Supplementary

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Summary of Treatment Options

Time	Height	Weight	Body surface	Treatment plan	Therapeutic drugs	Detailed treatment dosage
D1-28	160cm	45kg	1.40m^2	94 regimen	DXMS+VP-16	Dexamethasone (DXMS) 14mg（10mg/m^2）d1-14 7mg（5mg/m^2）d15-28 Etoposide (VP-16) 100mg（72mg/m^2）d1, 4, 8, 11, 14, 20
D26	160cm	45kg	1.40m^2	CD20 Monoclonal antibody regimen	R	Rituximab (R) 100mg d26
D28-48	160cm	45kg	1.40m^2	JAK inhibitor regimen	Jakavi	Ruxolitinib (Jakavi) 5mg po tid d28-48
D31-58	160cm	41.5kg	1.36m^2	LDEP DEP regimen	DOX+VP-16+ L+Pred	Doxorubicin liposome (DOX) 35mg（25mg/m2）d31, 45 Etoposide (VP-16) 100mg（72mg/m2）d31, 45 PEG-aspargase (L) 2448U（1800U/m2）d33 Methylprednisolone(Pred) 620mg（15mg/kg）d31-33 30mg（0.75mg/kg）d34-37 10mg（0.25mg/kg）d38-40 4mg（0.1mg/kg）d41-44 80mg（2mg/kg）d45-47 30mg（0.75mg/kg）d48-61 10mg（0.25mg/kg）d62-64 4mg（0.1mg/kg）d64-68
D43	160cm	41.5kg	1.36m^2	PD-1 Blockade regimen	PD-1 Blockade	Camrelizumab(PD-1 Blockade) 200mg d43

The patient was then discharged and started maintenance therapy monthly

Time	Height	Weight	Body surface	Treatment plan	Therapeutic drugs	Detailed treatment dosage
monthly	160cm	41.5kg	1.36m^2	PD-1 Blockade regimen	PD-1 Blockade	Camrelizumab(PD-1 Blockade) 200mg monthly until plasma EBV-DNA is undetectable
every 3 months	160cm	45kg	1.4m^2	PD-1 Blockade regimen	PD-1 Blockade	Camrelizumab(PD-1 Blockade) 100mg every 3 months for up to 1 year

the proportion of B lymphocytes increased in the fifth month after discharge

Time	Height	Weight	Body surface	Treatment plan	Therapeutic drugs	Detailed treatment dosage
D176、D209	160cm	45kg	1.4m2	CD20 Monoclonal antibody regimen	R	Rituximab (R) 100mg d176, 209

Hemophagocytic Syndrome and Relevant Genetic Testing of Immune Deficiency

Quality Contract Parameter for Testing					
Testing Method	High-throughput sequencing	Average Sequencing Depth	166X	Average Coverage of Targeted Region	99.99% (10X)-99.94% (20X)
Sequencing Type	Targeted sequencing	Testing Mutation Type	SNV, Indel, CNV		

Testing Result Description: Following genetic variations are tested within testing range		
Variation Type	proof Classification	Specific Variation
SNV (Single Nucleotide Variations)	Pathogenic	Undetected
	Likely pathogenic	Undetected
	VUS	Undetected
Indel (Insertion-deletion)	Pathogenic	Undetected
	Likely pathogenic	Undetected
	VUS	Undetected
CNV (Copy number variation)	Undetected	
Conclusion	Variation relating to clinical phenotype of patient: The clear pathogenic genetic variation for the sequencing results of the familial hemophagocytic syndrome and relevant genetic testing of immune deficiency (See genes list in detail) are not observed. Please make a comprehensive consideration with a combination of many aspects e.g. actual clinical condition, hereditary mode of disease, limitations of testing method etc. Please use the above results with discretion.	

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Detailed Analysis of Results

Variations relating to patient clinically (SNV or Indel):

Mutant gene	Chromosomal location	Transcript No./Exon	Nucleotide change	Amino acid change	Status	Variation classification	Relevant disease, hereditary mode	Genetic source
Undetected								

Recommended analysis:

The pathogenic variations of exon coding region for the familial hemophagocytic syndrome and relevant genetic testing of immune deficiency are not detected.

