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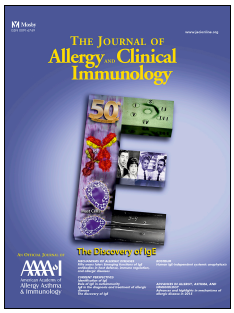

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TITLE PAGE

Letter to the Editor

Array-based sequencing of filaggrin gene for comprehensive detection of disease-associated variants

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Capsule Summary

Comprehensive sequencing of *FLG* is a challenging endeavour. We have developed a method using array-based amplicon PCR and NGS for a robust and cost efficient method to analyze this major atopic dermatitis risk factor.

Key words

Atopic dermatitis; filaggrin, mutations; microfluidics; NGS; skin

Abbreviations used

AD: Atopic dermatitis

CNV: Copy number variation

FLG: Filaggrin gene

IV: Ichthyosis vulgaris

IFC: Integrated fluidic circuit

LoF: Loss-of-function

NGS: Next generation sequencing

Word count: 1048

To the Editor:

The filaggrin gene (*FLG*) is essential for skin differentiation and epidermal barrier formation. *FLG* loss-of-function (LoF) variants are associated with ichthyosis vulgaris (IV) and the major genetic risk factor for developing atopic dermatitis (AD)[1-3]. Genetic stratification of AD patients according to *FLG* LoF risk is a common practice for both research and clinical studies however few studies comprehensively sequence the entire *FLG* coding region. The majority of studies that include *FLG* genotyping have screened for common predominant LoF variants to report allele frequencies after full Sanger-sequencing of a smaller batch of test patient samples or previous published data. This strategy potentially results in under-reporting of the genetic contribution especially in ethnicities where *FLG* LoF variants are highly diverse[4]. Distinct LoF variants have been reported for the majority of ethnicities studied to date. For example, two predominant sequence variants (p.R501X and c.2282del4) make up approximately 80% of the mutation burden in northern Europeans[5], whereas in East Asian ethnicities, a larger *FLG* LoF mutation spectrum is found with fewer predominating variants[6, 7]. However, routinely Sanger-sequencing the entire *FLG* coding region for large cohorts is not always feasible, although desirable as it is essential to correctly stratify patients. To address this we developed a robust and cost-effective high-throughput PCR-based method for analysing the entire coding region of *FLG* using Fluidigm microfluidics technology and next-generation sequencing (NGS). We have applied this method to fully resequence cohorts of Chinese, Malay and Indian AD patients from the Singaporean population.

We designed and optimized overlapping *FLG*-specific primer assays (containing NGS sequencing adapters) to span the entire *FLG* coding region including known intragenic copy number variation (CNV) (See Figure 1A and Table E1 in the Online Repository). 48 overlapping primer assays, with amplicons of maximum 500 base pairs, provided redundancy for sequencing reads across primer binding sites and 100% coverage of *FLG* exon bases (see Figure 1B). The Fluidigm Access Array 48.48 integrated fluidic circuit (IFC) chip (Fluidigm, US), generates 48 amplicons for 48 different DNA samples in parallel, simultaneously thermocycling 2304 PCR reactions at nanolitre volumes (See Figure E1 and Supplemental Methods in the Online Repository). Initially 96 DNA samples were assayed in IFC chips prior to Illumina MiSeq 2x250 bp read mode (4 samples failed). 14 samples from this batch of 96 were previously Sanger sequenced for the entire *FLG* coding region[5, 7]. The known *FLG* LoF variant profile was then used to validate LoF variant detection with the IFC and NGS method. We identified all *FLG* LoF variants originally identified by Sanger in the 14 samples as well as additional variants in two samples (see Table 1) and documented LoF variants in the remaining 78 samples that passed quality control testing (see Table E2 in the Online Repository). LoF variants were all confirmed by visual inspection using Integrated Genome Viewer before Sanger sequencing. Additionally we determined the *FLG* CNV of repeats 8 and 10 (an important risk factor for AD[8]) in the 92 samples using relative coverage based metrics (see Figure E2 in the Online Repository).

The IFC and NGS sequencing method was then used to analyse a further 334 Singapore IV and/or AD patient samples to obtain estimates of disease-associated LoF allele frequency in the three major ethnicities of Singapore - Chinese, Malay and Indian. In 279 Chinese Singaporean samples (see Table E3 in the Online Repository) we identified a further 11 additional LoF variants, raising the total number identified in this population to 33 (an increase

from 22 variants identified in our previous study[7]) with five not previously reported in the literature (see Figure 1C and 1D). 85 of these samples had also been previously Sanger sequenced[7] and the concordance profile was near identical (see Table E4 in the Online Repository). 14 LoF variants reached significance using Fisher's exact test ($p<0.05$) compared to population control data derived from ExAC (version 0.3.1) exome database (see Table E5 and Supplemental Methods in the Online Repository). The combined *FLG* LoF mutation allele frequency for Chinese Singaporean AD patients is now 32.3%, an increase from 20.2% in our previous survey of 425 patient samples further supporting the biological importance of *FLG* mutations in AD[7]. Smaller cohorts of 19 Indian and 36 Malay patients were analysed and we identified *FLG* LoF variants in nine Indian samples and nine Malay samples (See Figure 1D, Table E6 and Table E7 in the Online Repository). The identification of unreported *FLG* LoF variants in Indian and Malay ethnicities from Singapore confirms the diversity of *FLG* variants in AD between different ethnic groups. In total we identified 18 variants with limited overlap with the Chinese samples (4 out of 18) and a number of these are not present in the ExAC database highlighting the contribution of rare, family-specific mutations in AD (11 of 18). This small but well characterized study of Indian and Malay Singaporeans highlights the variation in combined allele frequencies between ethnicities with 47.4% of Indian AD patients and 25% of Malay AD patients harbouring *FLG* LoF variants. The presence of *FLG* LoF variants were strongly associated with AD, however, were not significantly associated with increasing severity in the 334 patients analyzed in this study, possibly due to the small number of mild cases analzyed (mild cases in this cohort 5.3%; see Table E8 in the Online Repository).

In conclusion, we describe a multiplexed targeted resequencing method to study the *FLG* coding region. We highlight that comprehensive sequencing improves accuracy estimates of

genetic contribution from *FLG* deleterious alleles and CNVs in AD. This strategy to study genetic variation does not rely on previous mutation spectrum information from any given population or ethnicity and therefore can identify rare, family-specific or de novo variants globally. This approach outperforms exome sequencing because of its throughput and ability to analyse known CNVs that are not currently reflected in NCBI RefSeq version of *FLG*. Amplicon resequencing is robust, reliable, unbiased and has the potential for scalable sample preparation for small and large research or clinical studies, increasing accurate genotyping for improved outcomes. We have developed a cost-efficient *FLG* genotyping method (approximately 10 times cheaper than exome sequencing) for researchers and clinicians studying patients from any ethnicity that is vital to advance a precision medicine approach to AD. This method can facilitate research studies immediately and be developed for clinical genetic diagnostics in the future.

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CONFLICT OF INTEREST

W. H. Irwin McLean and Frances J. D. Smith (University of Dundee) have registered patents for genetic testing and sequencing of *FLG*. The other authors have no competing interests.

DATA ACCESS

All sequencing files were submitted to NCBI SRA and are publicly available under BioProject ID “PRJNA360024”. Accession codes for the individual samples are included in Table E9.

CONTRIBUTIONS

J.E.A.C. and W.H.I.M designed the study components and planned the study. X.F.C.C.W., J.N.F., S.L.I.J.D., A.S.L.T., R.L.H. and H.J.C. designed and conducted all experiments under the guidance of E.B.L., J.L. and J.E.A.C. A.S. and F.J.D.S constructed the 12 repeat *FLG* sequence and designed the initial primer sets used for development of this assay. Patient samples collection was coordinated and supervised by M.B.Y.T. at National Skin Centre. X.F.C.C.W., S.L.I.J.D., E.B.L. and J.E.A.C. wrote the manuscript with inputs from all authors.

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208 **Table 1.** Concordance of *FLG* LoF variant detection for 14 previously fully Sanger-sequenced
209 AD samples using our MiSeq 2x250 bp protocol. Our NGS protocol detected additional LoF
210 variants in two samples (red text).

211

Sample ID	Sanger sequencing	Illumina MiSeq 2 x 250 assay
IA-P003	p.S1515X	p.S1515X
IA-P009	No LoF detected	No LoF detected
IA-P014	No LoF detected	No LoF detected
IA-P017	p.E2422X	p.E2422X
IA-P021	p.S406X; c.6950_6957del8	p.S406X; c.6950_6957del8
IA-P024	c.1640delG	c.1640delG
IA-P025	p.Q368X; c.3321delA	p.Q368X; c.3321delA
IA-P028	c.7945delA	c.7945delA
IA-P062	p.Q2417X	p.Q2417X
IA-P063	c.2952delC	c.2952delC
IA-P083	c.9040_9058dup19	c.9040_9058dup19; p.Q1790X
IA-P084	p.S1302X; ND	p.S1302X; p.S1515X
IA-P090	c.4004del2	c.4004del2
IA-P094	c.2282del4; p.R2447X	c.2282del4; p.R2447X

