Exome Sequencing Studies for Kids with Non-Familial Food Allergy

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ABSTRACT.

Introduction: Food allergies (FA) have been increasing dramatically over the past 25 years and the peanut allergy has been noticed more likely for a half-decade as well. The prevalence of FA in children was found to be 10%, which is high compared with adults. Limited periodic
reports are available of numerous food allergic reactions in children. Complete exome sequencing in this context had an opportunity to investigate Saudi children diagnosed with FA. However, the genetic mechanisms and their factors underlying FA are largely not recognized. Three non-familial cases were arbitrarily selected along with three matching control children.

**Results:** A total of 26 mutations were documented from the six samples; 20 mutations were confirmed through FA cases and 6 from control children. There are four mutations, namely rs35364374, rs2293404, rs9657362 and rs757387978, which had not been associated with FA in any prior study with children. This mutation was appearing in both the cases and controls. However, chr5: 109973901-TMEM322 gene and chr19: 39008235-RYRI gene appeared as novel mutations only in the control children. We could not find this mutation in any FA cases. Our study revealed four mutations which had not previously been connected with food allergy within the database and the rs35364374 and rs9657362 mutations were documented within the database with different diseases.

**Conclusion:** However, in the control children a couple of novel mutations were identified which have not been reported anywhere else. This study recommends screening all the six mutations in food allergy cases and their relevant controls to confirm the prevalence in the Saudi population. Future studies are recommended to study the four variants in detail.

**Keywords:** Mutation; Saudi children; Epigenetic mechanisms; Eczema

**INTRODUCTION**

Food allergy (FA) is defined as an adverse health effect arising from a precise immune response that ensues reproducibly upon exposure to a given food. Based on antigen-specific immunological mechanisms, food allergies are classified as it may be exposed to given food (Sato S, Yanagida N, Ebisawa M, 2018). One of the common mechanisms of food allergy is immunoglobulin E (Ig-E) mediated hypersensitivity reaction to food (Liu X et al., 2018). Food allergy may contribute to increase in mortality and origins life-threatening anaphylactic reactions (Bartuzi Z et al., 2018). The prevalence of food allergy in children is about 10% and 2-3% in adults (McWilliam VL et al., 2018), which is caused by cow milk and eggs. However, global studies have confirmed the connection of food allergy with cow milk and eggs (Martorell A et al., 2017). In children, food allergies are diversified with egg, cow milk, soya, tree nut, peanut, fish, wheat and the most common shellfish (Li J, 2016). Longitudinal studies have demonstrated the effects of exposure on heated eggs, which enhance eventual tolerance to a less heated form of eggs (Berin MC et al., 2018). The global prevalence of food allergies seems to be rapidly growing (Yu W, 2016).

A genetic component to food allergy is supported through numerous studies scrutinizing heritability in families (Carter CA and Frischmeyer-Guerrero PA, 2018). Earlier studies documented the connection between food allergies and eczema, asthma and allergic rhinitis, which are determined through genetic and environmental factors (Minami T et al., 2018; Prescott SL et al., 2013; Renz H et al., 2018). Earlier twin studies have documented that the extensive component of allergy risk is inherited significantly with the higher prevalence of allergic diseases such as food allergy, asthma, eczema, allergic rhinitis and atopic sensitization (Campbell D et al., 2015). Genetic factors are known to play an important role in the development of food allergy (Tan TT, 2012). Fast ripening of allergic diseases cannot be elucidated in terms of traditional Mendelian inheritance as the disease turns out to be multifactorial. Modern lifestyle has been implicated with an environmental factor and various genetic risk loci have
been documented, connected with epigenetic mechanisms and potential mediating for gene-environmental interactions (Neeland MR, 2015). Genetic studies documented with peanuts and flaggarin gene, Human Leukocyte antigen (HLA) -DQB1 loci were shown to be repeatedly associated with the food allergy disease (Brown SJ, 2011; Howell W et al., 1998).

