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Whole exome sequencing detects novel variants in Saudi children diagnosed with eczema

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ABSTRACT

Background: Eczema is also known as atopic dermatitis is well-known for the skin disease globally. In Saudi Arabia, exome sequencing studies have not been documented. The purpose of this study was to scrutinize the disease causing mutations in children affected with eczema with exome sequencing in the Saudi population.
Methods: We recruited randomly three sporadic cases of children diagnosed with eczema and simultaneously, three more cases were adopted for control samples. Exome sequencing was carried out by applying a pipeline that captures all the variants of concern related to the samples by using the Ion torrent.
Results: In this study, we have documented 49 variants, among which 37 variants were confirmed through eczema children and remaining 30 variants through control children. However, from the analysis of the 6 samples, we have identified rs10192157 (1646C > T; Thr549Ile), rs2899642 (27C > G; Asn9Lys), chr1:152127950 (1625 G > A; Gly542Asp) and chr1:152128041 (1534C > G; Gly512Arg) variants which are rarely linked to the disease eczema. In the rs10192157, we have documented these mutations in all three eczema children and one in the control; the rs2899642 mutation appeared in only a couple of eczema children, whereas the mutation in the chr1:152127950 regions appeared in only one eczema patient. However, the chr1:152128041 mutations appeared in only one case of eczema and also in two control children.
Conclusion: Our study revealed four mutations which had not previously been connected with eczema within the database. However, the rs10192157 and rs2899642 mutations were documented with asthma

Abbreviations: AD, atopic dermatitis; WES, whole exome sequencing; RPTN, repetin.

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disease. The remaining mutations such as chr1:152127950 and chr1:152128041 have not been reported anywhere else. This study recommends screening these 4 mutations in eczema cases and their relevant controls to confirm the prevalence in the Saudi population. It is recommended that future studies examine the 4 mutations in detail.

Introduction

Atopic dermatitis (AD; MIM603165) or eczema is classified as an allergic disease and chronic inflammatory skin disorder, associated with asthma and allergic rhinitis [1]. Eczema itself is defined as enlarged skin problems manifesting as rashes, which are itchy and blistered. Eczema is subdivided into two types. (i) Atopic eczema commonly affects the skin region in children due to an overactive immune system. The common factors for developing atopic eczema is due to sweat clothing which ripens for the rashes in the skin and flexor surfaces at the body. (ii) Contact eczema rarely affects the skin region and is allegedly associated with new clothes and the wearing of jewellery. Atopic eczema describes skin inflammation that results in allergy and it can be worsened with allergens, change in weather and stress [2]. Overall, 30–40% of allergies are affected in adults and children worldwide [3]. The precise cause of eczema is still not clear and it is documented as a complex disease linked with strong genetic predisposition. However, some genetic linkages have been demonstrated with particular genes whose pathophysiology is involved in atopy [4,5]. Gut microbiota play an important role in human immunological processes, potentially affecting eczema, one of the allergic diseases [6]. Currently, the therapeutic strategies are mainly based on moisturizers and corticosteroids [7]. The progression from eczema to other allergic disease has been documented and systematically synthesized [8]. Earlier studies have documented the treatment with immunoglobulin E and a central pathogenic role in eczema [9]. The genetic studies in eczema from the past few years have found a connection in converting with other diseases such as immune-mediated disorder and skin barrier impairment, although Th2 and Th22 immune dysfunction plays a significant role in the expansion of the disease eczema [10]. Genetic evidence has revealed the complex interaction between the epidermal barrier and dysregulation of both innate and adaptive immunities in the eczema disease. Mutations in the human filaggrin (FLG), SPINK5, SPRR3, and CLND1 are connected with the atopic dermatitis/eczema. Genetic variants contribute to both innate and adaptive responses with IL-1, a cytokines family with vitamin D pathway genes [11]. Recent advances in genome technology have greatly increased the capacity to analyse the genetic components of exposure to various genetic diseases. The available resources such as single nucleotide polymorphism, Sanger sequencing, TaqMan assay, genome-wide association studies in the large human cohort populations and meta-analysis studies in subsequent mapped for several genetic loci have been carried out in molecular biology [12]. Whole exome sequencing (WES) is the application of the next-generation sequencing technology to determine the variations in the exome, that is, all the coding regions of known genes in the genome. For example, more than 85% of disease-causing mutations in Mendelian diseases are found in the exome, and WES provides an unbiased approach to detect these variants in the era of personalized and precision medicine [13]. Recently, exome sequencing has been documented as having a potential role as a diagnostic tool in clinical practice. Earlier case reports and retrospective studies in paediatric neurology have been performed with WES as genetic tests [14]. Although some of the genetic studies have been implemented in Saudi Arabia [4,15], no WES studies have been conducted with eczema children. The current study aims to investigate the WES in eczema children in Saudi Arabia using Ion torrent equipment.

