

1 **Title page**

2 **Complete manuscript title:** mTOR complex 1 pathway activation in severe keratoconus; the
3 functional implications of GWAS identified loci

4 **Running title:** The role of cellular aging in keratoconus

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36 **Keywords:** keratoconus; GWAS; cellular aging; MTOR; RNA expression

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1 **Abstract**

2 Purpose: Keratoconus (KC) is an eye condition that can lead to a severe vision loss and may warrant a
3 corneal grafting procedure. Meta-analyses of genome wide association studies have identified several
4 genes that confer risks for differences in corneal curvature, corneal thickness, and developing
5 keratoconus. Currently, there is limited evidence of a functional role for the identified loci in the affected
6 corneal tissues.

7 Methods: We investigated the gene expression profiles of 4 GWAS confirmed risk loci and several related
8 pathways that function in cellular ageing and cell cycle control in corneal tissue of a discovery and
9 replication cohort comprising in total 27 keratoconus patients, 16 healthy controls, and 21 diseased
10 controls (failed corneal grafts).

11 Results: We confirmed the *MTOR* gene locus as differentially expressed in KC corneas in a discovery
12 cohort. Next, we replicated these results in a second cohort and found evidence of increased expression of
13 various mTORC1 pathway signature genes, namely *MTOR* itself ($P=0.040$), *AKT1* ($P=0.028$), *IGF1R*
14 ($P=0.022$) and *RAPTOR* ($P=0.007$).

15 Conclusions: Gene expression profiling in cornea tissues revealed robust up-regulation of the mTORC1
16 pathway in KC and substantiates a potential role for this pathway in its pathogenesis. Functional
17 implications should be further studied since biomarkers for disease activity are needed and selective
18 targeting of the mTOR pathway is a promising treatment concept.

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1 Introduction

2 Keratoconus (KC) is an eye condition that leads to poor visual acuity due to myopia (short-sightedness)
3 and irregular astigmatism, and in advanced cases to blindness due to corneal scarring. This is caused by
4 the loss of corneal tissue, which precedes an archetypical cone-like shape of the cornea in KC.¹ Sever cases
5 warrant corneal transplant surgery to restore visual acuity. The underlying etiology is considered a
6 complex interplay of corneal tissue remodeling, and activation of several proteases and inflammatory
7 pathways.⁴ Interestingly, the high degree of concordance in monozygotic twins, and a high prevalence of
8 KC in first degree relatives, indicates genetic predisposition as a predominant contribution to KC
9 susceptibility.^{6,7} Indeed, genome wide association studies (GWAS) have revealed susceptibility loci (*FOXO1*
10 and *FNDC3B*) linked to central corneal thickness and keratoconus.⁸ Additionally, meta-analyses of large
11 European and Asian cohorts reported that variants near *FNDC3B*,⁹ *FRAP1/MTOR*,⁹ and *PDGFRA*¹⁰ genes
12 conferred relatively large risks for corneal curvature aberrations- a hallmark of keratoconus.
13 Unfortunately, the translation from genetic studies to functional understanding, let alone targeted
14 therapeutic avenues, to combat corneal dysregulation in KC, is still in its infancy.

15 We hypothesized that the genetic changes put forward by GWAS in keratoconus and corneal curvature
16 might highlight pathways that are actually deregulated in the cornea, affecting its architecture. To test this
17 hypothesis we set out to map the expression of several associated risk genes in corneal tissues of patients
18 to assess the actual implications of previous genetic studies *ex vivo*. We first screened a a discovery cohort
19 (n= 36) for gene expression profiles of several genes and pathways highlighted by previous genetic
20 studies in corneal tissue from KC patients, healthy controls, and diseased controls (decompensated
21 corneal grafts). We technically validated the outcomes of the discovery cohort and used an independent
22 replication cohort (n= 27) to assess the robustness and reproducibility of our observations.

