UNIVERSITÀ DEGLI STUDI FIRENZE

DENOThe
Centro di Ricerca
Trasferimento e
Alta Formazione

DENOThe Meeting 2019

Martedì 1 Ottobre
Aula Magna (Ex-Presidenza di Medicina)

Strategie per lo studio della malattia vascolare e aterotrombotica

Rosina De Cario

Unità di Ricerca 4 – Prof.ssa Betti Giusti

Centro Malattie Aterotrombotiche

Obiettivi specifici sono:

Valutazione personalizzata in prevenzione primaria e secondaria del rischio vascolare aterotrombotico mediante marcatori bioumorali, genetici, cellulari e della funzione endoteliale;

Valutazione dell'efficacia della terapia antiaggregante in pazienti vascolari ad alto rischio;

Farmacogenetica di farmaci antiaggreganti, anticoagulanti ed ipolipidemizzanti;

Ricerca/diagnostica genetico-molecolare della Sindrome di Marfan e disordini correlati;

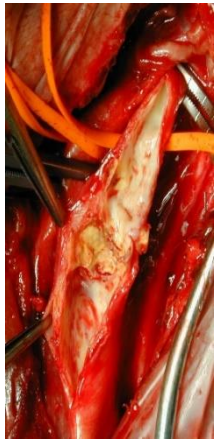
Ricerca/Diagnostica genetico-molecolare dei difetti ereditari della coagulazione e piastrinopatie;

Ricerca/Diagnostica genetico molecolare delle dislipidemie;

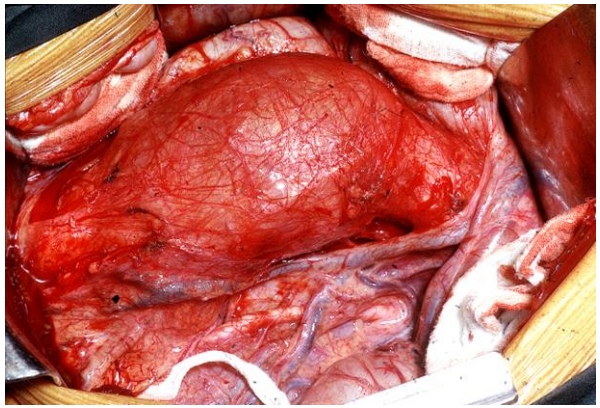
Implementazione dello studio della fibrinolisi con metodi globali;

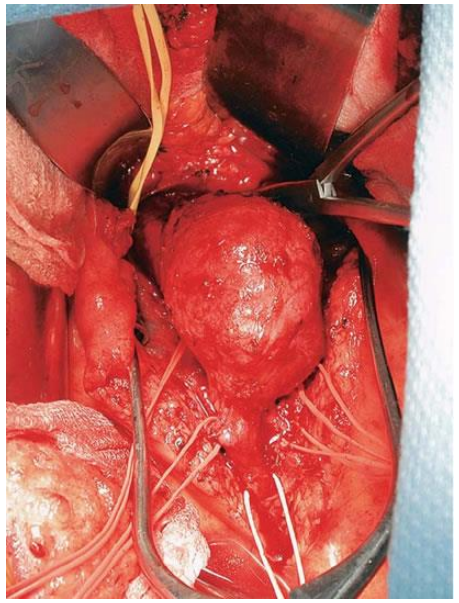
Coordinamento e manutenzione della Rete dell' AOU Careggi per l'utilizzo accurato dei POCT per l'emostasi