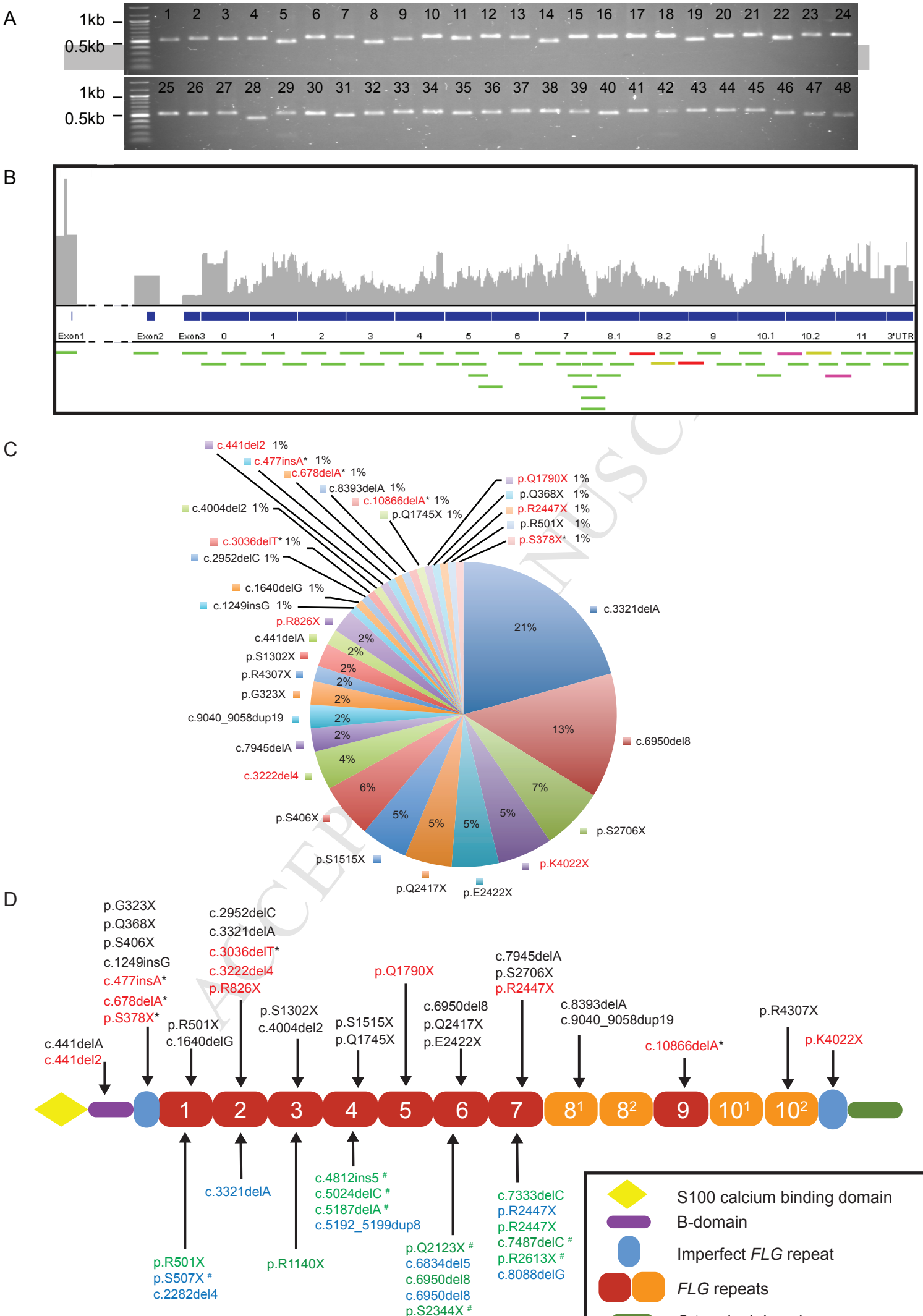
212

213 **FIGURE LEGEND**

214

215 **Figure 1. *FLG* primer validation, amplicon coverage and *FLG* LoF variant analysis in**
216 **Singaporean AD cohorts** (A) 48 *FLG*-specific primer assays were validated by PCR and gel
217 electrophoresis to confirm expected amplicon size and absence of non-specific products. (B)
218 Schematic visualization of overlapping amplicon design across 12-repeat *FLG* coding region
219 (green, yellow, red, purple bars). Primer assay 34 (yellow bars), 35 (red bars) and 41 (purple
220 bars) produced multiple distinct amplicons. (C) Spectrum of disease-associated *FLG* LoF
221 variants identified in the Singaporean Chinese IV and/or AD population. 11 additional variants
222 (highlighted in red) were identified in addition to those from our previous survey, of which 5
223 variants have not been previously reported (*). (D) Profilaggrin schematic showing LoF *FLG*
224 variants domain positions - Singaporean Chinese samples are present above the schematic,
225 variants not previously reported in Singapore Chinese samples are highlighted (red) including
226 *FLG* LoF variants not previously published (*). Positions of *FLG* LoF variants identified from
227 Singapore Malays are highlighted in blue and Singapore Indians in green below the schematic.
228 *FLG* LoF variants not previously identified in AD patient-based studies are marked with [#].

229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1kb
0.5kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
1kb
0.5kb
25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
B
Exon1
Exon2
Exon3
0 1 2 3 4 5 6 7 8.1 8.2 9 10.1 10.2 11 3'UTR
C
c.441del2 1%
c.477insA* 1%
c.678delA* 1%
c.8393delA 1%
c.10866delA* 1%
p.Q1745X 1%
p.Q1790X 1%
p.Q368X 1%
p.R2447X 1%
p.R501X 1%
p.S378X* 1%
c.4004del2 1%
c.3036delT* 1%
c.2952delC 1%
c.1640delG 1%
c.1249insG 1%
p.R826X
c.441delA
p.S1302X
p.R4307X
p.G323X
c.9040_9058dup19
c.7945delA
c.3222del4
p.S406X
p.S1515X
p.Q2417X
p.E2422X
c.3321delA
c.6950del8
p.S2706X
p.K4022X
21%
13%
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6%
5%
5%
5%
5%
4%
2%
2%
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2%
2%
2%
2%
D
p.G323X
p.Q368X
p.S406X
c.1249insG
c.477insA*
c.678delA*
p.S378X*
c.2952delC
c.3321delA
c.3036delT*
c.3222del4
p.R826X
p.Q1790X
c.7945delA
p.S2706X
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c.8393delA
c.9040_9058dup19
p.R4307X
c.441delA
c.441del2
p.R501X
c.1640delG
p.S1302X
c.4004del2
p.S1515X
p.Q1745X
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c.10866delA*
p.K4022X
1 2 3 4 5 6 7 8¹ 8² 9 10¹ 10²
c.3321delA
c.4812ins5 #
c.5024delC #
c.5187delA #
c.5192_5199dup8
c.7333delC
p.R2447X
p.R2447X
c.7487delC #
p.R2613X #
c.8088delG
p.R501X
p.S507X #
c.2282del4
p.R1140X
p.Q2123X #
c.6834del5
c.6950del8
c.6950del8
p.S2344X #
p.S2344X #
S100 calcium binding domain
B-domain
Imperfect FLG repeat
FLG repeats
C-terminal domain

Table E1. Primer assays with a maximum amplicon length of 500 base pairs used for multiplexed PCR amplification on Access Array IFC prior to Illumina MiSeq 2 x 250 sequencing. Primer assays in red text anneal to multiple regions of *FLG* CNV repeats. Primers assays in blue text have been previously published (Sandilands, et al., 2007). Universal CS1 (forward; ACACTGACGACATGGTTCTACA) or CS2 (reverse; TACGGTAGCAGAGACTTGGTCT) NGS adaptor sequences are synthesized 5' to the *FLG* target sequence.

Assay number	Primer name	Primer Sequence (5'-3')	Amplicon Size (bp)	*FLG* target region	Start position (HG19)	Primer binding location on *FLG* gene
1	1_F	CCTAAACTTCCAGAACCTTTTGCC	450	Exon 1	152297567	Intron 3' to exon 1
	1_R	CTGTCAAGCCAAAGTGGGGTTA			152298016	Intron 5' to exon 1
2	2_F	AAGAGCTCAAAATAACCCTTGCT	498	Exon 2	152287715	Intron 3' to exon 2
	2_R	ACACTGAGGTCTGTGAGACTACT			152288212	Intron 5' to exon 2
3	3_F	AGTCTTTCACTTAGCCTCTTCCT	500	RPT 0	152286751	RPT 0
	3_R	CTCCCTCTGTGACTTCCCTCTG			152287250	5' to exon 3
4	4_F	CATCTCTTGACTGCTCCCAC	500	RPT 0	152286368	RPT 0
	4_R	ACCTACTCATAGAGAAGAAGAATATGGA			152286867	RPT 0
5	5_F	GTCCAGACCGTTCCCCTGAC	487	RPT 0	152285972	RPT 0
	5_R	GACAGTGAGGGACACTCAGAAG			152286458	RPT 0
6	6_F	AAGCTTCATGATGACGTGACCC	498	RPT 0 & 1	152285588	RPT 1
	6_R	AGGGACATTCAGAAAACTCAGACA			152286085	RPT 0
7	7_F	CTGCTGACTGGAGCTGGTG	464	RPT 1	152285210	RPT 1
	7_R	ATCCAGAAGTGCAAGCAGACAAA			152285673	RPT 1
8	8_F	CTATCTACCGATTGCTCGTGGTG	483	RPT 1 & 2	152284774	RPT 2
	8_R	AAGTGCAGGAGAAAGACATGGAT			152285256	RPT 1
9	9_F	CCGGCTCTGTCTTCGTGAT	500	RPT 2	152284381	RPT 2
	9_R	AACTCCAGGCACTCAGCAT			152284880	RPT 2

10	10_F	TCACCTGGTAGATGAAAGACCCT	496	RPT 2	152283998	RPT 2
	10_R	GAAGACTCTGAGAGGTGGTCTGG			152284493	RPT 2
11	11_F	TAGAGCTGTCAGCCCAAGAGG	495	RPT 2 & 3	152283620	RPT 3
	11_R	CACAGTCAGTGTCAGGCCAT			152284114	RPT 2
12	12_F	GTGCCCAATGCCTGAGTGT	477	RPT 3	152283237	RPT 3
	12_R	AGCAGACAAACTCGTAAGGACAA			152283713	RPT 3
13	13_F	TCTACTGATTGCTCGTGGTAGGA	498	RPT 3 & 4	152282833	RPT 4
	13_R	ATCCCATGAACAGGCAAGATCAA			152283330	RPT 3
14	14_F	CTATCTTCTTGATGGGACCTGGG	498	RPT 4	152282443	RPT 4
	14_R	AAACAGCTCTAGGCACTCAGCAT			152282940	RPT 4
15	15_F	CTGGTAGAGGAAAGACCCTGAAC	471	RPT 4	152282058	RPT 4
	15_R	GAGTCGGCTTCCAGAAACCATTA			152282528	RPT 4
16	16_F	TTTCTCATTACGTGTTTGTCTGC	450	RPT 4 & 5	152281746	RPT 5
	16_R	GAGGGACACTCAGAAGAGTCAG			152282195	RPT 4
17	17_F	TCCATGTCTTTCTCCTGCACTTG	477	RPT 5	152281344	RPT 5
	17_R	TCCCAGGAAAGGTCTGATGTCT			152281820	RPT 5
18	18_F	TGAGTCTTCTGAATGTCCCTCAC	483	RPT 5	152281200	RPT 5
	18_R	AGCTCTAGACACTCGCAGGT			152281682	RPT 5
19	19_F	TTTCCCTGTGCTGACACTGA	400	RPT 5	152281171	RPT 5
	19_R	CTGAGAGGTGGTCTGGGTCT			152281570	RPT 5
20	20_F	TCCAGACCTATCTACCGATTGCT	485	RPT 5 & 6	152280876	RPT 6
	20_R	CAGGAGAAAGACATGGATCCCAC			152281360	RPT 5
21	21_F	CTCTGTCTTCGTGATGGGACCT	480	RPT 6	152280494	RPT 6
	21_R	CATCCCAAGAGGGTCAGGACA			152280973	RPT 6
22	22_F	GGCGGACTCAGACTGTTCAT	487	RPT 6	152280081	RPT 6/7 junction
	22_R	AAACCATCATGGATCTGCTCAGG			152280567	RPT 6
23	23_F	CGGCCCGAGAGGAAGC	500	RPT 6 & 7	152279738	RPT 7

	23_R	AGAAGACTCAGACACACAGTCAG			152280237	RPT 6
24	24_F	CTCTGACTGCAGATGAAGCTTGT	493	RPT7	152279321	RPT7
	24_R	ACTCGTAACGATGAACAATCAGGA			152279813	RPT7
25	25_F	CTCTTGGTGGCTCTGCTGAT	428	RPT7	152279196	RPT7
	25_R	CTGAGAGGTGGTCTGGGTCT			152279623	RPT7
26	26_F	TCCATGGGAGGACTCAGACT	489	RPT 7	152279103	RPT 7/8.1 junction
	26_R	CATCATGGATCTGCTCAGGA			152279591	RPT 7
27	27_F	GTGTCCACGAATGGTGTCCTG	482	RPT 7 & 8.1	152278995	RPT 8.1
	27_R	GTCACACACAGACTTCCTCTGG			152279476	RPT 7
28	28_F	GATGTGGTGTGGCTGTGATG	411	RPT 7 & 8.1	152278900	RPT 8.1
	28_R	CCGAGGGTACAGTGGTAGTCA			152279310	RPT 7
29	29_F	ACGTGTTGTTCTGCTTGCAC	483	RPT 7 & 8.1	152278836	RPT 8.1
	29_R	AGTGGACACCGAGGGTACAG			152279318	RPT 7
30	30_F	TTGTCTGCTTGCACTTCTGG	477	RPT 7 & 8.1	152278841	RPT 8.1
	30_R	AGTGGACACCGAGGGTACAG			152279318	RPT 7
31	31_F	GATGGTTTCTGGAAGCAGACCC	476	RPT 8.1	152278616	RPT 8.1
	31_R	CAGCACTAGAGGAAGACAAGGAT			152279091	RPT 8.1
32	32_F	CATGACCAGCTCTGCCTTCT	482	RPT 8.1	152278541	RPT 8.1
	32_R	AGACGGTCAGGACACCATTC			152279022	RPT 8.1
33	33_F	GTGGGCAGTCAGGATCCAGAA	403	RPT 8.2	-	RPT 8.2
	33_R	GCCTGTCCACCAGAGGAAGC			-	RPT 8.2
34	34_F	ACTGACTGTGTGTCTGAGTCTTC	474	RPT 8.1/8.2/10.2	152278270	RPT 8.1/8.2/10.2
	34_R	AGTCCAGGGACAATCAGAGGG			152278743	RPT 8.1/8.2/10.2
35	35_F	ATTACGTGTTGTTCTGCTTGCAC	500	RPT 8.1/8.2/9	152278833	RPT 8.2 & 9
	35_R	CTGCAGTCAGAGACAGTGGAC			152278360	RPT 8.1 & 8.2
36	36_F	CCAGAGGAAGTCTCTGCGTGA	468	RPT 9	152277511	RPT 9
	36_R	TTCGGTAGATAGCTCTGGACACT			152277978	RPT 9

