Supplementary Figures

Supplementary data include 6 Supplementary Figures (Figure S1-S6).

High-resolution analysis of the human retina miRNome reveals isomiR variations and novel microRNAs

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In each plot the samples are annotated based on potential sources of variation, namely gender (A), age (B), cause of death (C) and total post-mortem time (D). Symbols in B and D indicate ranges of years and hours, respectively. The contribution of PC1 and PC2 to expression data variance was 26% and 15 %, respectively.



Figure S2: Scatter plot of the coefficient of variation and the average normalised read count for each miRNA (indicated by a blue dot).

MIR182	CCCGI	TTTTGGCAATGGTAGAACTCACACTGGTGAGG	MIR22		AGCTAAAGCTGCCAGTTGAAGAACTGTTGCCCTC
hsa-miR-182-5p_can. hsa-miR-182-5p_is4 hsa-miR-182-5p_is6 hsa-miR-182-5p_is3	46.5% 19.6% 14.4% 11.8%	TTTGGCAATGGTAGAACTCACACT TTTGGCAATGGTAGAACTCACAC. TTTGGCAATGGTAGAACTCACAC. TTGGCAATGGTAGAACTCACAC.	hsa-miR-22-3p_can. hsa-miR-22-3p_is3 hsa-miR-22-3p_is2	96.2% 2.2% 1.6%	ARCTGCCAGTTGAAGAACTGT ARGCTGCCAGTTGAAGAACTG. ARGCTGCCAGTTGAAGAACT
			MIR30D		GTTGTTGTAAACATCCCCGACTGGAAGCTGTAAG
MIR183	CTGT	JTATGGCACTGGTAGAATTCACTGTGAACAGTC	hsa-miR-30d-5p_is27	34.9%	TGTAAACATCCCCGACTGGA
hsa-miR-183-5p can.	35.5%	TATGGCACTGGTAGAATTCACT	hsa-miR-30d-5p_is41	31.2%	TGTAAACATCCCCGACTGGAAGCT
hsa-miR-183-5p_is3	20.9%	. ATGGCACTGGTAGAATTCACT	hsa-miR-30d-5p_is36	9.3%	TGTAAACA TCCCCGACTGGAAGC
hsa-miR-183-5p_is4	13.6%	. ATGGCACTGGTAGAATTCACTG	hsa-miR-30d-5p_is28	8.4%	TGTAAACATCCCCGACTGGAA.
hsa-miR-183-5p_is16	13.5%	TATGGCACTGGTAGAATTCACTG	hsa-miR-30d-5p_is29	4.1%	TGTAAACA TCCCCGACTGGAAA
			hsa-miR-30d-5p_is33	3.1%	TGTAAACA TCCCCGACTGGAAGA
			hsa-miR-30d-5p_can.	2.3%	TGTAAACATCCCCGACTGGAAG
MIR181A1	TCAGTG	AACATTCAACGCTGTCGGTGAGTTTGGAATT			
hsa-miR-181a-5p_is5	46.0%	AACATTCAACGCTGTCGGTGAG.	MIR124	T	ACAATTAAGGCACGCGGTGAATGCCAAGAATGGG
hsa-miR-181a-5p_can	. 42.4%	AACATTCAACGCTGTCGGTGAGT	hsa-miR-124-3p_can.	46.7%	TAAGGCACGCGGTGAATGCC
			hsa-miR-124-3p_is6	14.0%	TAAGGCACGCGGTGAATGC.
			hsa-miR-124-3p_is27	12.6%	TTAAGGCACGCGGTGAATGCC
MIR26A	GCCTC	GTTCAAGTAATCCAGGATAGGCTGTGCAGGT			
hsa-miR-26a-5p can.	91.7%	TTCAAGTAATCCAGGATAGGCT			
hsa-miR-26a-5p is2	4.6%	TTCAAGTAATCCAGGATAGGC.	LETTA		GGATGAGGTAGTAGTTGTATAGTTTTAGGGTC
hsa-miR-26a-5p is4	3.7%	TTCAAGTAATCCAGGATAGGCTT	hsa-let-7a-5p_can.	73.1%	TGAGGTAGTAGGTTGTATAGTT
			hsa-let-7a-5p_is2	15.5%	TGAGGTAGTAGGTTGTATAGT.
A4/D204					
WIRZ04	CGTGGA	CTTCCCTTTGTCATCCTATGCCTGAGAATAT			
hsa-miR-204-5p_can.	85.8%	TTCCCTTTGTCATCCTATGCCT			
hsa-miR-204-5p_is3	10.8%	TTCCCTTTGTCATCCTATGCCTG			
MIR30A	GCGAC	TGTAAACATCCTCGACTGGAAGCTGTGAAG			
hsa-miR-30a-5p_is34	61.8%	TGTAAACATCCTCGACTGGA			
hsa-miR-30a-5p_is47	17.8%	TGTAAACA TCCTCGACTGGAAGCT			
hsa-miR-30a-5p_is35	5.5%	TGTAAACATCCTCGACTGGAA.			
hsa-miR-30a-5p_is42	3.9%	TGTAAACATCCTCGACTGGAAGC			
hsa-miR-30a-5p_is39	2.4%	TGTAAACATCCTCGACTGGAAGA			
hsa-miR-30a-5p can.	1.9%	TGTAAACATCCTCGACTGGAAG			

