
# POSTER PRESENTATION ABSTRACTS

# Frequency of Gangliosides Autoantibodies as Diagnostic Markers in Autoimune Neuropathies: A Malaysian Experience

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Gangliosides are known to play important roles in biological functions, such as cellular growth and differentiation, modulation of signal transduction, and immune reactions. Antibodies to gangliosides have been identified in patients suffering autoimmune neuropathies, especially in Guillain-Barré syndrome (GBS) and in motor-dominant acute polyradiculoneuropathy. Approximately 60% of patients with GBS have anti-ganglioside antibodies during the acute clinical phase of the disease.

**OBJECTIVE**: The aim of this study was to determine the frequency of gangliosides autoantibodies markers among patients suspected with autoimmune neuropathies.

**MATERIALS AND METHODS**: This retrospective study involved 254 patients in 2018 suspected with autoimmune neuropathies were tested for anti-GM1 antibodies, anti-GM-2 antibodies, anti- GM3 antibodies, anti-GM4 antibodies, anti-GD1a antibodies, anti-GD2 antibodies, anti-GD3 antibodies, anti-GD1a antibodies, anti- GD1b antibodies, anti-GT1a antibodies, anti-GT1b antibodies, anti- GQ1b antibodies and sulfatides by Immunoblot strips.

**RESULTS:** Out of 254 samples, 13% (33/254) were positive to at least one of the gangliosides autoantibodies biomarkers. The study population was predominantly male (male 66.7%; female 33.3%). Ethnicity distributions were Malays (69.7%), Chinese (18.2%), Indian (9.1%) and others (3.0%). From our data, 39.4% (13/33) had positive to anti-GQ 1b Ig G antibodies, 33.3% (11/33) had positive to anti-GT1a Ig G antibodies, 3.0% (1/33) to both anti-GM1 Ig G and Ig M antibodies. We also found that 12.1% (4/33) had positive to anti-GD1b IgG, 9.1% (3/33) had anti-GD1a IgG and 9.1% (3/33) positive to anti-GD3 Ig G. In our data base collection, we found that frequency of anti-GQ 1b Ig G, GT1a Ig G and GM 1 antibodies is higher detected among patient with suspected autoimmune neuropathies**.**

**CONCLUSION**: Our study shows that the anti-GQ1b IgG autoantibody was the most predominant biomarker in patients with suspected autoimmune neuropathies in Malaysia.

*Keywords: Gangliosides; autoantibodies; autoimmune neuropathies; Guillain-Barré syndrome;biomarker*

**PP02**

# IgE Reactivity and Cross-Reactivity of Group 2 Dust Mite Allergens

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**Purpose of study:** Dust-mites are the most common cause of sensitization among allergic patients worldwide. We report the IgE-reactivity of eight group 2 allergens (five published, three new) using the same patient population to understand their relative allergenicity and cross-reactivity profile.

**Methods:** Blo t 2 was amplified from the cDNA of *Blomia tropicalis* using degenerate primers from the conserved regions of group 2 allergens. Sui m 2 and Ale o 2 were identified from our in-house expressed sequence tag libraries. Remaining five published allergens were amplified from cDNA of the respective mites using specific primers. All allergens were expressed and purified as recombinant proteins. IgE-reactivities were tested using sera from 202 individuals from Singapore using immuno dot-blot assays. IgE cross-reactivity was tested using selected sera by competitive ELISA.

**Results:** From the 202 individuals tested, 116 showed IgE reactions to whole dust-mite protein extracts. Most of the dust-mite positive individuals had specific IgE binding to group 2 allergens from dust mites from the pyroglyphid mites, Der p 2 (78%), followed by Der f 2 (48%), with almost complete IgE inhibition between both allergens. Among group 2 allergens from the non-pyroglyphid mites, specific IgE-binding ranged between 34% (Blo t 2) to 22% (Gly d 2). Competitive IgE binding studies between group 2 allergens from non-pyroglyphid mites showed high inhibitions (>80%) using Blo t 2 and Tyr p 2 as representatives. The major allergen Der p 2 was only partially inhibited by Blo t 2 (52-88%) and Tyr p 2 (70-75%).

**Conclusion:** Group 2 allergens from *Dermatophagoides spp*. showed the highest IgE reactivity among the Singaporean dust-mite individuals, and were highly cross-reactive. Less than a third of the population react to group 2 allergens from other (non-pyroglyphid) dust mites. There was only partial cross-reactivity between group 2 allergens of pyroglyphid and non-pyroglyphid mites.