37	37_F	TCTCGTGCCTGCTCGT	499	RPT 9 & 10.1	152277103	RPT 10.1
	37_R	CCAGGTCCCATCACGAAGAC			152277601	RPT 9
38	38_F	TCAGAGTCTTCTGAGTGTCCC	461	RPT 10.1	152276704	RPT 10.1
	38_R	CATGGGCGGACCAGGA			152277164	RPT 10.1
39	39_F	TGTCTGGAGCCATCTCTTGA	400	RPT 10.1	152276632	RPT 10.1
	39_R	GAAGGCAGGGATCCCACT			152277031	RPT 10.1
40	40_F	CTGTCCGTGGGCTGACAC	491	RPT 10.1	152276310	RPT 10.1
	40_R	CCGGCCAGGGACAATCAG			152276800	RPT 10.1
41	41_F	TCATTACGTGTTTCTCTGCTTGC	499	RPT 10.1/10.2/11	152275915	RPT 10.2 & 11
	41_R	CAGTCAGAGACAGTGGACACC			152276413	RPT 10.1 & 10.2
42	42_F	GTCCACCCATGGACAGTCTGTG	430	RPT10.2	-	RPT10.2
	42_R	GCTAACACTGGATCCCTGGCG			-	RPT10.2
43	43_F	CAGAGAATTCCTCTGGTGGACAGC	406	RPT 10.2 & 11	152276149	RPT 10.2
	43_R	CGGGATCCTTGTCTTCCTCCAGTA			-	RPT 11
44	44_F	ACGATGGTTTCTGGAAGCAG	411	RPT 11	152275698	RPT 11
	44_R	CAAGAGGGTCAGGACACCAT			152276108	RPT 11
45	45_F	CCACGTGACTGTATTCCTGAGTG	486	RPT 11	152275519	RPT 11
	45_R	CCATCACAGCCACACCACAT			152276004	RPT 11
46	46_F	GAAAGTGAACTTGCTTCATTCTTCT	486	RPT 11 & 3'UTR	152275119	3'UTR
	46_R	TCAGACTCTAGTACCGCTAAGGA			152275604	RPT 11
47	47_F	GATGTGCTAGCCCTGATGTTGAT	500	RPT 11 & 3'UTR	152274758	3' UTR
	47_R	CCCAGGTTTATGTGGCCATTCTA			152275257	RPT 11
48	48_F	CATCTAATTCTGGCCATGGGGAA	482	3' UTR	152274569	Intron 3' to exon 3
	48_R	AGTATTTCATTAGTTTGGTGGTAGCTT			152275050	3' UTR

Table E2. *FLG* LoF mutation profile of the remaining 78 samples in the pilot run sequenced with the MiSeq 2x250 bp protocol

S/N	Sample ID	*FLG* mutations identified with Illumina MiSeq 2 x 250 assay
1	ADD1 NJR	No LoF detected
2	ADD1 TCF	No LoF detected
3	AD04	No LoF detected
4	AD05 YQY	No LoF detected
5	AD06 TGF	c.3321delA
6	AD7	No LoF detected
7	AD08	No LoF detected
8	AD09	No LoF detected
9	AD10	No LoF detected
10	C04-CX	No LoF detected
11	C03-VH	No LoF detected
12	C01-LRH	No LoF detected
13	EBL-K001	No LoF detected
14	EBL-K002	No LoF detected
15	EBL-K003	No LoF detected
16	EBL-K004	c.1249insG
17	EBL-K005	No LoF detected
18	EBL-K009	No LoF detected
19	EBL-K010	No LoF detected
20	EBL-K011	No LoF detected
21	EBL-K013	No LoF detected
22	EBL-K015	No LoF detected
23	EBL-K024	No LoF detected
24	EBL-K025	No LoF detected
25	EBL-K026	No LoF detected
26	13-S-K-6	No LoF detected
27	13-S-K-12	No LoF detected
28	13-S-K-13	No LoF detected
29	13-S-K-15	No LoF detected
30	13-S-K-16	No LoF detected
31	13-S-K-17	p.K4022X
32	IA-P102	No LoF detected
33	IA-P103	No LoF detected
34	IA-P104	No LoF detected
35	IA-P105	No LoF detected
36	IA-P106	No LoF detected
37	IA-P107	No LoF detected
38	IA-P108	p.S507X

39	IA-P109	c.6834del5
40	IA-P110	c.5024delC
41	IA-P111	No LoF detected
42	IA-P112	No LoF detected
43	IA-P113	No LoF detected
44	IA-P114	p.S2706X, p.R826X
45	IA-P115	p.R2447X, p.R501X
46	IA-P116	c.4812ins5
47	IA-P117	No LoF detected
48	IA-P119	No LoF detected
49	IA-P120	No LoF detected
50	IA-P121	No LoF detected
51	IA-P123	No LoF detected
52	IA-P124	c.5192_5199dup8
53	IA-P126	c.3321delA, p.R826X
54	IA-P127	No LoF detected
55	IA-P128	No LoF detected
56	IA-P129	No LoF detected
57	IA-P130	No LoF detected
58	IA-P131	No LoF detected
59	IA-P133	p.R2613X
60	IA-P135	c.7487delC, c.12856del2
61	IA-P136	p.Q2123X
62	IA-P137	No LoF detected
63	IA-P138	No LoF detected
64	JOR-RM	No LoF detected
65	IA-P140	No LoF detected
66	IA-P141	No LoF detected
67	IA-P142	p.S2344X
68	IA-P143	c.6950_6957del8
69	IA-P144	No LoF detected
70	IA-P148	No LoF detected
71	IA-P149	p.E2422X
72	IA-P150	c.6950_6957del8
73	IA-P151	p.E2422X
74	IA-P152	p.K4022X
75	IA-P153	No LoF detected
76	IA-P155	No LoF detected
77	IA-P166	p.K4022X
78	Rana. M	No LoF detected

Table E4. 85 of the 279 Singaporean Chinese AD samples were previously Sanger sequence and these samples have now also been sequenced with our microfluidics protocol to check for concordance. One difference was observed and highlighted in red text.

S/N	Sample ID	*FLG* mutation identified from previous Sanger sequencing	*FLG* mutation identified from Access Array/MiSeq sequencing
1	IA-P001	No LoF variant detected	No LoF variant detected
2	IA-P002	p.S1515X, c.8393delA	p.S1515X, c.8393delA
3	IA-P003	p.S1515X	p.S1515X
4	IA-P005	No LoF variant detected	No LoF variant detected
5	IA-P006	No LoF variant detected	No LoF variant detected
6	IA-P007	No LoF variant detected	No LoF variant detected
7	IA-P008	No LoF variant detected	No LoF variant detected
8	IA-P009	No LoF variant detected	No LoF variant detected
9	IA-P010	No LoF variant detected	No LoF variant detected
10	IA-P011	No LoF variant detected	No LoF variant detected
11	IA-P013	No LoF variant detected	No LoF variant detected
12	IA-P014	No LoF variant detected	No LoF variant detected
13	IA-P015	No LoF variant detected	No LoF variant detected
14	IA-P016	No LoF variant detected	No LoF variant detected
15	IA-P017	p.E2422X	p.E2422X
16	IA-P018	No LoF variant detected	No LoF variant detected
17	IA-P020	c.9040_9058dup19	c.9040_9058dup19
18	IA-P021	c.6950_6957del8, p.S406X	c.6950_6957del8, p.S406X
19	IA-P022	No LoF variant detected	No LoF variant detected
20	IA-P023	No LoF variant detected	No LoF variant detected

21	IA-P024	c.1640delG	c.1640delG
22	IA-P025	c.3321delA, p.Q368X	c.3321delA, p.Q368X
23	IA-P026	No LoF variant detected	No LoF variant detected
24	IA-P027	No LoF variant detected	No LoF variant detected
25	IA-P028	c.7945delA	c.7945delA
26	IA-P029	No LoF variant detected	No LoF variant detected
27	IA-P030	No LoF variant detected	No LoF variant detected
28	IA-P031	No LoF variant detected	No LoF variant detected
29	IA-P032	No LoF variant detected	No LoF variant detected
30	IA-P033	c.3321delA	c.3321delA
31	IA-P034	p.R4307X, p.S2706X	p.R4307X, p.S2706X
32	IA-P037	c.3321delA	c.3321delA
33	IA-P038	No LoF variant detected	No LoF variant detected
34	IA-P040	No LoF variant detected	No LoF variant detected
35	IA-P041	No LoF variant detected	No LoF variant detected
36	IA-P042	No LoF variant detected	No LoF variant detected
37	IA-P043	p.S406X, p.Q1745X	p.S406X, p.Q1745X
38	IA-P044	No LoF variant detected	No LoF variant detected
39	IA-P045	No LoF variant detected	No LoF variant detected
40	IA-P046	No LoF variant detected	No LoF variant detected
41	IA-P047	No LoF variant detected	No LoF variant detected
42	IA-P048	c.3321delA, c.3321delA	c.3321delA, c.3321delA
43	IA-P049	No LoF variant detected	No LoF variant detected
44	IA-P051	No LoF variant detected	No LoF variant detected
45	IA-P052	c.3321delA	c.3321delA
46	IA-P053	No LoF variant detected	No LoF variant detected

47	IA-P054	No LoF variant detected	No LoF variant detected
48	IA-P055	No LoF variant detected	No LoF variant detected
49	IA-P056	No LoF variant detected	No LoF variant detected
50	IA-P057	No LoF variant detected	No LoF variant detected
51	IA-P058	No LoF variant detected	No LoF variant detected
52	IA-P059	No LoF variant detected	No LoF variant detected
53	IA-P060	c.7945delA	c.7945delA
54	IA-P061	No LoF variant detected	No LoF variant detected
55	IA-P062	p.Q2417X	p.Q2417X
56	IA-P063	c.2952delC	c.2952delC
57	IA-P064	p.S2706X	p.S2706X
58	IA-P065	No LoF variant detected	No LoF variant detected
59	IA-P067	No LoF variant detected	No LoF variant detected
60	IA-P068	No LoF variant detected	No LoF variant detected
61	IA-P069	No LoF variant detected	No LoF variant detected
62	IA-P071	No LoF variant detected	No LoF variant detected
63	IA-P072	No LoF variant detected	No LoF variant detected
64	IA-P073	No LoF variant detected	No LoF variant detected
65	IA-P074	c.3321delA	c.3321delA
66	IA-P075	No LoF variant detected	No LoF variant detected
67	IA-P076	c.3321delA	c.3321delA
68	IA-P077	p.Q2417X	p.Q2417X
69	IA-P078	c.9040_9058dup19, p.G323X	c.9040_9058dup19, p.G323X
70	IA-P081	No LoF variant detected	No LoF variant detected
71	IA-P082	No LoF variant detected	No LoF variant detected
72	IA-P083	c.9040_9058dup19	p.1790X, c.9040_9058dup19

