Biomarker of DMR

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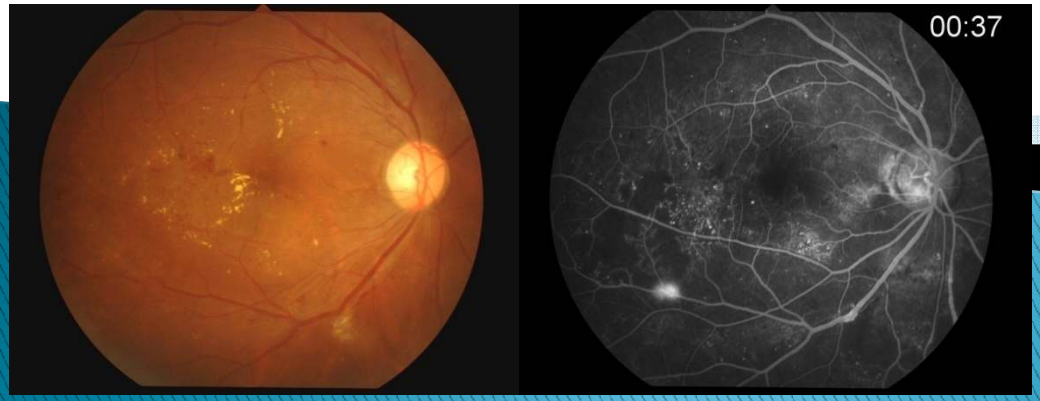


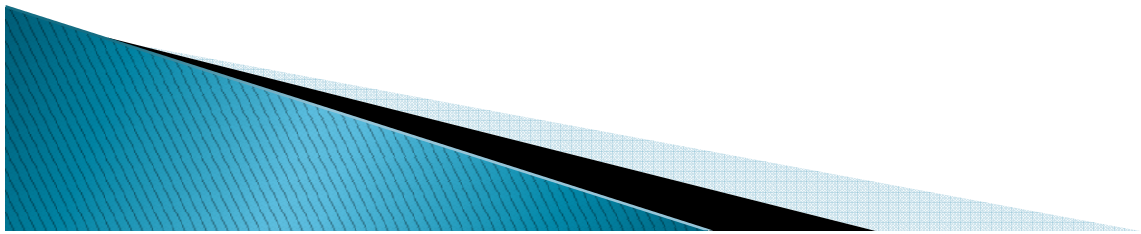
Diabetic Retinopathy

- Most common microvascular complication
- One of the leading causes of blindness
- Association with systemic vascular complications
  - CVA, coronary heart disease, heart failure, nephropathy

Role of Biomarker in DMR

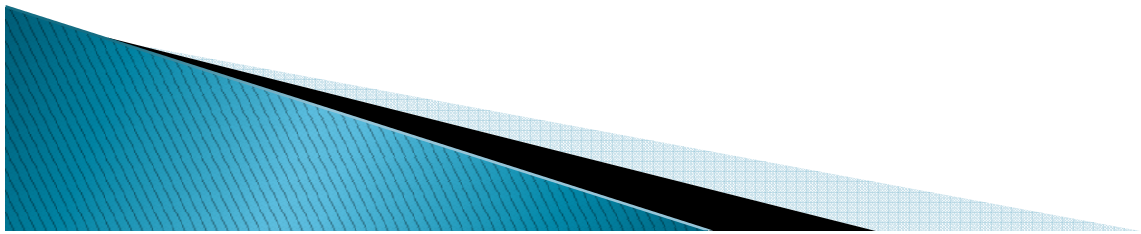
- General role of biomarker
  - Decision in therapy
  - Prognosis of disease

- Role of biomarker in DMR
  - Grading of DMR
  - Decision of laser photocoagulation
  - Prognosis of DMR

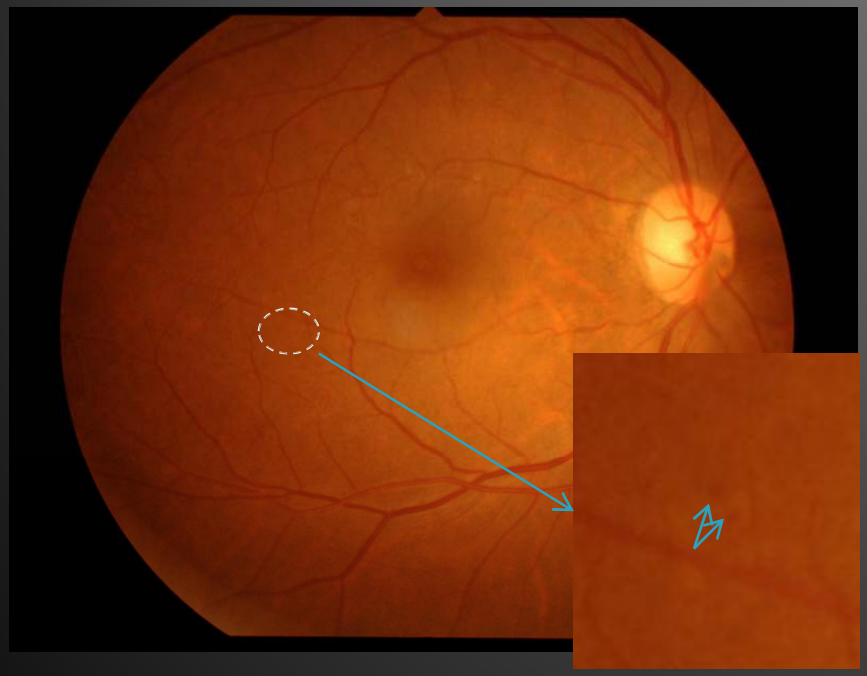


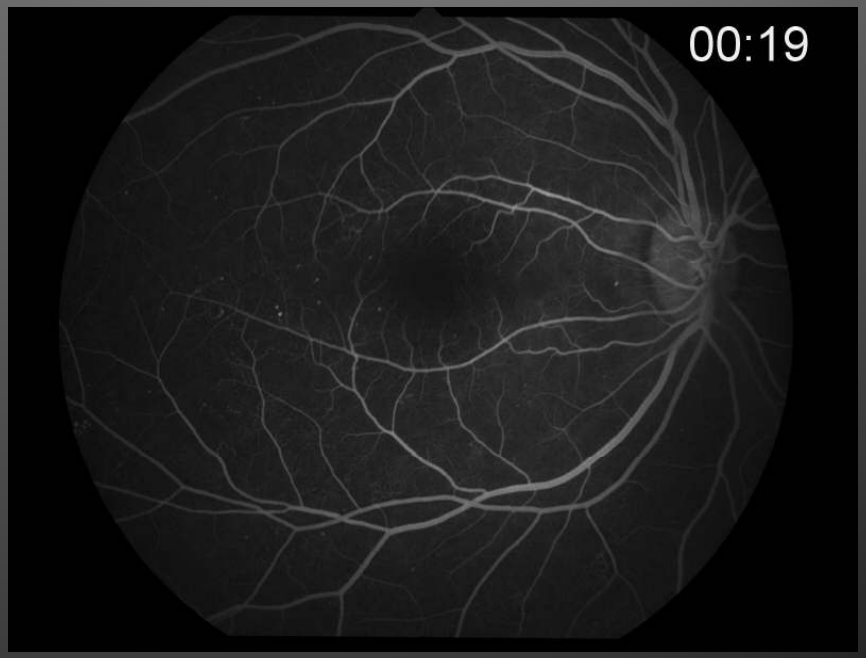
Biomarkers in DMR

- Fundoscopic and Angiographic biomarker
- Genetic biomarker
- Plasma biomarker
- Vitreous biomarker
- Proteomic approach for biomarker discovery



Microaneurysm

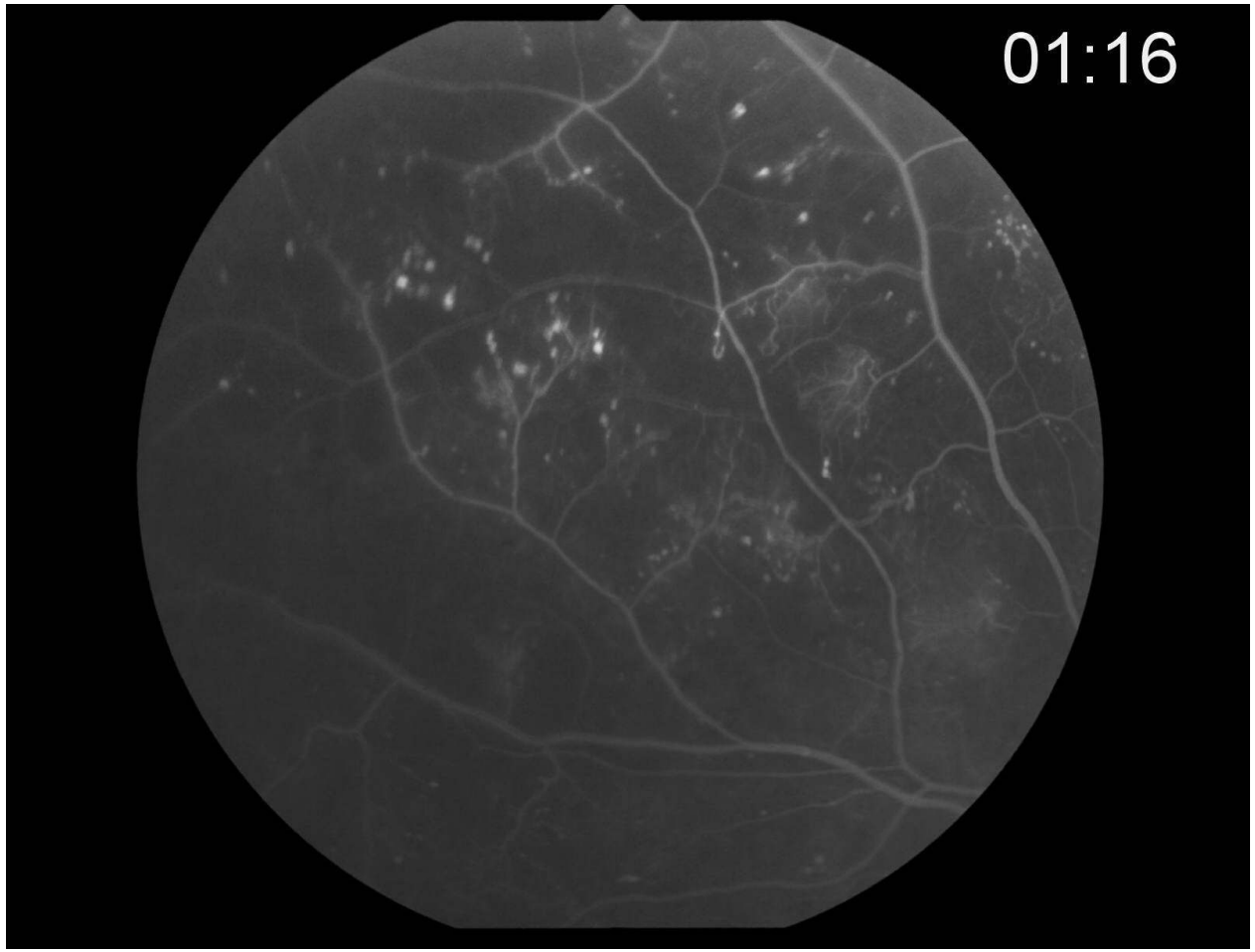




Microaneurysm

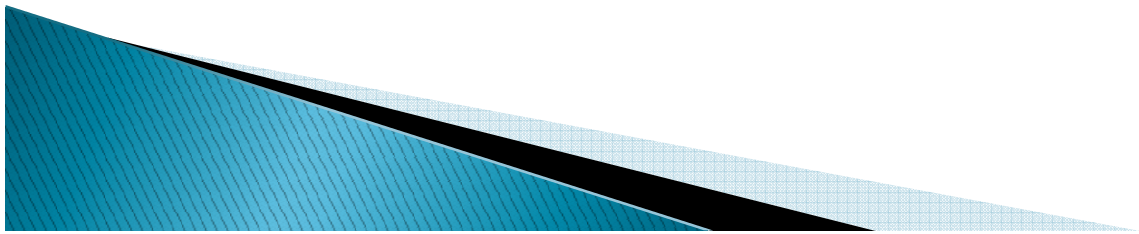
- Saccular outpouching of capillary wall
- The first visible sign of DR
- Staging biomarker as combined with punctate hemorrhage
- Leakage from Ma as a cause of macular edema
- Ma turnover as a biomarker for CSME

