

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<tr>
<th>S. No.</th>
<th>Position No.</th>
<th>Consolidated Emolument</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
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<tbody>
<tr>
<td>A.</td>
<td>Senior Research Fellow (Non-Medical)</td>
<td>@23,400/month (18000+30%HRA)</td>
<td>2,80,800</td>
<td>2,80,800</td>
<td>2,80,800</td>
<td>8,42,400</td>
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<tr>
<td>B.</td>
<td>Junior Research Fellow (Non-Medical)</td>
<td>@15,600/month (12000+30%HRA)</td>
<td>1,87,200</td>
<td>1,87,200</td>
<td>1,87,200</td>
<td>5,61,600</td>
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<td>C.</td>
<td>Project Assistant Level 1</td>
<td>@5,000/month</td>
<td>60,000</td>
<td>60,000</td>
<td>60,000</td>
<td>1,80,000</td>
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<tr>
<td></td>
<td>Total(A+B+C)</td>
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<td>5,28,000</td>
<td>5,28,000</td>
<td>5,28,000</td>
<td>15,84,000</td>
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Sub-Total (B.1) = 15,84,000
### B.2 Consumables

<table>
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<tr>
<th>S. No.</th>
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<tbody>
<tr>
<td>(A)</td>
<td>DNA Extraction Kits,</td>
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<td></td>
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<tr>
<td></td>
<td>Reagents for PCR</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>i</td>
<td>Primers</td>
<td></td>
<td></td>
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<tr>
<td>ii</td>
<td>Taq polymerase</td>
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<tr>
<td>iii</td>
<td>dNTPs</td>
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<td></td>
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<td></td>
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<tr>
<td>iv</td>
<td>Agarose</td>
<td></td>
<td></td>
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<tr>
<td>v</td>
<td>DNA marker</td>
<td></td>
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<tr>
<td></td>
<td>Mass Spectrometry Kits, columns and aminoacid standards</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>DGP (Deamide Glaidine Peptide), TTG ELISA kits</td>
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**Sub-Total (B.2) = 19, 47,000**

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<tr>
<th>Other items</th>
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<tr>
<td>B.3 Travel</td>
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<td>25,000</td>
<td>25,000</td>
<td>25,000</td>
<td>75,000</td>
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<td>B.4 Contingency</td>
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<td>30,000</td>
<td>30,000</td>
<td>30,000</td>
<td>90,000</td>
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<td>B.5 Overhead (5% of total)</td>
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<td>61,600</td>
<td>61,600</td>
<td>61,600</td>
<td>1,84,800</td>
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<tr>
<td>Sub-total of B (B.1+B.2+B.3+B.4+B.5)</td>
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<td>12,32,000</td>
<td>12,32,000</td>
<td>12,32,000</td>
<td>36,96,000</td>
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<tr>
<td>Grand Total (A + B)</td>
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<td></td>
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<td>51,60,800</td>
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</table>

**Justification for Manpower**

- Senior Research Fellow (Non-Medical) – For performing DNA and PCR analysis.
- Junior Research Fellow (Non-Medical) – For performing TTG & DGP assay and Citrulline analysis by HPLC.
- Project Assistant – For collection of Patient information from different hospitals to include in the study, to follow up relatives of index cases. To visit individual households in patients not completely inclined for follow up.
Justification for the Consumables.

Non recurring:

- Electrophoretic Unit – For analyzing DNA & PCR results.
- Gel Documentation System - For visualizing and analyzing PCR results.
- Minifuge- For centrifugation of PCR reagents.
- Weighing Machine (0.01mg) - For weighing chemical reagents.
- pH Meter- to check the pH of buffers and reagents.
- Pipettes- [0.1 µl -10µl, 2 µl-20 µl, 20 µl-200 µl, 100 µl-1000 µl] - For pipetting exact volume of reagents.

Recurring:

- DNA Extraction Kits - For extracting DNA from whole Blood of Celiac cases and controls.
- Reagents for PCR - For amplification of HLA and IL-18 genes of celiac cases and controls
- DGP (Deamide Glaidine Peptide) & TTG ELISA kits- For screening of celiac cases and controls.