73	IA-P084	p.S1515X, p.S1302X	p.S1515X, p.S1302X
74	IA-P085	No LoF variant detected	No LoF variant detected
75	IA-P086	c.3321delA	c.3321delA
76	IA-P087	No LoF variant detected	No LoF variant detected
77	IA-P088	c.3321delA, c.3321delA	c.3321delA, c.3321delA
78	IA-P089	c.6950_6957del8, p.S2317X	c.6950_6957del8, p.S2317X
79	IA-P090	c.4004del2	c.4004del2
80	IA-P091	c.3321delA	c.3321delA
81	IA-P092	No LoF variant detected	No LoF variant detected
82	IA-P093	No LoF variant detected	No LoF variant detected
83	IA-P096	c.7249C>T, p.Q2417X	c.7249C>T, p.Q2417X
84	IA-P098	p.E2422X, p.R501X	p.E2422X, p.R501X
85	IA-P100	No LoF variant detected	No LoF variant detected

Table E5. Fisher's exact test of Singaporean Chinese *FLG*-null mutations in the fully sequenced atopic dermatitis (AD) cohort of 279 patients compared with ExAC East Asian control data. False discovery rate is shown (FDR). 14 *FLG* LoF mutations reached individual significance of p<0.05 (**bold**)

***FLG* mutation**	**Cohort**	***FLG* Genotype**				***P*-value**	**FDR *P*-value**
		AA	**Aa**	**aa**	**Total**		
c.441delA	AD	277	2	0	279	0.020254	0.049855
	ExAC (East Asia)	4321	2	0	4323		
c.441del2	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
c.477insA	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
c.678delA	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
p.G323X	AD	276	3	0	279	0.002007	0.008028
	ExAC (East Asia)	4325	2	0	4327		
p.Q368X	AD	278	1	0	279	0.117490	0.134274
	ExAC (East Asia)	4326	1	0	4327		
p.S378X	AD	278	1	0	279	0.117490	0.134274
	ExAC (East Asia)	4326	1	0	4327		
p.S406X	AD	272	7	0	279	0.000012	0.000094
	ExAC (East Asia)	4319	8	0	4327		
c.1249insG	AD	278	1	0	279	0.430429	0.430429
	ExAC (East Asia)	4319	8	0	4327		
p.R501X	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
c.1640delG	AD	278	1	0	279	0.170969	0.188656

	ExAC (East Asia)	4325	2	0	4327		
p.R826X	AD	276	3	0	279	0.039975	0.084276
	ExAC (East Asia)	4317	10	0	4327		
c.2952delC	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
c.3036delT	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
c.3222del4	AD	274	5	0	279	0.003504	0.010638
	ExAC (East Asia)	4314	13	0	4327		
c.3321delA	AD	254	21	2	279	1.90×10^{-8}	2.03×10^{-7}
	ExAC (East Asia)	4245	80	2	4327		
p.S1302X	AD	276	3	0	279	0.000220	0.001173
	ExAC (East Asia)	4327	0	0	4327		
c.4004del2	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
p.S1515X	AD	273	6	0	279	0.003112	0.010638
	ExAC (East Asia)	4308	19	0	4327		
p.Q1745X	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
p.Q1790X	AD	278	1	0	279	0.354729	0.378377
	ExAC (East Asia)	4317	6	0	4327		
c.6950_6957del8	AD	264	15	0	279	3.78×10^{-19}	1.21×10^{-17}
	ExAC (East Asia)	4327	0	0	4327		
p.Q2417X	AD	273	6	0	279	0.000467	0.002137
	ExAC (East Asia)	4315	12	0	4327		

p.E2422X	AD	273	6	0	279	0.010488	0.027968
	ExAC (East Asia)	4304	22	1	4327		
p.R2447X	AD	278	1	0	279	0.117490	0.134274
	ExAC (East Asia)	4326	1	0	4327		
c.7945delA	AD	276	3	0	279	0.091426	0.121902
	ExAC (East Asia)	4312	15	0	4327		
p.S2706X	AD	271	8	0	279	1.65×10^{-10}	2.64×10^{-9}
	ExAC (East Asia)	4327	0	0	4327		
c.8393delA	AD	278	1	0	279	0.060573	0.084276
	ExAC (East Asia)	4327	0	0	4327		
c.9040_9058dup19	AD	276	3	0	279	0.000220	0.001173
	ExAC (East Asia)	4327	0	0	4327		
c.10866delA	AD	278	1	0	279	0.117490	0.134274
	ExAC (East Asia)	4326	1	0	4327		
p.R4307X	AD	277	2	0	279	0.003657	0.010638
	ExAC (East Asia)	4327	0	0	4327		
p.K4022X	AD	272	7	0	279	0.407330	0.420469
	ExAC (East Asia)	4162	163	2	4327		

Table E6. 36 Singaporean Malay and 19 Singaporean Indian AD patient demographics and clinical features. NR = Not recorded in clinic

S/N	Sample ID	BioSample ID	Age	Ethnicity	Gender	IV severity	AD Objective SCORAD	AD Total SCORAD	Onset of atopic eczema (years) 1=under 2, 2=2-4, 3= 5 or above 4=unknown	Asthma	Allergic conjuctivo-rhinitis	
											Recurrent sneezing or runny nose	Recurrent watery/ itchy eyes
1	IA-P019	SAMN06199218	16	Malay	Male	Moderate	52	66	1	Yes	No	Yes
2	IA-P036	SAMN06199139	45	Malay	Male	Moderate	31.7	42.7	3	No	No	No
3	IA-P066	SAMN06199168	28	Malay	Female	Moderate	39.5	48.5	3	No	Yes	No
4	IA-P079	SAMN06199180	21	Malay	Male	No IV	35.5	44.5	2	Yes	No	No
5	IA-P080	SAMN06199181	24	Malay	Male	No IV	44.5	61.5	2	Yes	Yes	Yes
6	IA-P094	SAMN06199195	21	Malay	Male	Mild	18.5	32.5	3	No	No	Yes
7	IA-P099	SAMN06199199	22	Malay	Male	Mild	19.5	32.5	2	Yes	No	No
8	IA-P102	SAMN06199202	21	Malay	Male	Moderate	55	74	3	Yes	No	No
9	IA-P103	SAMN06199203	24	Malay	Male	Mild	48.5	61.5	1	Yes	Yes	Yes
10	IA-P104	SAMN06199204	7	Malay	Female	Mild	25.1	30.1	1	No	No	No
11	IA-P105	SAMN06199205	36	Malay	Male	No IV	65	82	3	Yes	Yes	Yes

12	IA-P106	SAMN06199206	23	Malay	Male	Mild	26.1	28.1	1	No	No	No
13	IA-P107	SAMN06199207	18	Malay	Male	No IV	63	77	3	No	No	No
14	IA-P108	SAMN06199208	21	Malay	Male	Mild	59.5	76.5	1	Yes	No	No
15	IA-P109	SAMN06199209	19	Malay	Male	No IV	57	69	3	No	No	No
16	IA-P112	SAMN06199212	22	Malay	Female	No IV	46.5	50.5	3	No	No	No
17	IA-P117	SAMN06199217	39	Malay	Male	Moderate	47	53	2	Yes	No	No
18	IA-P124	SAMN06199222	21	Malay	Male	Mild	32.5	41.5	3	No	Yes	No
19	IA-P127	SAMN06199225	20	Malay	Female	No IV	44	54	3	Yes	No	No
20	IA-P128	SAMN06199226	31	Malay	Female	Mild	65	83	3	Yes	No	No
21	IA-P130	SAMN06199228	10	Malay	Male	Mild	14.2	17.2	3	Yes	Yes	No
22	IA-P131	SAMN06199229	21	Malay	Male	Mild	57	77	3	Yes	Yes	Yes
23	IA-P137	SAMN06199233	10	Malay	Female	Moderate	11.3	28.3	3	No	No	Yes
24	IA-P140	SAMN06199235	27	Malay	Male	No IV	38	45	1	Yes	Yes	No
25	IA-P141	SAMN06199236	8	Malay	Male	Mild	29.2	38.2	3	No	No	No
26	IA-P142	SAMN06199237	21	Malay	Female	Moderate	33.5	40.5	3	Yes	Yes	No
27	IA-P143	SAMN06199238	24	Malay	Male	Mild	52	67	3	No	No	No

28	IA-P144	SAMN06199239	22	Malay	Male	Severe	60	75	2	No	Yes	Yes
29	IA-P148	SAMN06199240	21	Malay	Male	Moderate	0	11	2	No	No	No
30	IA-P155	SAMN06199246	33	Malay	Male	No IV	49	64	3	No	No	No
31	IA-P165	SAMN06199256	18	Malay	Male	Mild	18.5	20.5	3	No	No	No
32	IA-P170	SAMN06199261	12	Malay	Male	No IV	19.5	36.5	3	Yes	Yes	No
33	IA-P181	SAMN06199271	26	Malay	Male	Moderate	32	51	2	No	Yes	Yes
34	IA-P198	SAMN06199288	28	Malay	Male	No IV	15	31	3	Yes	No	No
35	IA-P200	SAMN06199290	28	Malay	Male	Moderate	33	43	3	Yes	Yes	No
36	P016	SAMN06199306	18	Malay	Female	No IV	60	68	3	Yes	Yes	No
37	IA-P004	SAMN06199109	54	Indian	Female	Severe	42.4	55.4	3	Yes	No	No
38	IA-P050	SAMN06199152	19	Indian	Male	Moderate	36	48	3	Yes	Yes	No
39	IA-P095	SAMN06199196	8	Indian	Male	Mild	54	66	1	No	Yes	Yes
40	IA-P101	SAMN06199201	39	Indian	Female	Mild	24.9	36.9	3	Yes	No	No
41	IA-P110	SAMN06199210	17	Indian	Male	Moderate	48.5	54.5	2	Yes	No	No
42	IA-P115	SAMN06199215	18	Indian	Male	Mild	33.5	39.5	1	Yes	Yes	No
43	IA-P116	SAMN06199216	26	Indian	Male	No IV	38	38	3	No	Yes	Yes

44	IA-P119	SAMN06199218	8	Indian	Male	No IV	55	73	2	Yes	Yes	Yes
45	IA-P120	SAMN06199219	10	Indian	Male	No IV	26.1	36.1	3	No	Yes	Yes
46	IA-P121	SAMN06199220	34	Indian	Male	Severe	52	68	3	No	Yes	Yes
47	IA-P123	SAMN06199221	23	Indian	Male	NR	38	48	3	Yes	No	No
48	IA-P125	SAMN06199223	60	Indian	Female	Moderate	10.7	21.7	3	No	No	No
49	IA-P129	SAMN06199227	29	Indian	Male	Moderate	48	58	3	Yes	Yes	Yes
50	IA-P133	SAMN06199230	25	Indian	Female	Mild	44	63	3	No	Yes	No
51	IA-P135	SAMN06199231	8	Indian	Female	Moderate	50	70	1	Yes	Yes	Yes
52	IA-P136	SAMN06199232	13	Indian	Female	Mild	40.5	51.5	2	No	No	No
53	IA-P183	SAMN06199273	32	Indian	Male	NR	42	55	3	Yes	No	No
54	P001	SAMN06199292	8	Indian	Male	Mild	36	41	2	Yes	Yes	No
55	P148	SAMN06199433	17	Indian	Female	Moderate	28.2	38.2	1	No	Yes	Yes

Table E7. 18 *FLG* LoF variants identified in Singapore Malay and Indian IV and/or AD cohorts using MiSeq 2 x 250 bp protocol. Singaporean Malay and Indian ethnicities have both unique and recurrent *FLG* LoF variants compared to other published studies (see Table E1). * = not previously reported LoF variant in AD related published literature.