The initial results of genome-wide association studies (GWAS) confirm the association of HLA locus on chromosome 6 in children affected by peanut food allergy (Asai Y et al., 2018). A recent study by Marenholz et al., 2017. Through the GWAS has confirmed the five loci at genome-wide significance which includes the flaggarin and HLA genetic loci. Genomic information is accelerated through the initiation of new tool technologies as next-generation sequencing (NGS), exome-sequencing (ES) technologies are increasingly prevalent and genetics will significantly contribute to the prediction, prevention and treatment of food allergy (Hong X, 2009). Recent advances in second generation sequencing techniques have transformed the genetics study in human diseases. The targeted sequences of the protein-coding portion of the human genome have been documented as a powerful and cost-effective method for detection of the disease variants under Mendelian disorders (Wang Z, 2013). In the last decade, NGS and ES studies have been scrutinized in genetic and non-genetic diseases in the Saudi population and till date, no exome-sequencing studies have been carried out with the children affected by food allergy. Based on the prior studies (Yang M et al., 2017) implemented with exome sequencing, we speculated genetic susceptibility may ally with the development of food allergy and the current study aims to perform the exome sequence in 3 children affected with food allergy in the Saudi population.

MATERIALS AND METHODS

Children enrolment and samples collection

An ethical grant for this study has been received from the Institutional Review Board, Umm Al-Qura University (18-8/2/1453 H) and also from Wilada Maternity and Children's Hospitals (47/25/107862) from the Mecca premises. This study was carried out as per the Declaration of Helsinki. This study is designed with the selection of six children; (Table 1) three of the children was diagnosed and affected by food allergy and the remaining three children were normal. All the cases were part of the wide-scale project incorporated with allergy consisting of asthma, eczema and food allergy in the Saudi children, recruiting 333 cohort families with a total number of 1,000 patients. The children were opted from Paediatric Allergy and Immunology Clinic from Wilada Maternity and Children’s Hospital, Makkah during February 2016-July 2016. Based on the inclusion and exclusion criteria of the study plan, children were recruited. The food allergy cases were diagnosed based upon:

(i) The guidelines for the diagnosis and management of food allergy in the United States
(ii) Clinical history
(iii) Evaluation of IgE, reaction nature and
(iv) Skin prick-test.
(v) The exclusion criteria were based on tests that confirm the positive for food allergy. All the parents signed the informed consent form on behalf of their children to enrol in this study.

Table 1: Selected patients’ phenotypes and characteristics

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Diagnosis</th>
<th>Age at diagnosis (Year)</th>
<th>Blood eosinophil (%)</th>
<th>SPT*</th>
<th>Treatment</th>
<th>Others/diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case I</td>
<td>Food Allergy</td>
<td>1-5 years</td>
<td>10.6</td>
<td>Caw milk, egg , fish, peanut</td>
<td>NA</td>
<td>Asthma</td>
</tr>
<tr>
<td>Case II</td>
<td>Food Allergy</td>
<td>1-5 years</td>
<td>0.58</td>
<td>Egg, mango</td>
<td>NA</td>
<td>Eczema/Chin</td>
</tr>
<tr>
<td>Case III</td>
<td>Food Allergy</td>
<td>5-10 years</td>
<td>0.1</td>
<td>Egg</td>
<td>NA</td>
<td>Eczema</td>
</tr>
<tr>
<td>Control 1</td>
<td>Control</td>
<td>1-5 years</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Control 2</td>
<td>Control</td>
<td>10-15 Years</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Control 3</td>
<td>Control</td>
<td>10-15 Years</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

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From each child, 2 mL of the peripheral blood was collected in an EDTA vacutainer and stored in the deep freezer and later on genomic DNA was extracted using the QIAGEN kit as per the manufacturer’s instructions. Quantified genomic DNA using NanoDrop was further used for library preparation for the process of exome sequencing.

**Exome sequencing analysis**

Using ion torrent equipment, exome sequencing was carried out in six children (three were diagnosed as food allergy and the remaining three were controls). Using the Ion Proton platform, initially sequencing was accomplished with the whole exome AmpliSeq kit. A minimum of 10 cycles of amplification process was executed with Ampli Seq Hi-fi mixes and 12 diverse pools of exome primers by adding 100 ng of 6 different genomic DNA. After that, FUPA reagent was used to pool PCR products for further primer digestion and further followed for ligation with adapters and Ion barcodes. Libraries, which are purified and quantified through Ion library quantification kits, were processed for emulsion with the Ion One Touch System. For Ion One Touch ES, the enriched template on Ion Sphere particles was used for further sequencing process. Using the required sequences reads was mapped and variants were analysed through Ion Torrent software as well.

