Supplementary 1: Whole exome sequences (WES) procedure

Test requested: Whole Exome Sequencing (CentoXome GOLD®)

METHOD

RNA capture baits, against approximately 60 Mb of the Human Exome (targeting >99% of regions in CCDS, RefSeq, and Gencode databases), is used to enrich regions of interest from fragmented genomic DNA using the Agilent's SureSelect Human All Exon V6 kit.

The generated library is sequenced on an Illumina platform to obtain an average coverage depth of ~100x. Typically, ~97% of the targeted bases are covered >10x. An end-to-end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering out of low-quality reads and probable artefacts, and subsequent annotation of variants, is applied. All disease-causing variants reported in HGMD®, ClinVar, or CentoMD®, as well as all variants with minor allele frequency (MAF) of less than 1% in the gnomAD database are considered.

The evaluation is focused on the coding exons along with flanking +/-20 intronic bases. All pertinent inheritance patterns are considered. In addition, the provided familial history and clinical information are used to evaluate the eventually identified variants. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported.

Variants of relevance identified by NGS are continuously and individually in-house validated for quality aspects; those variants which meet our internal QC criteria (based on extensive validation processes) are not validated by Sanger.

Supplementary 2: CNV analysis

HGNC Syr	Chr.	hg19 Pos.	Isoform	Coding DNA	Protein	Effect	ACMG Insilic
DOCK8	9	289481	NA	NA	NA	intronic	Benign
DOCK8	9	325817	NA	NA	NA	intronic	Benign
DOCK8	9	325811	NA	NA	NA	intronic	Benign
DOCK8	9	439453	NA	NA	NA	intronic	Benign
DOCK8	9	379965	NA	NA	NA	intronic	Benign
DOCK8	9	439467	NA	NA	NA	intronic	Benign
DOCK8	9	215296	NA	NA	NA	intronic	Benign
DOCK8	9	215511	NA	NA	NA	intronic	Benign
DOCK8	9	327938	NA	NA	NA	intronic	Benign
DOCK8	9	316998	NA	NA	NA	intronic	Benign
DOCK8	9	328006	NA	NA	NA	intronic	Benign
DOCK8	9	327957	NA	NA	NA	intronic	Benign
DOCK8	9	376929	NA	NA	NA	intronic	Benign
DOCK8	9	317195	NA	NA	NA	intronic	Benign
DOCK8	9	432330	NM_20344	c.4785+6C>G	NA	splice dono	Benign
DOCK8	9	429719	NM_00119	c.4287T>C, c.4	p.Phe1429Phe,	synonymou	Benign
DOCK8	9	390512	NM_00119	c.2712C>T, c.2	p.Thr904Thr, p.	synonymou	Benign
DOCK8	9	421032	NM_00119	c.3903C>G, c.	p.Leu1301Leu,	synonymou	Benign
DOCK8	9	312124	NM_00119	c.495T>C, c.49	p.Asn165Asn, p	synonymou	Benign
DOCK8	9	286593	NM_00119	c.85C>A, c.85	p.Pro29Thr, p.P	nonsynonyı	Benign
DOCK8	9	334337	NM_00119	c.1034A>G, c.	p.Asn345Ser, p	nonsynonyı	Benign

ACMG Criteria Hits	Zygos	gnomAD AF (%)	ESP AF (%)	TGP AF (%	Loc.
BA1, BS2	het.	33.9	40.18	0	NA
BA1, BS2	hom.	88.41	0	88.36	NA
BA1, BS2	hom.	67.94	0	69.83	NA
BA1, BS2	hom.	59.69	0	61.56	NA
BA1, BS2	hom.	96.07	6.77	94.47	NA
BA1, BS2	hom.	65.27	0	68.51	NA
BA1, BS2	prob. h	3.65	3.79	3.53	NA
BA1, BS2	hom.	37.08	0	44.09	NA
BA1, BS2	hom.	63.58	0	67.55	NA
BA1, BS2	hom.	42.7	46.44	43.33	NA
BA1, BS2, BP6	hom.	68.55	33.16	69.59	NA
BA1, BS2	hom.	38.19	0	43.99	NA
BA1, BS2	hom.	63.65	48.86	56.89	NA
BA1, BS2	hom.	52.08	0	46.07	NA
BA1, BS2, BP4, BP6	poss. h	75.89	33.08	71.43	E37 (M.i.), E35, E36
BA1, BS2, BP6	hom.	99.77	0.32	99.94	E35, E34, E36 (M.i.)
BA1, BS2, BP6	het.	23.16	28.5	25.5	E23, E22, E24 (M.i.)
BA1, BS2, BP6	het.	50.2	46.26	39.18	E31, E30, E32 (M.i.)
BA1, BS2, BP6	hom.	16.75	21.67	16.43	E5, E5, E6 (M.i.)
PP2, PP3, BA1, BS2, BP6	hom.	52.14	46.42	52.98	E2, E2, E3 (M.i.)
PP2, BA1, BS2, BP4, BP6	hom.	24.57	28.2	19.97	E10, E10, E11 (M.i.)