ID	*FLG* mutation	Ethnicity	dbSNP ID	South Asian ExAC MAF	East Asian ExAC MAF
1	p.R501X	Indian	rs61816761	21/16512	0/8654
2	p.S507X *	Malay	-	Not reported	Not reported
3	c.2282del4	Malay	rs558269137	122/16510	0/8654
4	c.3321delA	Malay	rs200519781	0/16512	82/8654
5	p.R1140X	Indian	-	16/16512	0/8652
6	c.4812ins5 *	Indian	-	Not reported	Not reported
7	c.5024delC *	Indian	rs749542190	Not reported	Not reported
8	c.5187delA *	Indian	-	Not reported	Not reported
9	c.5192_5199dup8	Malay	rs754949514	Not reported	Not reported
10	p.Q2123X *	Indian	rs145119684	8/16512	0/8654
11	c.6834del5	Malay	rs772007167	Not reported	Not reported
12	c.6950del8	Malay; Indian	rs578184315	Not reported	Not reported
13	p.S2344X *	Malay, Indian	rs372754256	Not reported	Not reported
14	c.7333delC	Indian	-	Not reported	Not reported
15	p.R2447X	Malay, Indian	rs138726443	35/16512	1/8652
16	c.7487delC *	Indian	rs375277670	0/16512	5/8650
17	p.R2613X *	Indian	rs567795279	Not reported	Not reported
18	c.8088delG	Malay	-	Not reported	Not reported

Table E8. Summary of patient demographics and clinical features of the 3 ethnicities studied for *FLG* LoF variants. SCORAD (SCORing AD) index and objective SCORAD (oSCORAD) are used for clinical phenotyping. Complete metadata is provided in Table E3 and Table E6.

	Chinese	**Malay**	**Indian**
Subjects demographics			
Subjects (*n*)	279	36	19
Age, mean (range) in years	18.5 (2-70)	22.4 (7-45)	23.6 (8-60)
Male:female (*n*)	202:77	28:8	12:7
Ichthyosis vulgaris			
No ichthyosis vulgaris (*n*)	103	12	3
Mild (*n*)	80	13	6
Moderate-Severe (*n*)	84	11	8
Not recorded (*n*)	12	0	2
Number of IV patients with *FLG* LoF (%)	64 (39.0)	7 (29.2)	8 (57.1)
Atopic dermatitis			
Mean Total SCORAD (+/- SD)	47.2 (± 17.3)	50.6 (± 19.7)	50.6 (± 13.9)
Mean oSCORAD	37.6 (± 15.0)	38.8 (± 17.4)	39.4 (± 11.4)
No. of Mild AD / oSCORAD <15 (%)	14 (5.1)	3 (8.3)	1 (5.3)
No. of Moderate AD / oSCORAD 15-40 (%)	151 (54.9)	16 (44.4)	8 (42.1)
No. of Severe AD / oSCORAD >40 (%)	110 (40.0)	17 (47.2)	10 (52.6)
IV only, AD not reported (*n*)	4	0	0
Mean oSCORAD of samples with *FLG* LoF	37.5 (± 15.3)	38.9 (± 17.6)	38.2 (± 8.6)
Mean oSCORAD of samples with WT *FLG*	37.6 (± 15.0)	38.8 (± 17.4)	40.0 (± 11.3)
AD Age of onset<5 years age (*n*)	143	13	8
AD Age of onset>5 years age (*n*)	124	23	11
AD Age of onset unknown (*n*)	12	0	0

Table E9. Accession numbers and Biosample IDs for all 367 samples used in this study.

Sample_ID	Accession number	BioSampleID	LibraryType
IA-P021	SRR5140927	SAMN06199125	MiSeq 2x250bp
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IA-P062	SRR5140582	SAMN06199164	MiSeq 2x250bp
IA-P063	SRR5140846	SAMN06199165	MiSeq 2x250bp
IA-P083	SRR5140660	SAMN06199184	MiSeq 2x250bp
IA-P102	SRR5140912	SAMN06199202	MiSeq 2x250bp
IA-P103	SRR5140676	SAMN06199203	MiSeq 2x250bp
IA-P104	SRR5140639	SAMN06199204	MiSeq 2x250bp
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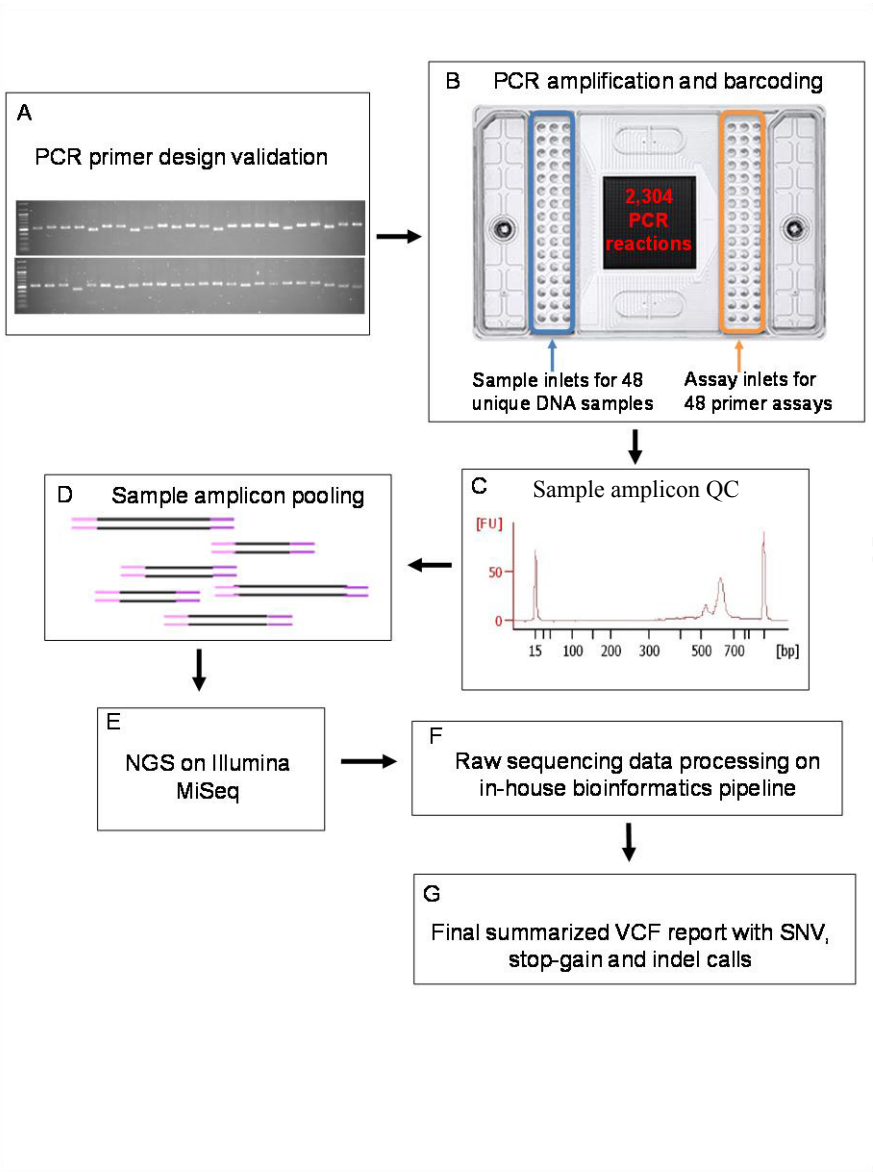
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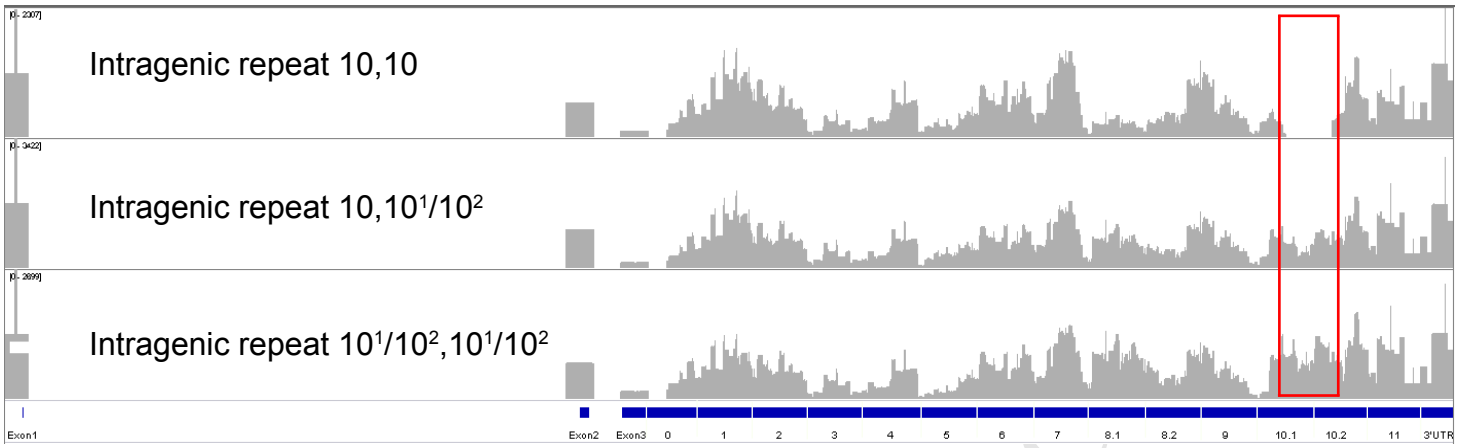
NGS on Illumina MiSeq
F
Raw sequencing data processing on in-house bioinformatics pipeline
G
Final summarized VCF report with SNV, stop-gain and indel calls

A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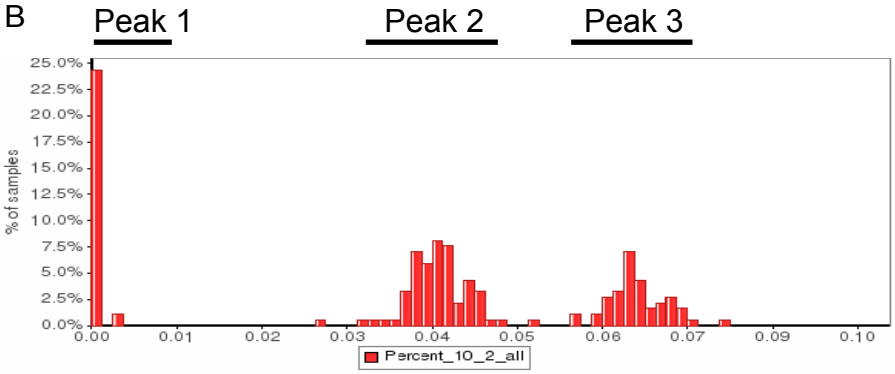
8.2
9
10.1
10.2
11
3'UTR