Complete exonic variants were analysed using Torrent Suite software in the children’s samples using the Ion Reporter tool (version 5.6) for functional consequences for identifying variants used for designing various filters to rescue several pathogenic variants in food allergic disease. Further, Thermofisher filters were created to opt children and DisGeNET databases were also used for built-in-filters for extra downstream analysis to eradicate all the variants continental results (Seo H et al., 2017). In addition, a heat map is used and performed in these studies, is a two-dimensional representation of data in which values are represented by colors. A simple heat map provides an immediate visual summary of information.

**RESULTS**

In this study, we have selected six children: three were diagnosed for food allergy and the remaining three were controls. The age range of all the six male children was 1-11 years. With the Ion Proton platform, exome sequencing was performed in all the children with an average read depth of 81x of targeted platforms. Almost 94% of the targeted regions were covered for more than 20x. Based on amino acid substitution, the SIFT score predicts the protein function of deleterious effects. The scale-invariant feature transform (SIFT) is a feature detection algorithm in computer vision to detect and describe local features in images. SIFT predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids. SIFT can be applied to naturally occurring non-synonymous polymorphisms and laboratory-induced missense mutations. The scores ranged from 0.0-1.0, with the variants scoring less than 0.05 being considered as deleterious. In Figure 1 is described as the heat map and illustrates six samples (Cases 1, 2 and 3 and Controls 1, 2 and 3) of diagnosed food allergy genes’ deleterious effects on protein function based on amino acid substitution (Ngak-Leng Sim et al., 2012).

Almost all, more than 332,000 variants per VCF files were annotated from all the samples. In this study a total of 52,744, 52,171 and 54,416 variants were found in the food allergy cases of the exon region with the incorporation of amino acid substitution, which is documented in Table 2. In the 3 control samples, 60261, 51243 and 61165 variants were documented in the exon region.

Various parameters such as coding region, protein effect/amino acid substitution, genetic locus, genomic position, pathogenicity, type of variant, transcript, allele frequencies (reference and observed), phylop, Sift, Grantham and polyphen were used to filter the variants. From the 6 samples, we have discovered 26 variants, among which the food allergy cases have confirmed 20 variants, whereas the controls have confirmed 18 variants. All the variants were found to be synonymous and missense.
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Figure 1: Details of SIFT score for 3 food allergy cases

Table 2: Identified variants in all the cases and controls.

<table>
<thead>
<tr>
<th>Location</th>
<th>sample 1</th>
<th>sample 2</th>
<th>Sample 3</th>
<th>Control 1</th>
<th>Control 2</th>
<th>Control 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB11: exonic : NM_003742.2___</td>
<td>A/G</td>
<td>A/G</td>
<td>A/G</td>
<td>A/G</td>
<td>A/G</td>
<td>G/G</td>
</tr>
<tr>
<td>TMEM232: exonic : NM_001039763.3___</td>
<td>C/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMEM232: exonic : NM_001039763.3___</td>
<td>C/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DPB1 : exonic : NM_002121.5___</td>
<td>C/A</td>
<td>C/A</td>
<td>C/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DPB1 : exonic : NM_002121.5___</td>
<td>G/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DPB1 : exonic : NM_002121.5___</td>
<td>A/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DPB1 : exonic : NM_002121.5___</td>
<td>C/T</td>
<td>C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DPB1 : exonic : NM_002121.5___</td>
<td>C/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQCE : exonic : NM_152558.4___</td>
<td>C/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQCE : exonic : NM_152558.4___</td>
<td>C/T</td>
<td>C/T</td>
<td>C/T</td>
<td>T/T</td>
<td>C/T</td>
<td>C/T</td>
</tr>
<tr>
<td>IQCE : exonic : NM_152558.4___</td>
<td>G/A</td>
<td>A/A</td>
<td></td>
<td>G/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQCE : exonic : NM_152558.4___</td>
<td>A/G</td>
<td>A/G</td>
<td>A/G</td>
<td>G/G</td>
<td>A/G</td>
<td>A/G</td>
</tr>
<tr>
<td>IQCE : exonic : NM_152558.4___</td>
<td>C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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The genetic mutations such as 1331T>C in \textit{ABCB11} gene, 1637C>T; 1786A>G in \textit{IQCE} genes; 1814C>T in \textit{COG7} gene and 1396T>A in \textit{ATP2C2} gene, 1238C>G in \textit{SERPINB7} gene and \textit{HLA}-\textit{DPB1}, \textit{HLA-DPA1} genes (Table 2). A minimum of 3-6 mutations appeared in both the cases and controls with 1331T>C in \textit{ABCB11} gene, 1637C>T; 1786A>G in \textit{IQCE} genes; 1814C>T in \textit{COG7} gene and 1396T>A in \textit{ATP2C2} gene. In the food allergy cases, single heterozygous mutations appeared in the \textit{c.1331T>C} in \textit{DPB1}, \textit{HLA} gene and 1396T>A in \textit{HLA}-\textit{DPB1}, \textit{HLA-DPA1} genes; 1894G>C in \textit{TMEM232} gene; 619C>A in \textit{ATP2C2} gene; a single homozygous mutant appeared in the c. 7259C>G, c.258G>C in \textit{IQCE} genes; 7293T>C, c.292A>G in \textit{ATP2C2} gene; a single homozygous variant appeared in 472G>A in \textit{MMP13} gene (Table 3). The variants listed in Table 4 have been documented in our database study in all the food allergy cases and controls. However, we have found 5 mutations in the food allergy children and the remaining 8 of them in the control children. Among the 13 mutations, 10 were found to be heterozygous and the remaining three were documented to be homozygous variants.

