Eye Banking and **Biobanking**

FONDAZIONE
BANCA DEGLI OCCHI
DEL VENETO - ONLUS

diego.ponzin@fbov.it
The Veneto Eye Bank Foundation, Venice

XVIII Congresso Nazionale SITraC

Padova, 20-22 febbraio 2014

Biobanking

- Biological sample collection for research, from:

✓ healthy subjects in epidemiological cohorts

✓ diseased tissues from clinical interventions

  - ✓ informing donors (patients ***/*** donor's relatives)
  - ✓ consent in compliance with local requirements
  - ✓ data acquisition
  - ✓ tissue procurement
  - ✓ preservation, processing, storage
  - ✓ quality control, cataloguing
  - ✓ distribution

- Samples maintained with minimal deterioration over time
- Eye bank ↔ biobank

Ferrari S et al. Advances in corneal surgery and cell therapy: challenges and perspectives for the eye banks. Exp Rev Ophthalmol 2009;4(3):317-29

Presidenza del Consiglio dei Ministri

Comitato Nazionale per la Biosicurezza e le Biotecnologie

LINEE GUIDA
PER L'ISTITUZIONE E L'ACCREDITAMENTO DELLE BIOBANCHE

Rapporto del Gruppo di lavoro
19 Aprile 2006

Presidente
Prof. Leonardo Santi

Coordinatore
Dr. Paolo Rebulla

Le fonti più comuni di tessuti e organi umani per dette banche sono:

- materiale derivato da interventi diagnostici (tra cui screening) o terapeutici (noto anche come surplus di materiale rispetto alle richieste cliniche)
- materiale specificamente donato per un progetto di ricerca e conservato per successivo uso
- materiale donato per trapianto e non utilizzato o ritenuto inadatto
- materiale proveniente da persone decedute e sottoposte ad autopsia

REGIONE VENETO
Centro Regionale per i Trapianti

FONDAZIONE BANCA DEGLI OCCHI DEL VENETO - ONLUS

Riferimenti di Fondazione Banca degli Occhi del Veneto

DICHIARAZIONE DI ASSENSO ALLA DONAZIONE DI CORNEE

(Ai sensi dell'art. 1 della legge del 12 agosto 1993 n. 301 e del provvedimento n. 31 del 9 gennaio 1995, Giunta Regionale, Regione del Veneto)

Il/La sottoscritto/a _____

Residente a _____ CAP _____

In via _____ n° _____ Telefono _____

☐ Coniuge non legalmente separato ☐ Genitore/i
☐ Convivente *more uxorio* ☐ Altro (specificare) _____
☐ Figlio di età non inferiore agli anni diciotto

Del Signor/Signora _____ nato/a a _____ Il _____
(nome e cognome del donatore) (Comune di nascita)

Residente a _____ C.F. _____
(comune di residenza) (Codice Fiscale)

Deceduto/a per _____ In data _____ Alle ore _____
(diagnosi)

Nel reparto di _____ Dell'Ospedale di _____

Medico di famiglia _____ Telefono _____

Dichiara di non essere a conoscenza che il congiunto avesse in vita manifestato per iscritto il rifiuto alla donazione **e**

☐ **Acconsente** ☐ **Non acconsente**

- **al prelievo delle cornee dalla salma del proprio congiunto;**
- che Fondazione Banca degli Occhi del Veneto svolga indagini anamnestiche sul proprio congiunto e accertamenti chimico-clinici (come per esempio: anticorpi anti HIV1 e HIV2, HBsAG, anticorpi anti HCV e anti HBc), volti a valutare l'idoneità del tessuto prelevato ed in particolare a prevenire la trasmissione di patologie da donatore a ricevente;

☐ **Acconsente** ☐ **Non acconsente**

che, nel caso il trapianto non sia possibile, i tessuti prelevati possano essere utilizzati per studi volti a migliorare la conoscenza e le possibilità di cura delle patologie oculari.

Data _____ ore _____ In fede _____
(firma)

Dati di chi ha raccolto la dichiarazione di assenso

_____ In fede _____
(nome e cognome in stampatello) (firma)

Informativa ai sensi del Decreto Legislativo 30 giugno 2003 n. 196, *Codice in materia di protezione dei dati personali*.

☐ **Acconsente** ☐ **Non acconsente**

Che i dati anagrafici di cui sopra siano utilizzati da Fondazione Banca degli Occhi del Veneto ONLUS per il perseguimento degli scopi statutari.

Vi informiamo che i Vostri dati personali, saranno da noi trattati secondo principi di correttezza, liceità e trasparenza. Il titolare del trattamento dei dati è Fondazione Banca degli Occhi del Veneto-ONLUS, in persona del suo legale rappresentante. Il trattamento avverrà con o senza l'ausilio di mezzi elettronici o comunque automatizzati e potrà comprendere tutte le operazioni e/o complesso di operazioni indicate alla lettera a) dell'art. 4 D.lgs. n. 196/2003 e nel rispetto di tutte le prescrizioni, indicate nel decreto citato. Vi informiamo altresì che, quale soggetto interessato alle operazioni di trattamento da Voi consentite e ai sensi dell'art. 7 D.lgs. n. 196/2003, Voi avete diritto di conoscere l'esistenza di trattamenti che possono riguardarVi, anche se non ancora registrati, avete altresì diritto ad ottenere l'indicazione dell'origine dei dati personali, delle finalità e modalità di trattamento, della logica applicata in caso di trattamento con mezzi elettronici, del titolare e dell'eventuale responsabile del trattamento nonché dei soggetti o categorie di soggetti ai quali i dati personali possono essere comunicati. Avete altresì diritto di ottenere l'aggiornamento, la rettificazione, l'integrazione, la cancellazione, la trasformazione o il blocco dei dati trattati in violazione della legge.