B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Peak 3
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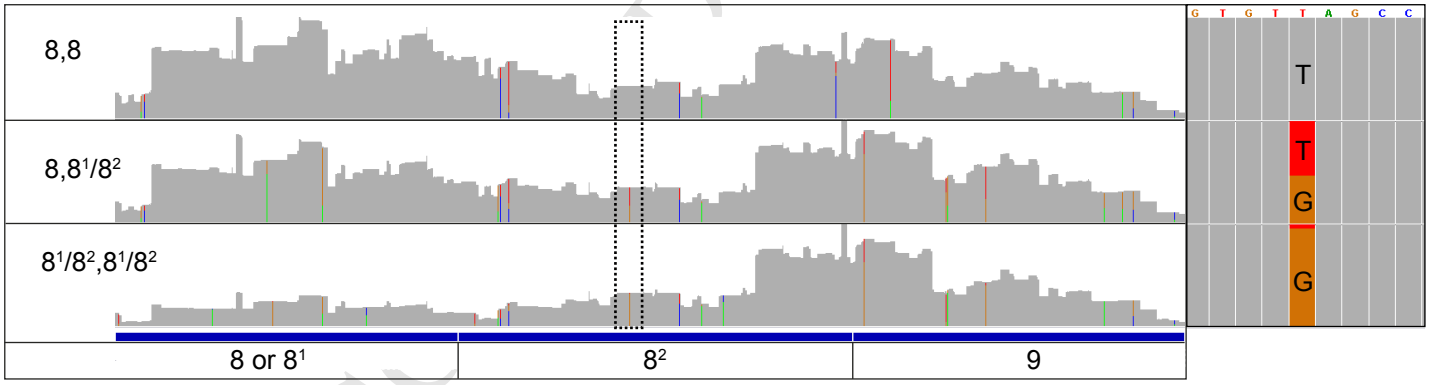
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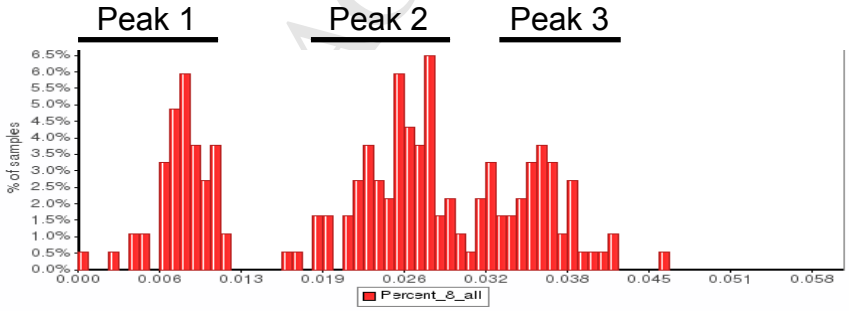
T
G
G

D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Peak 2
Peak 3
% of samples
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Figure E1. *FLG* sequencing workflow for Access Array 48.48 IFC and Illumina MiSeq.

Figure E2. Determination of *FLG* CNV for repeat 8 and 10. (A) The absence of a duplication of repeat 10 (repeat 10^2) manifests as a complete drop in coverage in this region (red box). (B) Based on a defined set of coverage ratios, three distinct peaks can be generated which corresponds to each repeat 10 CNV status in a set of screened samples (Peak 1=10,10; Peak 2=10, $10^1/10^2$; Peak 3=$10^1/10^2$, $10^1/10^2$). (C) Repeat 8 CNV status is correlated with a single nucleotide polymorphism at position c.9645 (dotted box) of the *FLG* 12-repeat reference sequence in South-East Asian populations. (D) Three distinct peaks corresponding to repeat 8 CNV status of a sample group can be calculated based on a set of coverage ratios (Peak 1=8,8; Peak 2=8, $8^1/8^2$; Peak 3=$8^1/8^2$, $8^1/8^2$).

SUPPLEMENTAL METHODS

Sample collection

367 DNA samples were extracted from blood or saliva using standard protocols (Oragene, DNA Genotek Inc.; Nucleon Illustra™ BACC2, GE Healthcare). This included 334 patients diagnosed with IV and/or AD, clinically scored with SCORAD and collected with local DSRB approval in accordance with the Declaration of Helsinki (see Table E9 in the Online Repository) [1].

Access Array 48.48 IFC target-specific primer design

Primer assays were specifically designed to produce amplicons for Illumina MiSeq 2x250 bp read mode sequencing using Reagent Kit-V2. Universal adapter sequences (CS1/CS2) were attached to 3' ends of primers for sample barcoding (see Table E1 in the Online Repository).

PCR-based *FLG* enrichment with the Access Array 48.48 IFC

PCR amplification of *FLG* was performed according to '4-Primer Amplicon Tagging on the 48.48 Access Array IFC' workflow (Chapter 5 Fluidigm user guide: Access Array System for Illumina Sequencing Systems – PN 100-3770 HI, Fluidigm).

Briefly, for each sample a 5 μL sample mix was prepared containing, (1) 50 ng of DNA sample, (2) 2 μM of unique barcoding primer pair (Illumina), (3) FastStart High Fidelity PCR reagents (Roche) and (4) dNTP mix (Bioline). 48 individual DNA containing sample mixtures were loaded into 'Sample Inlets' on the Access Array IFC according to manufacturer's guidelines (see Figure E1 in the Online Repository). Similarly, 48 *FLG* primer assays were loaded into 'Assay Inlets' of the Access Array IFC according to manufacturer's guidelines.

Thermocycling of the IFC was completed on Biomark HD PCR machine (Fluidigm) using cycling parameters stated in Table 5 Access Array user guide (PN 100-3770 HI, Fluidigm).

PCR product pools for each individual DNA sample were harvested and quality checked (Agilent DNA 1000 kit, Agilent). Samples were purified with Agencourt® AMPure® XP Reagent Beads (Beckman Coulter Genomics). Batches containing amplicon pools from 96 or 192 individual DNA samples were then further combined in a final pool and sequenced on the Illumina MiSeq (2 x 250 bp read mode).

Bioinformatics analysis of NGS data

Sequencing reads were mapped to a *FLG* reference sequence containing 12 repeats [2]. BWA-MEM (version 0.7.10-r789) was used to map sequencing reads. Mapped reads were stored as indexed BAM files and processed with GATK toolkit (v3.4-46-gbc02625, Java version 1.7.0_75). The *HaplotypeCaller* module was used to calculate SNVs and indels. Individual gVCF files were jointly processed with *GenotypeGVCFs* module to produce a VCF report for multiple samples (same quality cut-offs) to increase the sensitivity of SNV detection [3]. VCF files were annotated with SnpEff tool (version 4.2) for SNVs and assess their functional impact. These analysis steps were chained together with Pipeline Pilot 2016 (Biovia, Dassault Systems) for consistent data processing and results storage.

PCR and Sanger-sequencing validation of *FLG* LoF variants and intragenic CNVs

FLG LoF variants were validated with Sanger sequencing using previously published primers [2, 4]. *FLG* repeat 8 and 10 regions were PCR amplified using Expand High Fidelity^{PLUS} PCR System (Roche). Long-range PCR primers for repeat 8 were 5'-CCCAGGACAAGCAGGAACT-3' and

5'- GCTTCATGGTGATGCGACCA-3' [2] and for repeat 10 were 5'-GGGCCCAGGACAAGCAGGAAC-3' (in-house designed) and 5'-CTGCACTACCATAGCTGCC-3' [2]. Cycle conditions for repeat 8 PCR were: 95 °C enzyme activation for 5 min, followed by 36 cycles of denaturation (94 °C for 30 s), annealing (64.7 °C for 30 s) and elongation (72 °C for 4 min), followed by a final elongation at 72 °C for 7 min. Cycle conditions for repeat 10 PCR were: 95 °C enzyme activation for 5 min, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (62 °C for 30 s) and elongation (72 °C for 2 min 30 s), followed by a final elongation at 72 °C for 7 min. Intragenic CNVs were distinguished by long-range PCR product sizing on 0.9% w/v agarose gels.

Statistical analysis of *FLG*

Allele frequencies were analyzed with Fisher's exact test using a genotype based model (three categories). Population allele frequency control data was extracted from ExAC Database (version 0.3.1) [5] using the East Asia subset for comparison with Singaporean Chinese patients. South Asia and East Asia ExAC subset allele frequency data was provided as control data for the Singapore Indian and Malay patients as it was unclear which was the best genetic match. Fisher's exact test was not performed in Malay and Indian cohorts to avoid any over interpretation of results with small sample sizes. Analyses were performed in R (v3.3.2) with default settings for the Fisher's exact test function. The association of *FLG* genotype with AD severity – mild, moderate and severe grouping according to oSCORAD [1] – was evaluated both with logistic regression and Fisher's exact tests in R (v3.3.2).

SUPPLEMENTAL REFERENCES

1. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol 2007; 157:645-8.
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3. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010; 20:1297-303.
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5. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016; 536:285-91.

Table E3. 279 Chinese AD patient demographics and clinical features. NR = Not recorded in clinic

S/N	Sample ID	BioSample ID	Age (years)	Gender	IV severity	AD Objective SCORAD	AD Total SCORAD	Onset of atopic eczema (years) 1=under 2, 2=2-4 yrs, 3= 5 or above 4=unknown	Asthma	Allergic Conjuctivo-Rhinitis	
										Recurrent sneezing or runny nose	Recurrent watery/ itchy eyes
1	IA-P001	SAMN06199106	36	F	Moderate	25.5	39.5	3	No	Yes	No
2	IA-P002	SAMN06199107	17	M	Severe	39.5	56.5	3	No	Yes	Yes
3	IA-P003	SAMN06199108	33	F	Moderate	36	48	3	Yes	Yes	No
4	IA-P005	SAMN06199110	38	M	Moderate	50.5	66.5	3	Yes	Yes	No
5	IA-P006	SAMN06199111	30	M	Moderate	47	50	3	No	No	Yes
6	IA-P007	SAMN06199112	25	F	Mild	58	69	1	No	No	No
7	IA-P008	SAMN06199113	33	M	Moderate	42.5	53.5	3	No	Yes	Yes
8	IA-P009	SAMN06199114	25	F	No IV	26.1	28.1	4	Yes	Yes	Yes
9	IA-P010	SAMN06199115	29	M	Mild	49	58	1	Yes	No	No
10	IA-P011	SAMN06199116	23	F	Moderate	65	85	1	No	Yes	Yes
11	IA-P013	SAMN06199117	29	M	Mild	23	26	3	No	No	No
12	IA-P014	SAMN06199118	57	M	Severe	46	53	3	No	No	No
13	IA-P015	SAMN06199119	16	M	No IV	29	37	1	Yes	Yes	Yes
14	IA-P016	SAMN06199120	31	F	Moderate	46	53	2	Yes	Yes	Yes
15	IA-P017	SAMN06199121	33	M	Moderate	44.5	53.5	3	Yes	No	No
16	IA-P018	SAMN06199122	22	M	Mild	39	47	3	No	Yes	No
17	IA-P020	SAMN06199124	20	M	Severe	0	0	4	Yes	No	No
18	IA-P021	SAMN06199125	14	F	Moderate	26.5	32.5	3	No	No	No
19	IA-P022	SAMN06199126	43	M	Mild	39	48	3	No	Yes	No