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1 **Methods**

2 *Acquisition of corneal samples*

3 KC cornea samples were collected from patients receiving a corneal transplant for severe KC (N=25) or
4 pellucid marginal degeneration (N=1). Twenty-seven corneas from 27 patients were included in this
5 group. The group of diseased controls are composed of decompensated grafts (DG). Twenty-one samples
6 from 21 patients were included in this group. All aforementioned cornea buttons were processed using
7 Tissue-Tek (Sakura Finetek U.S.A., Inc.) immediately after resection, cut into five full thickness slices, and
8 stored at -80 °C. Healthy cornea (HC) controls were obtained from the Euro Cornea Bank (ECB),
9 Beverwijk, The Netherlands, and Department of Anatomy, University Medical Center Utrecht, The
10 Netherlands. A total of 16 corneas were prepared from post-mortem tissue within 24h of death and
11 prepared from eyes from unrelated Caucasian donors who had no history of keratoconus, ocular
12 inflammation, or vitreoretinal disease. The replication cohort comprised 11 KC, 11 DG, and 5 HC-samples,
13 see figure 1.

14

15 **Figure 1. Flowchart of gene expression analyses and technical validation in discovery and**
16 **replication cohort (N=64).**

17 Figure legend: GWAS = genome wide association study; mTORc1 = mammalian target of rapamycin 1
18 pathway.

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20 This study was approved by The Medical Research Ethics Committee of the UMC Utrecht. It reviews
21 research protocols in accordance with the Medical Research Involving Human Subjects Act (WMO). The
22 MREC of the UMC Utrecht is accredited by the Central Committee on Research Involving Human Subjects
23 (CCMO) since november 1999. The MREC of the UMC Utrecht is also member of the Dutch union of MRECs
24 (NVMETC). None of the donors were from a vulnerable population and all donors or next of kin provided
25 written informed consent that was freely given.

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2 *Clinical data extraction*

3 Patient records were reviewed for additional data collection, such as patient history and preoperative
4 assessment, including slit lamp evaluation, Schirmer's testing, and Scheimpflug corneal tomography
5 (Pentacam HR, Oculus GmbH). Available data for the healthy control group was limited to age, sex and
6 cause of death.

7 *RNA isolation*

8 RNA isolation from corneal buttons was performed using the TRIzol method (Life Technologies, Thermo
9 Fisher Scientific, USA) following the manufacturers protocol. RNA was isolated after dissolving the cornea
10 in TRIzol reagent. After checking RNA integrity, complementary-DNA synthesis was performed using the
11 iScript cDNA synthesis kit (Bio-Rad, USA).

12 *Gene expression analysis*

13 Gene expression analysis was performed by OpenArray quantitative real-time polymerase chain reaction
14 (qPCR), using *GUSB* and *GAPDH* as housekeeping genes for measuring relative expression levels. All qPCR
15 analyses were performed on the QuantStudio 12K Flex Real-Time PCR System (Life Technologies, Thermo
16 Fisher Scientific, USA). The qPCR data were interpreted using ExpressionSuite software version 1.0.3 (Life
17 Technologies, Thermo Fisher Scientific, USA). The genes *AHRR*, *FOXO3*, *IL6*, *IL10* and *hTERT* did not reach
18 detectable levels in our qPCR assay. Six healthy control samples, two keratoconus samples, and four
19 decompensated grafts did not meet qPCR quality control criteria for RNA expression analysis, thus
20 reducing the effective sample size from 64 to 51 corneas. Baseline characteristics of the non-viable KC and
21 DG samples did not differ from the mean group.

22 *Data analysis*

23 Gene expressions were presented as fold changes per sample and plotted in grouped scatter plots.
24 Statistical analysis are reported threefold; firstly a comparison of marker levels between KC vs. HC,
25 secondly a comparison of KC vs. both healthy and diseased control groups (HC+DG), and thirdly a
26 comparison of HC vs. both diseased groups (KC+DG). Differences in marker levels were statistically tested

1 using the one-way independent ANOVA for normal distributions or the Kruskal-Wallis Test for non-
2 normal distributions. We used either Tukey's or Dunn's Tests for Post Hoc multiple comparisons.
3 Thresholds for significance were corrected for multiple testing, based on the number of genes per analysis
4 (threshold for significance = $0.05/n$). Multiple imputation was performed using a multivariable
5 imputation method considering all entered variables. Statistical analyses were performed using SPSS 21.0
6 (IBM SPSS Statistics, USA) and graphs were made in Prism 6.02 (GraphPad Software Inc., USA).