**Table 3:** Complete analysis results of exome sequence analysis in children diagnosed in food allergy cases.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Ref.</th>
<th>Type</th>
<th>Variant Frequency</th>
<th>Genes</th>
<th>Location</th>
<th>Amino Acid Change</th>
<th>Coding</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr:169830328</td>
<td>A</td>
<td>SNV</td>
<td>1</td>
<td>\textit{ABCB11}</td>
<td>\textit{ABCB11} : exonic : NM_003742.2___</td>
<td>p.Val4444Ala</td>
<td>c.1331T&gt;C</td>
<td>A/G</td>
<td>A/G</td>
<td>A/G</td>
</tr>
<tr>
<td>chr:5109756361</td>
<td>C</td>
<td>SNV</td>
<td>0.33</td>
<td>\textit{TMEM232}</td>
<td>\textit{TMEM232} : exonic : NM_001039763.3___</td>
<td>p.Glu632Gln</td>
<td>c.1894G&gt;C</td>
<td>C/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chr:6330486459</td>
<td>TTT</td>
<td>MNV</td>
<td>0.33</td>
<td>\textit{HLA-DPB1}, \textit{HLA-DPA1}</td>
<td>\textit{HLA-DPB1} : exonic : NM_002121.5___, \textit{HLA-DPA1} : upstream : NM_001242524.1___</td>
<td>p.Glu632Gln</td>
<td>c.7112AAA&gt;TGC, c.1111G&gt;T, c.7115G&gt;A</td>
<td>TTT/CA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chr:6330486460</td>
<td>C</td>
<td>SNV</td>
<td>0.67</td>
<td>\textit{HLA-DPB1}, \textit{HLA-DPA1}</td>
<td>\textit{HLA-DPB1} : exonic : NM_002121.5___, \textit{HLA-DPA1} : upstream : NM_001242524.1___</td>
<td>p.Glu632Gln</td>
<td>c.7255G&gt;T, c.254C&gt;A</td>
<td>C/A</td>
<td>C/A</td>
<td></td>
</tr>
<tr>
<td>chr:6330486440</td>
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<td>SNV</td>
<td>0.33</td>
<td>\textit{HLA-DPB1}, \textit{HLA-DPA1}</td>
<td>\textit{HLA-DPB1} : exonic : NM_002121.5___, \textit{HLA-DPA1} : upstream : NM_001242524.1___</td>
<td>p.Lys996Glu</td>
<td>c.7293T&gt;C, c.292A&gt;G</td>
<td>A/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chr:633052981</td>
<td>C</td>
<td>SNV</td>
<td>0.33</td>
<td>\textit{HLA-DPB1}</td>
<td>\textit{HLA-DPB1} : exonic : NM_002121.5___</td>
<td>p.Leu207Met</td>
<td>c.619C&gt;A</td>
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<tr>
<td>chr:72644519</td>
<td>C</td>
<td>SNV</td>
<td>1</td>
<td>\textit{IQCE}</td>
<td>\textit{IQCE} : exonic : NM_152558.4___</td>
<td>p.Ala546Val</td>
<td>c.1637C&gt;T</td>
<td>C/T</td>
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<td>chr:72645526</td>
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<td>p.Arg587His</td>
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<td>SNV</td>
<td>1</td>
<td>\textit{IQCE}</td>
<td>\textit{IQCE} : exonic : NM_152558.4___</td>
<td>p.Thr596Ala</td>
<td>c.1768A&gt;G</td>
<td>A/G</td>
<td>A/G</td>
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</tr>
</tbody>
</table>