Data _____ **In fede** _____
(firma)

Note _____

_____ **Firma leggibile** _____

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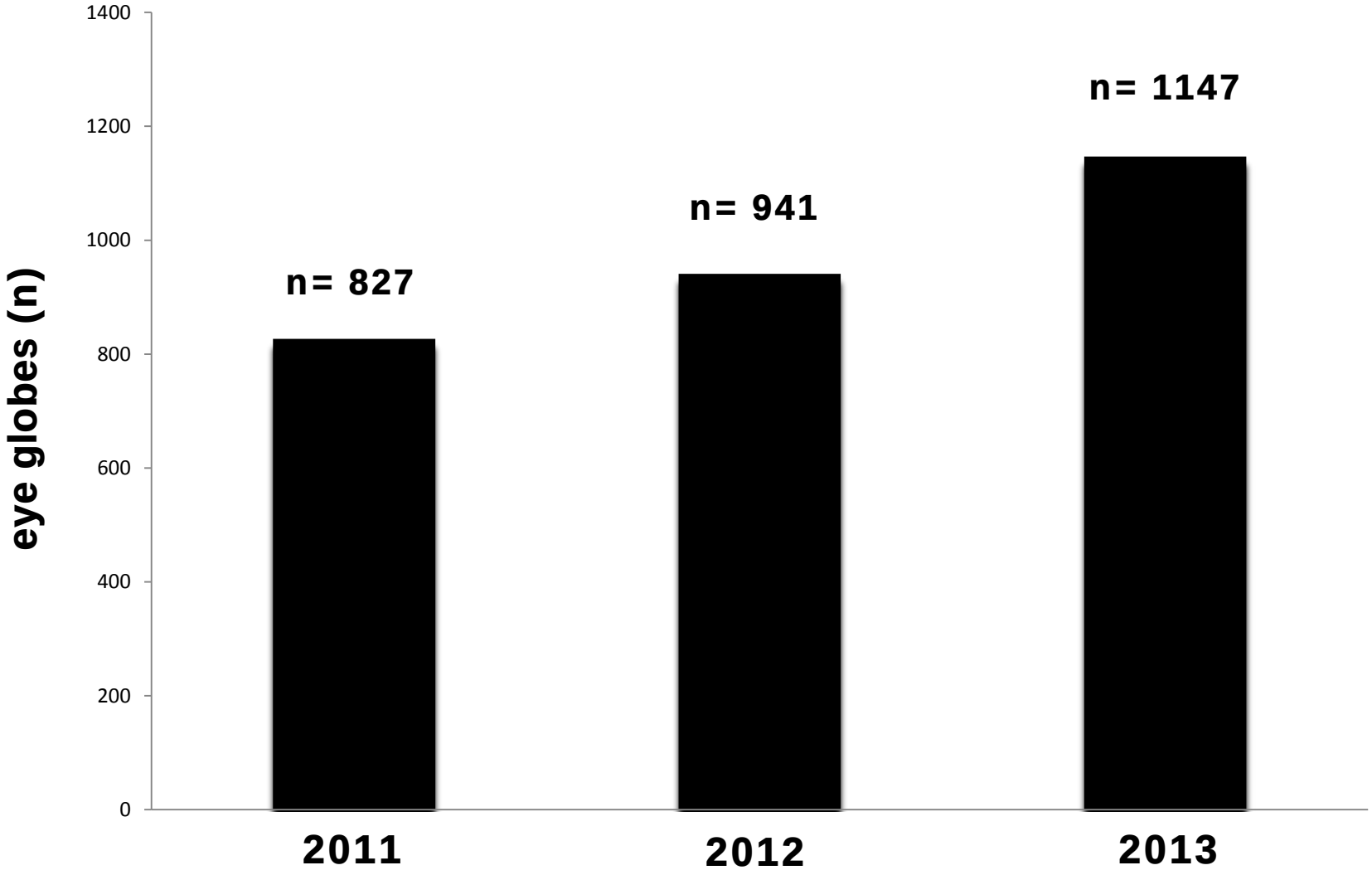
Donor eyes recovered / year

(potential for retina/choroid tissues)