PART V : EXISTING FACILITIES

Resources and additional information

Laboratory:

The Genetic laboratory at Maulana Azad Medical College was established as a center in the year 2007. It acquired state of art equipments for biochemical genetics; a burning need felt in India in the arena of genetics. Centers geared and dedicated to molecular and cytogenetics genetics existed in the country specifically in North India. The particular zeal for biochemical genetics was not only a passion but was also brought up not to duplicate efforts in the other fields of genetics

Manpower

Regular Staff
1. Biochemist : 1 post
2. Cytogenetecist : 1 post
3. Technical Assistant : 1 Post
4. Lab Technician : 1 Post
5. Lab Assistant : 1 Post

PhD students = 4 (Four)
### Equipments:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the equipment</th>
<th>Model Number and Make</th>
<th>Year of Installation</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>ELISA Reader</td>
<td>Biorad</td>
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<tr>
<td>2.</td>
<td>ELISA washer</td>
<td>Ranbaxy</td>
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<td>3.</td>
<td>UV double beam Spectrophotometer</td>
<td>ECI</td>
<td>2006</td>
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<tr>
<td>2.</td>
<td>ELICO Spectrofluometer</td>
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<td>2007</td>
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<tr>
<td>3.</td>
<td>Orbital Shaker</td>
<td>Widson scientific</td>
<td>2007</td>
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<td>5.</td>
<td>Deep Freezer (-20 c)</td>
<td>Remi</td>
<td>2007</td>
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<tr>
<td>7.</td>
<td>CO₂ incubator</td>
<td>New Brunswick</td>
<td>2008</td>
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<tr>
<td>8.</td>
<td>Thermal Cycler</td>
<td>Biorad</td>
<td>2008</td>
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<td>9.</td>
<td>Gel Doc system</td>
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<td>Fluka</td>
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<td>Dual base Thermal Cycler</td>
<td>Biorad</td>
<td>2012</td>
</tr>
</tbody>
</table>
PART VI: DECLARATION/CERTIFICATION

It is certified that
a) The research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.
b) The same project proposal has not been submitted to any other agency for financial support.
c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)
d) Necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.
e) If the project involves the utilization of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed into.
f) If the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.
g) It is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Department of Expenditure, as contained in Annexure-V.
h) We agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.
i) The institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.
j) The Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of the:
a) Principal Investigator
   Seema Kapoor

b) Co-Investigator(s)  (i)
   Lalav

   (ii)
   (iii)
   (iv)

c) Head of the Department
   Arun
   11.07.12

Dr. A. S. Puri
M.D., D.M., Professor & HOD
Department of Gastroenterology
G.B. Pant Hospital, N.Delhi-2

Dr. PRAVEEN KUMAR
Department of Pediatrics
Lady Harding Medical College &
Kalawati Saran Children’s Hospital
New Delhi-110001

Dr. P. A. DUBEY
Director Professor & Head
Department of Pediatrics
Maulana Azad Medical College &
Associated Lok Nayak Hospital, N. Delhi
PART VII: PROFORMA FOR BIOGRAPHICAL SKETCH OF INVESTIGATORS

Provide the following information for the key personnel in the order listed on PART II. Follow this format for each person.

Name: SEEMA KAPOOR
Designation: PROFESSOR
Department/Institute/University: Department of Pediatrics, Maulana Azad Medical College, New Delhi-110002
Date of Birth: 05-11-1963
Sex (M/F): Female

Education

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Institution Place</th>
<th>Degree Awarded</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Army Hospital NewDelhi</td>
<td>DNB (Pediatrics)</td>
<td>March, 1995-96.</td>
</tr>
<tr>
<td>2</td>
<td>PGIMS, Rohtak</td>
<td>DCH</td>
<td>December, 1991</td>
</tr>
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</table>

Position and Employment

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Institution Place</th>
<th>Position</th>
<th>From (Date)</th>
<th>To (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>Professor</td>
<td>2nd Feb 2008-</td>
<td>till date</td>
</tr>
<tr>
<td>2</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>Associate Professor</td>
<td>2nd Feb 2004</td>
<td>1st Feb 2008</td>
</tr>
<tr>
<td>3</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>Assistant Professor</td>
<td>18th April 2002</td>
<td>1st Feb 2004</td>
</tr>
<tr>
<td>4</td>
<td>PGIMS, Rohtak</td>
<td>Lecturer</td>
<td>July 2001</td>
<td>April 2002</td>
</tr>
<tr>
<td>5</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>Senior Research Associate</td>
<td>Sept 2000</td>
<td>July 2001</td>
</tr>
<tr>
<td>6</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>Senior Resident</td>
<td>July 1997</td>
<td>July 2000</td>
</tr>
</tbody>
</table>