Materials and methods

Children recruitment

The ethical approval grant for this study was adopted from the Institutional Review Board of Umm Al-Qura University (18 in 8/2/1435H) and the Wilada Maternity and Children Hospitals (47/25/107862) from the Mecca region as per the Declaration of Helsinki. In this study, we have selected six children: three had clinically proven eczema and the remaining three were controls. All the cases were selected from the Department of Paediatric Allergy and Immunology Clinic in the Wilada Maternity and Children Hospital. The subjects were selected in December 2016 in the Mecca premises. All the parents approved the signed inform consent for this study and none of the parents denied consent for broadcasting the clinical photographs. The diagnosis of eczema relied on the assessment of clinical features because there is no laboratory marker or definitive test that can be used to diagnose the condition. Itchy skin is a major criterion, along with three or more of the following five criteria: (i) history of flexural dermatitis; (ii) child's history of dry skin; (iii) onset under the age of 2 years; (iv) child's history of asthma; and (v) visible flexural dermatitis. As per the guidelines of the American Academy of Dermatology the essential features representing the eczema are: (i) pruritus; (ii) eczema (acute, subacute, chronic); (iii) typical morphology and age-specific patterns; and (iv) chronic or relapsing history. The eczema children were selected based on clinical symptoms such as rashes appearing in the flexor surfaces of the skin. The exclusion criteria were having any other genetic disease or syndrome. The control children were selected without eczema, or any other skin diseases, including genetic syndromes.

Exome sequencing analysis

From all the children, 2 mL of peripheral blood was collected in the EDTA vacutainer to carry out the exome sequencing analysis. Genomic DNA was isolated using the DNA extraction kit from Thermo Fisher, as per the manufacturer's recommendation. NanoDrop was used to quantify the genomic DNA and from each sample a minimum of 100 ng of genomic DNA was observed. Whole exome analysis or exome sequencing analysis was performed with the 6 samples (3 eczema cases and 3 controls) using Ion torrents equipment. The sequencing was performed with the Ion proton platform through the whole exome AmpliSeq kit. Along with 100 ng of genomic DNA, different primer pools of 12 exome primers and AmpliSeq Hifi Mix were all used for the amplification analysis for a minimum of 10 cycles. This step was performed with all 6 samples. Later on, using FUPA reagent, PCR products were pooled for further primer digestion and followed by further ligation using Ion barcodes and adapters. The purified and quantified libraries from the Ion library quantification kit were further pro-

Table 1
Demographic information of children participated in this study.

Children no	Gender	Age	Ethnicity	Family history of eczema
Eczema Case-1	Male	8 Years	Saudi	None
Eczema Case-2	Male	13 Years	Saudi	None
Eczema Case-3	Male	13 Years	Saudi	None
Control-1	Male	12 Years	Saudi	None
Control-2	Male	12 Years	Saudi	None
Control-3	Male	12 Years	Saudi	None

cessed for emulsion on an Ion one touch system. The enriched template of Ion Sphere particles was used for Ion one touch ES and further processed for sequencing. Reads were mapped using specific sequences and variants were also analysed with Ion torrent softwares/pipelines [16]. For this study torrent suite software was used to perceive the variants detected in the children's samples. All the exonic variants/mutations – stop loss, nonsense, missense, frameshift insertion/deletion and block substitutions – were identified. The ion reporter tool (version 5.6) was used for the functional consequences for variants and genes, which was applied and built in different filters to rescue pathogenic variants such as exonic variants, polyphen, SIFT score and disease research area were opted for eczema skin disease. These filters (Thermofisher) were curated with medical subject headings and DisGeNET databases which are also built-in filters for additional downstream analysis to eliminate specific variants as intronic, synonymous, and manually inferred the results.