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**DISCUSSION**

Genetics plays an important role in human health and after the grant success of the human genome project sequence has acutely amended the life science research (Murgia C and Adamski MM, 2017). Family-based studies provide unique opportunities to detect genetic variants that complement studies of unrelated individuals (Wang X et al., 2016). In the earlier era, in genetics, a genome-wide association study (GWA study, or GWAS) was basically focussed with moderate effects on common genetic and non-genetic variants as single nucleotide polymorphisms (SNPs) as well as with linkage disequilibrium. The second generation sequencing techniques such as NGS and ES have transformed genetics research through the accumulation of genomic sequencing. Sequencing interest has shifted towards categorizing rare variants connected with specific diseases (Khan IA et al., 2016). The aim of the present study was to identify the novel, candidate and genetic mutations for food allergic disease based on rare variants observed in exome sequencing analysis data in the Saudi population. We have identified 57 missense mutations in both the cases and controls after filtering the ES data for genetic and non-genetic mutations with strong functional effects and lower background frequencies (Hoebel A et al., 2017). However, with the

**Table 4:** Documented common variants present in both the food allergy cases and controls in the Saudi children

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>dbSNP</th>
<th>Locus</th>
<th>Allele</th>
<th>References</th>
<th>Amino acid Substitution</th>
<th>Coding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>TMEM232</td>
<td>Novel</td>
<td>chr5: 109973901</td>
<td>C/C</td>
<td></td>
<td>Lys167Glu</td>
<td>c.499A&gt;G</td>
</tr>
<tr>
<td>Case 1</td>
<td>RYR1</td>
<td>rs53564374</td>
<td>chr19: 38983180</td>
<td>G/T</td>
<td></td>
<td>Gly2060Cys</td>
<td>c.6178G&gt;T</td>
</tr>
<tr>
<td>Control 2</td>
<td>RYR1</td>
<td>rs53564374</td>
<td>chr19: 38983180</td>
<td>T/T</td>
<td>Gly2060Cys</td>
<td>c.6178G&gt;T</td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>IQCE</td>
<td>rs2293404</td>
<td>chr7: 2644519</td>
<td>C/T</td>
<td></td>
<td>Ala546Val</td>
<td>c.1637C&gt;T</td>
</tr>
<tr>
<td>Case 2</td>
<td>IQCE</td>
<td>rs2293404</td>
<td>chr7: 2644519</td>
<td>C/T</td>
<td></td>
<td>Ala546Val</td>
<td>c.1637C&gt;T</td>
</tr>
<tr>
<td>Case 3</td>
<td>IQCE</td>
<td>rs2293404</td>
<td>chr7: 2644519</td>
<td>C/T</td>
<td></td>
<td>Ala546Val</td>
<td>c.1637C&gt;T</td>
</tr>
<tr>
<td>Control 1</td>
<td>IQCE</td>
<td>rs2293404</td>
<td>chr7: 2644519</td>
<td>C/T</td>
<td></td>
<td>Ala546Val</td>
<td>c.1637C&gt;T</td>
</tr>
<tr>
<td>Control 2</td>
<td>IQCE</td>
<td>rs2293404</td>
<td>chr7: 2644519</td>
<td>C/T</td>
<td></td>
<td>Ala546Val</td>
<td>c.1637C&gt;T</td>
</tr>
<tr>
<td>Control 3</td>
<td>IQCE</td>
<td>rs2293404</td>
<td>chr7: 2644519</td>
<td>C/T</td>
<td></td>
<td>Ala546Val</td>
<td>c.1637C&gt;T</td>
</tr>
<tr>
<td>Case 3</td>
<td>ARHGEF10</td>
<td>rs9657362</td>
<td>chr8: 1833801</td>
<td>G/C</td>
<td></td>
<td>Leu370Phe</td>
<td>c.1110G&gt;C</td>
</tr>
<tr>
<td>Control 1</td>
<td>ARHGEF10</td>
<td>rs9657362</td>
<td>chr8: 1833801</td>
<td>G/C</td>
<td></td>
<td>Leu370Phe</td>
<td>c.1110G&gt;C</td>
</tr>
<tr>
<td>Control 1</td>
<td>ATP2C2</td>
<td>rs757387978</td>
<td>chr16: 84492758</td>
<td>A/G</td>
<td></td>
<td>Tyr727Cys</td>
<td>c.2180A&gt;G</td>
</tr>
<tr>
<td>Control 2</td>
<td>RYR1</td>
<td>Novel</td>
<td>chr19: 39080235</td>
<td>A/C</td>
<td></td>
<td>Thr3308Pro</td>
<td>c.9922A&gt;C</td>
</tr>
</tbody>
</table>
combined data for all the 6 children based on the gene prioritization strategy, 26 mutations remained identified in 9 genes (Table 2), which can now be considered as candidate variants in the involvement of food allergic disease in the Saudi population. However, in our study, we have documented two novel mutations in locus chr5: 109973901 in TMEM322 gene and chr19: 39008235 loci in RYR1 gene. Both these novel missense mutations involve amino acid substitutions and coding region in the precise locations (for TMEM322 gene, Lys167Glu; 499A>G and for RYR1 gene, Thr3308Pro; 9922A>C). The exact cause and role of this mutation has not been documented as this was the initial novel variants documented in the control children. The children diagnosed with food allergy cases have confirmed the family history of eczema in a couple of cases.