Honors/Awards

PRESIDENT’S MEDAL for the BEST LADY MEDICO for the year 1986
1. LATE Shri G R DEWAIKAR MEMORIAL PRIZE for the highest marks in Final MBBS
2. Smt MANORAMA GARDE GOLD MEDAL for the highest percentage of marks in final MBBS
3. Rajyapal of Gujrat Gold Medal For standing 1st in Final MBBS
4. Shri Bhaskar Chinniah Gold medal For standing 1st in Biochemistry
5. Shri L B Pandit Gold medal For standing 1st in Medicine
6. Dr. K. P. Sengupta Gold Medal For standing 1st in Final Professional
7. Shri Mahavir Madhav Rao Shinde Gold Medal For standing 1st in Final Professional
8. Shrimati Gangubai Jaiswal Gold Medal for standing 1st in Final Professional
9. Lalit B Mahajan Memorial Prize For standing 1st in Community Medicine
10. Shri V Sahnis Silver Medal For standing 1st in Surgery
11. Dr. B. K. Mahajan Silver Medal For standing 1st in Medicine
12. Shri Ramachandran Balakrishnan Deshpande Memorial Silver Medal For standing 1st in Medicine
13. Shri Yogendra Pal Silver Medal For standing 1st in Eye and ENT
15. Silver Medal For 1st in First MBBS
16. Silver Medal For 1st in Second MBBS
17. Silver Medal For 1st in Pathology
18. Silver Medal For 1st in Microbiology
19. Silver Medal For 1st in Physiology
20. V. Tirumala Prasad Silver Medal For 1st in 1st MBBS
22. Bronze Medal For standing 2nd in Anatomy
23. Bronze Medal For standing 2nd in Forensic Medicine
24. FIRST Prize For Short Paper Session For X Annual Conference Of Pediatric Gastroenterology

Professional Experience and Training relevant to the Project

The principal investigator was working in the field of pediatric gastroenterology as a senior research associate and learnt the basic principles of Pediatric gastroenterology including endoscopy. She has certified training from Applied biosystems from Foster city CA in the year 2009. (Copy of certificate enclosed as Annexure). The Lab is also quality assured from CDC Atlanta for amino acid analysis.

B. Publications (Numbers only): 64.
National:32
International:14

Books Edited: Clinical Manual on inborn errors of metabolism sponsored by Indian Council of medical Research
Chapters in books 14 (Fourteen),
Research Papers, Reports: 31
General articles: 11
Patents: None

Selected peer-reviewed publications (Ten best publications in chronological order)


List maximum of five recent publications relevant to the proposed area of work


## Ongoing Research Projects

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount</th>
<th>Date of sanction and Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Assessment of CCL18/PARC and Heparin-Thrombin II Cofactor expression in Indian Children with Lysosomal Storage Disorders</td>
<td>DBT</td>
<td>Rs. 27,45,000</td>
<td>8/10/2010 Three Years</td>
</tr>
<tr>
<td>2</td>
<td>Study of the Biochemical Profile and Polymorphisms of the Growth Hormone Receptor Gene in Patients with Idiopathic Short Stature and Proven Growth Hormone Deficiency</td>
<td>UGC</td>
<td>Rs. 10,39,800/-</td>
<td>1/7/2011 Three Years</td>
</tr>
<tr>
<td>3</td>
<td>Evaluation of solid phase &amp; solvent phase extraction procedures for urinary organic acid &amp; molecular characterization of MMA</td>
<td>ICMR</td>
<td>Rs. 35,92,280/-</td>
<td>20/6/2012 Three Years</td>
</tr>
<tr>
<td>4</td>
<td>Clinical application of array-based Comparative Genomic Hybridization (array CGH) in the genetic evaluation of stillbirths</td>
<td>DBT</td>
<td>Rs. 11100922</td>
<td>29/3/2012 Three Years</td>
</tr>
</tbody>
</table>
**Completed Research Projects** (State only major projects of last 3 years)

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount</th>
<th>Date of completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>To evaluate study the association of homocysteine, B12 and Folic acid and the polymorphisms of the MTHFR gene in osteoporosis</td>
<td>DBT</td>
<td>Rs 24 lakhs</td>
<td>29/3/07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Completed on 28/7/10</td>
</tr>
<tr>
<td>2.</td>
<td>To compare integrated screening for prenatal detection of Down’s Syndrome in the Indian context</td>
<td>DBT</td>
<td>Rs 22 Lakhs</td>
<td>29/7/2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Completed on 21/8/2012</td>
</tr>
<tr>
<td>3.</td>
<td>ICMR Taskforce study on newborn screening for congenital hypothyroidism and congenital adrenal hyperplasia</td>
<td>ICMR</td>
<td>Rs 3 crores</td>
<td>31/3/2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Completed on 28/7/2012</td>
</tr>
</tbody>
</table>
**BIODATA OF CO INVESTIGATORS (I)**