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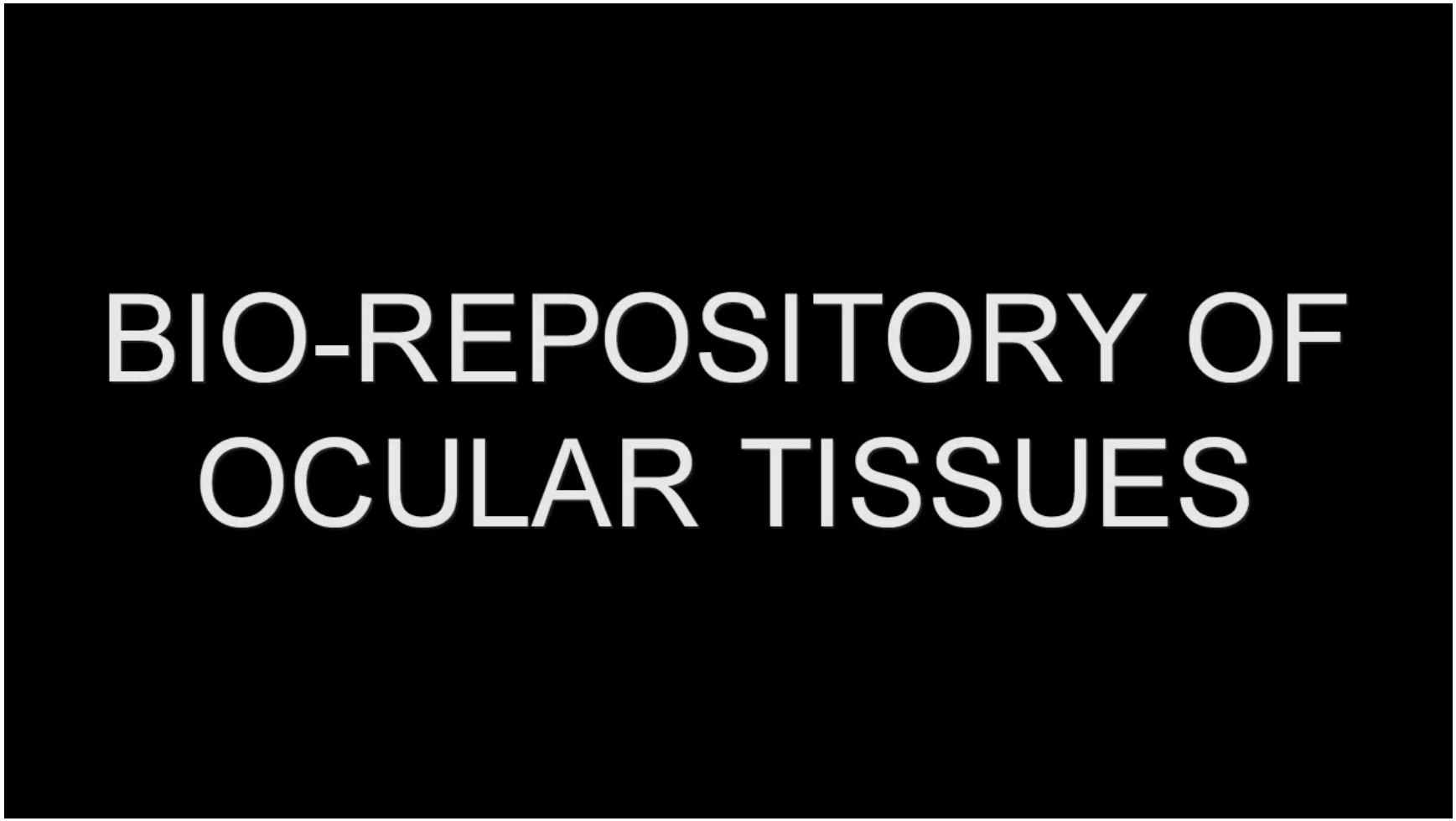
Isolation of RETINA and CHOROID



From: Parekh et al. A simplified technique for *in situ* excision of cornea and evisceration of retinal tissue from human ocular globe. *J Vis Exp* 2012; 64: e3765; URL for video: www.jove.com/video/3765/

Quality of RNAs isolated from retina and choroid samples (I)

Time from death to preservation of tissues in RNA-Later: <**25** hours

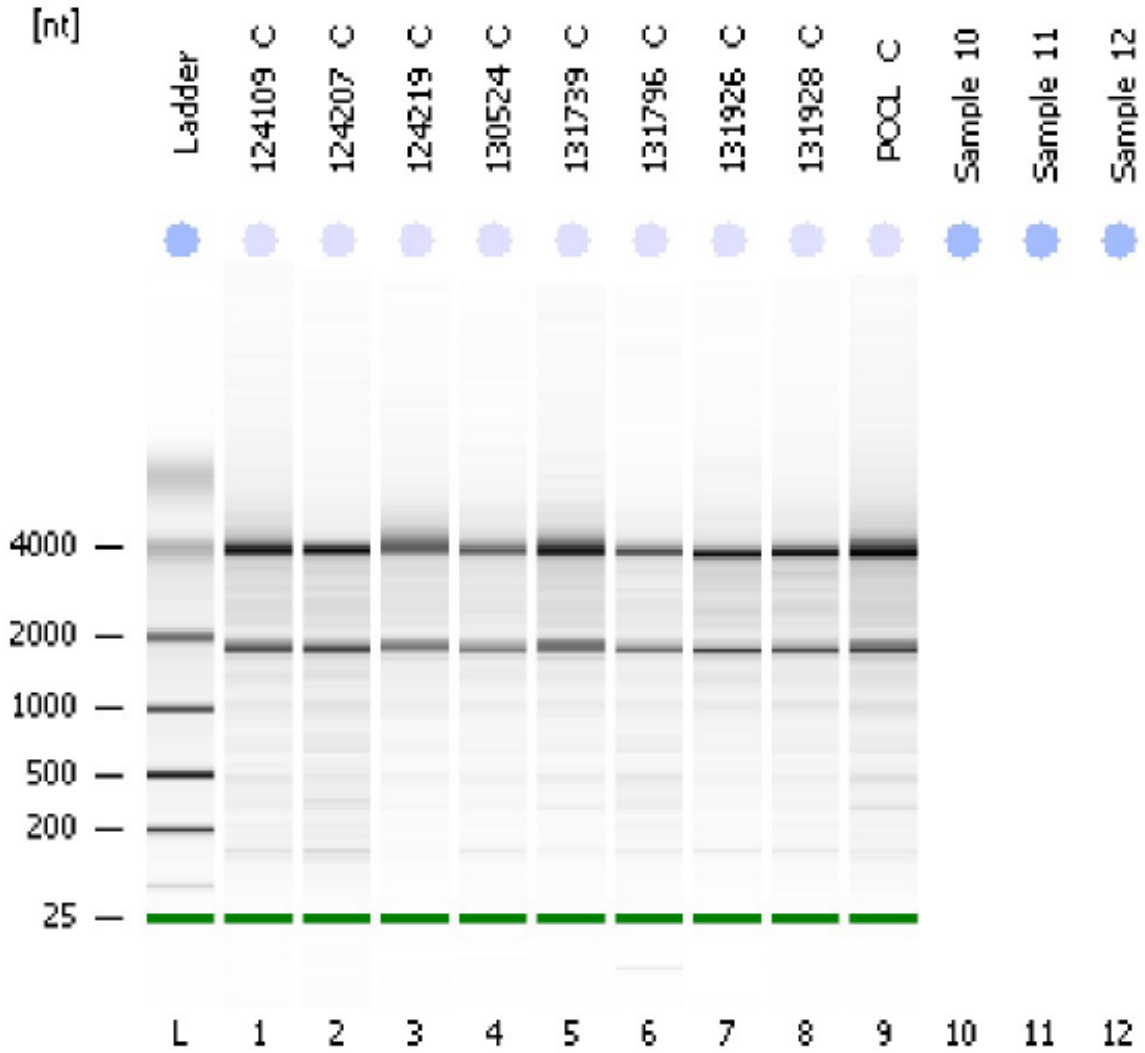
RNA **I**ntegrity **N**umber=**8.4** /10, from n=43 retinal tissues