7

1 Results

2 Study population

3 The mean age of the KC patients was 44.1±15.5 years, 66.9±12.2 years for the DG group, and 85.2±8.0
4 years for the healthy control group. Baseline characteristics (age, gender, atopy, contact lens use) of the
5 replication cohort did not differ materially from the samples used in the discovery cohort. Demographics
6 of the discovery and validation cohort are described in Table 1.

7 **Table 1. Study population characteristics**

	Keratoconus (KC)		Decompensated grafts (DG)		Healthy controls (HC)	
	Mean/N	Range/%	Mean/N	Range/%	Mean/N	Range/%
Age (years), mean	42,3	20-67	66.9	42-82	83.6	67-94
Male sex, N	16	62	3	38	9	56
Positive for atopic disease, N	11	42	6	27		
Contact lens wear <2 weeks before transplantation, N	14	54	2	10		
Intra ocular pressure (mmHg), mean	14,1	7-20	13,6	0-22		
Schirmer's test outcome (mm), mean	18,7	5-35	17,7	3-35		

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9 Gene expression profile

10 Fifteen genes were significantly different expressed between KC vs. HC, and most of these genes were also
11 affected in the diseased control group (DG). Of the four GWAS previously identified risk loci only
12 *FRAP1/MTOR* showed a significantly altered expression in KC samples ($P=0.005$). Subsequently, several
13 genes related to the mammalian target of rapamycin (mTORC1) pathway were significantly higher
14 expressed in the KC samples compared to healthy controls: *AKT1* (24.8x higher, $P<0.001$), *DEPTOR* (4.9x,
15 $P=0.006$), *FOXO4* (58.8x, $P<0.001$), *IGF1* (16.5x, $P<0.001$), *IGF1R* (20.4x, $P<0.001$), *MTOR* (6.5x, $P=0.004$),
16 and *RAPTOR* (4.8x, $P=0.010$). In contrast, the levels of *MDM2* (0.16x, $P=0.005$) decreased. The expression
17 of *RICTOR* (0.4, $P=0.331$) was not significantly altered. Strikingly, the aberrant gene expression profile of
18 KC largely overlaps with severely failed corneal grafts (DG). Finally, the levels of *NFKB1* (13.9x, $P<0.001$),
19 *SIRT7* (52.4x, $P<0.001$), and *WRN* (16.2x, $P<0.001$) were significantly lower in KC and DG when compared

1 to healthy controls. Table 2 highlights the gene expression of the GWAS conformed loci, and mTORC1-
 2 pathway associated genes. Gene expression profiles of all genes of the discovery cohort are indicated in
 3 appendix 1, and visually represented in appendix 2 and 3.

4 **Table 2: Gene expressions, median fold change and statistical analyses of GWAS identified genes**
 5 **and MTORC1-associated genes in the discovery cohort**

