
Contents

1	INTRODUCTION	1
1.1	MOLECULAR GENETICS OF HEART FAILURE AND CARDIOMYOPATHY	2
1.1.1	Genetics of dilated cardiomyopathy	3
1.1.2	Genetics of hypertrophic cardiomyopathy	3
1.1.3	Modifier and candidate genes of heart failure	4
1.1.4	Fatty acid metabolism in cardiac failure	9
1.1.5	Cd36 in fatty acid transport	10
1.2	THE RAT AS EXPERIMENTAL MODEL FOR STUDYING HEART FAILURE	12
1.2.1	The SHHF strain used for studying heart failure	12
1.2.2	Characteristics of the rat strains SHR, SHRSP, and WKY	13
1.3	EXPRESSION PROFILING FOR THE ANALYSIS OF HEART FAILURE	15
1.3.1	Expression profiling using cDNA array	15
1.3.2	Expression profiling using Affymetrix chips	16
1.4	OBJECTIVES	18
2	MATERIALS	19
2.1	LABORATORY EQUIPMENT	19
2.2	CHEMICALS, REAGENTS, ENZYMES, AND VECTORS	20
2.3	MEDIA AND SOLUTIONS	21
2.4	OLIGONUCLEOTIDES	24
2.5	ANIMALS	27
2.6	KITS AND OTHER MATERIALS	27
2.7	COMPUTER SOFTWARE AND DATABASES	28
3	METHODS	29
3.1	ANIMAL BREEDING AND PHENOTYPING	29
3.2	PREPARATION OF RNA FROM ANIMAL TISSUE	30
3.2.1	Isolation of total RNA	30
3.2.2	RNA cleanup	30
3.2.3	Preparation of poly A ⁺ from total RNA	31
3.3	FIRST STRAND CDNA SYNTHESIS	31
3.4	HYBRIDIZATION OF CDNA ARRAYS	32
3.4.1	Preparation of probe	32
3.4.2	Hybridization	33
3.4.3	Washing and scanning of the filters	33
3.4.4	Analysis of cDNA arrays	34
3.5	HYBRIDIZATION OF AFFYMETRIX CHIPS	34
3.5.1	Synthesis of double strand cDNA from total RNA	35
3.5.2	cDNA purification using phenol/chloroform extraction	36
3.5.3	Synthesis of Biotin-Labeled cRNA	36
3.5.4	Fragmentation of the cRNA and preparation of the hybridization cocktail	37
3.5.5	Targets cleanup and hybridization	37
3.5.6	Washing, staining, and scanning probe arrays	38
3.5.7	Analysis of Affymetrix chip	38
3.6	QUANTITATIVE REAL TIME PCR	42
3.6.1	Design of TaqMan probe and primer	42
3.6.2	Optimization of Real Time PCR condition	43
3.6.3	Quantitative RT-PCR using TaqMan probe	44

3.6.4	Quantitative RT-PCR using SYBR Green.....	44
3.6.5	Analysis of quantitative Real Time PCR.....	45
3.7	PREPARATION OF DNA.....	45
3.7.1	Isolation of genomic DNA from rat tissue.....	45
3.7.2	Isolation of genomic DNA from rat tail.....	46
3.7.3	Isolation of plasmid DNA.....	46
3.7.4	Quantification of DNA and quality assessment.....	47
3.7.5	Analysis of plasmid DNA using restriction enzyme digestion.....	47
3.8	PCR AND PURIFICATION OF PCR PRODUCTS.....	47
3.8.1	Standard PCR.....	47
3.8.2	Colony PCR.....	48
3.8.3	Purification of PCR products.....	48
3.9	DNA SEQUENCING.....	49
3.10	SOUTHERN BLOT HYBRIDIZATION ANALYSIS.....	49
3.10.1	DNA gel electrophoresis and blotting.....	49
3.10.2	Probe labeling by random hexamer priming.....	50
3.10.3	Hybridization.....	50
3.11	MOLECULAR ANALYSIS OF <i>Cd36</i> IN SHHF STRAIN.....	51
3.11.1	cDNA sequencing.....	51
3.11.2	Determination of 5' and 3' end of <i>Cd36</i> cDNA.....	51
3.11.3	Northern blot.....	53
3.11.4	Isolation of Cd36 protein from heart tissue.....	54
3.11.5	SDS-PAGE and Western blot.....	55
3.12	LINKAGE ANALYSIS OF CD36 IN HEART FAILURE MODEL SHHF.....	56
3.12.1	Phenotyping of F2 animals.....	56
3.12.2	Genotyping of F2 animals.....	56
3.12.3	Expression level of <i>Cd36</i> in F2 animals.....	57
3.12.4	Statistical analysis.....	57
4	RESULTS.....	59
4.1	GENE EXPRESSION ANALYSIS WITH CDNA MICROARRAYS.....	59
4.2	GENE EXPRESSION PROFILING WITH AFFYMETRIX CHIP.....	60
4.2.1	Targets preparation.....	60
4.2.2	Hybridization result with Test chips.....	61
4.2.3	Hybridization and analysis of RG-U34A and RG-U34B chips.....	62
4.2.4	Gene expression profiling in rat heart tissue.....	65
4.3	COMPARISON ANALYSIS OF CANDIDATE GENES USING REAL TIME PCR.....	67
4.4	MOLECULAR BASIS OF <i>Cd36</i> IN SHHF MODEL.....	69
4.4.1	Expression level of <i>Cd36</i> in SHHF strain.....	69
4.4.2	Different transcripts of <i>Cd36</i> in SHHF strain.....	70
4.4.3	SHHF and WKY <i>Cd36</i> cDNA sequences.....	70
4.4.4	Blast analysis of <i>Cd36</i>	74
4.4.5	<i>Cd36</i> copy number and deletion breakpoints in SHHF strain.....	76
4.4.6	Western blots of Cd36 protein in SHHF heart tissue.....	79
4.4.7	Comparison of predicted protein sequence of Cd36.....	80
4.5	FUNCTIONAL STUDY OF CD36 IN SHHF RAT USING LINKAGE ANALYSIS.....	81
4.5.1	Genotype of F2 animals.....	81
4.5.2	Expression of <i>Cd36</i> in F2 animals.....	81
4.5.3	linkage analysis by MapMaker.....	82
4.5.4	Comparison analyses by t-test and anova.....	83

5	DISCUSSION.....	85
5.1	CANDIDATE GENES SELECTED WITH CDNA ARRAYS.....	85
5.2	CANDIDATE GENES EXPLORED WITH AFFYMETRIX CHIPS.....	86
5.2.1	Genes with differential expression in SHHF train versus WKY or SHRSP.....	86
5.2.2	Genes with differential expression in SHHF and SHRSP strains versus WKY....	93
5.3	CONCLUSION FOR GENE EXPRESSION PROFILING.....	93
5.4	MOLECULAR BASIS OF <i>Cd36</i> LOCUS IN SHHF RAT.....	94
5.5	FUNCTION OF CD36 IN SHHF RAT.....	97
5.6	CONCLUSION AND OUTLOOK FOR CD36.....	98
6	SUMMARY.....	99
7	LITERATURES.....	101
8	CURRICULUM VITAE AND PUBLICATIONS.....	109
9	ACKNOWLEDGMENTS.....	111
10	ABBREVIATION INDEX.....	112
11	APPENDIX.....	115
11.1	CANDIDATE GENES SELECTED FROM AFFYMETRIX CHIP ANALYSIS.....	115
11.2	FORMULA AND DEFAULT VALUES FOR AFFYMETRIX CHIP ANALYSIS.....	123