IRMA



IRMA
(IntraRetinal microvascular Abnormality)

- Clusters of Ma and tortuous hypercellular vessels
- Adjacent to the nonperfused retina
- 70% of NVE from IRMA
- As a biomarker for progression to PDR

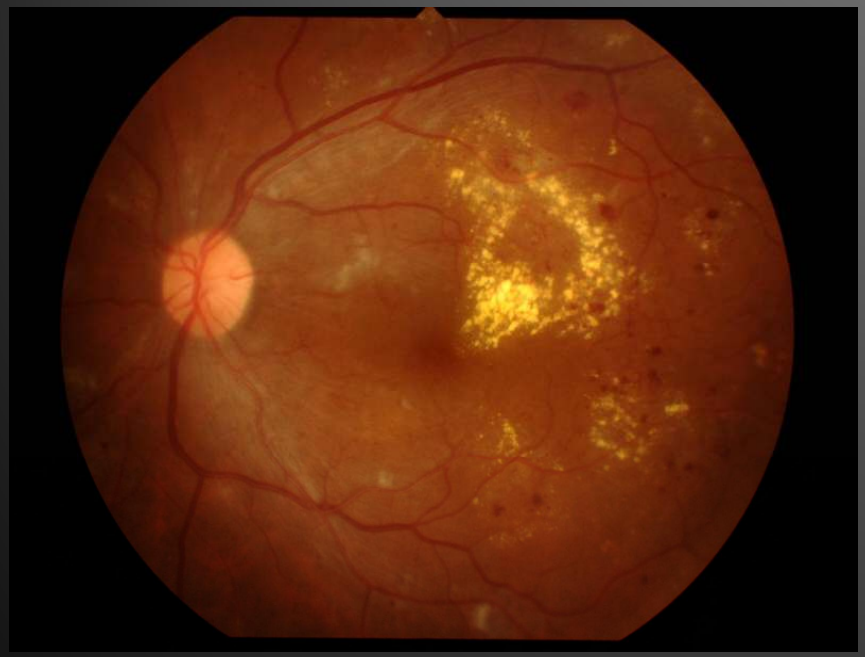


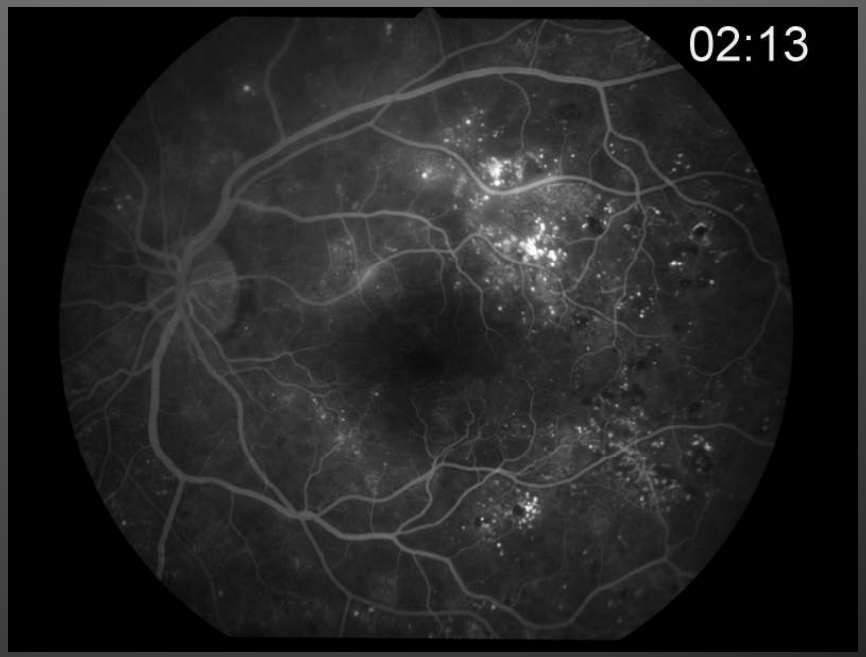
Venous beading

Venous beading

- Dilated segments of retinal veins
- Representative of retinal ischemia
- Biomarker most associated with progression to PDR

Hard exudate

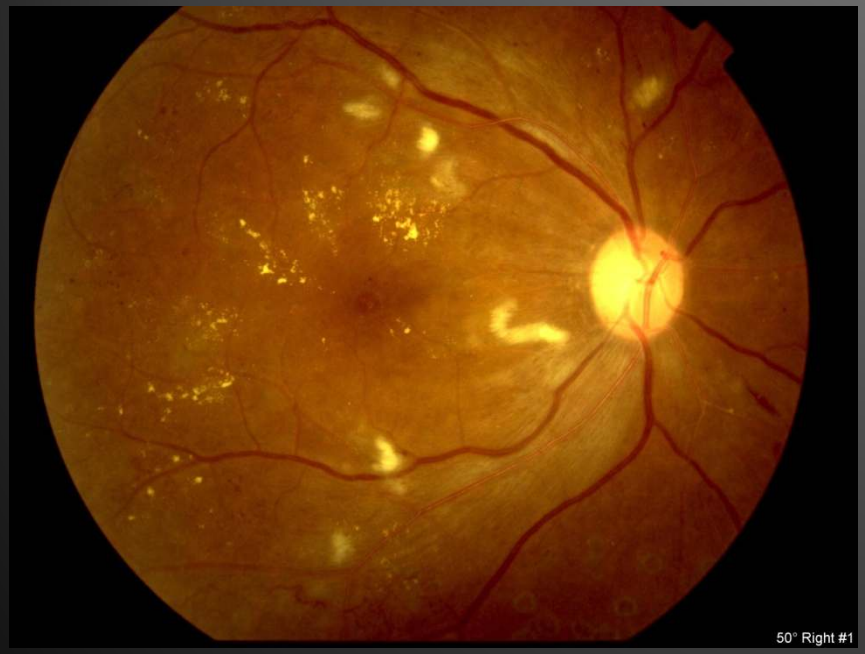


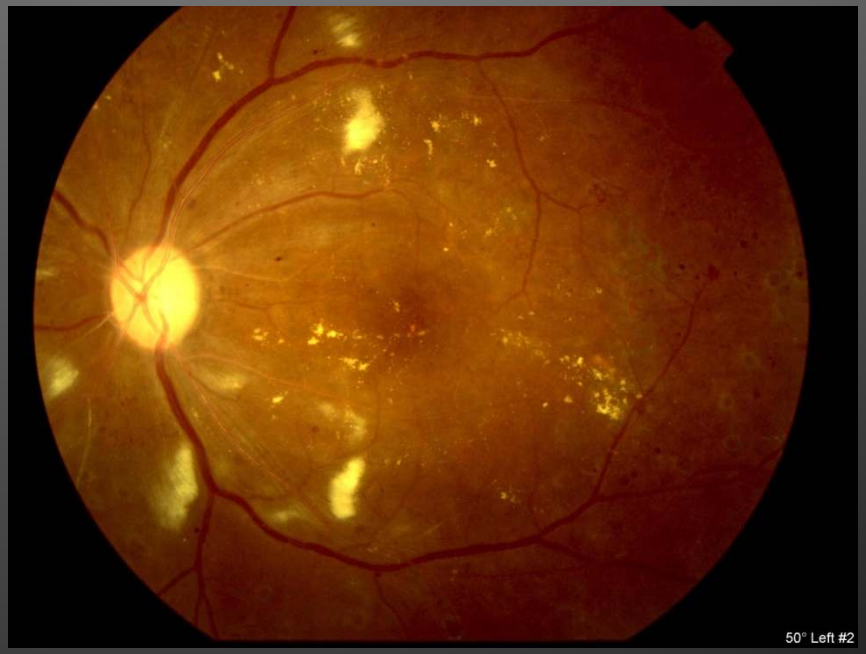


Hard exudate

- Lipid deposits in association with lipoprotein leakage
- Often accompanied with macular edema
- Associated with serum lipids level
- Not as a biomarker for progression to PDR