Figure S3: Alignment of the most abundant isomiR to the canonical sequence for the ten topranked miRNAs in the human retina.

The sequences of the isomiRs (_is) that contribute at least 10% to the total read counts of a given miRNA are aligned to the canonical sequence annotated in the miRBase (_can.; in blue). When the canonical form contributes with less than 10% to the total read counts (e.g. miR-30a-5p and miR-30d-5p), all isomiRs that are more abundant than the canonical miRNA are listed. In the cases where no isomiRs contributed to >10% (e.g. miR-26a-5p and miR-22-3p), the isomiRs that contribute with at least 1% are shown. The percentages represent the contribution of each isoform to the total read counts of a given miRNA. In green: isomiRs bearing the same seed as the canonical miRNA; in red: isomiRs with a modified seed sequence; in black: precursor sequence. The seed sequence (nucleotides 2 to 8) is shaded. The analysis was performed on isomiRs detected in all retina samples (n=16).





Figure S4: qRT-PCR analysis of nine putative novel miRNAs in the human retina and in other human tissues.

The expression of nine predicted novel miRNAs (see Table 2 for their genomic location) was validated by qRT-PCR using the miRCURY LNA[™] microRNA PCR System on total RNA from human retina, RPE/choroid, brain, liver, muscle and blood. The ubiquitous miR-26a was used as a reference endogenous control for normalisation in the context of each tissue. Values are shown as 2^{^-dCT}. The novel miRNAs are shown in the same order as in Figure 5A.



Figure S5: Characterisation of miRNA sequence variations and their contribution to miRNA expression levels in the RPE/choroid.

(A) Pie-chart illustrating the contribution of the different isomiR categories to total read counts in the RPE/choroid. (B) Contribution of canonical miRNAs (white bars) and of isomiRs with a canonical (grey bars) or modified seed (black bars) to overall miRNA expression in the human RPE/choroid. The bar reports the number of miRNAs for which the expression of each sequence class, relative to total counts, is within the five categories indicated on the x-axis. The data reported in this figure refer to the analysis of the isomiRs identified in the two human RPE/choroid samples.



miRetina	Browser isomi
isomiR :	
isomiR	Predicted Targets ⁽¹⁾
hsa-let-7a-5p_isomiR8	4
hsa-let-7b-5p_isomiR24	4
hsa-let-7c-5p_isomiR9	4
hsa-let-7d-5p_isomiR12	4
hsa-let-7e-5p_isomiR1	(III)