Montanini L, Ferrari S, Crafa P, Ghirardini S, Ponzin D, Orsoni JG, Mora P. Human RNA integrity after post-mortem retinal tissue recovery. *Ophthalmic Genetics* 2013;34:27-31.

Mora P et al. Correspondence. *Retina – Journ Ret Vitr Dis* 2010;**30**:1555.

Quality of RNAs from retina / choroid samples (II)

Electrophoresis File Run Summary



Status	Result Label
✔	RIN: 8.20
✔	RIN: 7.70
✔	RIN: 8.80
✔	RIN: 8.30
✔	RIN: 8.70
✔	RIN: 7.80
✔	RIN: 7.90
✔	RIN: 7.90
✔	RIN: 8.20

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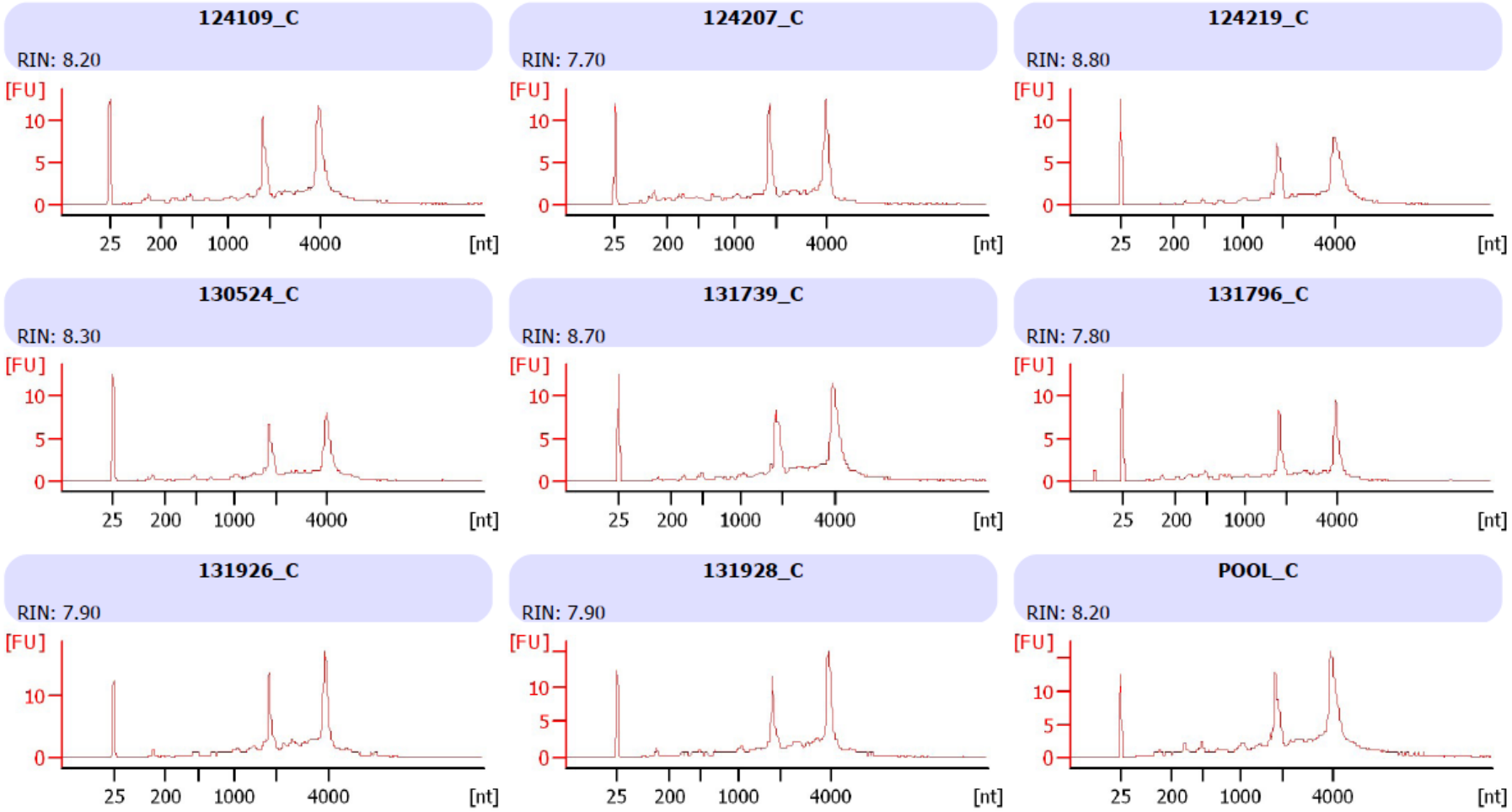
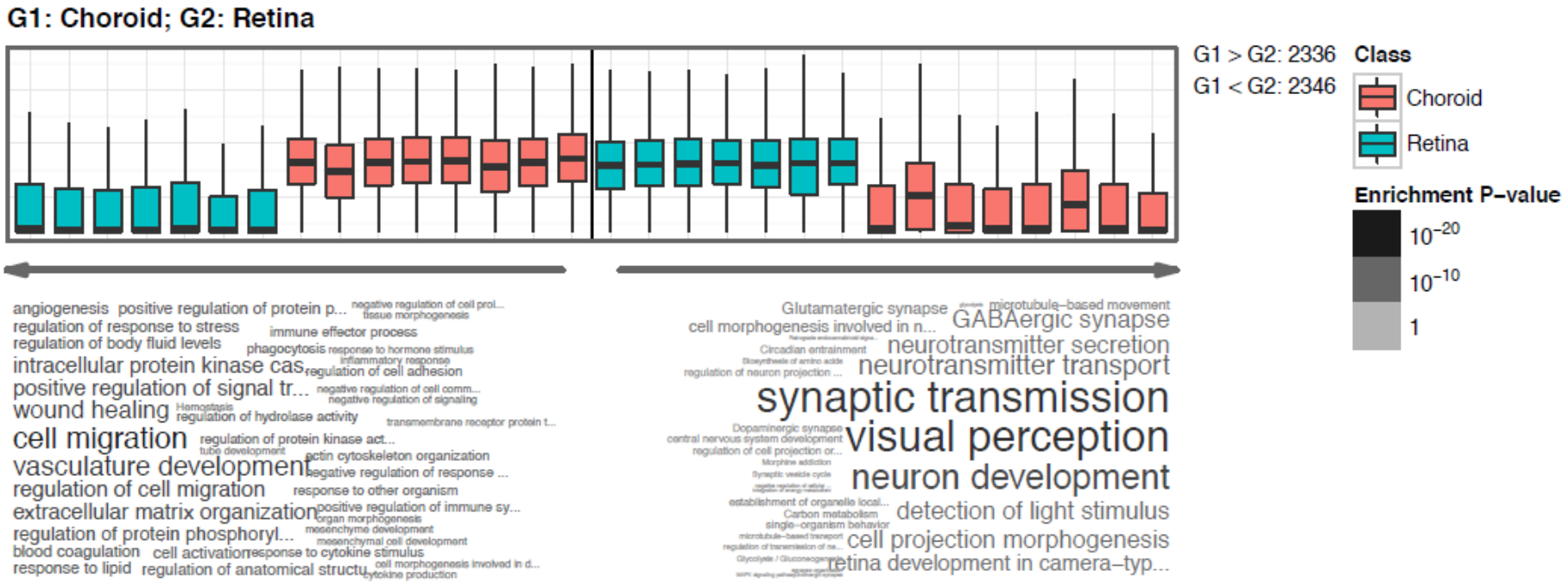
Quality of RNAs from retina / choroid samples (III)



Quality of RNAs: MICROARRAY (I)

- **DNA microarray**: collection of microscopic DNA spots attached to a solid surface
- Used to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome
- Each DNA spot contains picomoles (10^{-12} moles) of a specific DNA sequence, known as probes
- Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target

Quality of RNAs: MICROARRAY (II)



- Choroid: genes of **cell migration, vasculature development**, **extracellular matrix organization**
- Retina: genes of **synaptic transmission**, **visual perception** and **neuron development**
- Difference in gene expression between retina and choroid → protocol efficient, no cross contamination

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Age-Related Macular Degeneration:
analysis of gene expression in the human retina

- Donors ≥ 65 years (increased likelyhood ARMD)
- Time death - isolation of retina/choroid: <25 hours
- Tissues stored in:

✓ 10% formalin → histological signs of ARMD (drusen, neovascularization), **RT**

✓ RNA Later (to inhibit RNAse) → RNA extraction and microarray by Asper (Tartu, Estonia), stored and dispatched at **-80°C**

- Presence of drusen/neovascularization identifies **CASES** as opposed to **CONTROLS**