20	IA-P023	SAMN06199127	27	M	Mild	39	49	3	No	Yes	Yes
21	IA-P024	SAMN06199128	30	M	Moderate	50.5	61.5	2	No	Yes	No
22	IA-P025	SAMN06199129	20	M	Severe	38.7	42.7	2	Yes	Yes	No
23	IA-P026	SAMN06199130	52	F	Moderate	39.5	46.5	3	No	Yes	Yes
24	IA-P027	SAMN06199131	44	F	Severe	47.5	57.5	3	No	No	No
25	IA-P028	SAMN06199132	17	M	Moderate	57.5	61.5	3	Yes	Yes	No
26	IA-P029	SAMN06199133	21	M	Moderate	38.9	50.9	3	No	No	No
27	IA-P030	SAMN06199134	18	M	No IV	28.5	37.5	2	No	Yes	No
28	IA-P031	SAMN06199135	21	M	No IV	57	69	2	No	Yes	Yes
29	IA-P032	SAMN06199136	28	M	Moderate	68.5	86.5	3	No	Yes	No
30	IA-P033	SAMN06199137	30	M	Moderate	37	50	3	No	Yes	Yes
31	IA-P034	SAMN06199138	21	M	Moderate	39	46	3	No	Yes	No
32	IA-P037	SAMN06199140	60	F	Severe	46.6	56.6	3	Yes	Yes	Yes
33	IA-P038	SAMN06199141	25	F	Mild	21.4	27.4	3	No	Yes	No
34	IA-P040	SAMN06199142	24	M	Mild	62.5	72.5	2	No	Yes	No
35	IA-P041	SAMN06199143	22	M	Moderate	68.5	80.5	3	No	Yes	Yes
36	IA-P042	SAMN06199144	27	F	Mild	57.5	68.5	3	Yes	Yes	No
37	IA-P043	SAMN06199145	16	F	Moderate	24.9	30.9	2	No	No	No
38	IA-P044	SAMN06199146	27	M	No IV	41.5	51.5	3	No	No	No
39	IA-P045	SAMN06199147	18	M	Mild	21.6	36.6	2	Yes	Yes	No
40	IA-P046	SAMN06199148	41	F	Mild	0	0	4	No	Yes	No
41	IA-P047	SAMN06199149	34	F	Mild	37	45	1	Yes	Yes	Yes
42	IA-P048	SAMN06199150	37	M	Moderate	44	50	3	Yes	No	No
43	IA-P049	SAMN06199151	20	M	No IV	65	76	2	No	Yes	Yes
44	IA-P051	SAMN06199153	26	M	Moderate	68.5	77.5	2	Yes	No	No
45	IA-P052	SAMN06199154	24	F	Mild	54	70	2	No	Yes	No
46	IA-P053	SAMN06199155	36	M	Mild	47	57	3	Yes	Yes	Yes

47	IA-P054	SAMN06199156	28	F	Moderate	65	77	3	No	No	No
48	IA-P055	SAMN06199157	8	F	Mild	24.9	29.9	3	No	No	No
49	IA-P056	SAMN06199158	23	M	Moderate	43	46	3	No	Yes	No
50	IA-P057	SAMN06199159	28	M	No IV	40.5	43.5	2	Yes	No	No
51	IA-P058	SAMN06199160	31	M	No IV	29.6	33.6	3	Yes	No	No
52	IA-P059	SAMN06199161	31	M	Mild	38	55	3	Yes	Yes	Yes
53	IA-P060	SAMN06199162	33	M	No IV	34.5	48.5	3	Yes	No	No
54	IA-P061	SAMN06199163	22	M	No IV	21.4	27.4	3	Yes	Yes	No
55	IA-P062	SAMN06199164	23	M	No IV	29.6	42.6	3	No	Yes	No
56	IA-P063	SAMN06199165	20	M	Moderate	39	56	1	Yes	No	No
57	IA-P064	SAMN06199166	35	F	Mild	24.9	32.9	2	No	Yes	Yes
58	IA-P065	SAMN06199167	28	M	Mild	21.2	27.2	3	No	Yes	No
59	IA-P067	SAMN06199169	22	M	Mild	37	42	3	Yes	Yes	No
60	IA-P068	SAMN06199170	31	M	No IV	47.5	54.5	3	No	Yes	No
61	IA-P069	SAMN06199171	20	M	No IV	31	40	2	Yes	Yes	Yes
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63	IA-P072	SAMN06199173	29	M	Mild	26.5	43.5	3	Yes	Yes	No
64	IA-P073	SAMN06199174	25	M	No IV	37.5	43.5	3	No	Yes	No
65	IA-P074	SAMN06199175	26	M	Severe	25	36	3	No	No	No
66	IA-P075	SAMN06199176	33	F	No IV	21.6	23.6	3	No	No	No
67	IA-P076	SAMN06199177	20	F	Moderate	32.5	41.5	3	Yes	No	No
68	IA-P077	SAMN06199178	21	M	No IV	39	55	3	Yes	No	No
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70	IA-P081	SAMN06199182	14	F	Mild	33.5	48.5	3	Yes	Yes	No
71	IA-P082	SAMN06199183	30	M	No IV	44.5	59.5	2	No	No	Yes
72	IA-P083	SAMN06199184	21	M	Severe	66	82	3	No	Yes	Yes
73	IA-P084	SAMN06199185	22	M	No IV	75.5	83.5	1	Yes	Yes	No

74	IA-P085	SAMN06199186	30	F	No IV	68	84	3	No	Yes	Yes
75	IA-P086	SAMN06199187	35	M	No IV	35.5	40.5	3	No	No	No
76	IA-P087	SAMN06199188	21	M	No IV	37	45	3	Yes	Yes	Yes
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78	IA-P089	SAMN06199190	26	F	Mild	25.7	29.7	2	No	Yes	No
79	IA-P090	SAMN06199191	23	F	Mild	25.5	35.5	3	Yes	No	No
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81	IA-P092	SAMN06199193	25	F	No IV	31.9	38.9	3	No	No	No
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83	IA-P096	SAMN06199197	19	M	Moderate	44.5	46.5	2	No	No	No
84	IA-P098	SAMN06199198	21	F	Severe	0	0	1	No	Yes	Yes
85	IA-P100	SAMN06199200	38	M	Mild	53	70	3	Yes	No	No
86	IA-P111	SAMN06199211	38	F	Moderate	46	51	3	No	No	No
87	IA-P113	SAMN06199213	17	M	Moderate	0	0	3	Yes	Yes	No
88	IA-P114	SAMN06199214	14	M	Moderate	0	12	NR	No	Yes	Yes
89	IA-P126	SAMN06199224	31	M	NR	47.5	47.5	NR	NR	NR	NR
90	IA-P138	SAMN06199234	30	M	Moderate	44	49	3	Yes	Yes	No
91	IA-P149	SAMN06199241	24	M	Severe	17.7	34.7	3	No	No	No
92	IA-P150	SAMN06199242	27	F	NR	NR	NR	3	No	Yes	No
93	IA-P152	SAMN06199243	32	M	Mild	37	43	2	Yes	No	Yes
94	IA-P153	SAMN06199244	17	M	No IV	29	36	3	No	No	No
95	IA-P154	SAMN06199245	22	F	Mild	32	41	1	No	Yes	Yes
96	IA-P156	SAMN06199247	15	M	Moderate	27	31	1	No	Yes	No
97	IA-P157	SAMN06199248	37	M	Severe	NR	NR	1	No	No	No
98	IA-P158	SAMN06199249	38	M	Mild	47	58	3	Yes	No	No
99	IA-P159	SAMN06199250	37	F	Mild	42.5	46.5	1	No	No	No
100	IA-P160	SAMN06199251	16	M	Moderate	43	56	1	No	No	No

101	IA-P161	SAMN06199252	23	F	Moderate	30	35	2	No	No	No
102	IA-P162	SAMN06199253	21	M	No IV	19.5	27.5	4	No	Yes	Yes
103	IA-P163	SAMN06199254	15	F	Mild	42.5	60.5	1	No	Yes	Yes
104	IA-P164	SAMN06199255	21	M	Mild	16	22	3	Yes	Yes	Yes
105	IA-P166	SAMN06199257	27	M	Moderate	39	54	3	Yes	No	Yes
106	IA-P167	SAMN06199258	23	M	No IV	60	74	3	No	No	Yes
107	IA-P168	SAMN06199259	31	F	Moderate	29	43	3	Yes	Yes	Yes
108	IA-P169	SAMN06199260	18	M	No IV	15	24	3	Yes	Yes	No
109	IA-P171	SAMN06199262	22	M	Mild	32	39	2	No	No	No
110	IA-P172	SAMN06199263	32	M	Mild	37.5	49.5	3	No	Yes	Yes
111	IA-P173	SAMN06199264	22	M	No IV	35.5	51.5	1	Yes	Yes	Yes
112	IA-P174	SAMN06199265	50	M	Moderate	41	56	1	Yes	No	No
113	IA-P175	SAMN06199266	28	M	Moderate	46.5	64.5	2	No	Yes	No
114	IA-P176	SAMN06199267	21	M	Mild	54.5	68.5	2	No	No	Yes
115	IA-P177	SAMN06199268	53	M	Mild	38	44	3	No	Yes	Yes
116	IA-P178	SAMN06199269	21	M	No IV	33	53	3	Yes	Yes	No
117	IA-P179	SAMN06199270	23	M	Mild	38	51	3	Yes	No	No
118	IA-P182	SAMN06199272	28	F	Mild	16	30	3	Yes	Yes	Yes
119	IA-P184	SAMN06199274	21	F	Mild	23	26	1	No	No	No
120	IA-P185	SAMN06199275	28	M	Moderate	37.5	44.5	2	Yes	Yes	No
121	IA-P186	SAMN06199276	30	M	Severe	NR	NR	NR	NR	NR	NR
122	IA-P187	SAMN06199277	21	M	Mild	0	0	3	No	NR	NR
123	IA-P188	SAMN06199278	25	M	Severe	27.5	31.5	3	No	No	No
124	IA-P189	SAMN06199279	70	M	Moderate	26.5	35.5	3	No	No	No
125	IA-P190	SAMN06199280	27	M	No IV	28.5	36.5	1	Yes	Yes	Yes
126	IA-P191	SAMN06199281	22	M	Moderate	54	69	3	Yes	No	No
127	IA-P192	SAMN06199282	34	M	No IV	30.5	37.5	3	No	Yes	No

128	IA-P193	SAMN06199283	33	M	No IV	51.5	62.5	2	No	No	No
129	IA-P194	SAMN06199284	24	M	No IV	NR	NR	2	No	No	No
130	IA-P195	SAMN06199285	22	M	Mild	26.5	42.5	1	No	No	No
131	IA-P196	SAMN06199286	28	M	Mild	23	39	3	No	Yes	No
132	IA-P197	SAMN06199287	32	M	Moderate	30.5	38.5	2	Yes	No	No
133	IA-P199	SAMN06199289	15	M	No IV	26.5	38.5	2	No	Yes	No
134	IA-P201	SAMN06199291	28	M	No IV	NR	52.6	3	No	Yes	Yes
135	P002	SAMN06199293	5	M	Severe	39	43	1	No	Yes	No
136	P003	SAMN06199294	9	M	Severe	41	50	1	No	No	No
137	P004	SAMN06199295	6	F	No IV	51	61	1	Yes	Yes	No
138	P005	SAMN06199296	8	F	No IV	23	27	3	No	No	No
139	P006	SAMN06199297	7	F	No IV	54.5	65.5	2	No	No	No
140	P007	SAMN06199298	5	M	Severe	21.4	31.4	2	No	No	No
141	P008	SAMN06199299	10	M	Mild	28.8	29.8	2	Yes	Yes	No
142	P009	SAMN06199300	9	F	Mild	11.5	14.5	2	No	No	No
143	P010	SAMN06199301	5	M	Mild	21.4	24.4	2	No	Yes	Yes
144	P011	SAMN06199302	15	M	Moderate	50	52	1	No	Yes	Yes
145	P013	SAMN06199303	13	M	NR	36.4	40.4	3	No	Yes	No
146	P014	SAMN06199304	10	M	Severe	70.5	82.5	1	No	No	No
147	P015	SAMN06199305	10	F	No IV	11.5	16.5	3	No	No	No
148	P017	SAMN06199307	13	M	Moderate	40.1	49.1	3	Yes	Yes	No
149	P018	SAMN06199308	9	F	Severe	54.5	62.5	1	No	No	No
150	P019	SAMN06199309	5	M	Mild	22	27	2	No	No	No
151	P020	SAMN06199310	12	F	Moderate	39.6	50.6	2	No	No	No
152	P021	SAMN06199311	4	F	Severe	67	74	1	No	No	No
153	P022	SAMN06199312	14	M	Moderate	56.1	59.1	3	No	No	No
154	P023	SAMN06199313	8	M	Mild	37.4	49.4	2	Yes	No	No

