

Table of contents

Acknowledgements	iii
Abstract	v
Résumé	vii
Table of contents	ix
List of figures	xi
List of tables	xii
Chapter 1 Introduction	13
1.1 Genetic susceptibility to infectious diseases	13
1.2 Approaches to gene discovery	13
1.2.1 Genome-wide linkage and candidate-gene studies	13
1.2.2 Genome-wide association studies (GWAS)	14
1.2.3 High-throughput sequencing (HTS)	16
1.3 Genetic architecture of infectious diseases	20
1.4 Role of pathogen	22
1.5 Justification of research	23
1.6 References	24
Chapter 2 Exome sequencing reveals primary immunodeficiencies in children with <i>Pseudomonas aeruginosa</i> sepsis	35
2.1 Introduction	35
2.2 Material and methods	35
2.2.1 Patients	35
2.2.2 DNA extraction and exome sequencing	35
2.2.3 Alignment of sequencing reads, variant calling and annotation	36
2.2.4 Identification of potential causal variants	36
2.2.5 Gene-annotation enrichment analysis	36
2.3 Results	36
2.3.1 Clinical presentation and outcomes	36
2.3.2 Exome sequencing	37
2.3.3 Variant analysis	40
2.4 Conclusion and discussion	44
2.5 References	46
Chapter 3 Loss-of-function mutations in IFIH1 predispose to severe viral respiratory infections in children	47
3.1 Introduction	47
3.2 Material and methods	47
3.2.1 Subject recruitment and specimen collection	47
3.2.2 Screening of respiratory viruses	48
3.2.3 Exome sequencing and alignment	48
3.2.4 Variant calling	49

3.2.5	Variant annotation.....	49
3.2.6	RNA sequencing and alignment.....	49
3.2.7	Plasmids.....	49
3.2.8	Transfection & measure of interferon- β promoter activity.....	50
3.2.9	Viruses.....	51
3.2.10	Measure of protein stability by pulse chase.....	51
3.2.11	Recombinant IFIH1 expression.....	51
3.2.12	Measure of IFIH1 ATPase activity.....	51
3.2.13	Cell culture and transduction with lentivirus vectors.....	52
3.2.14	Quantitative RT-PCR.....	52
3.2.15	Statistical analysis.....	52
3.3	Results.....	52
3.3.1	Clinical presentation and outcomes.....	52
3.3.2	Exome sequencing and alignment of short reads.....	54
3.3.3	Variant calling and annotation.....	54
3.3.4	Loss of function variants.....	55
3.3.5	IFIH1 role in innate immunity.....	55
3.3.6	Description of <i>IFIH1</i> LoF carriers.....	56
3.3.7	Description of <i>IFIH1</i> LoF mutations.....	56
3.3.8	RNA sequencing and read mapping of IFIH1 LoFs.....	57
3.3.9	Functional characterization of IFIH1 LoF mutations.....	59
3.3.10	Role of IFIH1 in controlling respiratory viruses.....	61
3.4	Conclusion and discussion.....	63
3.5	References.....	64
Chapter 4	Deep sequencing and <i>de novo</i> assembly of two respiratory syncytial virus genomes isolated from previously healthy children with severe infection.....	67
4.1	Introduction.....	67
4.2	Material and methods.....	68
4.2.1	Subject recruitment and specimen collection.....	68
4.2.2	Viral RNA sequencing.....	68
4.2.3	De novo assembly and analysis.....	68
4.2.4	Maximum likelihood phylogeny analysis.....	69
4.2.5	Protein similarity analysis.....	69
4.3	Results.....	69
4.3.1	Study subjects.....	69
4.3.2	Viral RNA sequencing.....	70
4.3.3	<i>de novo</i> assembly and analysis.....	70
4.3.4	Maximum likelihood tree generation.....	78
4.3.5	Protein similarity analysis.....	81
4.4	Conclusion and discussion.....	85
4.5	References.....	86
Chapter 5	Conclusion.....	89
Curriculum Vitae.....		91