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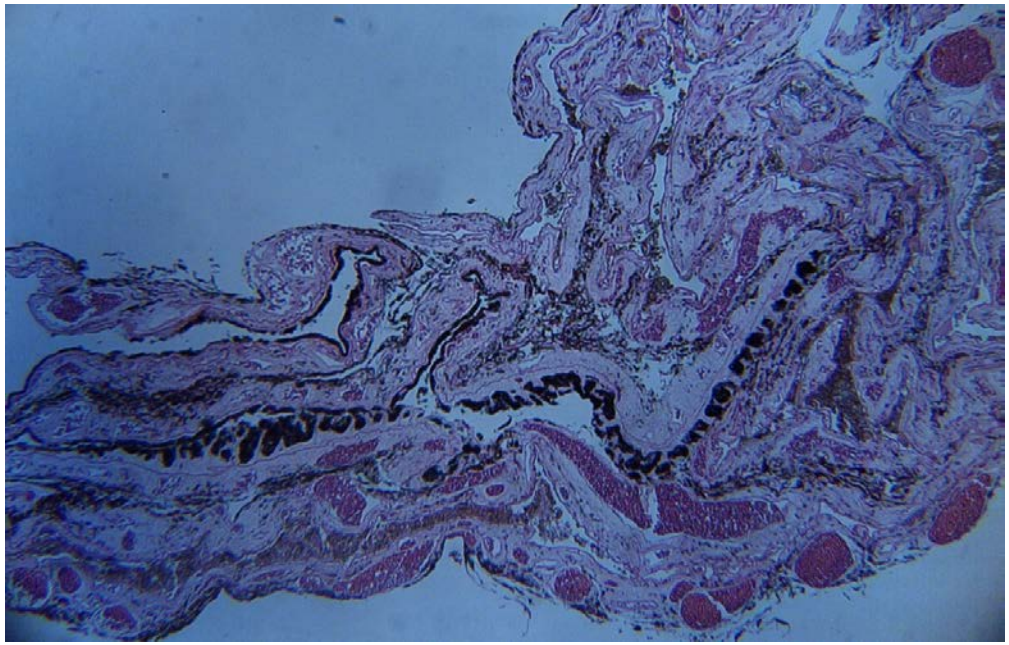
Results (2013)

- 64 donor eyes, 32 donors, mean age 68.5 years
- 32 → histological identification of ARMD
- 32 → genetic analyses
- Mean death – isolation interval: **21 hours**
- **8 donors**: **drusen** (no clinical signs, as confirmed by GP and donor's relatives) = **CASES**



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Microarray

- RNAs extracted from the retina & choroid tissues
- N=8 retina CASES
- N=8 choroid CASES
- N=1 pooled retina CONTROL
- N=1 pooled choroid CONTROL
- Illumina Whole Genome Expression BeadChips for microarray analyses
- **34370** different probes
- Values from the 8 CASES statistically compared to the pooled CONTROLS

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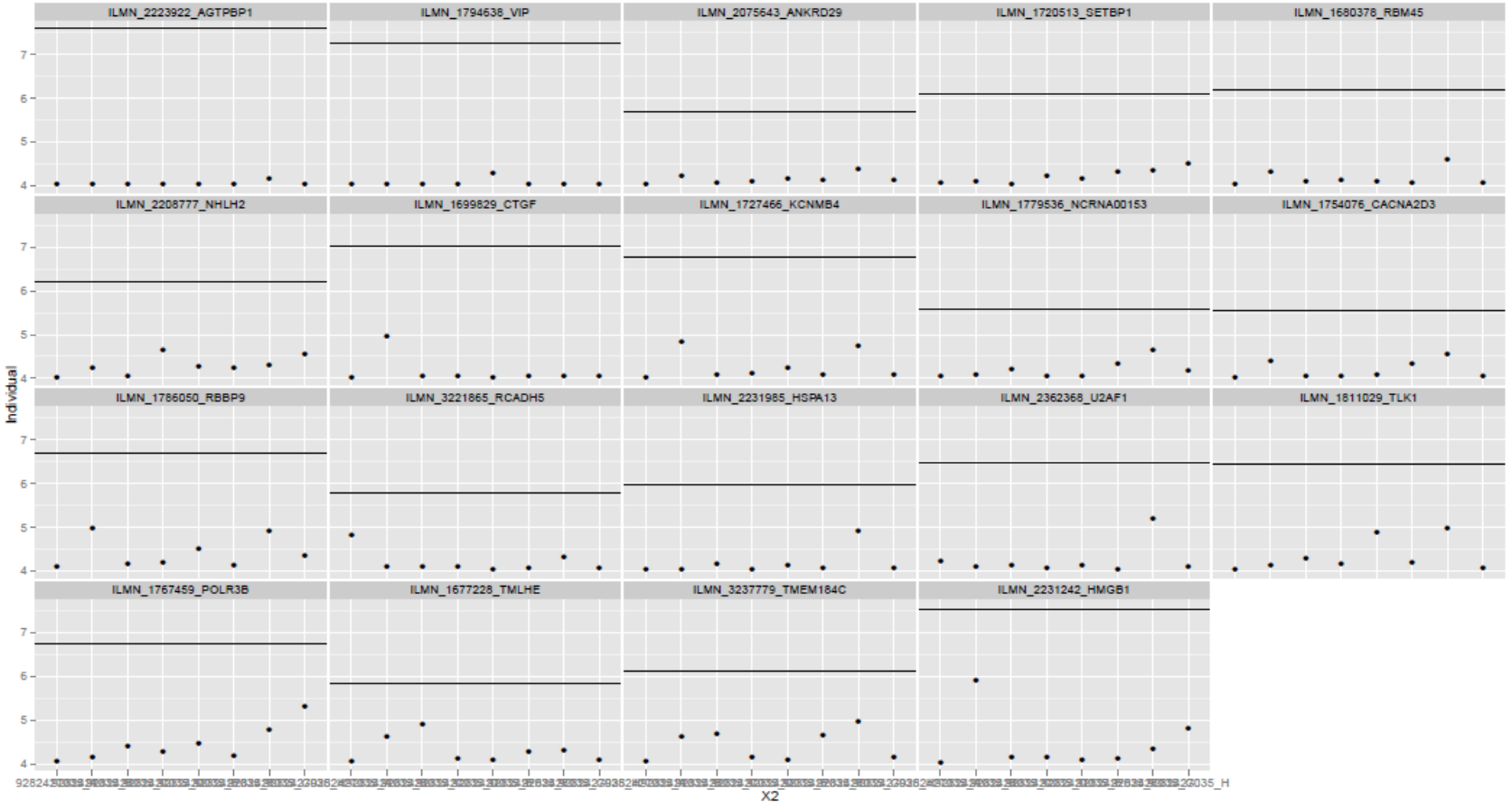
Microarray (example)