Tuen	Real Brow	rser isomiR	t Novel Help				miRetina		Browser isomiR	Novel Help
loomiR Ter	get Prediction						Bradicted novel mil	2NAr		
- Re		760	miR-182-5p_komiR14				Predicited Hover His	003		
uiR- cific	GeneTitle	Shared	GeneTitle	Canonical mRNA- specific	GeneTitle		Novel mIRNA ID1	Genomic coordinates	Predicted targets ⁽¹⁾	Predicted hairpin structure (7)
12	ecto-NOX disuffice-thiol exchanger 2	MYRP	myosin VIA and Rab interacting protein	MITF	microphthalmia-associated transcription factor		nem1	chr1:142850083-142850134	A	6
	homeodomain interscring protein kinase 1	CO2AP	C02-associated protein	ACTTR	ANP2 actin-related protein 2 homolog (yeasi)				v	
	quaking homolog, KH domain RNA binding (mouse)	FOXOD	forkhead box 03	MFAPS	microfibrillar-associated protein 3		nrm2	chr0.48357870.48357894	G	G
	mesoderm induction early response 1 homolog (Xanopus laenits)	PCDH8	protocodherin 8	CTIN	oortactin				0	
1/2	microtubule associated protein 2	CHMP28	ohvometin modilying protein 28	NCALD	neurocolon della		nm3	chr7.x1687430-41687404	U	U
DATH	lysophosphatidylglycensi acyltransferase 1	SANDS	sterile sighs motif domain containing 5	FR52	fibroblast growth factor receptor substrate 2					
GA3	cyclic nucleoticle gated channel alpha 3	CULS	eutin 5	EPAS1	endothelial PAS domain pratein 1		nm4/5	chr6.65351159-65351217	6	
KR012	ankyrin repeat domain 12	ONECUT2	one out homeobox 2	CAMEAP1L1	calmodulin regulated spectrin-associated protein	n 1-lika 1			-	
DRS	chemokine (C-C motif) receptor 6	RARG	retinoic adid receptor, gamma	NRGAM	neuronal cell achesion molecule		am6	chr10:105362131-105362190	6	
1.9	X-linked inhibitor of apoptosis	CCDC131	colled-coll domain containing 131	MT881	metastasis suppressor 1				-	-
ne .	eta variant gene 6-(TEL proogene)	6/402	besorucin 2	RAPOEFS	Rap guarrine nucleotide exchange factor (SEP)	5	nem7	chr13:27260471-27260537	A	A
AR20	activin A receptor, type 10	FORM	forkhead box N3	NPTRI	neuronal pentraxin I				-	-
ABPCD	poly(A) binding pratein, cytoplasmic 3	PANZ	PIM2 polyA specific ribonuclease subunit homolog (5). serevisies)	CD47	CD47 molecule		nem8	chr19:57950479-57950542	G	6
UNIPOI	THUMP domain containing 1	FAMIO881	family with sequence similarity 108, member 81	ZNF2808	zine finger protein 2508				-	-
r.	bromodomain PHD finger transcription factor	EVIS	ecotropic vital integration site 5	WWC2	WW and C2 domain containing 2					
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Figure S6: The miRetina database.

potassium voltage-gated channel, delayed-rectifier, subfamily S, member 1 potassium voltage-gated channel, delayed-rectifier, subfamily S, member 2

(A) miRetina enables queries by miRNA gene name (e.g. MIR182 or miR-182) or genomic coordinates and provides graphical information on the genomic location by presenting an alignment of the reference miRNA in a Genome Browser window along with the respective isomiRs. Only sequence variants that were identified in all 16 individuals are depicted. The relative expression level of each miRNA variant in the retina is shown as relative frequency (RF,%) out of total miRNA reads. A colour code discriminates between the reference miRNA sequence (in red) and the isomiRs (green for the isomiRs with a canonical seed, blue for the ones with a modified seed). Nucleotide substitutions are indicated within the isomiR field (see a C to A substitution within the blue coloured isomiR182-5p). miRNA variants that contribute with more than 10% to the total miRNA reads are labelled in darker shade (e.g. see RF of light and dark green isomiRs).

(B, C) For the isomiRs of the 100 top-ranked miRNAs that bear a modified seed and have an RF \geq 1%, the user can download the list of predicted targets, generated using the TargetScan custom tool. For each isomiR, the database provides information on the genes predicted to be targeted only by the isomiR, those targeted both by the isomiR and its respective reference miRNA and genes predicted to be targeted only by the reference miRNA.

(D) The 51 predicted novel miRNAs are listed in a tabular format and can be visualised in the genome browser interface of miRetina.

(E) The user can retrieve the list of predicted target genes for each novel miRNA, obtained using the TargetScan custom software, and download this information as a table with active hyperlinks to NCBI.

(F) Information on the predicted secondary structure of the hairpin and alignment of reads across the predicted precursor (as provided by the miRDeep2 software) is also downloadable.