It means, there is no pathogenic variation observed within the detectable range of this method. Please pay attention that almost all genetic testing methods are having some limitations without exception. Please see the “Methodology Instruction” part in detail for the description of method performance. Especially when the pathogenic variation is not detected for the extremely likely cases clinically.

The negative results reduce the possibility for patients to have genetic pathogenic variation. However, due to the clinical manifestation, relevant genetic variation type for this disease are all diversified, it is difficult to detect all types of variations by the current technology.

Variations relating to patient clinically (CNV):

Variation Name and Length	Relevant OMIM Gene within This Region	Variation Classification
Undetected		

Note: CNV (copy number variation) results are largely affected by the sequencing quality with a possibility of false negative; As for the reported positive result, which can be used as the basis of diagnosis and clinical decisions upon the testing verification for copy number variation. Please see the Second Point of Methods and Limitations in this Report.

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Other Results

Mutant gene	Chromosomal location	Transcript No./Exon	Nucleotide change	Amino acid change	Status	Variation classification	Relevant disease, hereditary mode	Genetic source
Undetected								

Variations reported in “Other Results” part can include the following situations:

1. As for the genes in a complete compliance or partial compliance with the clinical phenotype, the variation is classified as ambiguity according to ACMG, and it is not recorded in the database of public health group but recorded in the database of independent property rights of Kingmed.

2. Including the genes relating to some recessive genetic diseases within the package, the pathogenic or likely pathogenic variations are tested, and the patient doesn’t have relevant phenotype.

3. Including the genes relating to some dominant genetic diseases within the package (especially the explicit is not complete in the early stage), the pathogenic or likely pathogenic variations are tested, but not compliant with the clinical phenotype of patient or the clinical phenotype information of patient is insufficient, which requires clinical doctor to assess and consider the overall information of patient.

4. The result interpretation and verification with parental relevant variable sites will be performed in a selective manner for these variations.

5. The clinical doctor shall make a comprehensive consideration with combination of patient’s clinical manifestation, and use this variation results with discretion.

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Methods and Limitations

1. This detection applies the column chromatography method to abstract DNA from peripheral blood. Through high throughput sequencing technology, perform the direct sequencing to the exon region of genes. Make a comparison with hg19 reference sequencing, and analyze the site mutation and CNV (copy number variation) of genes.

2. CNV (copy number variation)'s sensitivity to copy number variation larger than 300kb is 99%. the detectable rate for copy number variation smaller than 300kb depends on genomic structure and sequencing quality of relevant regions. The accuracy and range of copy number variation obtained by this method has not been confirmed by independent copy number analysis experiment. Therefore, this result shall only be for reference for the clinical doctor, which can be used as the basis of diagnosis and clinical decisions upon experimental verification for copy number test. The sequencing depth and mutation analysis level are affected by the type and quality of sample with a certain vibration; Analysis level of CNV is largely affected by the type and quality of sample.

3. This sequencing method covers exon encoding region and the genetic mutation at interface of intron-exon, failing to cover the regions e.g. intron depth and gene control region; Meanwhile, there may have small possible leak detection of mutation occurred at exon region (encoding region) sequencing in the following circumstances (unknown mutation in primer binding region causes difficult binding between primer and template; High GC content region; Sequencing high iteron; Other locations of genome have highly homologous sequencing regions etc.). Due to the clinical manifestations and relevant genetic variation types of disease are diversified, it is difficult to detect the variation of all types by the current technology.

4. This laboratory performs the genetic variation classification and interpretation according to the relevant provisions of ACMG, only report the pathogenic variations relating to patient's clinical manifestations within package, likely pathogenic variations (including VUS - tending to pathogenic variations) and some equivocal variations (Reporter believes that the variations that doctor may need to note within combination with the clinical conditions).