Results

In this study, six children were randomly selected: three were diagnosed with eczema and other three were selected as controls. All the children were male in an age range of 8–13 years (Table 1). Exome sequencing was performed in all the children samples without a family history of eczema by Ion proton platform. The average read depth for the targeted platforms was 82×, with 94% of targeted regions covered at greater than 20×. SIFT score predicts protein function deleterious effects based on amino acid substitution. Scores ranges from 0.0 to 1.0, variants with scores less than 0.05 are considered as deleterious. However, below the heat map illustrates three samples (Samples 1–3) genes deleterious effects in protein function based on amino acid substitution (Fig. 1).

Almost all, more than 160,000 variants per VCF files were annotated from all the samples. In this study a total of 57,970, 33,052 and 52,217 variants were found in the eczema cases of the exon region with the incorporation of amino acid substitution, which is documented in Table 2. In the 3 control samples, 60,272, 51,251 and 50,284 variants were documented on 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 49 variants, among which the eczema cases have confirmed 37 variants, whereas the controls have confirmed 30 variants. All the variants were found to be synonymous and missense. The genetic mutations such as 1534 G > C in RPTN gene, rs174221133, rs194520, rs16853305, rs1607017/rs34441478, rs113327860/rs773766721 and rs748786614/rs76015112/rs770518514 were documented with 1 mutation appearing in the eczema cases and 2 in the control samples. We have documented the 2 novel mutations appearing on the RPTN gene, locus at chr1:152127950 (1652 G > A; Gly542Asp) & chr1:152128041 (1534 G > C; Gly512Arg). The rs11583410/rs10192157/rs1130499/rs2287922 polymorphisms appeared in all the eczema cases and in 1 control; however, rs1064213 was found in only 2 cases of eczema but in all 3 control subjects (Table 2). However, Table 3 describes the maximum number of variants observed in all the 6 samples. All the 37 variants were found to be in both exonic and missense mutations with both the coding and amino acid substitutions. The reference and observed alleles were documented, which was confirmed in this study. The variants listed in Table 4 have been never documented in any of the databases. However, we have found 7 mutations in eczema patients and 3 of them in the control subjects. Among the 10 mutations, 9 were found to be heterozygous and one of the homozygous variants was documented in the control sample.

Discussion

In this study, we documented 4 rare mutations which were linked with eczema cases as well as controls. The identified variants rs10192157 (1646C > T; Thr549Ile), rs2899642 (27C > G; Asn9Lys), chr1:152127950 (1625 G > A; Gly542Asp) and chr1:152128041 (1534C > G; Gly512Arg) were found in the six samples subjected as 10 mutations, which have rarely been linked with the disease eczema when performing the exome sequencing analysis. In the rs10192157, we have documented these mutations in all three eczema children and in one control; the rs2899642 mutation appeared in only a couple of eczema children, whereas the mutation in the chr1:152127950 regions appeared in only one eczema child. However, chr1:152128041 mutations appeared in only one case of eczema and also in two control children. Altogether, in this study we have documented 49 variants, among which 44 variants were confirmed through eczema children and the remaining 15 variants through control children. Familial, genetic and twin studies have standardized the relation between eczema and their family histories as well as with the concordance rate in twins [17,18]. Eczema is also commonly termed as atopic eczema and atopic dermatitis is a skin disease commonly affecting the inflammation of humans which affects children as well as adults. Clausen et al. [19] have confirmed from their studies that skin microbiome significantly varies in AD/eczema subjects as well as in controls. Moreover, the microbiome composition varied within the AD/Eczema patients. From this study, we have confirmed a couple of novel mutations present on the repetin (*RPTN*) gene. In the earlier literature, only a single study documented AD/eczema, which was from the Polish population. The real-time PCR results have confirmed the RPTN gene was not associated with rs28441202, rs12117644 polymorphisms and the CC genotype from rs3001978 was shown to be nominally associated [20]. The *RPTN* gene consists of 784 amino-acid proteins which are rich in glutamine. The RPTN protein is not expressed highly in homeostasis epidermis; however, it can be upregulated based on the modification of the epidermal barrier. In both the loricrin-deficient and Klf4-null mices, the transcription in RPTN was upregulated [20–24].