	Group	Mean Δ CT \pm SD	Median Fold Change	Multiple comparison [†]	Post Hoc tests				
					Post hoc KC vs. HC [‡]	Post hoc KC vs. DG [‡]	Post hoc DG vs. HC [‡]	Post hoc HC vs. KC+DG [‡]	Post hoc KC vs. HC+DG [‡]
<i>FOXO1</i>	HC	43.26 \pm 23.00	0.000						
	DG	60.52 \pm 77.74	0.000	0.783	-	-	-	-	-
	KC	50.96 \pm 34.58	0.000						
<i>FNDC3B</i>	HC	12.79 \pm 15.24	0.009						
	DG	22.75 \pm 18.66	0.000	0.021*	0.150	0.220	0.766	0.011*	0.044
	KC	39.32 \pm 29.19	0.000						
<i>PDGFRA</i>	HC	50.89 \pm 76.23	0.000						
	DG	39.64 \pm 62.21	0.000	0.001**	0.304	0.086	0.998	< 0.001**	0.143
	KC	336.99 \pm 464.68	0.000						
<i>FRAP1/MTOR</i>	HC	4.299 \pm 0.637	0.048						
	DG	2.754 \pm 0.970	0.148	0.005*	0.004*	0.182	0.081	0.005*	0.013*
	KC	1.813 \pm 0.966	0.313						
<i>AKT1</i>	HC	2.130 \pm 0.926	0.159						
	DG	-1.713 \pm 0.514	3.638	< 0.001**	< 0.001**	0.843	< 0.001**	< 0.001**	0.047
	KC	-1.901 \pm 0.738	3.950						
<i>DEPTOR</i>	HC	5.971 \pm 0.732	0.015						
	DG	2.308 \pm 0.883	0.190	< 0.001**	0.006*	0.026	< 0.001**	0.001**	0.815
	KC	3.751 \pm 0.635	0.074						
<i>IGF1</i>	HC	9.513 \pm 2.257	0.002						
	DG	2.815 \pm 1.308	0.084	< 0.001**	0.005*	0.124	< 0.001**	< 0.001**	0.826
	KC	4.887 \pm 0.472	0.033						
<i>IGF1R</i>	HC	0.681 \pm 1.040	0.621						
	DG	-3.704 \pm 0.737	14.470	< 0.001**	< 0.001*	0.837	< 0.001**	< 0.001**	0.049
	KC	-3.952 \pm 0.956	12.676						
<i>RAPTOR</i>	HC	6.565 \pm 0.346	0.012						
	DG	3.699 \pm 1.729	0.075	0.010*	0.018*	0.927	0.014*	0.002**	0.306
	KC	3.955 \pm 0.308	0.058						
<i>RICTOR</i>	HC	2.755 \pm 0.580	0.169						
	DG	4.363 \pm 0.596	0.044	0.109	-	-	-	-	-
	KC	3.732 \pm 1.241	0.069						

GWAS: Genome Wide Association Study, see references 7,8,9. MTORC1: Mammalian Target Of Rapamycin Complex 1. Δ CT: delta cycle threshold. SD: standard deviation. HC: healthy control. DG: decompensated graft/diseased control. KC: keratoconus. *: significance < 0.05, **: significance <0.002 (corrected for multiple testing). †: One-way ANOVA in normal distributions and Kruskal-Wallis for non-normal distribution. ‡: post-hoc Tukey. V-Akt Murine Thymoma Viral Oncogene Homolog 1 (*AKT1*), DEP domain containing MTOR-interacting protein (*DEPTOR*), fibronectin type III domain containing 3B (*FNDC3B*), Forkhead Box O1 (*FOXO1*), Insulin-Like Growth Factor 1 (*IGF1*), Insulin-Like Growth Factor 1 Receptor (*IGF1R*), Mammalian Target Of Rapamycin (*MTOR*), Platelet Derived Growth Factor Receptor Alpha (*PDGFRA*), Rapamycin-Insensitive Companion Of MTOR (*RICTOR*), Regulatory Associated Protein Of MTOR (*RAPTOR*).

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7

1 *Replication study*

2 Since the mTOR gene was the only GWAS locus that displayed differential expression in corneas, we

3 selected the mTORC1-pathway for replication analysis. In an independent cohort *AKT1*, *DEPTOR*, *IGF1*,

4 *IGF1R*, *MTOR*, *RAPTOR*, *REPTOR*, and *RICTOR* expression levels were assessed in 27 additional corneal

5 samples (11 KC, 11 diseased controls and 5 healthy control samples). A solid difference in expression was

6 noted for 4 out of 7 genes involved in the mTORC1 pathway. A significantly altered gene-expression among

7 all three sample groups in total was identified for *AKT1* ($P=0.028$), *IGF1R* ($P=0.022$), *MTOR* ($P=0.040$),

8 and *RAPTOR* ($P=0.007$). Gene expression differences were less pronounced in the replication analysis,

9 and mTORC1 pathway dysregulation was comparable between KC and diseased control samples, as was

10 reported in the discovery cohort. Details on post-hoc tests are given in Table 3.