5. Limited by the unicity of tested sample, germline variations and somatic cell variations are difficult to distinguish. As for the likely cell variations, this report is only attached with the suggested interpretation relating to the somatic cell variations without identification for germline and somatic cell variations.

6. As for the SNP sites detected in this sample, through the literature review and combination with previous testing results in this Center, these sites can also exit in the sample of normal person, confirmed to have no crucial linkage with the clinical manifestations of patient, thus it is not listed in this Report. If you have interest in these variations, please consult to us.

7. The bioinformatics analysis means applied by this Center include:

Missense mutation analysis: PolyPhen2; SIFT; LRT; Mutation Taster; Mutation Assessor; FATHMM; GERP; Phylop; SiPhy;

Splicing mutation analysis: NetGene 2 Server; AUGUSTUS

8. Interpretation of regular hereditary mode:

Autosomal dominant (AD): Patient of heterozygous mutations has 50% possibility to inherit pathogenic variations to descendants.

Autosomal recessive (AR): Person with one heterozygous pathogenic variations will not be developed to a patient, but this testing can't detect all variation types (Please see the Methodology Instruction in detail). patient's parents are always carrying the pathogenic variations. The parents carrying pathogenic variations have 25% possibility of developing to a patient when having children each time. Other relatives of patient's parents are also having the risk of carrying same pathogenic variations.

X chromosome recessive (XLR): As for the genetic diseases of X chromosome recessive, patients are mostly male. Female carriers have 50% possibility to inherit the pathogenic variations to descendants. Among the descendants who are inherited the pathogenic variations, sons are always the patient and daughters are the carrier. If mother's testing results are normal, the pathogenic variations carried by her may be a novel mutation, and it is unable to rule out the possibility of mother carrying

hybrid mutation.

Declaration

1. In terms of the interpretation for this testing report, it is recommended to make a genetic consulting to the authorized agent.
2. This detection shall only take responsibility for receiving sample. If you have any doubt to results, please contact us within 7 working days upon the reception of results.
3. This laboratory has been recognized by the College of American Pathologists (CAP).

Reference and Database

1. HGMD (Human Gene Mutation Database), Professional, Cardiff University UK;
2. Locus Specific Mutation Databases (http:// www.hgvs.org/dblist/glsdb.htm);
3. ClinVar, National Center for Biotechnology Information (NCBI, www.ncbi.nlm.gov/clinvar/);
4. ESP 6500, America's National Heart, Lung and Blood Institute (NHLBI);
5. 1000 genomics (Genomes Project);
6. dbSNP, National Center for Biotechnology Information (NCBI, www.ncbi.nlm.gov/clinvar/);
7. UniProt (http://www.uniprot.org.uniprot.).
8. Standards and guidelines for the interpretation of sequence variants ACMG recommendation. Genet Med. 2016.
9. Hemophagocytic Lymphohistiocytosis, Familial. GeneReviews. [Last Updata: January 17, 2013].

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Appendix 1: Genes List

This detection covers the genetic variations on all exons of totally 62 genes for the familial hemophagocytic syndrome and relevant genetic testing of immune deficiency (including the point mutation, small segment insertion/deletion mutation, copy number variations).

ADA	AK2	AP3B1	ATM	BLM	BLOC1S6	CARD11	CASP10	CASP8	CD27
CD40LG	C0G1	C0G6	C0R01A	DCLRE1C	DKC1	DNMT3B	D0CK8	FADD	FAS
FALSG	FCGR3A	GATA2	IL21R	IL2RA	IRF8	ITK	JAK3	LRBA	LYST
MAGT1	MCM4	MVK	MY05A	NCF1	NCF2	NCF4	NLRC4	NLPR12	0STM1
PIK3CD	PIK3R1	PLCG2	PRF1	PRKCD	RAB27A	RAG1	RAG2	RECQL4	SH2D1A
SH3BP2	SLC29A3	SLC7A7	STX11	STXBP2	TCIRG1	TNFRF11A	TNFRSF13B	UNC13D	UNG
XIAP	ZAP70								

Remarks:

1. The red and bold parts are the genes testing recommended by China's Hemophagocytic Syndrome Union;
2. Others are the genes relating to immune deficiency that can cause the hematologic disorder (OMIM Database).