Fig. 2 presents the combination of common mutations observed in all the 3 samples of eczema disease. The rs10192157 variant was found to be in all 3 cases of eczema. The variant in the rs11583410 was confirmed in the first and second samples of eczema. In the combination of the second and third sample, we have established the single mutation in the rs2287922 region. Three mutations in the regions of rs1064213, rs11430499 and rs289964 were documented in the first and last samples. In total, we have observed 37 common mutations that appeared in all the eczema cases. In this study, 315,134 non-singleton variants were present in 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. The novel mutations such as (i) chr1:152127950-1652 G > A; Gly542Asp, and (ii) chr1:152128041-1534 G > C; Gly512Arg were present in both the cases and controls. The mutation on locus chr1:152127950 was present in only one eczema case whereas in the locus chr1:152128041 was docu-

Table 2
List of all variants observed in all the cases and controls of eczema children's.

Genes	dbSNP	Variant frequency	Locus	Amino acid change	Coding	PhyloP	SIFT	Grantham	PolyPhen	FATHMM	Ref	Case 1	Case 2	Case 3	Control 1	Control 2	Control 3	South Asian Genome	SIMONDiver-sityProject	StatGen	1000GSAS	WellnessGeno-micsProjec
RPTN	rs75957773	0.33	chr1:152127455	p.Glu707Gly	c.2120A > G	1.33	0.05	98	0.356	0.06	T			T/C	T/C			0.11429	0.07706	0.09722	0.07084	
RPTN	rs752917382	0.17	chr1:152127917	p.Gln553Arg	c.1658A > G	0.07	0	43	0.09		T	T/C						No data				
RPTN		0.17	chr1:152127950	p.Gly542Asp	c.1625 G > A	−0.09	0.01	94	0.034		C	C/T										
RPTN		0.5	chr1:152128041	p.Gly512Arg	c.1534 G > C	−1.06	0	125	0.991		C	C/G				G/G	C/G					
RPTN	rs748786614	0.5	chr1:152129094	p.Ser161Pro	c.481 T > C	−1	0	74	0.005	0.016	A			A/G	A/G		A/G	No data				
RPTN	rs113327860	0.5	chr1:152129115	p.Arg154Gly	c.460A > G	0.07	0	125	0.745	0.062	T			T/C	T/C		T/C					0.22727
ITPKB	rs149227914	0.17	chr1:226923466	p.Asn565Ser	c.1694A > G	−0.61	0.05	46	0.002		T				T/C							0.0000
OR2B11	rs12070953	0.17	chr1:247614386	p.Asp300Gly	c.899A > G	5.02	0	94	0.001		T						T/C	0.06966	0.09319	0.05556	0.04312	0.50000
OR2B11	rs4925663	0.33	chr1:247614617	p.Gly223Asp	c.668 G > A	−0.3	0.01	94	0.351	0.075	C	C/T				C/T		0.45975	0.41039	0.54167	0.51643	0.50806