**PP03**

# Chronic Urticaria and Angioedema Associated with Toxocariasis

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**Introduction:** Toxocariasis is a zoonotic disease that occurs when humans are infected by the parasites Toxocaracanis(dog) and Toxocaracati (cat). Both are highly prevalent in tropical areas, industrialized countries and especially rural areas (Dureault,et al 2017). Clinical features include ocularand cutaneous larva migrans, fever, malaise and inducing chronic allergy-like symptoms such as urticaria, angioedema, dermatitis, asthma and conjunctivitis (Qualizza, R et al,2009). Toxocara antigens induced TH2 lymphocyte to produceIL-4 (interleukin 4) and B cells to synthesize IgE (Immunoglobulin E) andIL-5(interleukin 5) to activate eosinophils leading to allergic inflammation. We report two cases of chronic urticaria associated with angioedema treated as allergy and both are positive for anti-Toxocara antibodies detected by ELISA (enzyme-linked immunosorbent assay) method. Both patients were students, have no history of atopy and had no pets. There was no history of travelling to other Asian or tropical countries. **Case 1:** 24-year-old man with history of chronic urticaria for 6 years (since age 18year old) associated with periorbital and lip oedema. He gave history of studying and having lived in 3 different states, beginning with Kota Bharu Kelantan, Jitra Kedah and currently in Kuala Lumpur. Total IgE was normal (52.5ku/l). Very low specific IgEto house dust mites (0.10 ku/l) andanisakis (0.11ku/l). Parasitologic ELISA test showed positive IgG (Immunoglobulin G)1.293 to toxocara. **Case 2:** 11-year-old student with history of chronic urticaria for two years associated with angioedema. He stayed in Beranang, Hulu Langat Selangor and gave history of playing football barefoot in the fields. Total IgE was raised (463ku/l). Very low specific IgE to house dust mites (0.12 ku/l) and to wheat (0.2ku/l). Parasitologic ELISA test showed positive IgG 0.3425 to toxocara. Both patients showed improvement in clinical symptoms after given treatment with Albendazole (antihelminthics) and antihistamines. In summary, our findings suggestsdespite being rare, Toxocara may present as chronic allergy-like symptoms and maybe missed to be diagnosed in most urticaria cases.

# Very Early Onset Inflammatory Bowel Disease due to NOD2 Mutation: A Case Report

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## Introduction

Inflammatory bowel disease (IBD) is a complex disorder characterized by chronic relapsing intestinal inflammation. IBD presents in three forms namely, ulcerative colitis (UC), Crohn's Disease (CD) and inflammatory bowel disease unclassified (IBDU). The exact etiology is unknown, but data from previous studies indicated that IBD arises from complex interaction of genetic, immunological and environmental factors. Several *NOD2* risk variants has been associated with an increased risk of developing IBD in Caucasian population. However, the genetic etiology of IBD among Asian population has not been clearly defined.

## Purpose of the study

The purpose of this study is to elucidate the underlying genetic etiology for IBD using whole exome sequencing (WES) technology.

## Materials and method

A 6-year-old Chinese boy presented with three episodes of hemophagocytic lymphohistiocytosis between the ages of 14 months and 3 years. Subsequently he developed altered bowel habit and was found to have Crohn's disease through colonoscopy. His abnormal immunologic parameters led to a suspicion of an underlying primary immunodeficiency, hence WES was offered. Genomic DNA was extracted from whole blood sample. WES was performed using Illumina paired-end sequencing platform with 100X coverage and exome capture kit Agilant SureSelect V4 was used. WES data were mapped to human reference genome (hg38). Candidate genes were selected based on disease phenotype and in-silico pathogenicity prediction tools. Bidirectional Sanger sequencing were performed to validate WES findings.

## Results

A c.1753G>A, p.585A>T variant in *NOD2* was detected using WES. This variant is predicted to be damaging by three in silico-pathogenicity tools; SIFT, PolyPhen 2 and Mutation Taster. We validated this variant by bidirectional Sanger sequencing.

## Conclusion

Our report highlights the usefulness of WES technology in disentangling a complex disease with multiple possible genetic etiologies.

Keywords
Inflammatory Bowel Disease, Chron's Disease, *NOD2*

# Whole Exome Sequencing reveals NLRP12 and CARD14 Mutations in a Patient with Poorly-defined Primary Immunodeficiency Phenotype

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**Introduction**: Diagnosing primary immunodeficiencies (PIDs) is challenging because single gene variant can result in different clinical manifestations (genetic pleiotropism) whereas multiple genetic variants can result in similar clinical picture (phenotypic heterogeneity). In cases with poorly defined PID phenotype, whole exome sequencing (WES) can aid in elucidating the underlying genetic variants.

