

The methodology of the GUSTO cohort study: a novel approach in studying pediatric allergy

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Growing Up in Singapore Towards healthy Outcomes (GUSTO) is Singapore's largest birth cohort study to date. The main aim of GUSTO is to evaluate the role of developmental factors in the early pathways to metabolic compromise. Detailed data is collected for a range of environmental exposures in the parents and offspring, and allergic disorders are among a number of outcomes assessed in infancy and childhood. Under the Allergy domain of GUSTO, this integrated study will describe the epidemiology of allergic manifestations and different phenotypes in the Asian context and help shed light on the association of metabolic disease to allergy. Epigenetic mechanisms and associations with other childhood disorders will also be explored. The aim of this report is to focus on methodology of GUSTO, and to suggest similar approaches (i.e., integrated cohort studies on pediatric allergy) worldwide. Recruitment commenced in 2009 with a cohort of 1,163 pregnant mothers in their first trimester. The mothers and children were followed throughout pregnancy and follow-up will continue until the child reaches 3 years of age. Preliminary results showed that 39.8% of the mothers had a personal history of having at least one allergic disease, which included asthma, eczema and allergic rhinitis. Further data collection and analyses are still ongoing. Allergy is a complex spectrum of disorders with numerous poorly-understood aspects. The ongoing GUSTO cohort study, with its longitudinal design and multi-disciplinary nature, may provide new insights into developmental influences on allergy. As a Singapore-based study, it will be the first integrated allergy cohort in Southeast Asia, of which recruitment started during pregnancy.

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INTRODUCTION

Allergic diseases such as asthma, rhinitis, and atopic eczema are considered the most common group of diseases in children and are an important cause of somatic and psychosocial morbidity, affecting quality of life of patients and their families [1]. Moreover, a lot of aspects of allergic diseases are still unknown. As a result there is a need to better understand the pathogenesis, natural course, prevention and treatment of allergic disease.

It is now generally accepted that allergic disorders result from the complex interplay between genetic factors and the environment. However, the exact reasons for the increase of allergic diseases during the last three decades are still largely unknown, and the Hygiene Hypothesis only explains part of the increase [2]. Other environmental and lifestyle factors have been implicated but the exact underlying mechanisms and triggers remain speculative [3]. In addition, it still needs to be elucidated whether there are any exposures that have causal roles in the similar increases in allergic and metabolic diseases such as diabetes [4, 5] and obesity [6] over recent decades. The Growing Up in Singapore Towards healthy Outcomes (GUSTO) cohort study is well placed to explore this hypothesis. Additionally, the GUSTO study will examine a number of other unanswered questions about different aspects of allergy. These include: the role of epigenetic mechanisms in the pathogenesis of allergic diseases, the underlying mechanisms of heterogeneous presentations of allergy within families, even among identical twins [7], and the mechanisms behind the dynamics of allergic symptoms in young children [8] and the growing-out of an allergy.

Singapore is a modern Southeast Asian city state with a westernised population. The disease profile of the nation is dominated by ailments such as cancer, metabolic syndrome and allergy. A local cross-sectional study in the year 2001 showed that 27.4% of 12 to 15 year-olds had been medically diagnosed with asthma [9]. Another study found that atopic dermatitis affected 20.8% of Singaporean school children [10]. In fact, symptoms related to allergy were found to be already prevalent at a younger age, with a significant 42.2% of a cohort having had chronic itchy skin, wheeze or rhino-conjunctivitis by 24 months of life [11]. The high prevalence of allergy comes with an economic burden, with the total healthcare cost of asthma alone in Singapore estimated to be US\$33.93 million per annum [12].

Aim

Because of the complexity of allergy and because of its possible association with other pathologies, it was decided to set up an innovative Singaporean cohort (as part of GUSTO – see below) to study different aspects of allergic diseases, such as its epidemiology and clinical presentations, with a focus on different phenotypes of allergic manifestations. Within the study we aim to analyse the association of metabolic diseases to allergy.

In addition, disease mechanisms, including epigenetic mechanisms and associations with other childhood diseases and features will be explored. GUSTO is the first birth cohort in Southeast Asia that aims to study multiple types of allergy. This is significant as Asia is the most populous continent, and an increase in the prevalence of allergy will result in tremendous burden [13]. The aim of this report is to focus on methodology of GUSTO, being an innovative approach to research in pediatric allergy.

Methodology

An introduction to GUSTO

GUSTO is Singapore's largest and most comprehensive birth cohort study to date [14]. With recruitment commencing in 2009, it is a currently ongoing cohort study of pregnant women and their offspring, with recruitment beginning in the first trimester. The last GUSTO baby was born in May 2011. Mothers have been followed throughout pregnancy and their offspring will be seen until the child reaches 3 years of age. It is currently in plan to set up a longer follow-up through childhood and into adulthood.