OR2B11	rs11583410	0.67	chr1:247614896	p.Ile130Ser	c.389 T > G	6.29	0	142	0.998	0.984	A	A/C	A/C			A/C	A/C	0.55882	0.37276	0.56944	0.54620	0.64130
CD207	rs115727537	0.17	chr2:71061091	p.Arg84His	c.251 G > A	0.36	0.01	29	0.293		C						C/T	0.01703	0.00896		0.00821	
IL1RL1	rs10192157	0.67	chr2:102968356	p.Thr549Ile	c.1646C > T	−2.38	0.04	89	0.063	0.009	C	C/T	C/T	C/T	C/T			0.17492		0.11111	0.20637	0.50000
XIRP2	rs143400009	0.17	chr2:168099316	p.Ser472Pro	c.1414 T > C	2.22	0	74	1		T		T/C					0.02857	0.00717	0.01389	0.01643	0.02419
XIRP2	rs16853305	0.5	chr2:168099775	p.Pro625Ala	c.1873C > G	1.73	0.01	27	1	0.983	C		C/G		C/G	C/G		0.20743	0.23297	0.33333	0.23717	0.24286
XIRP2	rs556174062	0.17	chr2:168100505	p.Ile868Arg	c.2603 T > G	5.97	0	97	1		T	T/G									0.00103	
XIRP2	rs16853309	0.5	chr2:168103304	p.Arg1801His	c.5402 G > A	2.75	0	29	0.119	0.94	G		G/A		G/A	G/A		0.19814	0.17025	0.30556	0.22895	0.20714
XIRP2	rs16853326	0.33	chr2:168105695	p.Leu2598Arg	c.7793 T > G	−0.03	0.02	102	0.137		T		T/G		T/G			0.05714	0.02867	0.09722	0.05749	0.04762
XIRP2	rs16853333	0.33	chr2:168108032	p.Gly3377Glu	c.10130 G > A	2.59	0.01	98	0.922		G		G/A		G/A			0.05714	0.01971	0.09722	0.05749	0.04762
PLCL1	rs754994541	0.17	chr2:198949644	p.Phe468Tyr	c.1403 T > A	8.03	0	22	1		T					T/A						
PLCL1	rs1064213	0.83	chr2:198950240	p.Val667Ile	c.1999 G > A	9.65	0	29	1	0.989	G	G/A		G/A	G/A	G/A	A/A	0.61920	0.38351	0.61111	0.56982	0.58025
MANBA	rs75826658	0.17	chr4:103553372	p.Val828Ile	c.2482 G > A	5.32	0	29	0.999		C					C/T			0.00538		0.00000	0.50000
KIAA1109	rs149346321	0.17	chr4:123249358	p.Pro3699Thr	c.11095C > A	4.6	0	38	0.798		C				C/A				0.00538		0.00000	
IL7R	rs2229232	0.17	chr5:35876449	p.Thr414Met	c.1241C > T	−0.67	0.02	81	0.723		C			C/T				0.04286	0.01254	0.02778	0.03491	0.03750
TNXB, CYP21A2	rs17421133	0.5	chr6:32010272	p.Asn4055Ile	c.*1361T > A	0.05	0	149	0.989		T		T/A			T/A	T/A				0.24025	0.58333
TNXB	rs62402693	0.17	chr6:32012987	p.Ser3573Pro	c.10717 T > C	1.53	0.01	74	0.998	0.753	A				A/G							0.55000
TNXB	rs9267795	0.17	chr6:32017163	p.Gly3212Val	c.9635 G > T	0.66	0.05	109	0		C						C/A	0.02041	0.02688		0.01745	0.50000
TNXB	rs440160	0.17	chr6:32023903	p.Pro2731Arg	c.8192C > G	0.61	0.05	103	0.989		G			G/C							0.00000	
TNXB	rs10947230	0.17	chr6:32024395	p.Arg2704His	c.8111 G > A	2.36	0.01	29	1		C	C/T						0.03274	0.05914		0.03285	0.50000