11 **Table 3: Gene expression of the mTORC1-pathway in keratoconus corneas, healthy controls and**

12 **diseased controls in the pooled discovery and replication cohort**

	Group	Mean Δ CT \pm SD	Median Fold Change	Multiple comparison ^a	Post Hoc tests				
					Post hoc KC vs. HC ^b	Post hoc KC vs. DG ^b	Post hoc DG vs HC ^b	Post hoc HC vs. KC+DG ^a	Post hoc KC vs. HC+DG ^a
<i>AKT1</i>	HC	2.952 \pm 2.949	0.062						
	DG	5.100 \pm 2.852	0.031	0.028*	0.110	0.699	0.024*	0.010*	0.616
	KC	4.533 \pm 1.124	0.064						
<i>DEPTOR</i>	HC	4.889 \pm 3.542	0.065						
	DG	7.109 \pm 3.471	0.009	0.074	-	-	-	-	-
	KC	6.828 \pm 2.059	0.008						
<i>IGF1</i>	HC	5.856 \pm 4.192	0.608						
	DG	7.457 \pm 3.649	0.654	0.345	-	-	-	-	-
	KC	6.954 \pm 1.945	1.065						
<i>IGF1R</i>	HC	0.785 \pm 2.975	0.239						
	DG	3.204 \pm 2.995	0.153	0.022*	0.081	0.749	0.020*	0.007**	0.533
	KC	2.646 \pm 1.808	0.241						
<i>MTOR</i>	HC	4.610 \pm 3.082	0.013						
	DG	6.814 \pm 2.981	0.011	0.040*	0.324	0.373	0.031*	0.031*	0.894
	KC	5.861 \pm 2.608	0.023						
<i>RICTOR</i>	HC	5.894 \pm 3.657	0.004						
	DG	7.967 \pm 3.187	0.003	0.098	-	-	-	-	-
	KC	7.544 \pm 1.934	0.008						
<i>RPTOR</i>	HC	2.952 \pm 2.949	0.052						
	DG	5.100 \pm 2.852	0.020	0.007**	0.058	0.699	0.024*	0.003**	0.894
	KC	4.533 \pm 1.124	0.047						

Δ CT: delta cycle threshold. SD: standard deviation. HC: healthy control. DG: decompensated graft/diseased control. KC: keratoconus. *: significance < 0.05, **: significance < 0.008 (corrected for multiple testing). a: One-way ANOVA in normal distributions and Kruskal-Wallis for non-normal distribution. b: post-hoc Tukey. V-Akt Murine Thymoma Viral Oncogene Homolog 1 (*AKT1*), DEP domain containing MTOR-interacting protein (*DEPTOR*), Insulin-Like Growth Factor 1 (*IGF1*), Insulin-Like Growth Factor 1 Receptor (*IGF1R*), Mammalian Target Of Rapamycin (*MTOR*), Rapamycin-Insensitive Companion Of MTOR (*RICTOR*), Regulatory Associated Protein Of MTOR (*RPTOR*).

1 *Cluster analysis and visual representation of mTORC1 pathway expression*

2 To reveal the underlying structure of mTORC1 pathway dysregulation, all corneal samples were subjected
3 to unsupervised hierarchical clustering. Global comparisons by hierarchical cluster analysis discerned
4 three overarching groups labeled C1, C2 & C3 (Figure 2). The three clusters roughly corresponded with
5 each of the three investigated sampled specimens (keratoconus, decompensated grafts, healthy controls).
6 C1 is the most homogenous set and contains largely healthy controls, whereas most keratoconus cases
7 were found in C2 (17/25). Strikingly, keratoconus cases were also well represented in the C3 group, to a
8 level comparable to the diseased controls. In the latter mTORC1 pathway dysregulation was most
9 outspoken. A classification based on mTORC1 pathway activation was not readily feasible, though it
10 should be noted that this clustering was performed only within the mTORC1 pathway.

11

1 **Figure 2. Heatmap of mTORC1 pathway gene expression in corneal samples of keratoconus**
2 **patients, decompensated grafts, and healthy controls.**

3 Figure legend: Heatmap of the unsupervised hierarchical clustering of the expression level data of genes of
4 the mTOR pathway in corneal tissues of 24 patients with Keratoconus (KC), 21 patients with
5 decompensated grafts (DG), and 15 healthy controls (HC). Heatmap colors represent the normalized
6 expression data in a color-coded way: From yellow (low) to dark red (high). Dendrograms indicating the
7 clustering relationships are shown to the left and above the heatmap. Pareto scaling is applied to rows.
8 Both rows and columns are clustered using Euclidean distance and Ward linkage.