**Objective**: To identify the genetic variant(s) harboured by a boy suspected of PID with poorly-defined phenotype using WES.

**Patients and Methods**: We report a one-year-old Malay boy of consanguinous conception who presented with bicytopenia, hepatosplenomegaly and multiple lymphadenopathies. This boy was initially thought to suffer from Common Variable Immunodeficiency (CVID) in view of low serum immunoglobulins and was treated with intravenous immunoglobulin (IVIG). There was no improvement of symptoms and later developed disemminated tuberculosis which warranted further investigation to identify the underlying genetic variant(s). WES was performed on patient's genomic DNA extracted from peripheral blood mononuclear cells (PBMCs) using HiSeq 4000 (Illumina platform) with 100x coverage and exome capture of 51Mb by Agilent SureSelect V4.

**Results**: We performed bioinformatic analysis using in-house pipeline and identified two genetic variants, namely NLRP12 (c.A1820C) and CARD14 (c.G1118A). These genetic variants have been classified as auto-inflammatory disorders under the PID phenotype categorization and were very rare according to gnomAD (genomic reference database). We validated the WES findings using Sanger sequencing. GeneMania pathway analysis was performed and revealed that both genes resulted in upregulation of NFKB signalling. The NLRP12 and CARD14 genes were associated with auto-inflammatory and auto-immunity respectively.

**Conclusion**: Two pathogenic variants of NLRP12 and CARD14 genes were identified in a patient with poorly-defined phenotype using WES. We are the first to report these two pathogenic genetic variants to be seen in a single patient.

**PP06**

# Detection of Serum Coeliac Antibodies in Suspected Coeliac Disease Patients in Malaysia

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**Introduction:**

Coeliac Disease (CD) is an autoimmune enteropathy related to gluten and linked to a strong genetic susceptibilty background. This disease is characterised by inflammation of the intestinal mucosa causing total or subtotal villous atrophy. Serological tests for anti-gliadin antibody, anti-tissue transglutaminase antibody (tTG) and anti-human endomysial IgA antibodies have been identified to help in diagnosing CD.

**Objective:**

This study aims to determine the frequency of CD antibodies in suspected CD patients in Malaysia.

**Methods:**

This is a cross sectional study involving 492 suspected CD patients whose blood samples were sent for CD antibodies tests from January 2015 until December 2018. Serum samples were tested for anti-gliadin antibody immunoglobulin A/immunoglobulin G (IgA/IgG), anti-tissue transglutaminase antibody (tTG) immunoglobulin A/immunoglobulin G (IgA/IgG) using fluoroenzyme immunoassay method and anti-human endomysial IgA antibodies using indirect immunofluorescence method.

**Results:**

16 patients (3.3%) with age ranging from 2 to 58 years old were positive for at least 1 antibody. 69% (11/16) of these patients were above 18 years old. Out of 16 patients, 68.75% have anti-human endomysial IgA antibodies, followed by anti-gliadin IgA (50%), anti-gliadin IgG (25%), anti tTG IgA (18.75%) and anti-tTG IgG (12.5%).

**Conclusion:**

In this study, majority of the patients are above 18 years old and the anti-human endomysial IgA antibody is the most prevalent autoantibodies among CD patients.

**Keywords:** Coeliac Disease, anti-gliadin, anti-tissue transglutaminase, anti-endomysia.

**PP07**

# Toxoplasmosis Exposure with Urticaria

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Toxoplasmosis is one of the most common parasitic infections found worldwide caused by Toxoplasma *gondii* (Robert-Gangreux F & Darde ML, 2012). It spreads mainly by foodborne, animal-to-human (zoonotic) transmission, mother-to-child (congenital) transmission, and rarely via organ transplant or blood transfusion. The oocysts survive better in freezing compared to higher temperature, humid climates, lower altitudes and also resistant to chemical disinfectants (Chao Y et al, 2016). The clinical manifestations are flu-like symptoms; fever, headache, muscle aches, sore throat, swollen lymph nodes and to some extend chronic urticaria or cold urticaria (Fernandez-Figares V et al, 2015 and Miralles Lopez JC et al, 2005). Thirty-five year old Malay male with previous history of intermittent urticaria for some time was suddenly referred to Emergency Department (ED) for acute generalized urticaria associated with choking sensation in the throat. He responded well to *intravenous* (IV) hydrocortisone, maxolon and ranitidine, was subsequently referred to Allergy Clinic Hospital Kuala Lumpur (HKL) for skin prick test (SPT). Patient had previously stayed in Sarawak for 1 year working as government officer in the department of road transport (JPJ) and was exposed to roadsides and environment in rural areas. SPT was done in 2017 which was positive for house dust mites (HDM) and seafood. Total Immunoglobulin E (tIgE) by flouroenzymeimmunoassay (FEIA) was raised (1102 ku/l). Mast cell tryptase was normal. Full blood count showed raised eosinophils (9%), serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were normal). HIV screening was non- reactive. Parasitologic serology test showed positive for enzyme-linked fluorescent assay (ELFA) immunoglobulin G (IgG) 45IU/ml with indirect fluorescent antibody test (IFAT) IgG 1:32 to toxoplasma and was reported as possible infection with Toxoplasma gondii. Filiariasis and schistosomiasis test were negative. Patient was referred to physician clinic for further treatment and observation In summary, the presence of urticaria with eosinophilia and positive serology test to toxoplasma gondii suggests the most likely cause of urticaria in this man is due to toxoplasmosis.

