

WHOLE GENE SEQUENCING OF KIR-3DL1 WITH SMRT® SEQUENCING AND THE DISTRIBUTION OF ALLELIC VARIANTS IN DIFFERENT ETHNIC GROUPS

NEZIH CEREB¹, HWARAN KIM¹, JAEJUN RYU¹, EUNSIL KIM¹, VIKAS RAI¹, SEHO CHOI¹, CYNTHIA VIERRA-GREEN²,
STEPHEN SPELLMAN², JEONGOK JEON¹, JANGYOUNG KWON¹, SOO YOUNG YANG¹

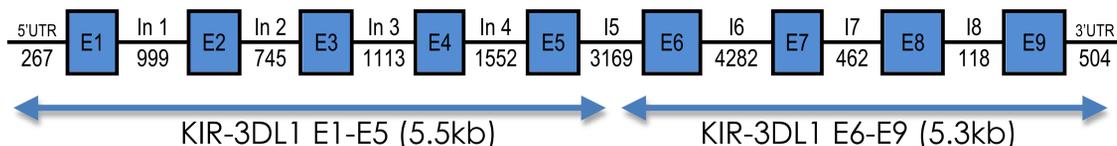
¹ HISTOGENETICS, OSSINING, NY USA ²CENTER FOR INTERNATIONAL BLOOD AND MARROW TRANSPLANT RESEARCH, MINNEAPOLIS, MN USA

The killer-cell immunoglobulin-like receptor (KIR) gene family are involved in immune modulation during viral infection, autoimmune disease and in allogeneic stem cell transplantation. Most KIR gene diversity studies and their impact on the transplant outcome is performed by gene absence/presence assays. However it is well known that KIR gene allelic variations have biological significance. Allele level typing of KIR genes has been very challenging until recently due to the homologous nature of those genes and very long intronic sequences. SMRT® (Single Molecule Real-Time) Sequencing generates average long reads of 10 to 15 kb and allows us to obtain in-phase long sequence reads. We have developed a PCR assay for SMRT® Sequencing on the PacBio RSII platform in our lab for 3DL1 whole gene sequencing. This approach allows us to obtain allele level typing for 3DL1 genes and could serve as a model to type other KIR genes at allelic level.

MATERIAL & METHODS

DNA samples were obtained from previously typed 41 cell lines of Caucasian ethnicity and 97 volunteers with Korean and South Asian origin. Gene absence/presence assays were done on Illumina MiSeq platform using our in-house protocol.

We performed whole gene 3DL1 sequencing on the genomic DNA of samples using two gene-specific primers to get two amplicons (5.5kb and 5.3 kb, respectively) that will span all the exons and most of Intron 5. These amplicons were sequenced on the PacBio RSII platform. We analyzed the data based on current KIR DB v 2.6.0 using our in-house program.



Samples Tested	All	Ethnicity Background					
		Cau/Euro	Korean	So. Asian	Philipino	Mediterranean	Mexican
	91	53	26	9	1	1	1
3DL1 negative samples	7	5	2	-	-	-	-
3DL1 positive samples	84	48	24	9	1	1	1

RESULTS

A total of 123 out of 138 samples had 3DL1 gene by gene absence/presence assay. Based on the exon sequences alone, 123 samples had 47 of the 110 known alleles. However when we included intron sequences to our analysis only 23 sequences out of 47 unique alleles had a match to the database for intronic sequences. The remainder 24 sequences had novel variations in the introns and the majority being in intron 6. 3DL1*002 that has stronger inhibitory receptor was found in 13 samples and mostly in CAU/EURO samples in this study. Some ethnic clustering of certain alleles (ie:3DL1*01501/2 group)were observed in Korean samples.

3DL1 allele typed by Histogenetics		All	Matched to DB	New alleles
		alleles typed	127	87
unique alleles	40	19	21	

110 3DL1 alleles exist in IPD-KIR DB ver 2.6.0

Allele distribution based on Ethnic Background

	CAU/EURO	Korean	South Indian	Philipino	Mexican	Mediterranean	Occurrence
3DL1*0010102	1						1
3DL1*002	11						11
3DL1*0040101	7						7
3DL1*00402					1		1
3DL1*00403	1						1
3DL1*0050101/3DL1*0050103	18	5	1			1	25
3DL1*0070101	2	1	2				5
3DL1*0070104		1					1
3DL1*008	4		2		1	1	8
3DL1*009	1		1				2
3DL1*0150101		1					1
3DL1*0150203/3DL1*0150209		3		1			4
3DL1*0150204/3DL1*0150205	1	7	1				9
3DL1*0150207		1					1
3DL1*0150208		2					2
3DL1*019	1						1
3DL1*0200102	3	2					5
3DL1*033	1						1
3DL1*052	1						1
Total							87

3DL1*0150203 (IPD Acc No: KIR00480, Known ethnic origin: Oriental)

New Intronic Sequence pattern and occurrence based on Ethnicity

Reference	Pattern	Occurrence	Frequency
3DL1*0010101	7	19	CAU/EURO (14), Korean (2), South Indian (3)
3DL1*0040101	3	4	CAU/EURO (4)
3DL1*008	1	2	CAU/EURO (1), South Indian(1)
3DL1*0150201	1	3	South Indian (1), Korean (1), Thai (1)
3DL1*0150203	6	11	Korean (7), Thai (4)
3DL1*002	1	1	CAU/EURO (1)
3DL1*0040101	3	5	CAU/EURO (5)
3DL1*0050101	1	1	Korean (1)
3DL1*0150202	1	1	Korean (1)
3DL1*0150203	6	11	Korean (7), Thai (4)
3DL1*0290101	1	1	Korean (1)

Stronger inhibitory receptors 3DL1*002(11) and 3DL1*002New(1) were found only in CAU/EURO samples in this study.

Residue 238 in the D2 domain	Residue 320 in the transmembrane region	All	Ethnicity Background						Inhibition
			CAU/EURO	Korean	South Indian	Philipino	Mediterranean	Mexican	
R	I	12	12						Strong
G	I	93	46	33	10	1	2	1	Weak
G	V	22	17	2	2			1	Weak

KIR3DL1 Polymorphisms That Affect NK Cell Inhibition by HLA-BW4 Ligand. Carr et al.

CONCLUSION

Whole gene sequencing of KIR genes is now feasible. Allelic typing of KIR genes will allow us to better dissect their modulatory role during immune response and their distribution among diverse populations.