Clinical Significance

1. The familial hemophagocytic syndrome is always accompanied with infection or induction from infection. 70% FHLH will have an onset within 1 year old, some even have an onset before birth, who may have the clinical manifestations at the time of birth. However, some may have an onset as late as 8 years old. In the same family, its onset age is similar. Almost all patients have fever at the beginning, mostly have a high fever, and the fever type is in a wave type with a protracted course of disease. Only few have a fever at the late period of onset. Most patients' liver and spleen become a progressive enlargement with an obvious enlargement. About 50% patients have a lymphadenectasis, especially for those hiv-infected patients. The incidence rate for rash is about 6% to 43%, always appearing during high fever and transient with nonspecific type but in a diversity.
2. The pathogenesis for familial hemophagocytic syndrome is extremely complex, and the main relevant genes include:

Gene	Introduction
PRF1	Genes encode a perforin protein, which is present in immune cells such as T cells and natural killer cells, destroying other cells, and whose mutations mainly cause familial Hemophagocytic Syndrome Type II;
UNC13D	Genes encode one protein involved in cytolysis and regulation of the immune system, and its mutation can cause familial Hemophagocytic Syndrome Type III;
STX11	Genetic encoding mutation blends a member of syntaxin family, and its mutation can cause familial Hemophagocytic Syndrome Type IV;
STXBP2	Protein of genetic encoding belongs to STXBP/unc-18/SEC1 family, and its mutation can cause familial Hemophagocytic Syndrome Type V;
SH2D1A	Genes encodes a protein involved in signaling lymphocyte activation, and the XIAP gene encodes a protein existing in many types of cells including immune cells, it can protect cells by blocking the action of certain enzymes. Mutation of these two genes can cause X linked lymphoproliferative disorder (XLP);
RAB27A	Genes encode a protein in sac-like moving cells and protein of other molecular structures, it is associated with Grisselli Syndrome Type II;
AP3B1	Genes encode a protein that may play a role in organelles related to the bio-genesis of melanosomes,

	dense granules of platelets, lysosomes, it is associated with Hermansky-Pudlak Syndrome Type II;
LYSY	Encoded protein transports regular protein for lysosomal, and associated with Chediak-Higashi Syndrome.

3. The familial hemophagocytic syndrome shall be distinguished from several diseases, such as Virus-related hemophagocytic syndrome, malignant histiocytosis, X-Chain lymphoproliferative disease, macrophage activation syndrome and other similar diseases.

4. Partial relevant genes included in this detection and their corresponding diseases:

Disease	Relevant Genes
Hemocytopenia caused by immune deficiency	ADA, AK2, ATM, BLM 、 BLOC1S6, CD27, CD40LG, COG1, COG6, DCLRE1C, DKC1, DNMT3B, FAS, GATA2, ITK, MAGT1, MVK, OSTM1, PIK3R1, PNP, RAG1, RAG2, TCIRG1, TNFRSF11A, CASP10、FASLG、LRBA, NLRC4, XIAP
Abnormal lymphocyte or NK cell function caused by immune deficiency	CASP8, DKC1, DOCK8、FCGR3A, IL21R, IRF8, JAK3, RECQL4, ZAP70, PLCG2, SH3BP2
Epstein-Barr virus caused by immunodeficiency predisposes	CORO1A, IRF8, ITK, MAGT1, PIK3CD, LRBA, MCM4
Granulomatous disease caused by immune deficiency	NCF1, NCF2, NCF4, RAG1, RAG2
Proliferation of lymphoid system caused by immune deficiency	PRKCD, SLC29A3, TNFRSF13B, UNG, CASP10, FASLG, IL2RA, NLRP12, LRBA, CARD11, FADD, CASP8, CD27, DCLRE1C, FAS, ITK, MVK, PIK3R1
Inflammasome activation	NLRC4