9

1 Discussion

2 In this study we confirm our hypothesis that susceptibility loci for keratoconus and corneal curvature
3 point towards deregulation of pathways in the pathophysiology of keratoconus. Our results highlight
4 especially the implication of the mTOR pathway. Insights provided by meta-analysis of GWAS data from
5 large European and Asian KC cohorts have revealed susceptibility loci near *FOXO1* and *FNDC3B*, and
6 *MTOR/FRAP1*⁹ and *PDGFRA*¹⁰ in European and Asian cohorts that confer relatively large risk for the
7 development of KC or biometric properties linked with KC (corneal thickness & corneal curvature). We
8 only found the *mTOR* gene being differentially expressed in cornea's from keratoconus patients compared
9 to healthy controls. Interestingly, the levels of KC risk loci *FOXO1*, *FNDC3B* and *PDGFRA* were not
10 significantly altered between the groups and suggest that the associated SNPs do not alter gene
11 expression. Indeed, publicly available expression quantitative trait locus (eQTL) database Genevar
12 revealed that the previously reported SNPs near *FOXO1*, *FNDC3B* or *PDGFRA* do not function in terms of
13 transcript regulation of these genes.^{8,11,12}

14 In addition to the *mTOR* gene itself, we identified several key components of the mTORC1 pathway to be
15 significantly and reproducibly upregulated in KC corneas, including MTORC1 complex constituent
16 *RAPTOR*, the gene coding the major growth factor *IGF1*, its receptor *IGF1R*, and *AKT1*, a potent stimulator
17 of the *mTOR* pathway.¹³ These functional implications strengthen the previous genetic association with
18 *MTOR/FRAP1* identified by genome-wide studies.

19 Although the absolute number of corneas investigated in this study is relatively small, flowing from the
20 scarce availability of ex vivo corneas, we were able to reveal distinct gene expression profiles. Of note, the
21 diseased corneal samples were all obtained during a grafting procedure of more severe keratoconus
22 patients. Therefore our results are mainly applicable to more severe KC. To reduce technical bias all
23 samples were handled and analyzed in the same laboratory utilizing the same protocol and apparatus.

24 Previous studies indicated a role for *mTOR* signaling in maintaining the corneal homeostasis. For instance,
25 corneal wound healing in murine corneas seems to be mediated through *PDGF-BB* in a *mTOR* dependent
26 matter.¹⁴ Since decompensated grafts exhibit an ongoing inflammatory process, this might be an
27 explanation for the increased *mTOR* signaling observed in decompensated graft corneas compared to
28 healthy controls. In addition, corneal scarring is common in the severe cases of keratoconus, while less

1 advanced cases can also show the archetypical conical shape, though with a completely clear cornea.
2 Importantly, the sample set in this study also included clear corneas and revealed mTORC pathway
3 activation for these corneas as well. This might indicate that the increased mTOR signaling precedes the
4 scarring and therefore might underlie the progressive tissue loss associated with advanced keratoconus.
5 In this mouse model, the process of ongoing wound-repair responses was successfully inhibited by
6 administering rapamycin intra-ocularly.¹⁴ In addition, *in vivo* and *in vitro* mTOR pathway inhibition has
7 showed less corneal TGF- β and myofibroblast activation¹⁵. Increased TGF-b signaling has been shown to
8 be an important part of KC pathogenesis.¹⁶

9 The combination of mTOR signaling in the corneal wound repair and the implication of this pathway
10 found in GWAS and our translational study, makes it tempting to speculate that the mTOR pathway opens
11 novel diagnostic and therapeutic avenues in keratoconus.¹⁷ We believe that a future prospective clinical
12 study could manifest mTOR as a biomarker for disease activity and its inhibition as an alternative for
13 invasive corneal surgery.