TNXB	rs12524664	0.17	chr6:32029431	p.Pro2412Leu	c.7235C > T	2.76	0	98	0.999		G	G/A									0.03285	0.66667
TNXB	rs9469081	0.17	chr6:32035603	p.Val2127Met	c.6379 G > A	−2.07	0.01	21	0.907	0.012	C		C/T							0.27778	0.27721	0.66667
TNXB	rs204900	0.17	chr6:32056580	p.Ser921Ala	c.2761 T > G	2.75	0	99	0.032		A		A/C					0.35714	0.20789	0.27778	0.27721	0.63636
TNXB	rs17201602	0.17	chr6:32063681	p.Arg650His	c.1949 G > A	−1.26	0.01	29	1		C	C/T						0.15410	0.07885	0.09722	0.07906	0.50000
TNXB	rs204896	0.17	chr6:32064098	p.Arg511His	c.1532 G > A	−0.04	0.05	29	0.585	0.032	C		C/T					0.34354	0.19713	0.26389	0.27207	0.68182
TNXB	rs780536658	0.17	chr6:32064372	p.Val420Met	c.1258 G > A	0.21	0	21	1		C	C/T										
TNXB	rs778734003	0.17	chr6:32064972	p.Gly220Arg	c.658 G > C	−0.4	0	125	0.732		C	C/G										
TNXB	rs41270461	0.17	chr6:32065023	p.Val203Met	c.607 G > A	0.47	0	21	0.997		C		C/T					0.11682	0.07168	0.09722	0.06571	0.50000
HLA-DPB1	rs1042153	0.33	chr6:33048663	p.Met105Ile	c.315 G > A	0.18	0.04	10	0	0.045	G			G/A			G/A	0.22401	0.37500	0.32854	0.55556	
C7orf72	rs1456908	0.17	chr7:50180972	p.Thr358Ile	c.1073C > T	3.85	0	89	1	0.347	C	C/T						0.42879	0.32079	0.58333	0.46817	0.48780
STEAP2	rs194520	0.5	chr7:89854446	p.Phe17Cys	c.50 T > G	5.04	0.02	205	0.999	0.958	T			T/G		T/G	T/G					
PTPRN2	rs1130499	0.67	chr7:157931144	p.Ser325Asn	c.974 G > A	0.5	0.02	46	0.01		C	C/T		C/T	C/T		C/T					
PTPRN2	rs3800855	0.17	chr7:157985149	p.Ser140Thr	c.419 G > C	−0.67	0.01	58	0.001		C				C/G							
RAD51B	rs34094401	0.17	chr14:68352648	p.Leu172Trp	c.515 T > G	0.27	0.05	61	0.991		T					T/G						
RNF111	rs2899642	0.33	chr15:59323048	p.Asn9Lys	c.27C > G	0.74	0.02	94	0.449	0.774	C	C/G		C/G								
SCAPER	rs1607017	0.5	chr15:76726465	p.Pro1089Thr	c.3265C > A	0.69	0.01	38	0	0.706	G			G/T		G/T	G/T					
CLEC16A	rs758110635	0.17	chr16:11137918	p.His602Tyr	c.1804C > T	2.2	0.02	83	0.009		C			C/T								
CLEC16A	rs201524313	0.17	chr16:11272395	p.Val1004Ile	c.3010 G > A	5.23	0	29	0.981		G		G/A									
GSDMB	rs16965388	0.17	chr17:38062139	p.Arg330Cys	c.988C > T	−0.14	0.02	180	0.128		G				G/A							
CHST8		0.17	chr19:34263955	p.Ala421Val	c.1262C > T	3.56	0.04	64	0.005		C					C/T						
RASIP1	rs2287922	0.67	chr19:49232226	p.Arg601Cys	c.1801C > T	3.18	0	180	0.999		G		G/A	A/A		G/A	G/A					