Cotton wool patch

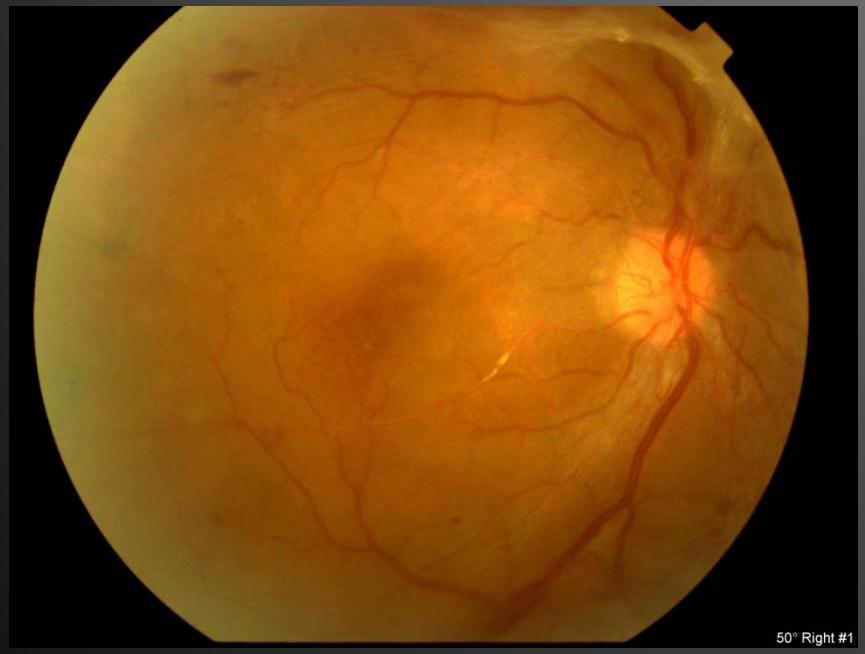


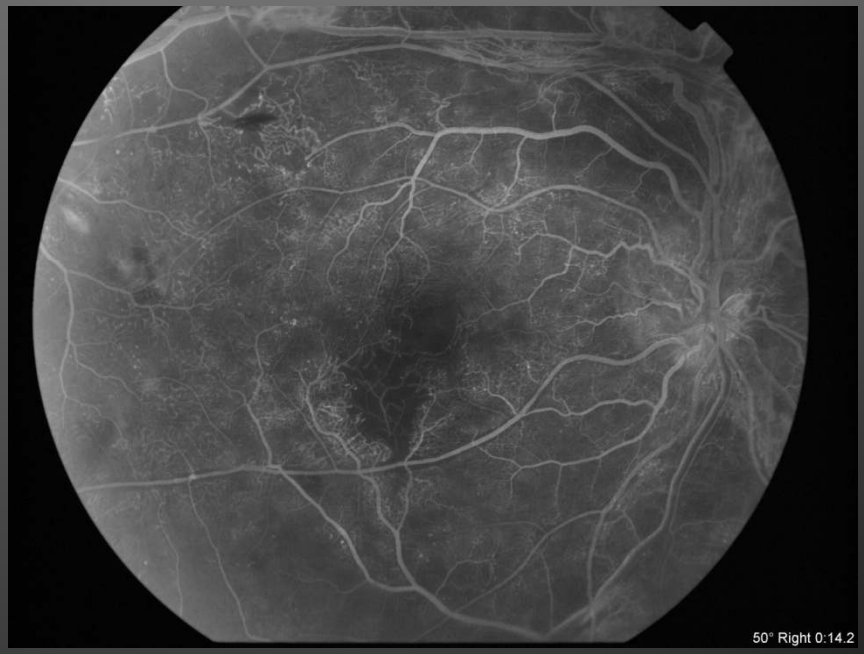


Cotton wool patch

- Defect in axonal transport from microinfarcts in the retinal nerve fiber layer
- Not as a biomarker for progression of DR

PDR

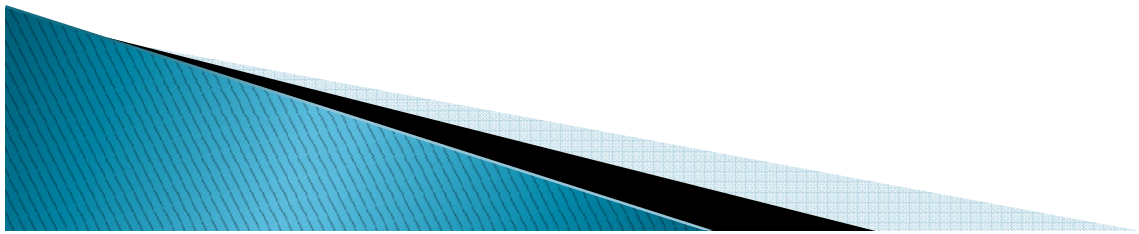




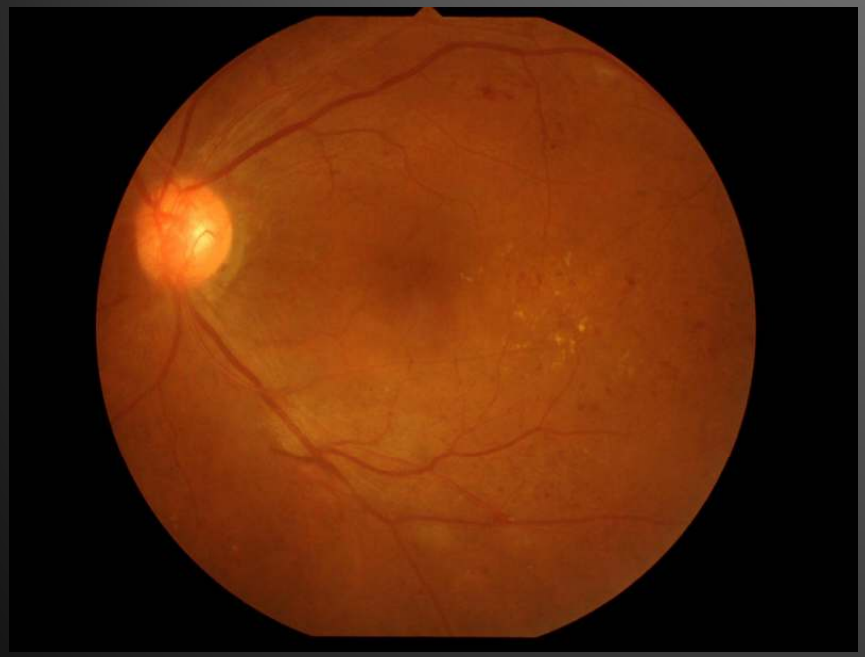
PDR

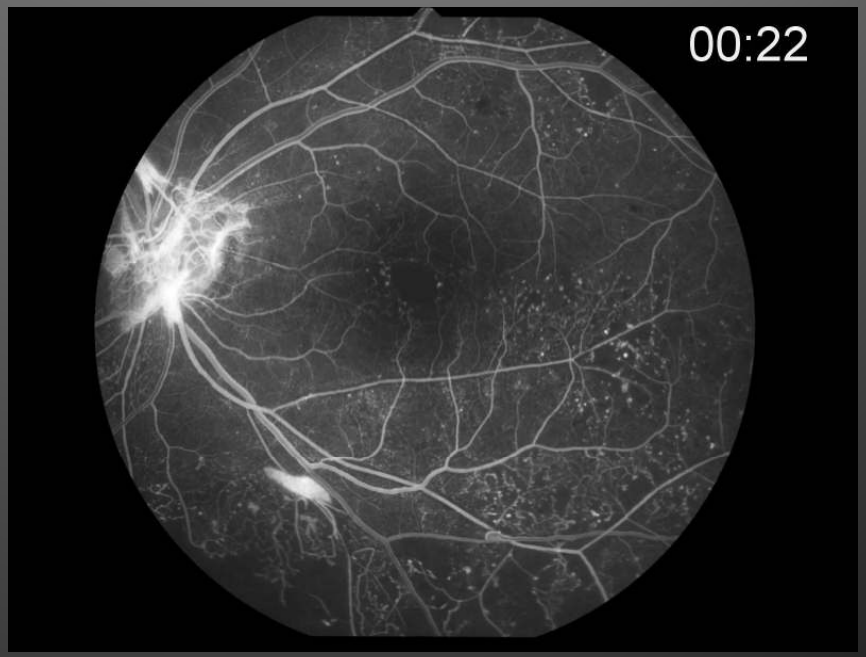
- PDR, accompanying
  - Neovascularization: NVD, NVE
  - Fibrous proliferation

- NVD (New Vessels on Disc)
  - Biomarker most associated with severe visual loss

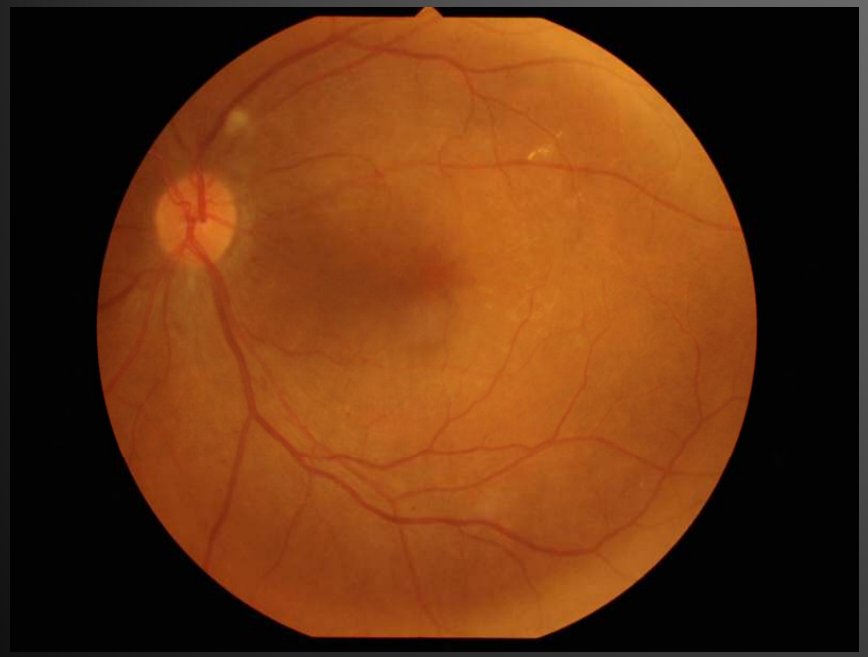


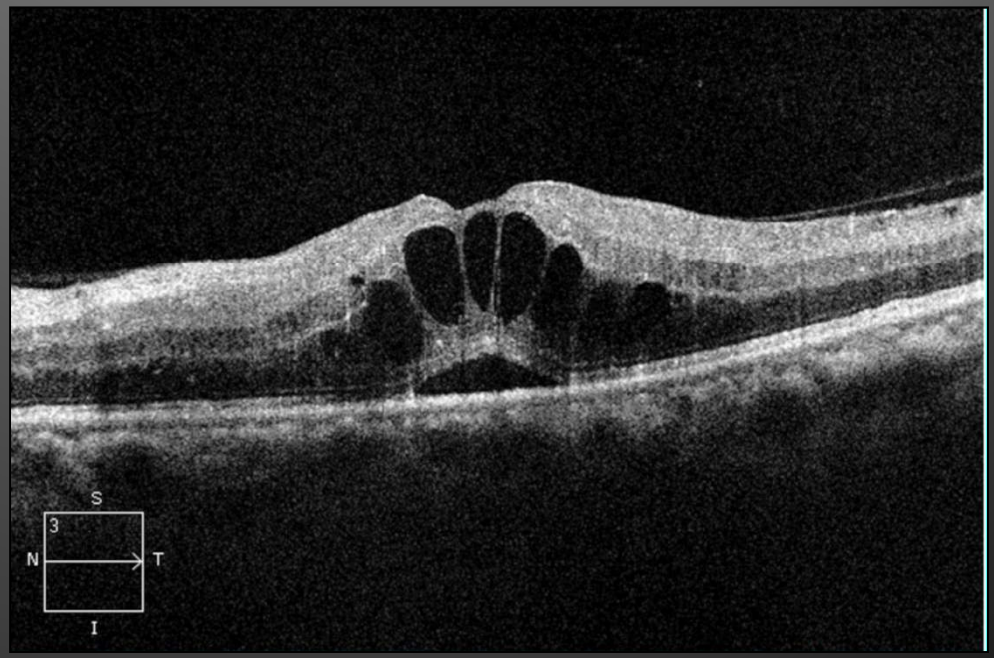
Neovascularization





Macular edema





Macular edema

- Retinal thickening from accumulation of fluid within 1 DD of the macula
- Most common cause of visual loss in DR
- CSME (Clinically Significant Macular Edema)
  - Macular edema involving or threatening fovea