**PP08**

# Perioral Angioedema and Rash to Multiple Food Allergies (Egg, Wheat and Cow's Milk Allergy) in a Toddler

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Food allergies affect up to 8% of children and about 3% in adults (Rima R, et al 2017). We report a one-year-old child, ~~who was~~ fully breastfed till 6 months of age, who presented with recurrent lip swelling and facial rash for four months. She first presented with acute lip swelling and rash at the age of 8 months ~~old~~ after taking half boiled egg together with a mixture of breast milk and formula milk (cow's milk). At 10 months ~~old~~ of age, vomited after eating cereal, cheese cake, pasta and fried egg. However, she was able to eat rice, porridge, fish, chicken and fruits. Her total Immunoglobulin E (IgE) was elevated to 71.0 kU/l. The specific IgE was moderately raised to wheat (10kU/L) and egg white (8.24kU/L). We observed low Ig E to cow's milk (1.41kU/L) and peanut (0.18kU/L). Aeroallergens were negative. Skin prick test was not performed due to history of angioedema. Her medical history revealed that she may have suffered from allergic reactions due to eating food containing hidden wheat in cereal, cakes and pasta. Furthermore, she was allergic to egg and dairy products such as cheese cake and mixed breast milk and cow's milk. At present, she is still unable to tolerate egg and needs total avoidance of egg in her diet. The treatment for her condition includes maintaining a food diary, elimination diet, food challenge and in the near future, food allergy immunotherapy i.e. oral immunotherapy (OIT), epicutaneous immunotherapy (EPIT) and sublingual immunotherapy (SLIT) (Rima R, et al 2017).

**PP09**

# Diagnosis of Chronic Granulomatous Disease (CGD) by Dihydrorhodamine (DHR) Test: A Single Centre Experience

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**Introduction**: Chronic granulomatous disease (CGD) is a heterogenous group of disorders that usually manifests in the first few years of life with recurrent pyogenic infections such as superficial or deep seated abscesses, lymphadenitis, septic arthritis or osteomyelitis. Clinically highly suspicious patients subjected to the Dihydrorhodamine (DHR) test would allow the quantitative determination of neutrophil oxidative burst rapidly.

**Objective**: To describe a one year findings of CGD screening in Malaysia.

**Patients and Methods**: A total of 52 samples received for DHR test on appointment basis over a one-year period (2018). Patient's samples were received in lithium heparin tubes along with a control sample from various hospitals. These whole blood samples were processed using commercial reagent kit PHAGOBURST™ (Glycotope Biotechnology, Germany) according to the manufacturer's protocol. The kit contains three stimulants, namely opsonized bacteria (*E.coli*), phorbol 12-myristate 13-acetate (PMA) and N-formyl-Met-Leu-Phe (fMLP). Samples were then analyzed in BD FACSCanto II flowcytometry analyzer whereby only neutrophils were gated and analyzed.

**Results**: Four (4) cases showed impaired neutrophil oxidative burst activity and four (4) more cases were inconclusive. All the inconclusive DHR was from the East Malaysian hospitals and the sample integrity (age of sample) was the main contributing factor. Among the positive DHR tests, three were detected in females and one in male. Methicillin Sensitive *Staphylococcus aureus* (MSSA) predominates most of the culture and sensitivity isolates. One case was supported with the findings of 'inflammed granuloma' on histopathological report. The only male patient had a strong family history and the DHR test was also done for the mother which suggests she is a carrier.

**Conclusion**: Dihydrorhodamine (DHR) test is a rapid tool to detect both the diseased patient as well as the X-linked carrier parent and is the preferred test to diagnose CGD. Female patients with impaired DHR test could be autosomal recessive inherited, although X-linked CGD is more common in this part of the world. Aged sample contributes to an inconclusive DHR test outcome and hence a timely analysis of patient sample is crucial for best results.