dbSNP- database single nucleotide polymorphism; Polyphen- polymorphism phenotyping; Phylop; FATHMM; Statgen.

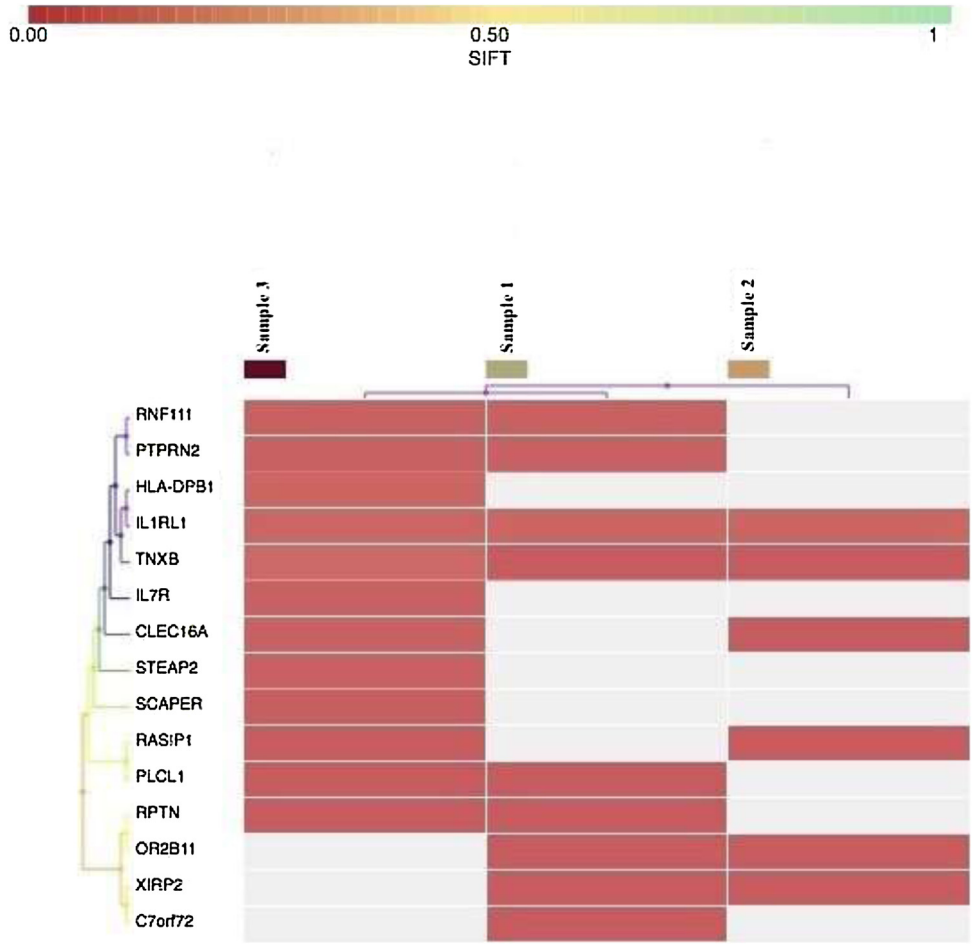

Fig. 1. Details of Sift score for all the 3 eczema cases.

Table 3
List of common variants observed in the eczema cases.

Gene	rsID	Region	Mutation	Coding	Amino acid change	Reference allele	Observed allele	Case 1	Case 2	Case 3
IL1RL1	rs10192157	Exonic	Missense	C1646T	Thr549Ile	C	T	CT	CT	CT
PLCL1	rs1064213	Exonic	Missense	G1999A	Val667Ile	G	A	GA	–	GA
PTPRN2	rs1130499	Exonic	Missense	G974A	Ser325Asn	C	T	CT	–	CT
RNF1111	rs2899642	Exonic	Missense	C27G	Asn9Lys	C	G	CG	–	CG
RAS1P1	rs2287922	Exonic	Missense	C1801T	Arg601Cys	G	A	–	GA	AA
OR2B11	rs11583410	Exonic	Missense	C389T	Ile130Ser	A	C	AC	AC	–

Table 4
Rare variants observed in both the cases and controls of eczema disease.

Gene	rsID	Locus	Region	Mutation	Coding	Amino acid change	Reference allele	Observed allele	Case 1	Case 2	Case 3	Control 1	Control 2	Control 3
IL1RL1	rs10192157	chr2:102968356	Exonic	Missense	C1646T	Thr549Ile	C	T	CT	CT	CT	CT	CC	CC
RNF111	rs2899642	chr15:59323048	Exonic	Missense	C27G	Asn9Lys	C	G	CG	CC	CG	CC	CC	CC
RPTN	–	chr1:152127950	Exonic	Missense	G1625A	Gly542Asp	C	T	CT	CC	CC	CC	CC	CC
RPTN	–	chr1:152128041	Exonic	Missense	G1534C	Gly512Arg	C	G	CG	CC	CC	CC	GG	CG

mented in one eczema case/two controls, which may be due to the fact that in the controls, we have documented 161,807 variants, while in the cases only 143,239 variants were present. This may not be the only reason and our study strongly recommends conducting the case-control study with the two identified novel variants.