14

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1 **Abbreviations**

- 2 Δ CT = delta cycle threshold
- 3 AHR = Aryl Hydrocarbon Receptor gene
- 4 AHRR = Aryl-Hydrocarbon Receptor Repressor gene
- 5 AKT1 = V-Akt Murine Thymoma Viral Oncogene Homolog 1 gene
- 6 CDKN2A = Cyclin-Dependent Kinase Inhibitor 2A gene
- 7 CT = cycle threshold
- 8 DEPTOR = DEP domain containing MTOR-interacting protein
- 9 DG = decompensated graft
- 10 ECB = European Cornea Bank, Beverwijk, The Netherlands
- 11 FNDC3B = fibronectin type III domain containing 3B
- 12 FOXO1 = Forkhead Box O1 gene
- 13 FOXO3 = Forkhead Box O3 gene
- 14 FOXO4 = Forkhead Box O4 gene
- 15 FRAP1 = MTOR = Mechanistic Target Of Rapamycin gene
- 16 GWAS = Genome Wide Association Study
- 17 H2AFX = H2A Histone Family, Member X gene
- 18 HC = healthy control
- 19 HDAC9 = Histone Deacetylase 9 gene
- 20 IGF1 = Insulin-Like Growth Factor 1 gene
- 21 IGF1R = Insulin-Like Growth Factor 1 Receptor gene
- 22 IL6 = Interleukin 6 gene
- 23 IL10 = Interleukin 10 gene
- 24 KC = Keratoconus
- 25 MDM2 = Mouse Double Minute 2 homolog gene
- 26 MTOR = FRAP1 = Mechanistic Target Of Rapamycin gene
- 27 mTOR = mammalian target of rapamycin protein
- 28 mTORC1 = mammalian target of rapamycin complex 1
- 29 mTORC2 = mammalian target of rapamycin complex 2
- 30 NFATC1 = Nuclear Factor of Activated T-cells, Cytoplasmic 1 gene
- 31 NFKB1 = Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells 1 gene
- 32 PDGFRA = Platelet Derived Growth Factor Receptor Alpha
- 33 PTEN = Phosphatase And Tensin Homolog gene
- 34 qPCR = quantitative real-time polymerase chain reaction
- 35 RICTOR = Rapamycin-Insensitive Companion Of MTOR gene
- 36 RAPTOR = Regulatory Associated Protein Of MTOR gene
- 37 SIRT1 = Sirtuin 1 gene
- 38 SIRT6 = Sirtuin 6 gene
- 39 SIRT7 = Sirtuin 7 gene

- 1 TERT = Telomerase Reverse Transcriptase gene
- 2 TP53 = Tumor Protein P53 gene
- 3 WRN = Werner syndrome, RecQ helicase-like, gene

