The MR1 gene encompasses at least 6 alleles

Non-classical MHC class I genes, also known as MHC class Ib genes and MHC-I like genes, have few alleles and are considered oligo- or even mono-morphic. We developed a MR1 specific PCR assay and sequenced DNA samples from a diverse set of HLA genotypes. In this relatively small panel we found multiple alleles encoding for different MR1 proteins. Here we present new allele variants for MR1 and map the variant residues to the protein structure.

Materials & Methods

MR1 is a MHC-I like gene that is on chromosome 1. The structure of MR1 is that of a typical MHC class I gene, with exon 1 coding for a leader peptide and exons 2-4 coding for extracellular domains that can bind an antigen. Exon 5 and 6 are coding sequences for the transmembrane and cytoplasmic domains (Figure 1A). An unusual feature of MR1 is a large intron 1 of 15kb. To study variation of MR1 we developed a PCR assay that amplifies a region spanning intron 1 to the 3' UTR, so that the resulting amplicon of 8 kb includes all exons encoding for the MR1 protein (Figure 1B). We performed PCR reactions for 56 DNA samples from the genetic testing reference material from the Coriell Cell Repositories.

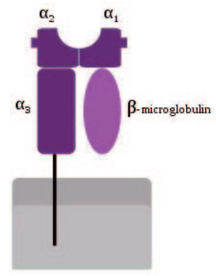
Sequencing results

We sequenced the 56 amplicons on an Illumina Miseq and analyzed the data using NGSengine software (Figure 2A). The sequence data was of high quality. A diverse set of heterozygous genotypes was discovered (Figure 2B). In all cases the minor fraction of the heterozygous positions was between 43-50%, indicating a balanced amplification of both alleles in each sample.

To obtain fully phased MR1 allele sequences, MR1 was re-amplified from 5 samples containing alleles encoding for novel MR1 proteins. These amplicons were sequenced on a PacBio Sequel II System. This method resulted in high-quality long reads that covered the complete amplicons and in which phasing of all heterozygous positions could be achieved (Figure 3).

Structure analysis

Figure 4 shows a cartoon display of MR1 with the variant residues in 5 novel alleles highlighted in teal. R9 represents a TCR contact in crystal structures of a MAIT TCR complexed with MR1 presenting the drug diclofenac or its metabolite 5-OH-diclofenac 11. Accordingly, R9H might be relevant for TCR recognition of MR1 ligands whose presentation is not disrupted by R9H. E52G and H90Q are non-conservative changes and could be of consequence to the function or folding of MR1 but this should be tested in functional studies. I244V represents a conservative change in a region that is not known and unlikely to be of functional importance. R304K represents a conservative change in the cytoplasmic tail that has been observed in other species as well.



A exon 1; leader peptide

exons 2-4; extracellular domains that binds antigen.

Exon 5 and 6; transmembrane and cytoplasmic domains

B

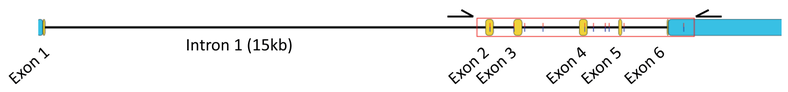
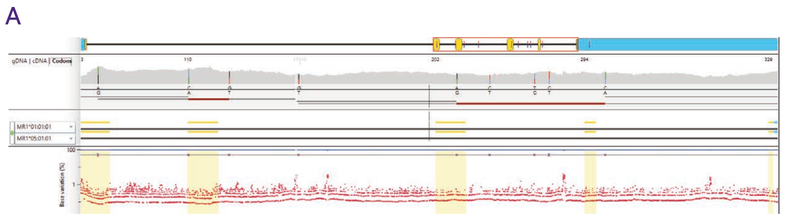


Figure 1: MHC class I-related protein 1 (MR1) shares great homology with MHC-I genes. (A) Protein structure (B) genomic structure and primer binding sites.