Only a limited number of studies have been carried out in the Saudi population with AD/AE/eczema [4,15,25–27]. No genetic sequencing studies have been documented in the Saudi population with eczema disease. Indeed, in the global population, no genetic studies have been carried out with eczema, with exome or next-generation sequencing studies. However, Nylund et al. [28] have conducted a microarray study in eczema children and revealed the marked intestinal microbiota aberrancy and concluded it may subsidise the perpetuation of eczema. Next-generation sequencing studies have documented the connection between eczema and *S. aureus* colonization. These studies proved that flare disease is associated with a significant fall in skin microbiota diversity and increases the relative abundance with *S. aureus* and epidermidis. For eczema, directly, *S. aureus* does not seem to be the major reason

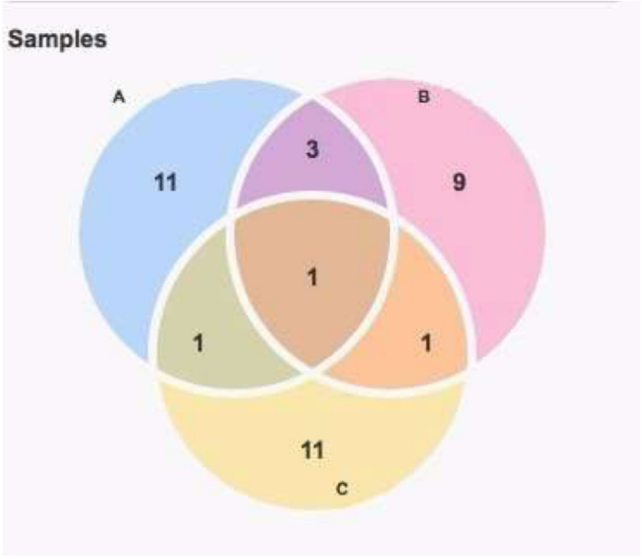

Fig. 2. Common variants identified in eczema cases through exome-sequencing. In figure A denotes as Sample 1; Sample B denotes as Sample 2 and Sample C denotes as Sample C.

that improves a flare, as antimicrobial and anti-inflammatory therapy enhances bacterial diversity [29]. We can confirm our study as the initial exome sequencing studies in the global population and from the Kingdom of Saudi Arabia; we have documented the 2 novel mutations in both the cases of eczema and control children.

Strength of our current study is that we selected accurately diagnosed eczema cases along with the matching controls. Performing exome sequencing was another strength. One limitation of this study is that we skipped the Sanger sequencing for the validation of the four documented mutations that appear in this study. Another limitation was incorporating only three each of the eczema cases and controls.

Conclusions

In conclusion, we have identified a couple of novel mutations in the *RPTN* gene in two different eczema children with exome sequencing analysis. Our current results revealed four mutations which had not previously been linked with eczema within the database. However, rs10192157 and rs2899642 mutations were documented with asthma disease. Remaining novel mutations such as chr1:152127950 and chr1:152128041 have not been reported anywhere else. This study recommends screening these 4 mutations in eczema cases and their relevant controls to confirm the prevalence in the Saudi population. It is recommended future studies examine the four variants in detail.

Authors' contribution

NMB, KKA led the studies concept. AAA, AA, FAA, AF design the study. HHR, AAS, HA, AM, RB, FJ, FM, MA has granted the ethical approval from the hospital and confirmed, collected the cases and controls. AD, HAS, AA, MB has performed the experiment. MMT, AB is part of this project. URGK analysed the bioinformatics data IAK has written the manuscript and communicated the manuscript. All the authors have read and approved final version of the manuscript.

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Ethical approval and consent to participate

Ethical grant for this study has been approved from the Institutional Review Board of Umm Al-Qura University (18 in 8/2/1435 H) and the Wilada Maternity and Children Hospitals (47/25/107862) from the Mecca region as per the Declaration of Helsinki. This study has benne explained to the parents and with their permission, all the participants has provided the inform consent form and parents sign the consent form.

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