Discovery Cohort

4 GWAS identified genes
25 ageing related genes

N = 36

Technical Validation

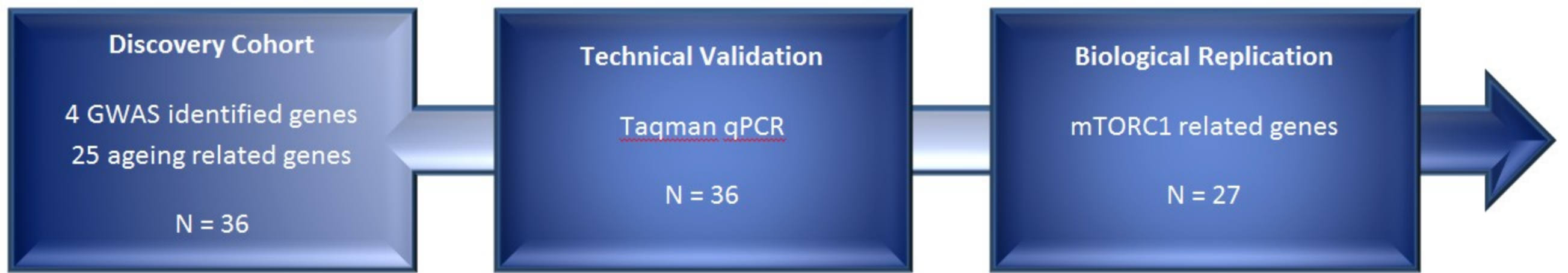
Taqman qPCR

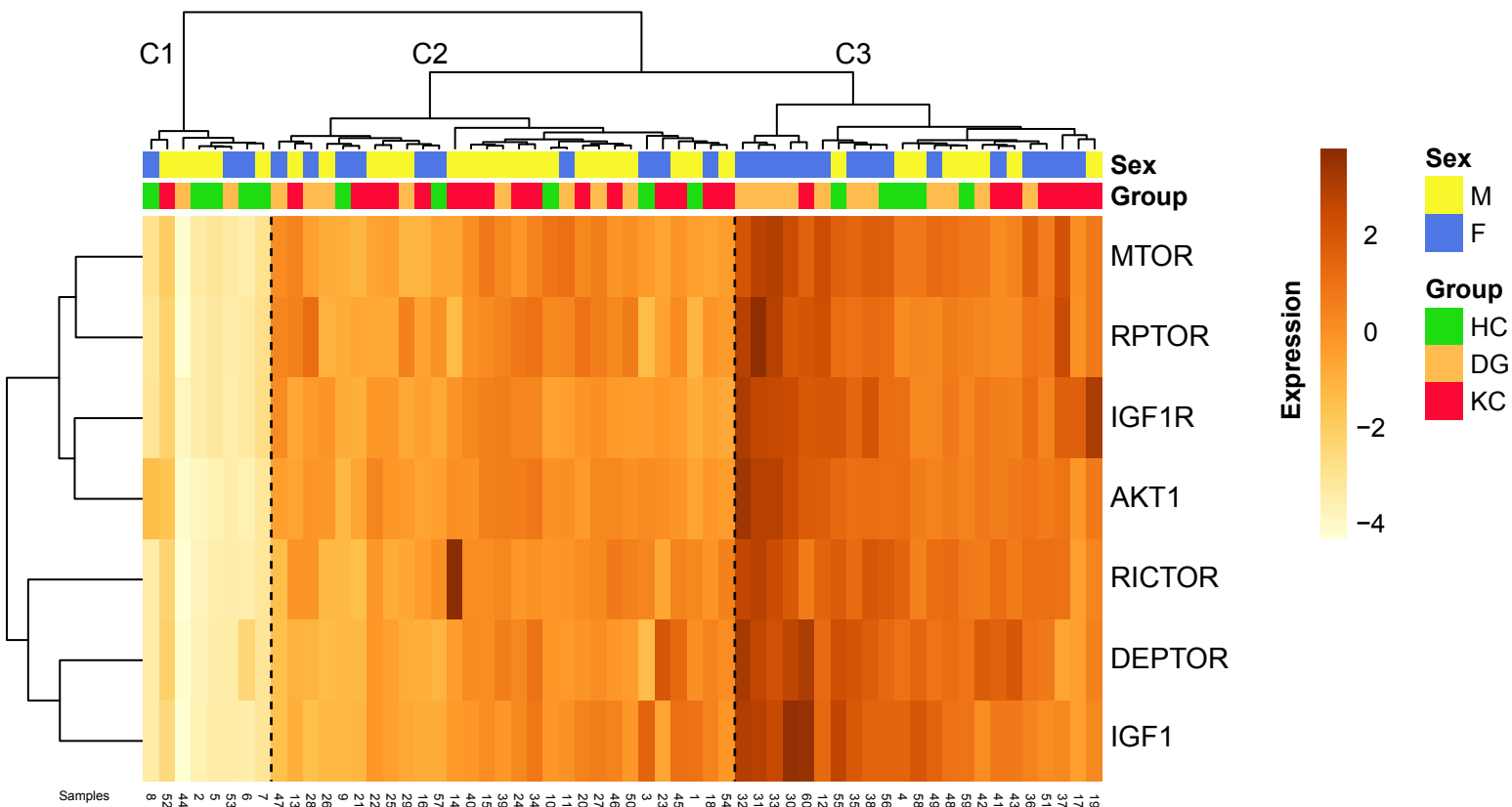
N = 36

Biological Replication

mTORC1 related genes

N = 27





Heatmap of the unsupervised hierarchical clustering of the expression level data of genes of the mTOR pathway in corneal tissues of 24 patients with Keratoconus (KC), 21 patients with decompensated grafts (DG), and 15 healthy controls (HC). Heatmap colors represent the normalized expression data in a color-coded way: From yellow (low) to dark red (high). Dendrograms indicating the clustering relationships are shown to the left and above the heatmap. Pareto scaling is applied to rows. Both rows and columns are clustered using Euclidean distance and Ward linkage.