ClinVar Cli	rs ID	AF Pred.	ClinVar Disease	ClinVar Allele ID	COSMIC ID
NA	rs34722895	common	NA	NA	NA
NA	rs2360277	common	NA	NA	COSN14792919
NA	rs3831138	common	NA	NA	NA
NA	rs10758598	common	NA	NA	NA
NA	rs10758420	common	NA	NA	NA
NA	rs10217568	common	NA	NA	NA
NA	rs73370597	common	NA	NA	NA
NA	rs2023402	common	NA	NA	NA
NA	rs2296825	common	NA	NA	NA
NA	rs2296824	common	NA	NA	COSN14795504
Benign	rs2296828	common	not specified	253508	COSN26732699
NA	rs2296826	common	NA	NA	NA
NA	rs7020921	common	NA	NA	NA
NA	rs10813355	common	NA	NA	NA
Benign	rs7036567	common	not specified; Hyper-IgE s	174825	NA
Benign	rs7854035	common	not specified	174698	NA
Benign	rs2297075	common	not specified; Hyper-IgE s	140844	COSM1179612, C
Benign	rs2297079	common	Hyperimmunoglobulin E re	175111	COSM3763946, C
Benign	rs2039045	common	not specified; Hyper-IgE s	140860	COSM6851474, C
Benign	rs529208	common	Hyperimmunoglobulin E re	175103	COSM3763874, C
Benign	rs10970979	common	not specified; Hyper-IgE s	174691	COSM3765451, C

OMIM-P	VCF Coordinates
614113; 243700	9:289481-C-CAT
614113; 243700	9:325817-T-C
614113; 243700	9:325811-T-TC
614113; 243700	9:439453-G-C
614113; 243700	9:379965-T-C
614113; 243700	9:439467-A-C
614113; 243700	9:215296-A-G
614113; 243700	9:215511-C-T
614113; 243700	9:327938-G-C
614113; 243700	9:316998-C-T
614113; 243700	9:328006-T-C
614113; 243700	9:327957-T-G
614113; 243700	9:376929-A-G
614113; 243700	9:317195-G-A
614113; 243700	9:432330-C-G
614113; 243700	9:429719-T-C
614113; 243700	9:390512-C-T
614113; 243700	9:421032-C-G
614113; 243700	9:312124-T-C
614113; 243700	9:286593-C-A
614113; 243700	9:334337-A-G

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LABOR INGELHEIM

Final Report

Patient		
Patient-ID	Visit code	Add ID
938082		
Date of birth (Age)	Gender	
13.08.2011 (7)	male	

Order	Customer-Code
JEL402O	**K0033316 (000000000)**
Date received	Reportdate
07.11.2018 14:09	27.07.2022 10:03

Primary Materials	
EDTA-Blut (EB)	
Date of sampling	Laborcode

Bioscientia Institut für Medizinische Diagnostik GmbH

Post

National Guard Health Affairs
Prince Mohammed Bin Abdul
Aziz Hospital (PMBAH)
Dr. Mohammad Afzal

00000 Al Madinah Al Munawwarah

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Examination	Result	Unit	Ref.-Range
See Special Report	done		
hyper-IgE syndrome (NGS)	done		

report validation: hartigvali 12.02.2019 12:07 Uhr

20190211 3 21 00005

BIOSCIENTIA
HUMAN GENETICS

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National Guard Health Affairs
Prince Mohammed Bin Abdul
Aziz Hospital (PMBAH)
Department of Pathology
Al Madinah Al Munawarah
Saudi Arabia

Client No: 0033316

Report date: 07.02.2019

CC:

Request code	Date of receipt	Specimen type	Sample date	Client No
JEL402O	07.11.2018	EDTA blood	04.11.2018	0033316

Patient		Date of birth	ID number	Sample number
Sanan, Abdulrahman	/ M	13.08.2011	938082	J12756/18

Indication: chronic failure to thrive, high IgE syndrome, eosinophilia recurrent viral infection, no fever, skin rash

Analysis performed:
Next-generation sequencing of the *DOCK8* gene

Molecular genetic analysis of the *DOCK8* gene by next-generation sequencing (NGS)

Analysis: The coding exons of the *DOCK8* gene was enriched using Roche/NimbleGen sequence capture technology and sequenced on an Illumina system (next-generation sequencing, NGS) (details of the method at the end of the report). Resulting sequence data were compared with the reference sequence NM_203447.3.