The primary objective of the GUSTO birth cohort study is to evaluate the role of developmental factors in the early pathways to metabolic compromise, namely obesity and type 2 diabetes mellitus.

With the intention of being a holistic cohort study not limited to a single field, GUSTO is further subdivided into several secondary domains with individual objectives to maximise the research opportunities that have arisen (Fig. 1). Such a domain is Allergy—the concern of this article. Researchers will lead work in their respective domains, in addition to the possibility of co-operating with other domains in a multi-disciplinary approach.

Design of the GUSTO allergy studies

All pregnant mothers (and their offspring) enrolled in the main GUSTO study were eligible for recruitment into the allergy domain. To qualify for GUSTO, mothers had to be Singapore citizens or

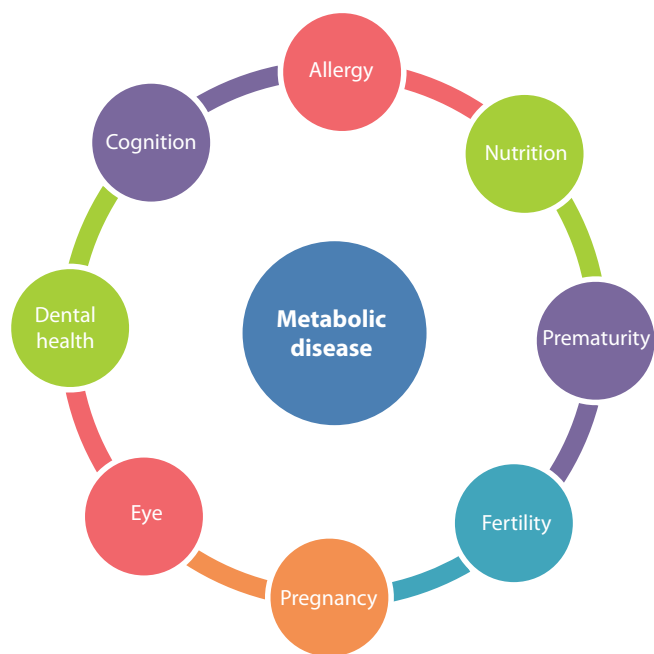


Fig. 1. The main and surrounding secondary domains of Growing Up in Singapore Towards healthy Outcomes.

permanent residents aged 18 years and above who are Chinese, Malay or Indian with homogenous parental ethnic background and have the intention to eventually deliver in the 2 major public maternity units (National University Hospital and KK Women's and Children's Hospital) and to reside in Singapore for the next 5 years. Only healthy women were recruited and thus mothers on chemotherapy, psychotropic drugs or with type 1 diabetes mellitus were excluded. In addition, only women who agreed to donate birth tissues (i.e., cord blood, cord and placenta) at delivery were included. These tissues would then be utilized to study the epigenetic processes associated with the development of allergy.

Women who were recruited in the first trimester before 14 weeks returned to the hospital for 4 follow-up visits. Detailed interviews were conducted in the clinic during the recruitment visit and at 26-28 weeks gestation. Information gathered during interviews included demographics, social data, lifestyle, diet, medications, personal health and family history of disease (including allergy).

Upon delivery, birth tissues were obtained and anthropometric measurements of the newborn were made. During infancy, the babies are examined at home at 3 weeks, 3 months and every 3 months thereafter till 15 months of age. The toddler will be then seen at the study clinic site at 18 months, 24 months and 36 months.

A personal history of allergy was taken from the mother in the 3rd week post-delivery. Information gathered during the abovementioned visits included but was not limited to the child's diet, medications (and supplements), immunisations and environment. The child will be also screened for the onset of symptoms in addition to other illnesses, and biological samples (e.g., breast-milk, stool and nasal swabs) will be also taken at designated visits. Signs indicative of allergy will be recorded in a way that was not temporally restricted to scheduled follow-ups. For example, in the assessment of eczema, photographs of the rash are taken by the mother and sent to pediatric allergists for verification.

Throughout the study, case definitions of atopic eczema, rhinitis, and asthma, adapted and validated for young children from The International Study of Asthma and Allergy in Children definitions [15], were standardized in the questionnaires to ensure consistency during the interviews and house visits. Symptomatology of the conditions was assessed thoroughly. Photos of skin conditions like hives and urticaria were attached to help parents identify and report these conditions.

An extensive assessment of the toddler is planned at age 18 months. This will include a detailed history-taking, a clinical examination of the skin, upper and lower respiratory tract systems and allergy testing. Proposed investigations at this age include standard skin prick testing for common allergens. Longer term follow-up through childhood is also planned which would include repeat skin prick assessment and lung function test.