155	P024	SAMN06199314	5	M	No IV	41.5	54.5	1	No	No	No
156	P025	SAMN06199315	12	M	No IV	25.5	38.5	1	No	Yes	No
157	P026	SAMN06199316	7	M	Moderate	37.5	45.5	1	Yes	Yes	Yes
158	P027	SAMN06199317	5	M	Moderate	48.9	56.9	2	No	Yes	No
159	P028	SAMN06199318	5	M	No IV	18.5	26.5	2	No	No	No
160	P029	SAMN06199319	7	F	No IV	32.5	38.5	2	No	No	No
161	P030	SAMN06199320	11	M	Mild	37	48	2	Yes	Yes	Yes
162	P031	SAMN06199321	5	M	Moderate	33.5	43.5	1	Yes	No	Yes
163	P032	SAMN06199322	19	M	No IV	21.5	32.5	3	Yes	No	No
164	P033	SAMN06199323	4	M	Severe	67	83	1	No	No	No
165	P034	SAMN06199324	11	M	Moderate	35.5	37.5	3	Yes	Yes	No
166	P035	SAMN06199325	9	M	Moderate	34	46	2	No	No	No
167	P036	SAMN06199326	12	F	No IV	29.5	41.5	1	Yes	Yes	No
168	P037	SAMN06199327	19	F	Mild	10.9	14.9	3	No	Yes	No
169	P038	SAMN06199328	6	M	Moderate	26.5	34.5	3	Yes	No	No
170	P039	SAMN06199329	8	F	NR	7.4	9.4	1	No	Yes	NR
171	P040	SAMN06199330	9	F	NR	17	23	2	Yes	No	No
172	P041	SAMN06199331	10	M	No IV	21.4	28.4	3	No	Yes	Yes
173	P042	SAMN06199332	17	M	No IV	36	48	3	Yes	No	No
174	P043	SAMN06199333	17	M	No IV	69	80	3	No	Yes	No
175	P044	SAMN06199334	11	F	NR	23	33	2	No	No	No
176	P045	SAMN06199335	13	F	No IV	33.1	43.1	3	No	Yes	No
177	P046	SAMN06199336	4	M	No IV	38.5	54.5	1	No	Yes	No
178	P047	SAMN06199337	17	M	No IV	50.5	66.5	1	No	Yes	No
179	P048	SAMN06199338	8	M	No IV	63	73	1	Yes	Yes	No
180	P049	SAMN06199339	7	F	Mild	21.8	26.8	2	No	Yes	No
181	P050	SAMN06199340	4	M	NR	32.5	45.5	1	No	No	No

182	P051	SAMN06199341	16	F	No IV	26.5	34.5	3	No	Yes	Yes
183	P052	SAMN06199342	9	M	NR	52	62	1	No	No	No
184	P053	SAMN06199343	4	F	NR	36.5	45.5	1	No	Yes	Yes
185	P054	SAMN06199344	7	M	No IV	53	60	1	No	No	No
186	P055	SAMN06199345	20	M	Severe	14.4	17.4	4	No	Yes	No
187	P056	SAMN06199346	10	M	No IV	60	78	2	Yes	Yes	Yes
188	P057	SAMN06199347	19	M	No IV	33.5	43.5	3	No	Yes	Yes
189	P058	SAMN06199348	6	M	No IV	49.5	58.5	2	No	No	No
190	P059	SAMN06199349	14	M	No IV	43.5	48.5	3	No	Yes	No
191	P060	SAMN06199350	9	M	Mild	25.3	27.3	1	No	Yes	No
192	P061	SAMN06199351	5	M	Mild	21.6	27.6	1	Yes	Yes	Yes
193	P062	SAMN06199352	5	M	Mild	42	54	2	No	Yes	No
194	P063	SAMN06199353	6	F	Mild	35.5	53.5	2	No	Yes	No
195	P064	SAMN06199354	19	M	Severe	37.5	51.5	1	No	Yes	No
196	P065	SAMN06199355	14	M	Severe	59.5	69.5	3	No	Yes	No
197	P066	SAMN06199356	17	M	Mild	56	66	1	No	No	No
198	P067	SAMN06199357	10	F	Mild	33.1	39.1	2	No	Yes	Yes
199	P068	SAMN06199358	5	M	No IV	36	46	1	No	Yes	No
200	P069	SAMN06199359	11	M	Mild	32.5	42.5	3	No	Yes	Yes
201	P070	SAMN06199360	15	M	No IV	62.5	69.5	3	No	Yes	No
202	P071	SAMN06199361	9	M	Moderate	50.5	57.5	1	No	Yes	No
203	P072	SAMN06199362	19	M	Mild	50.1	66.1	3	No	Yes	No
204	P073	SAMN06199363	19	M	No IV	59.5	68.5	3	No	No	No
205	P074	SAMN06199364	19	M	Mild	27	35	3	No	Yes	Yes
206	P075	SAMN06199365	20	M	No IV	68	84	3	No	No	No
207	P076	SAMN06199366	20	M	No IV	59	75	4	No	No	No
208	P077	SAMN06199367	13	F	No IV	38.2	46.2	3	No	No	No

209	P078	SAMN06199368	6	M	No IV	33.9	47.9	2	No	Yes	No
210	P079	SAMN06199369	11	F	NR	60	74	1	No	Yes	No
211	P080	SAMN06199370	9	M	Mild	44.5	58.5	1	No	No	No
212	P081	SAMN06199371	6	M	No IV	25.5	32.5	2	No	Yes	Yes
213	P082	SAMN06199372	20	M	No IV	33.5	43.5	3	No	Yes	No
214	P083	SAMN06199373	8	M	No IV	34.5	39.5	3	Yes	No	No
215	P084	SAMN06199374	19	M	No IV	57	73	1	Yes	Yes	Yes
216	P085	SAMN06199375	8	F	No IV	46.5	53.5	2	No	Yes	No
217	P086	SAMN06199376	14	F	Mild	40.5	46.5	2	No	No	No
218	P087	SAMN06199377	17	M	No IV	55.2	65.2	1	No	Yes	No
219	P088	SAMN06199378	3	F	Mild	21.2	23.2	1	Yes	Yes	No
220	P089	SAMN06199379	18	M	No IV	21.8	26.8	1	No	No	No
221	P090	SAMN06199380	3	M	Severe	53	69	1	No	No	No
222	P091	SAMN06199381	7	F	No IV	38.9	50.9	1	No	Yes	Yes
223	P092	SAMN06199382	10	M	NR	29	41	1	Yes	Yes	No
224	P093	SAMN06199383	10	M	No IV	31	37	3	No	Yes	Yes
225	P094	SAMN06199384	3	M	Mild	22	23	1	No	No	No
226	P095	SAMN06199385	11	M	Mild	49.5	57.5	1	Yes	Yes	No
227	P096	SAMN06199386	2	M	Mild	42.5	58.5	1	No	No	No
228	P097	SAMN06199387	5	M	Mild	36.1	49.1	1	No	Yes	Yes
229	P098	SAMN06199388	4	F	Mild	25.3	39.3	1	No	No	No
230	P099	SAMN06199389	12	M	Moderate	62.5	68.5	4	No	No	No
231	P100	SAMN06199390	11	M	No IV	21.4	30.4	2	No	Yes	No
232	P101	SAMN06199391	6	M	No IV	22	42	2	Yes	Yes	Yes
233	P102	SAMN06199392	6	M	Mild	18.5	25.5	2	No	No	No
234	P103	SAMN06199393	13	M	No IV	63	83	3	Yes	Yes	Yes
235	P104	SAMN06199394	18	F	No IV	23	31	1	Yes	Yes	Yes

236	P105	SAMN06199395	17	M	No IV	53	69	3	Yes	Yes	Yes
237	P106	SAMN06199396	15	M	Severe	37	44	1	No	No	No
238	P107	SAMN06199397	20	M	No IV	46.9	62.9	4	No	Yes	No
239	P108	SAMN06199398	8	M	Mild	25.3	36.3	1	No	Yes	No
240	P109	SAMN06199399	13	M	No IV	45.6	51.6	3	NR	Yes	Yes
241	P110	SAMN06199400	12	M	Mild	44	57	1	No	Yes	No
242	P111	SAMN06199401	15	M	Severe	51	61	1	No	Yes	No
243	P112	SAMN06199402	5	M	Mild	44	58	2	Yes	No	No
244	P113	SAMN06199403	15	F	No IV	22	26	3	No	Yes	Yes
245	P114	SAMN06199404	5	M	No IV	35.5	43.5	2	No	No	No
246	P116	SAMN06199405	13	M	No IV	25.5	34.5	2	Yes	No	No
247	P117	SAMN06199406	16	F	Moderate	30	34	1	NR	Yes	No
248	P118	SAMN06199407	8	F	No IV	21.4	29.4	2	NR	Yes	No
249	P119	SAMN06199408	7	F	No IV	21.2	31.2	3	NR	No	No
250	P120	SAMN06199409	12	F	No IV	20.5	34.5	1	Yes	Yes	No
251	P122	SAMN06199410	13	M	Moderate	35.6	40.6	3	No	Yes	No
252	P123	SAMN06199411	4	F	No IV	41.3	49.3	1	NR	Yes	Yes
253	P124	SAMN06199412	19	M	No IV	21.4	35.4	3	Yes	Yes	No
254	P125	SAMN06199413	9	M	Moderate	55.5	73.5	1	Yes	Yes	Yes
255	P126	SAMN06199414	3	M	Mild	29.6	41.6	1	Yes	No	No
256	P127	SAMN06199415	5	F	Mild	42.5	51.5	1	Yes	Yes	No
257	P129	SAMN06199416	12	F	NR	34.1	46.1	3	No	Yes	No
258	P130	SAMN06199417	14	M	No IV	40.5	52.5	1	No	Yes	No
259	P131	SAMN06199418	2	M	Severe	54.5	64.5	1	No	No	No
260	P132	SAMN06199419	20	M	Mild	34	41	3	Yes	Yes	No
261	P133	SAMN06199420	7	F	No IV	33.5	45.5	1	Yes	Yes	Yes
262	P134	SAMN06199421	18	M	Mild	36	47	3	No	Yes	No

263	P135	SAMN06199422	19	M	Mild	37.5	39.5	3	No	Yes	No
264	P136	SAMN06199423	20	M	No IV	41	50	3	Yes	No	No
265	P137	SAMN06199424	17	M	No IV	40	51	1	Yes	No	No
266	P138	SAMN06199425	2	M	No IV	40.6	52.6	1	No	No	No
267	P140	SAMN06199426	6	M	No IV	44	61	3	No	Yes	Yes
268	P141	SAMN06199427	6	M	No IV	2.8	7.8	2	No	Yes	Yes
269	P142	SAMN06199428	13	M	Mild	59	68	2	No	Yes	No
270	P143	SAMN06199429	7	M	Mild	40.5	56.5	2	No	No	No
271	P144	SAMN06199430	3	F	Mild	36.9	49.9	2	No	Yes	No
272	P146	SAMN06199431	11	F	No IV	35.4	43.4	3	No	No	No
273	P147	SAMN06199432	13	M	No IV	42.5	57.5	3	No	Yes	No
274	P149	SAMN06199434	12	M	No IV	32.3	41.3	3	Yes	Yes	No
275	P150	SAMN06199435	7	M	No IV	29.2	39.2	2	No	No	No
276	P151	SAMN06199436	12	M	No IV	27.5	39.5	1	Yes	No	No
277	P152	SAMN06199437	8	F	No IV	47.1	51.1	3	No	Yes	No
278	P157	SAMN06199438	14	M	Mild	41	54	3	No	Yes	No
279	P158	SAMN06199439	17	F	Mild	63	73	1	No	No	No