Grading of DMR

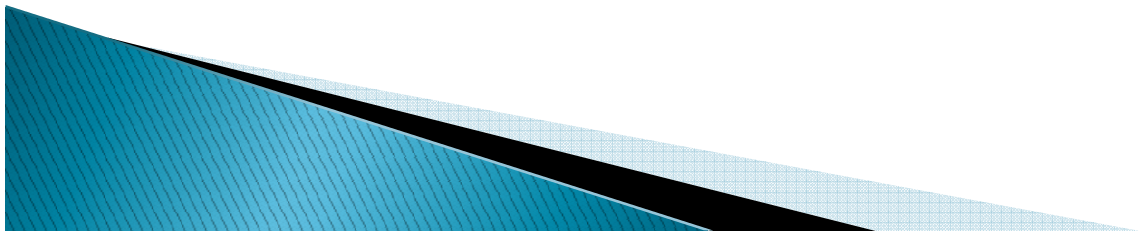
Severity	Lesions present
Mild NPDR	Ma +/- retinal hemorrhage, hard exudates
Moderate NPDR	Mild NPDR + cotton wool spots and/or IRMA
Severe NPDR	Presence of one of the following features 1)H/Ma≥ standard photograph 2A in 4 Q 2)marked venous beading in 2 Q 3)moderate IRMA in 1 Q
Very severe NPDR	≥ 2 of the above features in severe NPDR

Prognosis of DMR

Grading	Follow-up	Severe visual loss (%)
NPDR	2-year	3.2
	4-year	12.8
PDR without high risk markers	2-year	7.0
	4-year	20.9
PDR with high risk markers	2-year	26.2
	4-year	44.0
All eyes	2-year	14.0
	4-year	28.5

Treatment of DR

- Diabetic Control
- Laser photocoagulation
  - Considered over very severe NPDR
  - Focal/grid photocoagulation
    - Macular edema
- Medication
  - Antiplatelet
  - Lipid lowering agents
  - Antioxidants



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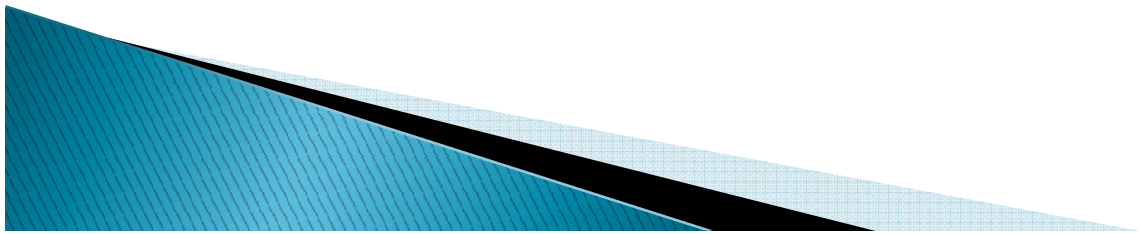
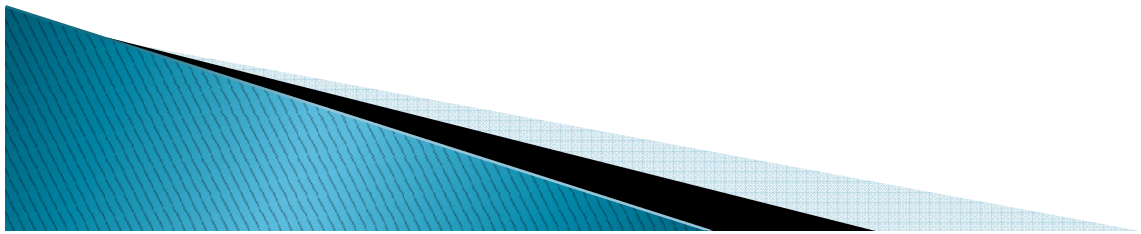


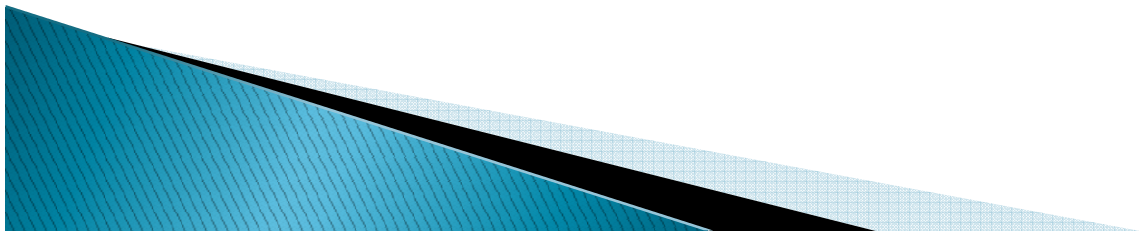
Aldose Reductase 2 Gene

- Aldose Reductase 2
  - 1st and rate limiting enzyme in polyol pathway
- (A-C)n repeat polymorphism
  - found to be associated with DR in
    - Hong Kong-Chineses, Japaneses, Indians, Chinese
- (AC)23 allele in ALR2-gene
  - associated with early onset of DR
  - Rapid progression of DR



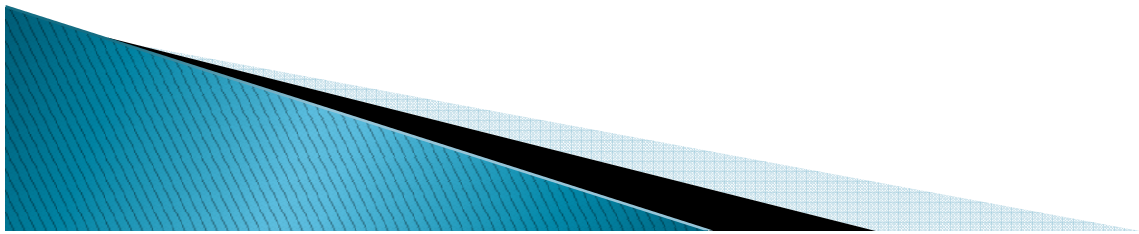
AGE Receptor (RAGE) gene

- Receptor-mediated activation and secretion of various cytokines
  - Progression of DR
- Gly82 Ser polymorphism
  - Decreased RAGE expression
  - lower risk for development of DR
    - Pros: Kumaramanickavel et al., Hudson et al.
    - Cons: JiXiong et al., Kankova et al., Liu and Xiang, Petrovic et al.



Renin-angiotensinogen system

- ATR1 gene
  - Angiotensin II Type 1 Receptor
  - ATR1 polymorphisms
    - No association with the development of retinopathy in type 2 diabetes

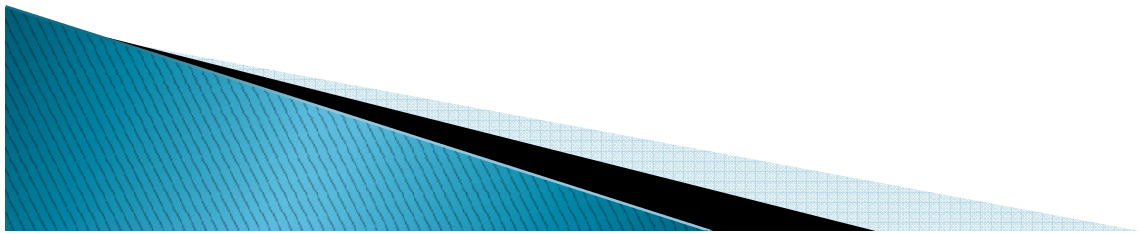


RAS (cont'd)

- ACE gene
  - ACE I/D (insertion/deletion) polymorphism
    - Association with DR
      - demonstrated only in one study performed with Japanese
- Angiotensinogen gene
  - No association with DR
    - Chinese, Caucasians

VEGF gene

- C(-634)G polymorphism
  - -634C allele
    - Increased in DR patients
  - CC genotype
    - Higher VEGF serum levels in healthy subjects



MTHFR gene

- Methylenetetrahydrofolate reductase
  - Remethylation of homocysteine to methionine
  - Hyperhomocysteinemia
    - independent risk factor for macroangiopathy
    - Activation of vascular inflammation through inflammatory cytokines, including VEGF
  - Polymorphic mutation (C677T)
    - Impaired enzyme activity, resulting in hyperhomocysteinemia
    - can contribute to the progression of DMR

Other Genes

- TNF-α, nitrate oxidase gene, GLUT1, PAI-1, PON-1 (paroxonase-1), HFE (hemochromatosis), TGF β1, EDN1 (Endothelin-1), PPARγ, or α2β1 integrin, matrix metalloproteinase, basic fibroblast growth factor gene, manganese superoxide dismutase gene, SUMO4 gene, IGF-1 gene, PEDF

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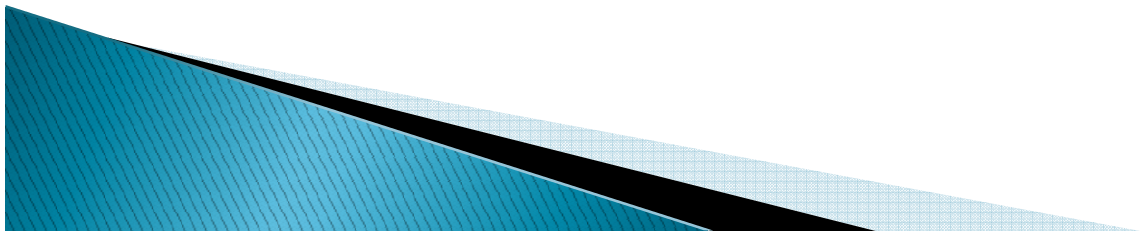
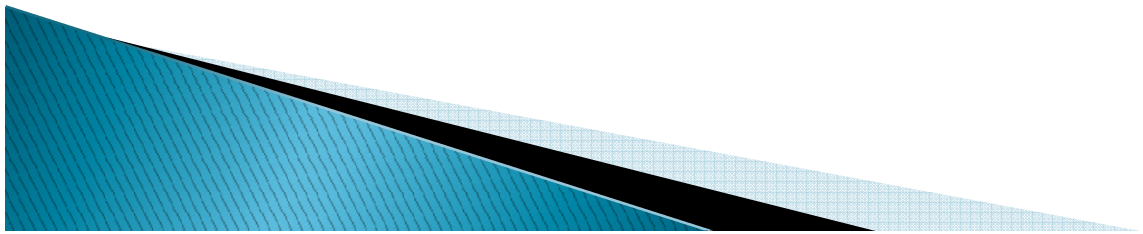


Candidate Plasma Biomarker

- Risk markers of
  - inflammation
  - Hemostatic disturbance
  - endothelial dysfunction
  - Hyperhomocyeteinemia
- Current plasma risk factors of DR
  - Inconsistent
  - limited clinical use