Figure 2 presents the combination of common mutations observed in all the 3 samples of food allergy cases. The 1396A>T, 1786A>G, 1637C>T and 1331T>C mutations commonly appeared in all three cases. In the initial sample, 5 single mutations (2105A>G, 1231G>A, 1238C>G, 5360C>T and 6178G>T) were documented. We could not find a common mutation with the combination of first and second samples. However, the 1396A>T common mutation appeared in the second and third samples. Three single mutations in the c.-7112AAA>TGC, c.111_113delTTTinsGCA, 7259C>G, 258C>G and 7293T>C, 292A>G were documented in the second sample. Only the 7255G>T, 254C>A mutation was present in the second and third samples.

The 1894G>C, 619C>A, 1760G>A, 2069C>T, 1110G>C and 472G>A single mutation was found in the third sample. We could find common mutations with the first (1396A>T) and second (7255G>T, 254C>A) samples (Figure 2). From all three samples, we have documented the 30 mutations and divided them as 10 mutations in the first sample; in the second sample, we have documented 8 mutations; and in the third sample, 12 mutations have been.

![Figure 2](https://example.com/figure2.png)

**Figure 2:** Regular variants identified in food allergy cases through exome-sequencing. This presents the combination of common mutations observed in all the 3 samples of food allergy cases. Sample 1 is indicated with light blue colour, sample 2 with yellow and pink is indicated with the sample 3. Sample 1 consists of 5 unshared and 5 shared variants. Sample 2 consists of 5 shared and 3 uncommon variants. Sample 3 is involved with 6 mutual and non-communal variants.
In the documented controls (Figure 3), we have observed 28 mutations (first control–11 mutations, second control–8 mutations and in final control–9 mutations). In this study, 315,134 non-singleton variants were present in the 6 children, with a minimum of 2 alleles observed per variant. Filtering to retain only coding mutations resulted in 305,046 variants for exome-wide analysis. Three common mutations in the region of 1331T>C, 1637C>T and 1786A>G in all the three samples have been documented. In the initial control, the 499A>G, 7255G>T, 254C>A, 1110G>C and 2180A>G common mutations were present. The combination of the first and second sample showed 1396A>T mutations, whereas the second and third sample combination displayed 797G>A, 1238C>G and 1760G>A mutation. The second sample consisted of 5360C>T, 6178G>T and 9922A>C mutations and among this 9922A>C was found to be novel, one which appeared only in the control subjects. The combination with the first sample showed 1396A>T as a common mutation and 596C>T mutation in the combination of the second and third samples. The final sample consisted of 7112AAA>TGC-113delTTT, 985C>G and 2069C>T single mutations. The 596C>T combinational mutation was present with the first and third sample, whereas 797G>A, 1238C>G and 1760G>A common mutations appeared in the combination of the second and third sample. We have documented 161,807 variants in the controls, while only 143,239 variants were present in the cases. This may not be the only reason and our study strongly recommends conducting the case-control study with the two identified novel variants.