Result: Abdulrahman Sanan carries a homozygous deletion of exon 23 to 48 of the *DOCK8* gene.

Interpretation: Mutations in the genes *STAT3, TYK2* and *DOCK8* are known to cause hyper IgE syndrome (1). NGS analysis of these genes did not reveal any pathogenic mutation in Abdulrahman Sanan.

Next-generation sequencing of the *DOCK8* gene did not reveal clearly pathogenic point mutations in the analysed regions. However, CNV (copy number variation) analysis of the NGS data suggested a homozygous deletion of the exons 23 to 48 of the *DOCK8* gene. This homozygous deletion was confirmed by MLPA analysis (SALSA P386-A2DOCK8-STAT3 Vs.04 kit, probes in exon 1, 2, 9, 13, 17, 19, 23, 28, 31, 32, 38, 42, and 48). The exact chromosomal breakpoints of the deletion can not be determined by the methods applied here. Homozygous deletions of more than one exon of the *DOCK8* gene have already been described in the literature (2). Considering the available information the variant is classified as pathogenic .

In summary, considering the homozygous pathogenic deletion in the *DOK8* gene and the phenotype of the patient a diagnosis of hyper IgE syndrome is confirmed by molecular genetics.

We recommend genetic counseling of the parents for discussion of the results. If you have any further questions please do not hesitate to contact us.

Sincerely,

Dr. med. Monika Hartig
(Clinical Geneticist)

Dr. rer. nat. Melanie Kuhn

Method: Genomic DNA was fragmented, and the coding exons of the *DOCK8* gene as well as the corresponding exon-intron boundaries were enriched using the Roche/NimbleGen sequence capture approach, amplified and sequenced simultaneously by Illumina technology (next-generation sequencing, NGS) using an Illumina system. The target regions were sequenced with an average coverage of 133-fold and for all coding exons of the analyzed genes a 20-fold coverage was obtained. NGS data analysis was performed using bioinformatic analysis tools as well as JSI Medical Systems software (version 4.1.2). Identified variants and indels were filtered against external and internal databases and filtered depending on their allele frequency focusing on rare variants with a minor allele frequency (MAF) of 1% or less. Nonsense, frameshift and canonical splice site variants were primarily considered likely pathogenic. Assessment of pathogenicity of identified non-synonymous variants was performed using bioinformatic prediction programmes like Mutation Taster, Polyphen-2, MutationAssessor, FATHMM etc. Only those variants were considered likely pathogenic which were predicted probably damaging by the majority of the used algorithms. *In silico* analysis of splice site effects was performed by bioinformatic programmes like Fruitfly, NetGene2, SpliceView, Mutation Taster and ESE-Finder. Variants that have been annotated as common polymorphisms in databases or in the literature are not being described in this report but are available on request.

20190211 3 21 00006

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JEL402O Sanan, Abdulrahman 938082

Putatively pathogenic differences between the wildtype sequence (human reference genome according to UCSC Genome Browser: hg19, GRCh37) and the patient's sequence mentioned and interpreted in this report were validated using polymerase chain reaction (PCR) amplification followed by conventional Sanger sequencing. Limitations of the NGS method: Mutations in insufficiently covered regions and rare alterations in other parts of the genes (e.g. regulatory regions), low level mosaics or deep intronic splice mutations cannot be excluded with this analysis. In principle, high coverage of NGS data enables copy number variation (CNV) analysis indicating deletions or duplications in the analyzed genomic regions, but not completely ruling out such structural variations. Positive CNV results are validated by independent approaches such as MLPA (multiplex ligation-dependent probe amplification), if available. Insufficiently covered (e.g. GC-rich) regions or coding regions for which highly homologous sequences exist in the genome and which are partially difficult to interpret due to missing specificity in the sequence capture approach were not analyzed by Sanger sequencing. The percentage of such regions in the analyzed genes most often is <1%. Therefore, Sanger sequencing of these regions is not being conducted routinely, but can be performed upon request. On NGS panels for most heterogenous diseases genes for allied indications unrelated to the current patient are sequenced in parallel. Carriership for mutations in these genes is not being reported routinely.

Reference:

(1) Hsu AP, Davis J, Puck JM, et al. Autosomal Dominant Hyper IgE Syndrome. 2010 Feb 23 [Updated 2012 Jun 7]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2016. Available from: https://www.ncbi.nlm.nih.gov/books/NBK25507/

(2) Engelhardt KR, et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J Allergy Clin Immunol. 2009 Dec;124(6):1289-302.e4.

This test was developed and its performance characteristics determined by Bioscientia, Ingelheim, Germany. It has not been cleared or approved by the US Food and Drug Administration.