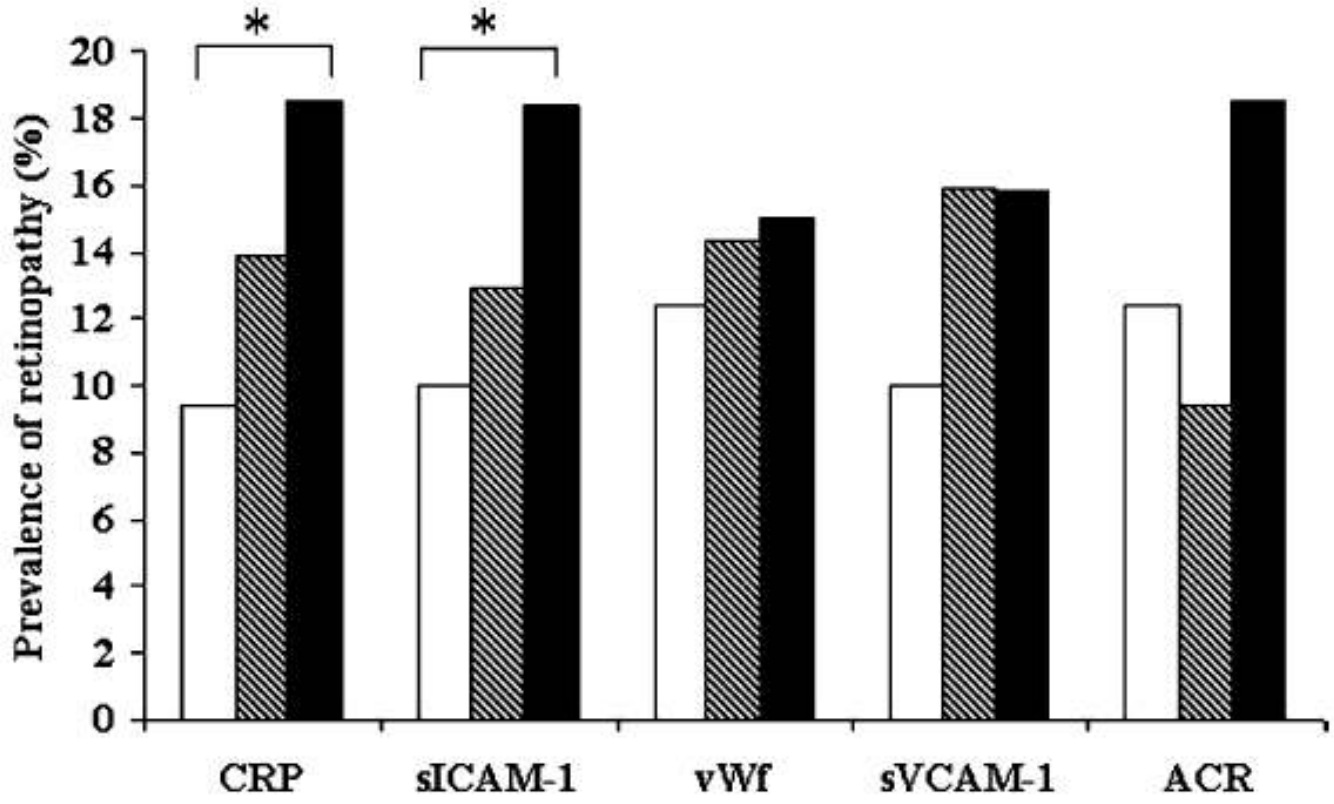


the Hoorn Study

- 625 individuals aged 50 to 74 years
- Levels of CRP, soluble intercellular adhesion molecule-1 (sICAM-1), von Willebrand factor, soluble vascular adhesion molecule-1 (sVCAM-1), urinary albumin : creatinine (ACR)
- investigated the association of the markers with prevalent retinopathy

van Hecke et al. Diabetologia 2005

Prevalence of retinopathy according to tertiles of CRP, sICAM-1,vWf, sVCAM-1 and ACR



van Hecke et al. Diabetologia 2005

Wisconsin Epidemiologic Study of Diabetic Retinopathy

- type 1 DM
- hsCRP, IL-6, sVCAM-1, sICAM-1, TNF, total homocysteine
- Prevalence data
  - sVCAM, TNF, and homocysteine levels were associated with increased odds of more severe DR in those with kidney disease
  - total homocysteine level was associated with increased odds of ME, irrespective of kidney disease.
- Incidence data
  - None of the markers were associated with incidence of proliferative DR, ME, or progression of DR 15 years later.

Klein et al. Arch Ophthalmol 2009

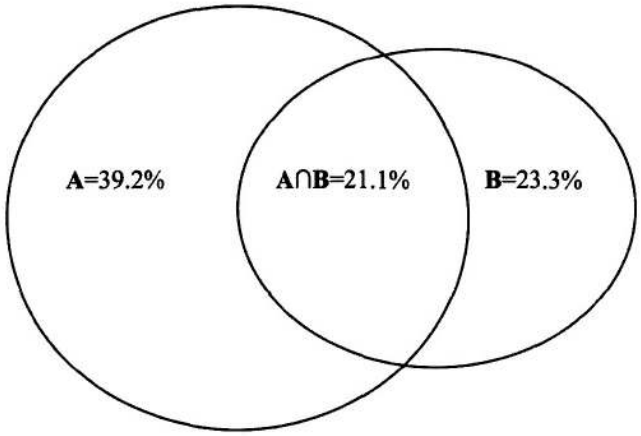
Multi-Ethnic Study of Atherosclerosis

- 921 patients with DM
- established risk factors
  - diabetes duration, HbA1C, systolic blood pressure, waist-to-hip ratio, use of diabetes medications
- novel markers
  - C-reactive protein, homocysteine, fibrinogen, plasmin-alpha(2)-antiplasmin complex (PAP), interleukin-6, d-dimer, factor VIII, serum creatinine, and urinary albumin-to-creatinine (UAC) ratio
  - After adjusting for established risk factors
    - Fibrinogen and PAP were associated with any DR
    - PAP & homocysteine were associated with vision-threatening DR

Nguyen *et al. Diabetes Care 2009*

Multi-Ethnic Study of Atherosclerosis

- Area under receiver-operator characteristic curve (AUC) for DR for established and novel risk factors
  - Established risk factors accounted for a 39.2% increase of the AUC, whereas **novel markers only accounted for an additional 2.2%.**



☞ suggest that there is limited clinical use of these biomarkers for prediction of diabetic retinopathy

Nguyen *et al. Diabetes Care 2009*

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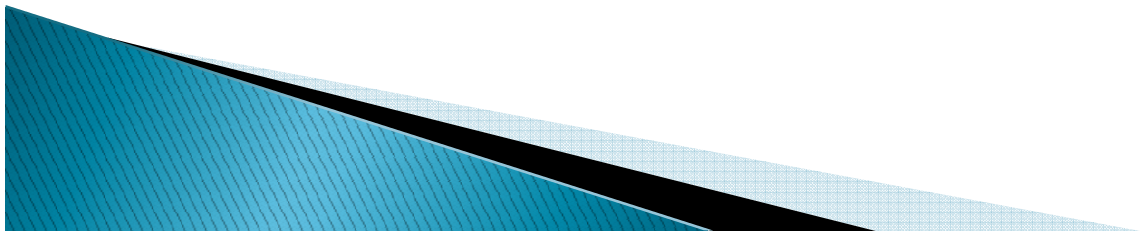
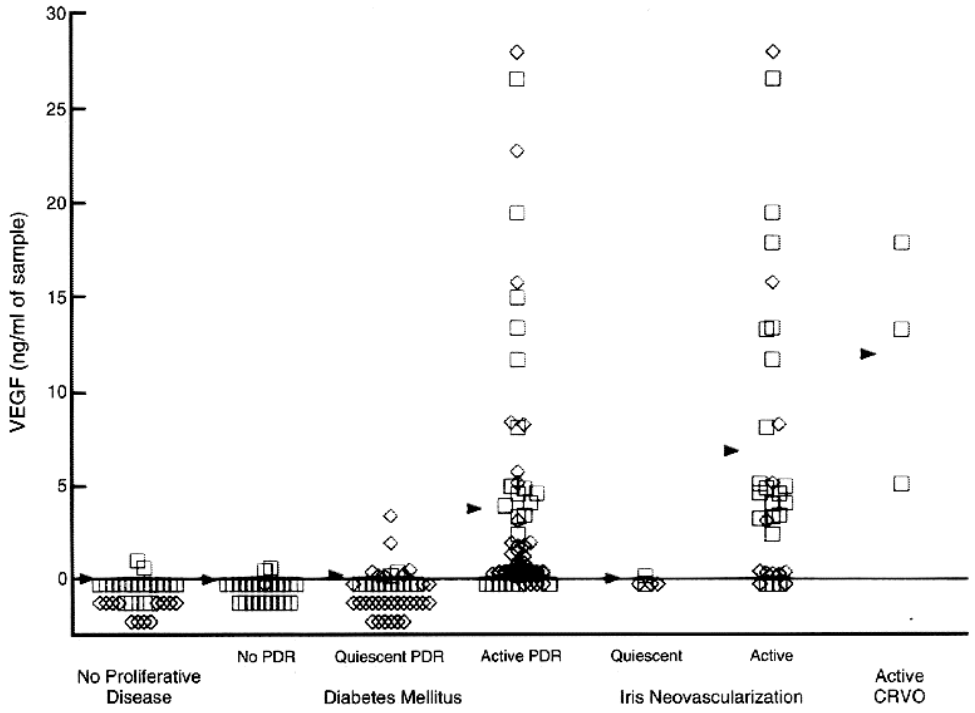


Changes in vitreous proteins

- Significant changes in diabetic retinopathy
  - **VEGF**, SDF-1, HGF, IGF-1, IGF-2, angiogenin, angiopoietin, angiotensin II, endothelin-1, erythropoietin, PEDF, angiostatin, endostatin



Aiello et al. NEJM 1994

Vitreous inflammatory factors and DME

- Vitreous fluid levels of **VEGF, ICAM-1, IL-6, and MCP-1** were significantly **higher** in patients with DME than in nondiabetic patients or diabetic patients without retinopathy.
- In contrast, the **PEDF** level was significantly **lower** in patients with DME than in nondiabetic patients or diabetic patients without retinopathy

	DME	Non-DM	Non-DR
VEGF (pg/ml)	1086.4 (15.6–3450.0)	20.4 (15.6–69.6)*	35.6 (15.6–86.4)†
ICAM-1 (ng/ml)	18.6 (5.84–52.6)	6.44 (5.00–16.2)*	8.42 (5.00–18.4)†
IL-6 (pg/ml)	192.4 (18.0–823.4.)	8.74 (4.00–23.2)*	18.8 (4.00–66.4)†
MCP-1 (pg/ml)	1764.4 (176.4–3298.6)	426.3 (116.4–1128.6)*	678.4 (143.8–1654.2)†
PEDF (ng/ml)	3.20 (1.95–18.8)	23.4 (7.84–56.3)*	24.6 (16.6–39.3)†