**CURRICULUM VITAE**

**Name:** Dr. Vineeta Vijay Batra  
**Designation:** Professor  
**Department/Institute/University:** Department of Pathology, G.B Pant Hospital, New Delhi  
**Date of Birth:** 27th August 1966  
**Sex (M/F):** Female  
**Email address:** vvbatra@rediffmail.com

**EDUCATIONAL QUALIFICATION**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Degree</th>
<th>Place</th>
<th>Year</th>
</tr>
</thead>
</table>

**PROFESSIONAL EXPERIENCE:**

<table>
<thead>
<tr>
<th>No.</th>
<th>Post</th>
<th>Place of work</th>
<th>Time</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Professor</td>
<td>GB Pant Hospital, New Delhi</td>
<td>07.11.06 till date</td>
<td>5 years</td>
</tr>
<tr>
<td>2.</td>
<td>Associate Professor</td>
<td>GB Pant Hospital, New Delhi</td>
<td>10.4.06 - 7.11.06</td>
<td>7 months</td>
</tr>
<tr>
<td>3.</td>
<td>Associate Professor</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>7.11.02 – 10.4.06</td>
<td>3 years 5mths</td>
</tr>
<tr>
<td>4.</td>
<td>Assistant Professor</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>7.11.2000- 7.11.2002</td>
<td>2 years</td>
</tr>
<tr>
<td>5.</td>
<td>Assistant Professor</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>7.03.1998–7.11.2000</td>
<td>2 years 9mths</td>
</tr>
<tr>
<td>7.</td>
<td>Senior Resident</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>23.06.1994-2.12.1994</td>
<td>6 mths</td>
</tr>
</tbody>
</table>

**AWARDS RECEIVED:**

1. International Society of Nephrology Fellowship for training in renal pathology at Kidney Pathology Section, Dept of Pathology, “Brigham and Women’s Hospital, Harvard Medical University, Boston, USA from 18.04.07 to 15.08.07.

2. UGC travel grant training in renal pathology at Kidney Pathology Section, Dept of Pathology, “Brigham and Women’s Hospital, Harvard Medical University, Boston, USA from 16.03.09 to 12.06.09.

**PROFESSIONAL TRAINING IN INDIA**

1. Workshop on autoimmunity at SGPGI, Lucknow 1.07.08 to 6.07.08
PUBLICATIONS

Total no. of Publications: 19
National: 8
International: 11

Selected peer-reviewed publications (Ten best publications in chronological order)


Funded Research Projects as Principal Investigator

<table>
<thead>
<tr>
<th>S.No</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Date of sanction and Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Predictors of advanced fibrosis in non –alcoholic steatohepatitis (NASH)</td>
<td>ICMR</td>
<td>2005 –2008 Three Years</td>
</tr>
<tr>
<td>2</td>
<td>Diagnosis of autoimmune diseases with particular reference to SLE and autoimmune hepatitis</td>
<td>Ministry of Health, Govt. of Delhi</td>
<td>2003 -2004 2 Years</td>
</tr>
<tr>
<td>3</td>
<td>Role of cytodiagnosis in urine examination</td>
<td>ICMR</td>
<td>2005</td>
</tr>
<tr>
<td>S.No</td>
<td>Title of Project</td>
<td>Agency</td>
<td>Date of sanction and Duration</td>
</tr>
<tr>
<td>------</td>
<td>---------------------------------------------------------------------------------</td>
<td>--------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>To estimate the SLEDAI (SLE disease activity index) and correlate serum levels of bioactivity markers as IL-18, IL-10, IL-6 and TNF-α with the active form of disease</td>
<td>KVPY</td>
<td>2011</td>
</tr>
<tr>
<td>2</td>
<td>To evaluate the role of urine microscopy in differential diagnosis of acute kidney injury and risk stratification of acute kidney injury</td>
<td>ICMR</td>
<td>2011</td>
</tr>
<tr>
<td>3</td>
<td>Studying the efficacy of nuclear antigen line assay in the diagnosis of autoimmune rheumatological disorders</td>
<td>KVPY</td>
<td>2012</td>
</tr>
</tbody>
</table>
BIODATA OF THE CO INVESTIGATORS (II)