Results after statistical analysis are given as differences between dots (CASES) and lines (CONTROL)

A **Pubmed search was needed to understand which genes were more interesting in the ARMD setting**

Genes DOWN-regulated in retina

GENE	CHARACTERISTICS
AGTPBP1	Also known as NNA1, zinc carboxypeptidase. Retinal degeneration was found in the ataxia and male sterility (AMS) mouse, a mutant of the NNA1 gene (Araki et al., Pathol Int 2012; 62: 719-727)
VIP	Protective effects of the Vasoactive Intestinal Peptide in ischemic retinal degeneration. VIP is protective against several types of retinal injuries. Down-regulation of VIP might lead to degeneration
CTGF	Connective Tissue Growth Factor. Mitogen secreted by vascular endothelial cells. In AMD, abnormal CTGF synthesis regulation may play a role in fibrous peri-retinal membrane formation (proliferative vitreoretinopathy). Bevacizumab exerts pro-fibrotic effects on human RPE cells at clinical doses by up-regulation of CTGF expression via an Fc-FcR interaction
TLK1	Gene associated with age, which is a risk factor in ARMD.
POLR3B	Polymerase (RNA) III (DNA directed) polypeptide B. Associated with leukodystrophies, inherited neurodegenerative disorders characterised by abnormal central nervous system white matter
HMGB1	In dsRNA-induced retinal degeneration, decreased levels of pro-inflammatory cytokines (such as TNF-α and IL-6) in the retina, and attenuated intravitreal release of high-mobility group box-1 (HMGB1) were seen

Genes UP-regulated in retina

GENE	CHARACTERISTICS
RRAS2	Involved in Huntington's disease. Pharmacological inhibition of RRAS signaling may confer therapeutic benefit in Huntington's disease
APBB3	Involved in Alzheimer's disease
MAB21L2	Involved in retina development in mouse and zebrafish.
CPLX3	Absence of Complexin 3 and Complexin 4 differentially impacts the ON and OFF pathways in mouse retina
SEMA4	Involved in optic nerve development
SCYL1	May be associated with glaucoma
OPN1MW	Opsin 1 (cone pigments), medium-wave-sensitive. Human X-linked blue-cone monochromacy (BCM), disabling congenital visual disorder of cone photoreceptors, caused by mutations/alterations in the red (OPN1LW) and green (OPN1MW) cone photoreceptor opsin gene array
SIGMAR1	Involved in Alzheimer's disease
TRPC4AP	Involved in Alzheimer's disease

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Genes DOWN-regulated in choroid

GENE	CHARACTERISTICS
GCNT3	Expressed in goblet cells of the conjunctival epithelium
GPX2	Glutathione peroxidase 2. Data showing increased oxidative damage in baseline GPx-deficient retina give rise to the hypothesis that increased oxidative stress provides a "preconditioning" environment in which protective mechanisms paradoxically render GPx1-deficient retinas less vulnerable to light-induced oxidative damage.
ALDH3A1	Antioxidant aldehyde dehydrogenase 3A1, expression modulated in stress conditions.

Genes UP-regulated in choroid

GENE	CHARACTERISTICS
PLEKHB2	Pleckstrin homology domain containing, family B (evectins) member 2. Its restricted cellular distribution and genetic locus make it a candidate gene for the inherited human retinopathy autosomal dominant familial exudative vitreoretinopathy. It also may be a susceptibility gene for multiple sclerosis
MCM3	Involved in eye development?
IL10RB	IL-10 receptor, beta. In animal model of ARMD, low-dose LPS pretreatment suppresses laser-induced CNV IL-10 secretion by peritoneal macrophages. Macrophages stimulated by environmental agents, like pathogens, may play a protective role in the pathogenesis of AMD
TBRG4	TGF-beta regulator 4. Known as a neovascular activator in choroidal neovascularization
NCL	Nucleolin binding peptide can deliver small and large molecules into retinal and corneal cells and plasmid DNA into retinal cells. May be useful for the delivery of therapeutics to the eye
TFCP2	The gene product interacts with certain inflammatory response factors, and polymorphisms may be involved in the pathogenesis of Alzheimer's disease.
CHST12	Involved in multiple sclerosis?
SIRT5	SIRT1 levels elevated in human choroidal neovascularization membranes. Inhibition of SIRT1 activity correlated with decreased secretion of potent proangiogenic cytokines. Potential role for SIRT1 in the pathogenesis of neovascular age-related macular degeneration
VLDLR	Oxidative stress and inflammation: pathological mechanisms in many neurodegenerative diseases, including ARMD. The very low-density lipoprotein receptor knockout mouse (Vldlr-/-) is a model for retinal angiomatous proliferation in AMD
UBQLN1	UBQLN1 variants associated with increased risk for late-onset Alzheimer's disease
SEMA4D	SEMA5A and SEMA5B inhibit retinal neurite outgrowth through PlexinA1 and PlexinA3 receptors, *in vitro* and *in vivo*. These findings define a set of ligands and receptors required for the establishment of inner retinal lamination and function
PXDN	Peroxidasin is essential for development of the anterior chamber of the eye, where it may have a structural role in supporting cornea and lens architecture as well as an enzymatic role as an antioxidant enzyme in protecting the lens, trabecular meshwork, and cornea against oxidative damage
LRP1	Expression of this gene decreases with age, is lower in brain tissue from Alzheimer patients. LRP1 plays a role in ischemic neovascular diseases
SAMD11	Mouse SAMD11: predominantly expressed in developing retinal photoreceptors