Funatsu et al. Ophthalmology 2009

Vitreous Proteome profiling

- one-dimensional SDS-PAGE and nano-LC/MS/MS
- 17 independent vitreous samples
  - **252 proteins from human vitreous** were identified.
  - 56 proteins were differentially abundant in no DR and PDR vitreous compared with no DM vitreous
  - PDR vs no DR
    - increased levels of **angiotensinogen**
    - decreased levels of **calsyntenin-1, interphotoreceptor retinoid-binding protein, and neuroserpin.**
  - PDR vs no DM
    - Increased **complement C3, complement factor I, prothrombin, alpha-1-antitrypsin**, and **antithrombin III**

Gao et al. J Proteome Res 2008

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Proteome Analysis

- Proteome
  - PROTEin expressed by a genOME

- Proteomics
  - 같은 genome을 지닌 생명체의 특정한 생리적인 조건하에서 존재하는 전체 단백질을 대상으로 종류, 분포, 존재량, 성질, 상호 연결망 및 기능 등을 체계적으로 총체적 수준에서 연구하는 기법

- Proteomics 분석 방법의 장점
  - 대량 분석 : 수십 ~ 수천개 단백질 동시 분석
  - 항체 없이 동정 가능 : peptide sequencing 이용

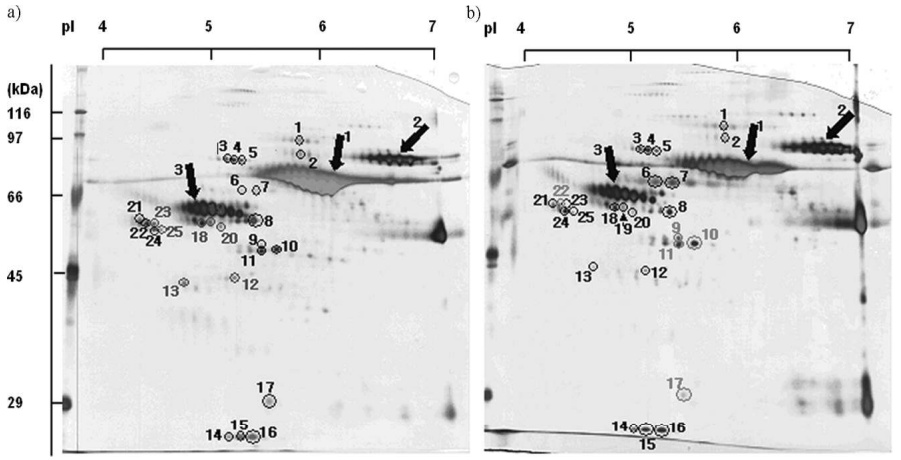
☞ **새로운 biomarker 발굴에 유용**

Vitreous Proteome of PDR

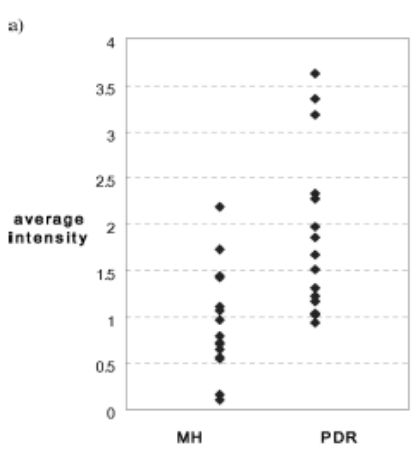
- **Undiluted vitreous** specimen
- 15 active PDR & 15 macular hole

- Proteome analysis
  - **2D electrophoresis and mass spectrometry (MALDI-TOF)**

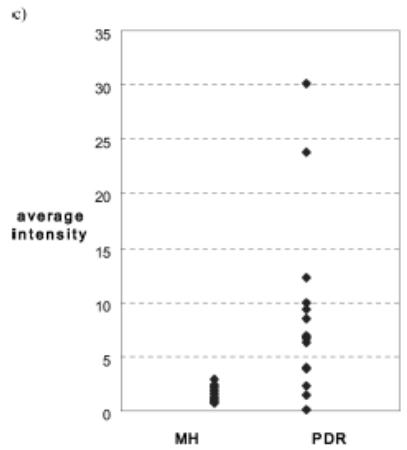
- **25 protein spots** were identified in the 2D gel electrophoresis gels.
- **8 proteins** including PEDF, serine protease inhibitor, apolipoprotein A-IV precursor, and PGH2 d-isomerase were **differentially expressed**.



Macular hole PDR



PEDF



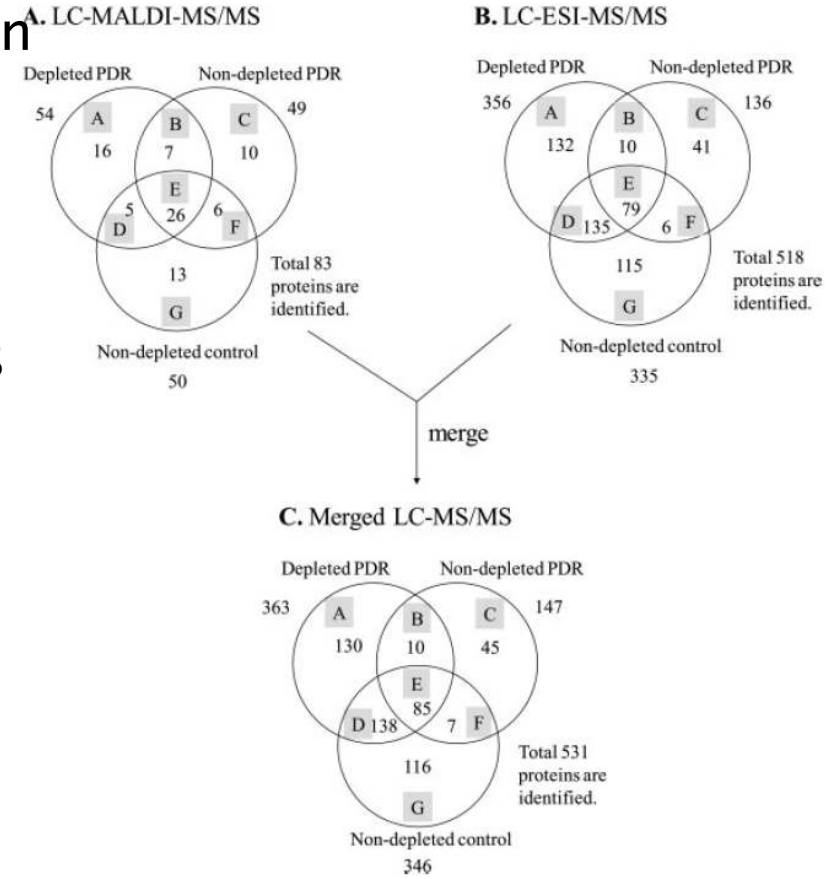
PGH2

Kim et al. Curr Eye Res 2006.

Vitreous Proteome of PDR

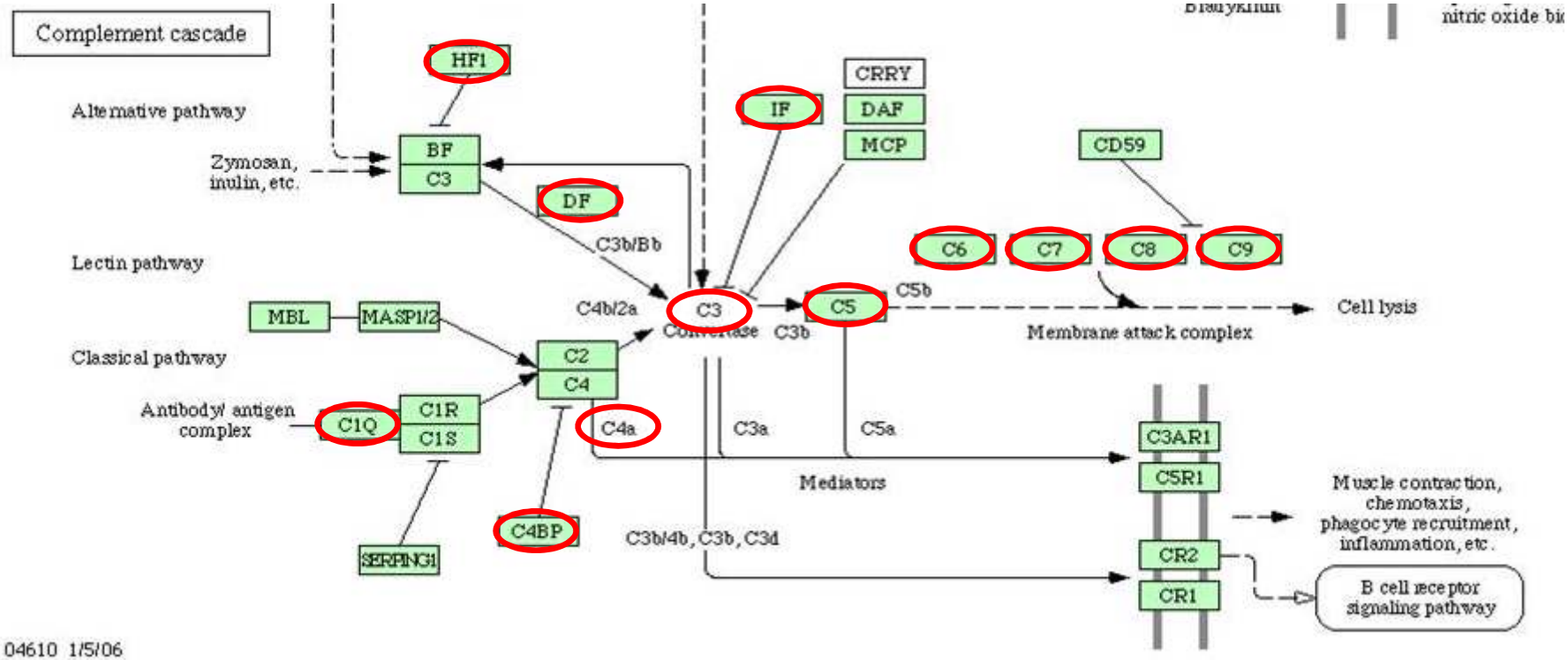
- Undiluted pooled vitreous specimen
- from 14 active PDR & 11 macular hole patients.
- Proteome analysis
  - 1D SDS-PAGE, nano-LC, mass spectrometry (offline LC-MALDI-MS/MS & LTQ LC-ESI-MS/MS)

- **531 proteins were identified**
  - 415 from PDR and 346 from nondiabetic control vitreous