DR. MALOBIKA BHATTACHARYA

Name: Dr. Malobika Bhattacharya
Designation: Assistant Professor
Department/Institute/University: Department of Pediatrics, Maulana Azad Medical College, New Delhi
Date of Birth: 19th March 1979
Sex (M/F): Female

Education
Senior Residency: 14/08/06-13/08/09 at Deptt of Pediatrics, Maulana Azad Medical College

Professional Qualification

<table>
<thead>
<tr>
<th>DEGREE</th>
<th>YEAR</th>
<th>INSTITUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD (Pediatrics)</td>
<td>May 2003-April 2006</td>
<td>Maulana Azad Medical College, Delhi University</td>
</tr>
<tr>
<td>Internship</td>
<td>Jan –Dec 2001</td>
<td>Lady Hardinge Medical College</td>
</tr>
</tbody>
</table>

Current Position: Assistant Professor, Deptt of Pediatrics, Maulana Azad Medical College.

Experience and Training relevant to the Project

Post MD training
Underwent short course training programme in gastrointestinal endoscopy from 13th August to 17th September, 2010 under Dr A S Puri, Professor & Head, Department of Gastroenterology, G B Pant Hospital.

Research work during residency:

Study of the Prevalence of Celiac Disease in North Indian Children Using Anti-Tissue Transglutaminase Assay. MD Thesis. Guide: Dr AP Dubey, Dir Professor of Pediatrics and Head of the Department. Co-Guides: Dr NB Mathur, Professor of Pediatrics, Dr V Malhotra, Director Professor of Pathology and Head of the Department GB Pant Hospital.

Research work during pool job:

Randomised double-blinded controlled trial on the effect of zinc supplementation on duration of acute diarrhoea and subsequent growth and morbidity in infants 1-6 months of age. Supervisor: Dr AP Dubey, Dir Professor & Head, Department of Pediatrics.

Publications

Total no. of Publications: 17
National: 9
International: 8
Selected peer-reviewed publications (Ten best publications in chronological order)


9. Bhattacharya M, Joshi N. Spinal epidural abscess with myelitis and meningitis caused by Streptococcus pneumonia in young child. Accepted for publication in the Journal of Spinal Cord Medicine

List maximum of five recent publications relevant to the proposed area of work

Name: Dr. Amarendra Singh Puri  
Designation: Professor & Head  
Department/Institute/University: Dept. of Medical Gastroenterology, G.B. Pant Hospital, New Delhi.  
Date of Birth: 14/08/1961  
Sex (M/F): Male  
Education (Post-Graduation onwards & Professional Career)  

Post Graduate Degrees:  
- DM Gastroenterology, SGPGI Lucknow 1992  
- MD Internal Medicine, Punjab University, Chandigarh 1988  

Faculty Appointments:  
- Professor of Gastroenterology 2002 – Present  
  - GB Pant Hospital (Delhi University) 1998 - 2002  
- Associate Professor of Gastroenterology 1998  
  - GB Pant Hospital (Delhi University) 1998  
- Assistant Professor of Gastroenterology 1988 – 1990  
  - Lecturer in Medicine  
    - CMC Ludhiana (Punjab University)  

Honors:  
1. President of India Medal for best student 1978  
2. Governors Gold Medal for best graduate 1984 MBBS  

Affiliations:  
- Society of Gastro-intestinal Endoscopy of India  
- Indian Society of Gastroenterology  
- Indian Society for study of Liver disease  

B. Publications  
Research Papers, Reports: 54  
National: 34  
International: 20  

Selected peer-reviewed publications  


**List maximum of five recent publications relevant to the proposed area of work**


BIODATA OF CO INVESTIGATORS (IV)

Name: Dr. Praveen Kumar
Designation: Professor, Department of Pediatrics,
Department/Institute/University: Lady Hardinge Medical College, Kalawati Saran Children’s Hospital
Date of Birth: 18th April, 1966
Sex (M/F): Male

Education (Post-Graduation onwards & Professional Career)

<table>
<thead>
<tr>
<th>Position and Employment (Starting with the most recent employment)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S.No</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
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<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Honors/Awards
1. Dr. V.K. Gujral Award for best senior resident 2000.

B. Publications

Books: 1
Research Papers, Reports: 26
General articles: 8

Selected peer-reviewed publications (Ten best publications in chronological order)

List maximum of five recent publications relevant to the proposed area of work.