A

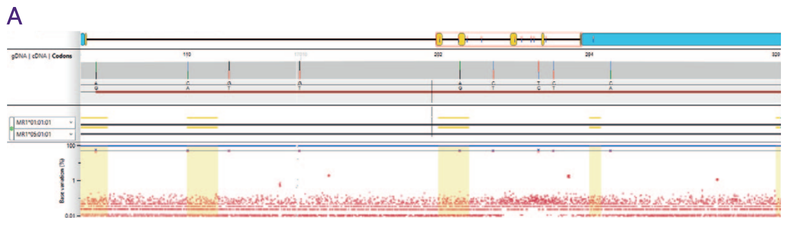


B

Sample	Reads	Map%	[Exon+] Noise%	[Exon+] ESA%	[Exon+] HP	[Amplicon] HP	Sample	Reads	Map%	[Exon+] Noise%	[Exon+] ESA%	[Exon+] HP	[Amplicon] HP
NA07439	28339	91	1,2	46,72	1	3	NA17219	33107	90	1,63		0	1
NA09301	21876	90	1,45		0	8	NA17222	28440	87	1,79	48,19	2	9
NA10005	22940	89	1,64		0	4	NA17226	29321	90	2,19		0	0
NA12244	19904	87	1,97	47,04	1	3	NA17228	30207	86	2,92	49,28	2	10
NA16688	29860	90	1,85		0	11	NA17229	29556	92	1,12	47,77	1	2
NA16689	26998	88	2		0	0	NA17230	26944	91	1,73	48,12	1	8
NA17019	24333	91	1,87	45,96	1	8	NA17232	30134	92	1,68		0	1
NA17020	23918	86	1,58		0	0	NA17234	30441	88	1,85		0	0
NA17039	29395	92	1,56		0	4	NA17237	19567	91	1,88	46,75	1	6
NA17057	27034	88	1,34	44,17	1	8	NA17240	17483	91	1,92		0	6
NA17058	27231	91	1,41	44,22	1	9	NA17246	17055	88	2,06		0	0
NA17078	23598	90	2,63	43,79	1	10	NA17247	15286	90	1,59	48,45	1	9
NA17084	21898	90	1,36	46,9	1	5	NA17256	20780	90	1,48	46,28	1	8
NA17114	17885	90	1,59	49,6	1	4	NA17261	17234	91	2,06	48,33	1	1
NA17115	19316	89	1,86	47,54	4	15	NA17264	20280	87	1,59	48,77	2	3
NA17119	18755	88	1,9	47,48	4	11	NA17267	18975	92	1,42	41,48	1	2
NA17129	23455	91	1,31	45,55	1	2	NA17269	22885	90	1,8		0	0
NA17130	19493	90	1,55		0	5	NA17274	24179	91	1,4		0	0
NA17201	20172	90	2,39	46,58	1	8	NA17282	20639	91	1,36	47,23	1	2
NA17203	19119	89	1,93		0	0	NA17285	23421	89	1,39	44,92	1	8
NA17204	26351	91	1,86		0	0	NA17286	25701	87	1,41		0	0
NA17205	25495	91	2,15	47,49	3	10	NA17289	23993	90	1,39		0	11
NA17206	23477	90	2,66		0	0	NA17290	27217	91	1,7		0	10
NA17210	27078	91	1,09		0	0	NA17295	26253	90	1,61		0	5
NA17211	28286	88	2,48	47,99	1	2	NA17296	28872	91	1,37		0	1
NA17212	29607	90	1,68		0	8	NA17440	25068	90	1,64	46,49	1	1
NA17213	27959	90	1,26		0	1	NA17466	23387	88	1,68	48,94	1	1
NA17214	34375	88	1,84	45,65	1	3	NA17618	23189	84	2,35		0	0

Figure 2: Considerable heterozygous positions in MR1 in 56 sample panel. (A) NGSengine coverage and base variation plot (B) sequence quality metrics from illumine sequence data. Shown are the mappability % (Map%), exon region maximum noise %, exon region estimated second allele fraction (ESA %), exon region heterozygous positions (HP) and heterozygous positions in the complete amplicon region ([amplicon] HP). 0 heterozygous positions is colored green and >0 heterozygous positions is colored yellow.

A



B

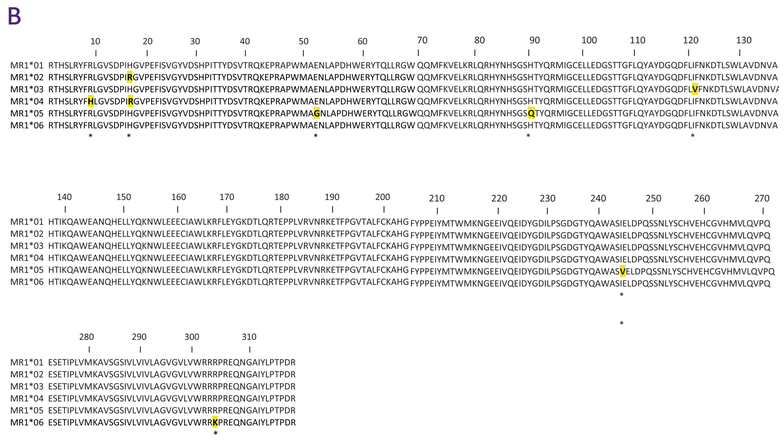


Figure 3: Six fully phased MR1 alleles. (A) NGSengine coverage and base variation plot from Pacbio sequence data from samples (B) novel MR1 protein variants.

A. Polymorphism in the a1/a2- domains

B. Polymorphism in the a3-domain/cyptoplasmictail

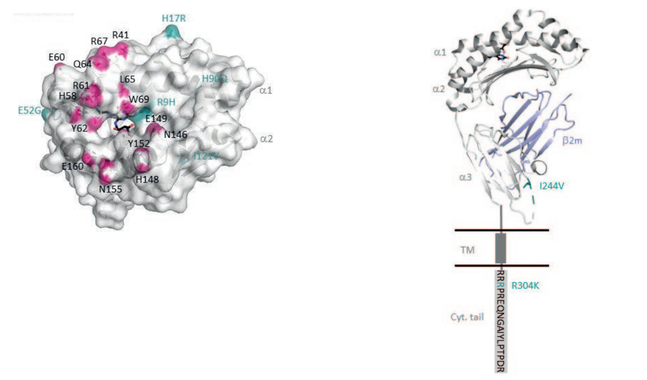


Figure 4: Cartoon and surface display of MR1 protein with variant positions

Conclusion

Despite the monomorphic classification of MR1, a diverse set of heterozygous genotypes was discovered in a panel of 56 samples, including 5 new alleles encoding for protein variants. The data presented here is consistent with marked variation in MR1.

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Erik Rozemuller[1], Sidonia Barbara Guiomar Eckle[2], Ian McLaughlin[3], Sake van Wageningen[1]

1 GenDx, Utrecht, The Netherlands
2 Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia
3 Pacific Biosciences, Menlo Park, California, USA

experts in transplant diagnostics