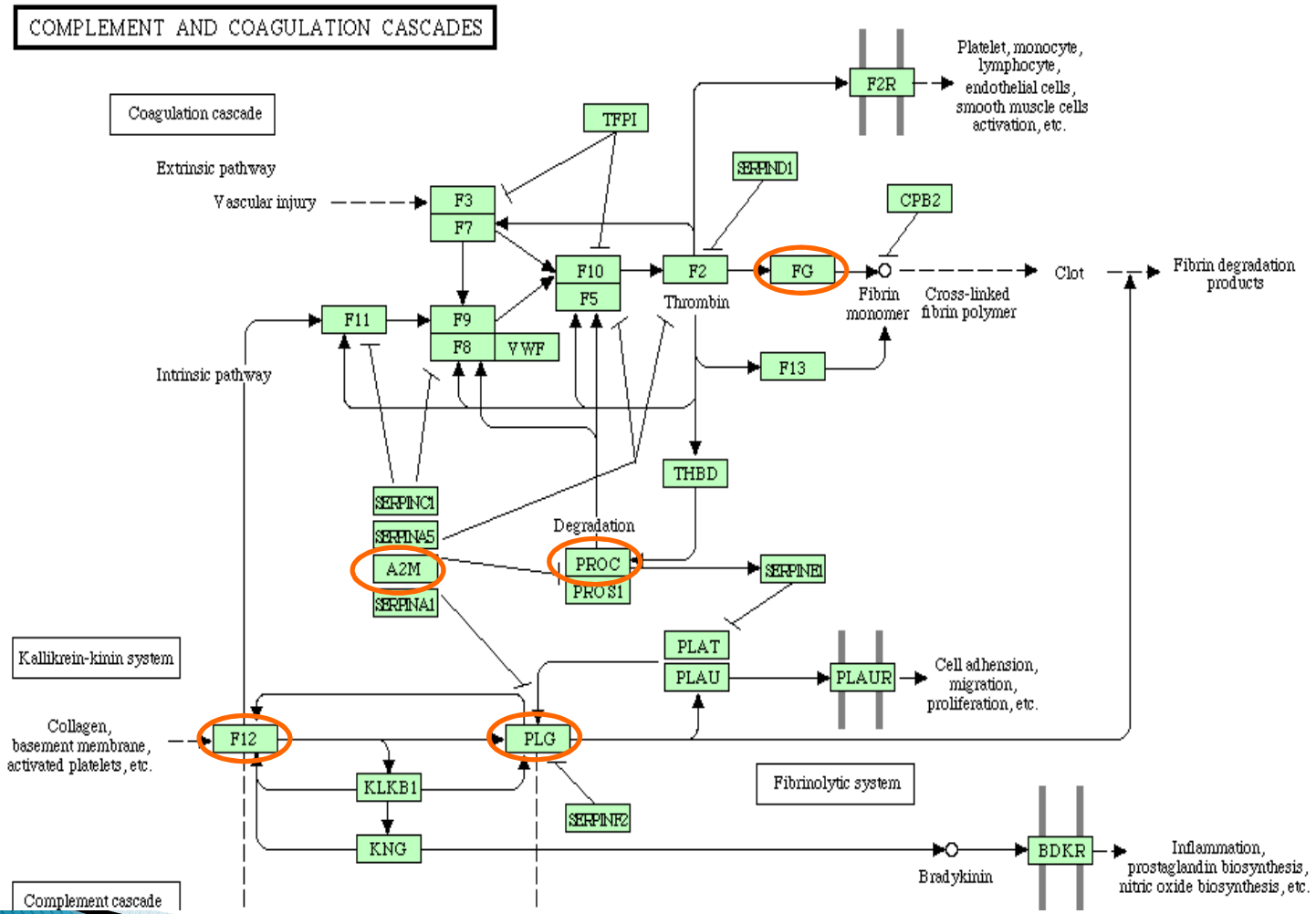


Kim et al. Proteomics. 2007

Identified proteins of complement cascade



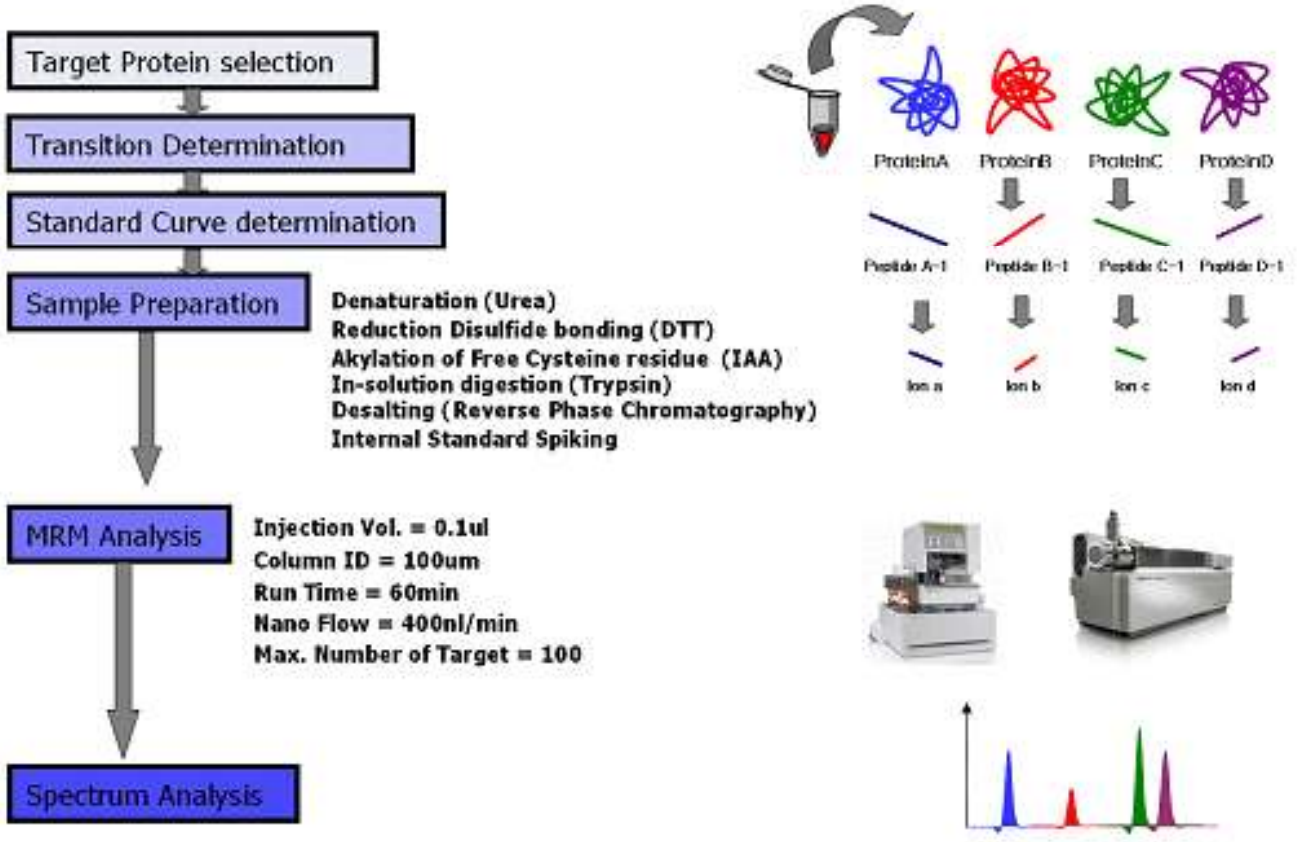
Identified proteins of coagulation cascade & kallikrein-kinin system



Protein Quantification using multiple reaction monitoring (MRM)

- Protein quantification from individual vitreous & plasma

Procedure for MRM



Verification of Vitreous & Plasma Biomarkers using Multiple reaction montoring (MRM)

- Subjects
  - Vitreous & plasma
    - NPDR with ME 15명, PDR 15명, non-diabetic Macular hole 19명
  - plasma
    - DM patients without DR 16명, non-diabetic control 16명
- Methods
  - Relative Quantification (12 proteins)
    - multiple reaction monitoring (MRM) using triple quadrupole LC-MS/MS

- 12 Target proteins
  - thyroxine binding globulin (TBG)
  - gamma-Glutamyl Hydrolase
  - Kallistatin
  - von Willebrand factor
  - Hepatocyte growth factor activator
  - Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)
  - coagulation factor IX Precursor
  - Myocilin
  - Peroxiredoxin 2
  - Haptoglobin
  - Apolipoprotein B100
  - PEDF

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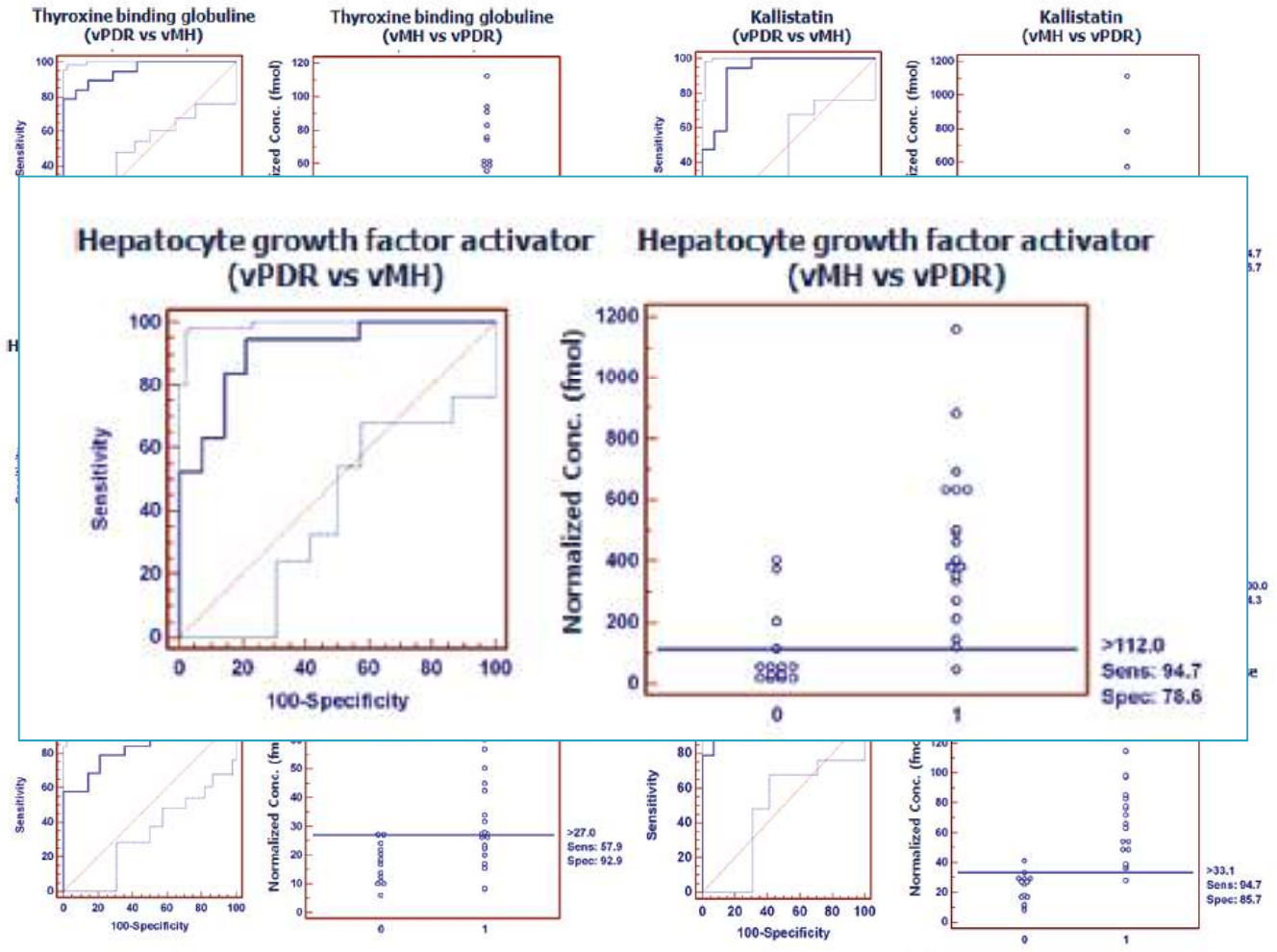
ROC curves and interactive plots of MRM in PDR versus MH vitreous