Figure 3: Normal variants identified in control children through exome-sequencing.

From our samples, we could not find any symptoms connected with allergy-gastrointestinal linked with KAT6A mutations. However, Elinius et al. (Elinius V, 2017) have documented this mutation in their case study in the 16 months of the children. This novel mutation KAT6A is also known as MOZ/MYST3. The functional role of the KAT6A mutation is to identify the syndromes appearing in hypotonia, microcephali, developmental delay, early feeding problems and cardiac defects. However, in our study, a novel mutation appeared in a couple of control subjects rather than the food allergy cases. The clinical features in the control children seemed to be normal. In Table 4, a total of 6 mutations, altogether four common (rs35364374-RYRI, rs2293404-IQCE, rs9657362-ARHGEF10 and rs757387978-ATP2C2) and a couple of novel mutations (chr5: 109973901-TMEM322 & chr19: 39008235-RYRI) were documented. The rs35364347 mutation was connected with central core disease (Kraeva N et al., 2013) and Hendrix et al., (Hendrix P et al., 2017) study failed to show the association with this mutation in RYRI gene. The rs9657362 mutation was documented in a couple of studies with Charcot-Marie-Tooth disease (Beutler AS et al., 2014; Boora GK et al., 2015). Both the rs2293404 and rs757387978 mutations were not
documented with any of the human diseases. Limited studies in Saudi Arabia have been conducted in genetics and non-genetic studies on food allergy (Aba-Alkhaiil BA and El-Gamal FM, 2000; Alduraywish SA et al., 2016; Al-Hussaini A, 2013; Almogren A, 2013; El-Rab MO, 1998; Vandenplas Y et al., 2014). Very limited studies through globally were documented on case-control, hospital-based, genetics and non-genetic studies. Limited exome and next-generation sequencing studies were documented (Li J, 2016; Yang M et al., 2017; Hoebel A et al., 2017).

The strength of our present study is to opt the precisely diagnosed diseased matching case-controls. Execution of complete exome sequencing for the cases and controls is another of its strengths. A final strength is to opt purely Saudi children. However, in this study, we have certain limitations; one of the current limitations involved in this study is not documenting the other disease in the control subjects. Skipping the Sanger sequencing for validation is another current limitation of this study. Finally, incorporating only three of each food allergy cases and controls is a major limitation.

CONCLUSION

In conclusion, a couple of novel mutations in TMEM322 and RPTN genes were recognized only in the control children and this suggests the presence of this mutation indicates the non-risk in any Saudi subject/children/population. Our current results exposed four mutations (rs35364374, rs2293404, rs9657362 and rs757387978) which were not already connected with food allergy within the database. However, rs35364374 and rs9657362 mutations were documented within the database with different diseases. The remaining novel mutations such as chr5: 109973901 and chr19: 39008235 have not been reported anywhere else. This study recommends screening all the six mutations in food allergy cases and their relevant controls to confirm the prevalence in the Saudi population. It is recommended future studies examine the four variants in detail.

CONFLICT OF INTEREST

Authors Mr. UDAYA RAJA GK and Mr. MOHAMMAD ADIL were employed by Integrated Gulf Biosystems. All the authors declare no competing interests.

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Husni H Rayes: Diagnose patient and monitored data collection in hospital - Project Co-investigator

Ahmed Abdelmotelb: Research design

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Exome Sequencing Studies for Kids with Non-Familial Food Allergy

Anas Dannoun: Monitored data collection for the whole trial -Practical genetic analysis

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Ahmed Fawzy: Research design and contacting scientific journal steps for publishing

Imran Ali Khan: Drafting the article and submission

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