Implementation of the GUSTO allergy studies

Enrolment of mothers started in June 2009 up till September 2010, with a total of 1,163 recruited mothers out of the 3,335 assessed for eligibility (1,880 were eligible). The study protocol was approved by the Institutional Review Board and all participants gave their written, informed consent. Questionnaires were administered and samples were taken by trained personnel to ensure validity of information collected. All mothers have since delivered by May 2011.

The investigators of the GUSTO allergy study are pediatric allergists from the National University Hospital and KK Women's and Children's Hospital, working in collaboration with colleagues and specialists from other departments.

Preliminary findings

Recruitment for the study was completed in September 2010

with 1,163 pregnant women recruited into the main GUSTO cohort (current drop-out rate = 12.1%). All of the women have delivered but data collection and analyses are still ongoing. Amongst the participants recruited, 56% were Chinese, 26% Malay and 18% Indian. The women were on average 30 years of age (range, 18-46 years). At least 55% of them had completed pre-university education and 70% were in paid employment at the time of recruitment. Results showed that 39.8% of the mothers had a personal history of having at least one allergic disease, which included asthma, eczema and allergic rhinitis.

Of the babies delivered, at least 945 have reached 3 months of age by end-May 2011 and preliminary data showed that 10.4% have had symptoms suggestive of chronic rhinitis (defined as running nose, blocked or congested nose, snoring or noisy breathing during sleep or when awake that has lasted for 2 or more weeks duration), 10.3% have had a possible eczematous rash with 3.6% already diagnosed with atopic eczema, and 5.6% have had wheezing episodes. Further analyses are ongoing.

DISCUSSION

There have been numerous cohort studies carried out on various aspects of allergy. Birth cohorts such as those in Australia [16], the USA [17] and cohorts that comprise the GA²LEN project in Europe [18] have identified numerous potential risk factors for the development of allergic diseases. These include gene associations and a variety of environmental factors in early life.

The advantage of the longitudinal nature birth cohorts is the ability to detect cause-effect relationships. Besides sharing this advantage with previous cohorts, GUSTO also includes new and exciting opportunities in the study of pediatric allergy due to its unique nature, as elaborated below.

Firstly, GUSTO is a cohort study with multiple domains, with a primary aim of evaluating early factors leading to metabolic compromise. The multi-domain nature of GUSTO allows researchers to utilise findings from and draw on the expertise of other GUSTO domains through collaborations with other specialists. This creates the prospect of exploring a wider variety of associations and also in greater depth, which is needed to unlock the multi-factorial nature of allergic disease.

Secondly, GUSTO aims to explore the role of epigenetics in allergy, through conducting studies on different tissues. There is increasing evidence pointing to the influence of prenatal and early

life exposures on the development of allergic disease, in particular that transient environmental pressures can have permanent effects on gene regulation and expression through epigenetic mechanisms [19]. The GUSTO design offers the opportunity to examine (in relation to clinical outcomes) the epigenetic effects of maternal diet, in addition to epigenetic markers at birth as a function of fetal growth and subsequently over development and its effect on allergy.

The GUSTO allergy domain, like other birth cohorts of allergy, uses standard case definitions of asthma, rhinitis and eczema to ascertain the presence of allergy in subjects. Interviewers were well versed in clinical symptoms and signs in assessing for allergy. Standard skin prick tests will also help in identifying specific allergens prevalent in Singapore such as house dust mites [20]. This will help shed light on mechanisms underlying different types of allergy.

There is a need to obtain local epidemiological data on allergy in Singaporeans and to detail the natural history of the local disease phenotype. A unique feature of GUSTO is that the three distinct ethnic groups, Chinese, Malays and Indians, in the Singaporean population allows us to examine the extent to which genomic variation influences allergic phenotype. Singapore's advanced infrastructure provides advantages for population-based research studies. Every citizen has a unique identity card number which promises the potential for linkages using national databases. In addition, being a small country, there is ease of subject follow-up.

A large number of specific sub-studies on pediatric allergy are now planned in GUSTO; some of them have started, and preliminary data are now gathered. These include studies on the role of vitamin D in allergic sensitization, house dust mite sensitisation through life, the role of bacterial colonisation in the development of eczema, epigenetic mechanisms of expression of allergy genes at birth, and its association with prenatal environmental conditions (diet, allergen exposure, and smoking), early studies of flaggrin, and phenotypes of rhinitis during the first year of life and the role of respiratory viruses.

In conclusion, allergy is a complex spectrum of diseases with numerous unexplored aspects. The GUSTO allergy study is a major birth cohort study with an innovative design and multi-disciplinary nature. It has the potential to shed light on the unanswered questions in allergy. As a Singapore-based study, it will be the first major Southeast Asian cohort on allergy to be conducted.

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