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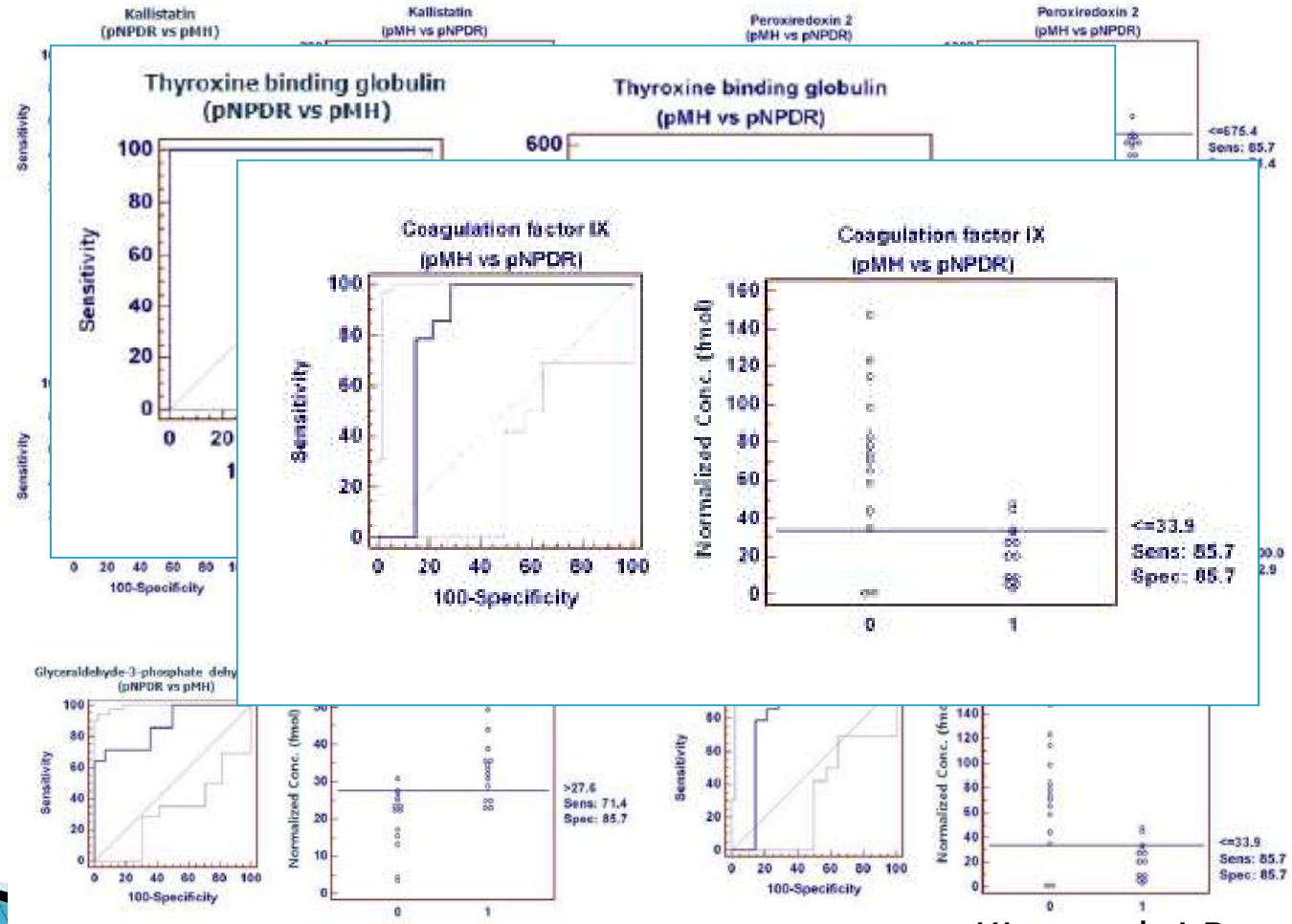
ROC curves and interactive plots of MRM in NPDR versus MH plasma



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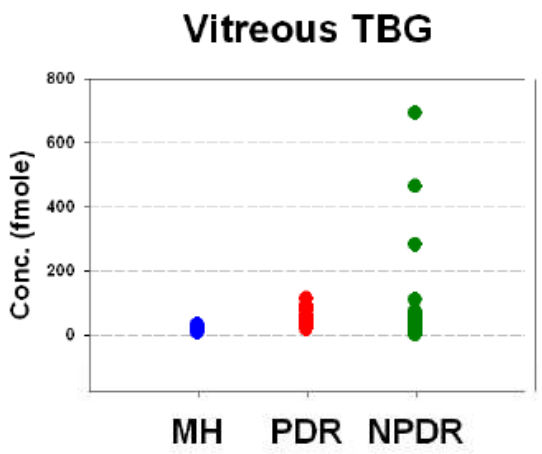
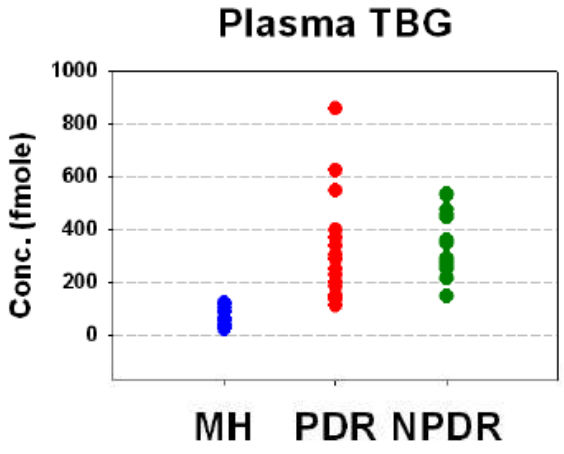
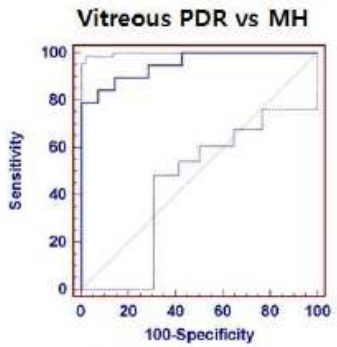
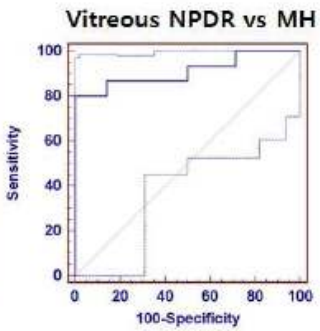
Interactive plots and ROC curves of TBG in vitreous and plasma



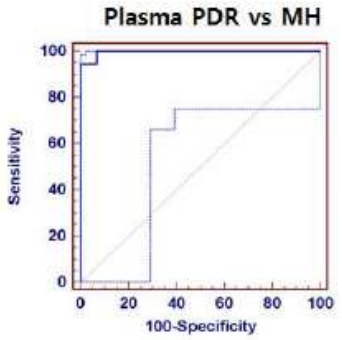




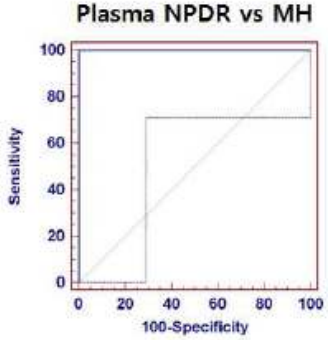
- AUC : 0.951
- Specificity : 100
- Sensitivity: 70
- *p*-value : 0.00011



- AUC : 0.933
- Specificity : 100
- Sensitivity: 73.3
- *p*-value : 0.0117



- AUC : 0.996
- Specificity : 93.3
- Sensitivity: 94.4
- *p*-value : 0.0001

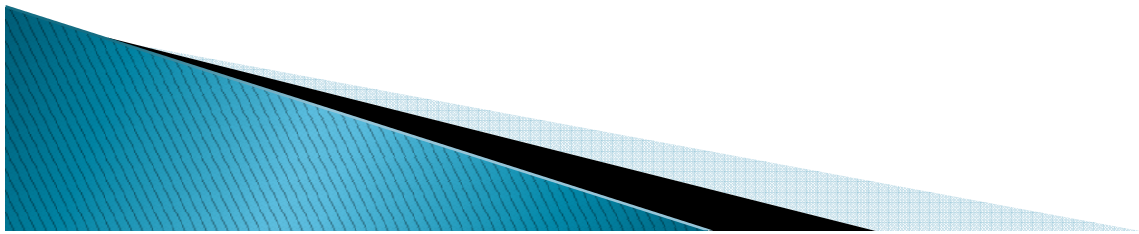


- AUC : 0.999
- Specificity : 93.3
- Sensitivity: 100
- *p*-value : > 0.0001

Kim et al. J Proteome Res 2009

Vitreous & Plasma Biomarker in DR – summary of our results

- **Vitreous proteome profiling** *(Kim et al. Proteomics 2007)*
  - Search for candidate biomarker of diabetic retinopathy
  - 531 vitreous proteins were identified
- Selection of candidate : 12 proteins
- **Quantification of 12 proteins in vitreous & plasma** *(Kim et al. J Proteome Res 2009)*
  - in patients with PDR, NPDR, and nondiabetic patients
  - Quantification using Multiple reaction monitoring (MRM)
    - Vitreous TBG, kallistatin, HGF activator, vWF, GAPDH
      : increased in NPDR & PDR compared to non-diabetic controls
    - Plasma TBG, GAPDH, kallistatin
      : increased in DR compared to non-diabetic controls
    - Plasma coagulation factor IX, haptoglobin, peroxiredoxin 2, vWF
      : decreased in DR compared to non-diabetic controls



Conclusion

- Development of Biomarker for diabetic retinopathy
  - To identify susceptible individuals
  - For early diagnosis
  - To decide treatment methods
  - To predict treatment outcome
  - To predict visual prognosis

- Multimodal approach for development of biomarkers
  - Ophthalmic : fundoscopic, angiographic, and from ocular specimens
  - Genetic
  - Systemic factors including plasma protein