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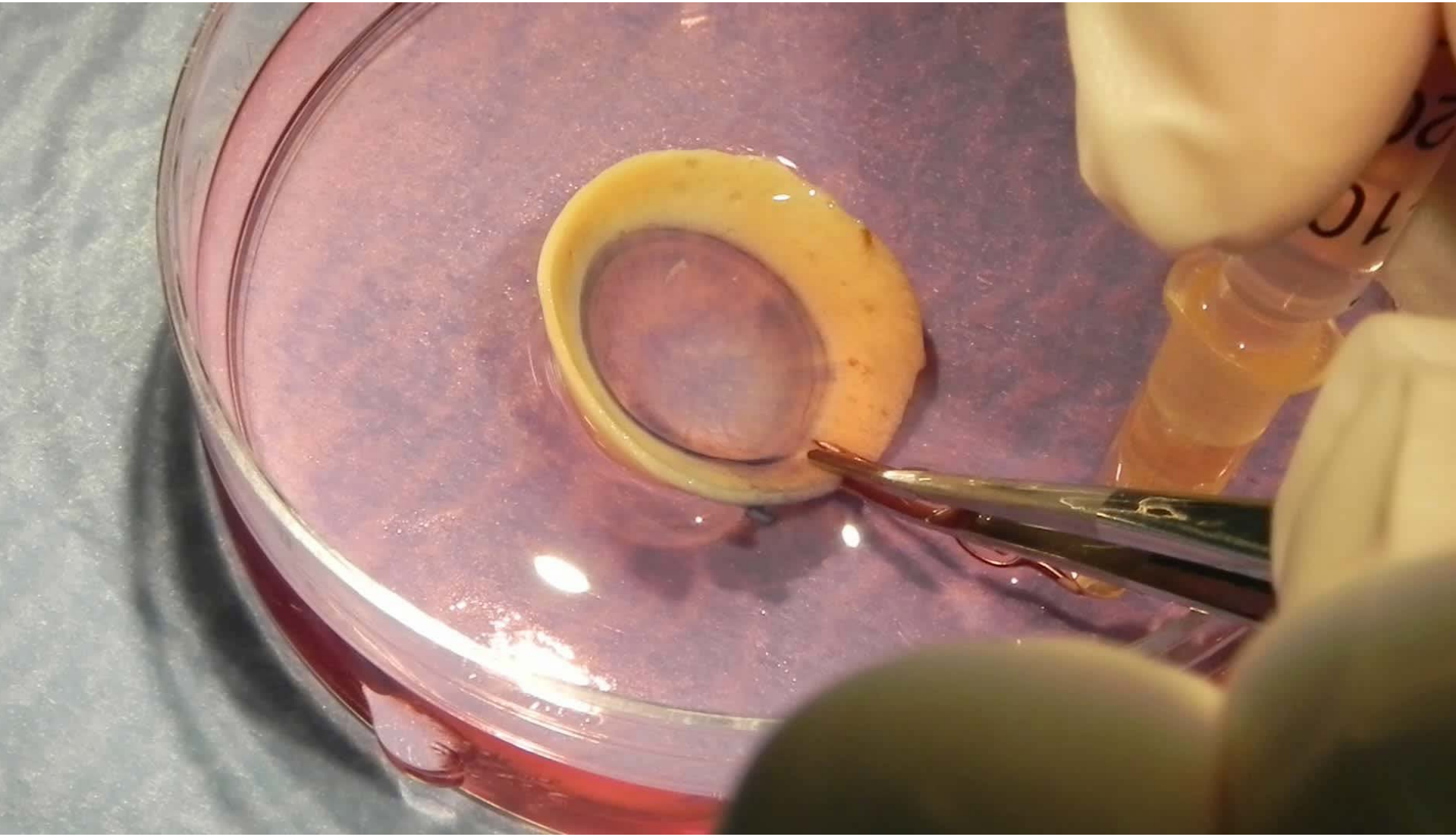
Summary of the research project

- CTGF (down-regulated in retina) and VLDLR (up-regulated in choroid): genes already involved in ARMD. **The strategy is correct, might lead to some of the genes to be included in diagnostic tools**
- Some of the **genes identified are also involved in Alzheimer's disease**. There are studies trying to evaluate whether there is an association between ARMD and Alzheimer's disease or common risk factors
- The **majority of genes identified are involved in inflammatory patterns, oxidative stress and vascularization**, thus suggesting the pathogenic role of these patterns and the potential usefulness of therapeutic strategies based on anti-oxidants, anti-inflammatory and anti-angiogenic factors
- Peripheral blood testing for putative gene expression in AMD



Busin M, Scorcia V, Patel A, Salvalaio G, Ponzin D. Pneumatic dissection of donor endothelial tissue add storage for descemet membrane endothelial keratoplasty. *Ophthalmology* 2010;117(8):1517-20.

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Conclusions

- Bio-banking of human ocular tissues: feasible
- Certified donor and tissue characteristics (age, sex, cause of death, post mortem interval)
- Procedures validated for optimal preservation of RNA (for gene expression studies by means of real-time PCR, microarray, etc.) and of the histological studies
- Tissues available for research, education, training
- Collaborations are welcome

Acknowledgments

FBOV: Ferrari S, Parekh M, Salvalaio G

UniPR: Montanini L, Crafa P, Mora P, Orsoni J

2012 Global Ophthalmology Awards Program from BAYER

Camera di Commercio, Venice

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