Carotid artery stenosis

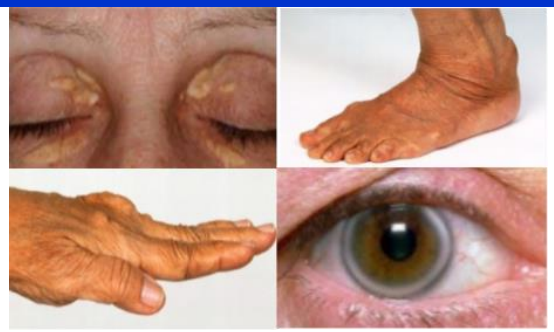


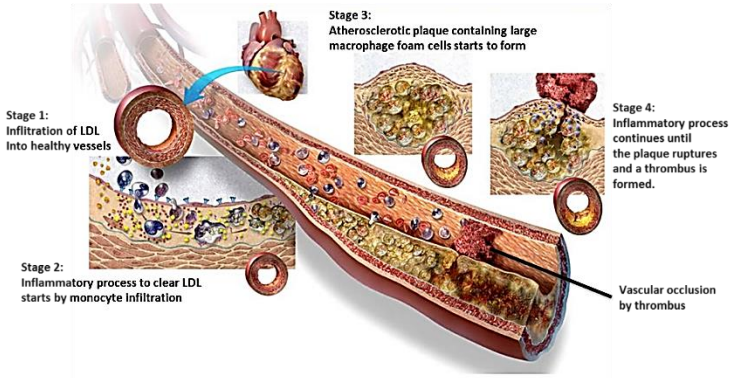
Thoracic/Abdominal Aortic Aneurysm



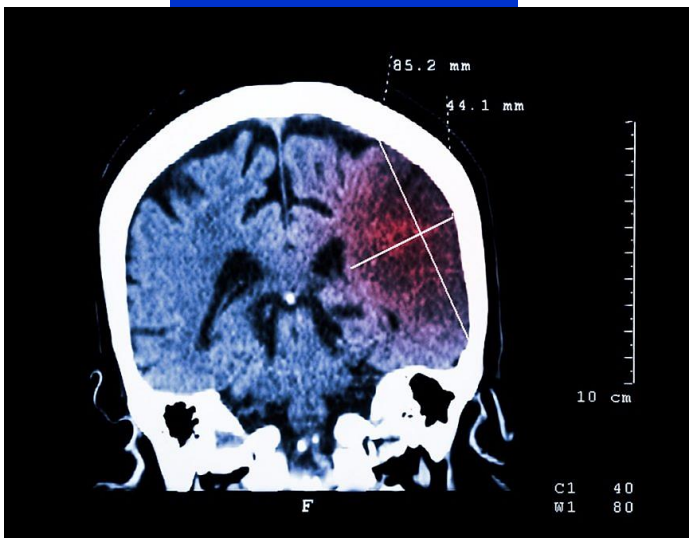


Familial Hypercholesterolemia





Ischemic Stroke



Tasks

1. Deepening knowledge on the pathophysiological mechanisms of cardiovascular and atherothrombotic diseases

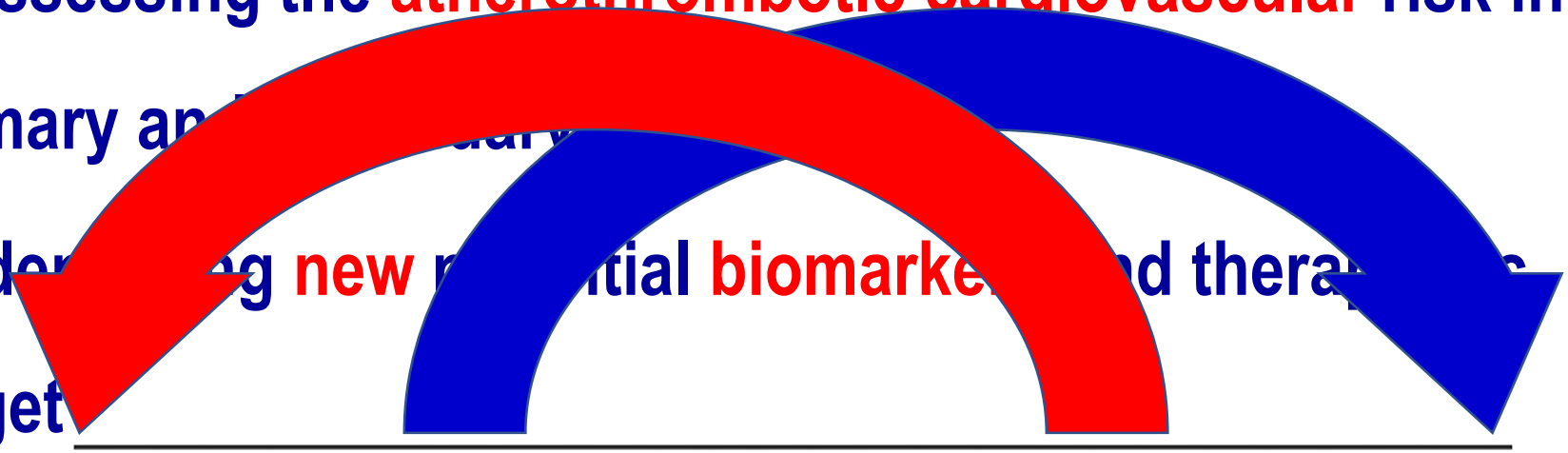
DIAGNOSTICS & RESEARCH

RESEARCH

DIAGNOSTIC

2. assessing the atherothrombotic cardiovascular risk in primary an

3. ide g new p tial biomarke d thera s target



4. evaluating the efficacy of medical therapies

Aree Laboratorio Integrato

Biologia Molecolare

Genomica e Trascrittomica

Estrazione Acidi Nucleici (DNA, RNA, miRNA)

manuale, automazione piccoli e grandi volumi

Analisi quantitativa e qualitativa acidi nucleici

Genotipizzazione

RFLP in parziale automazione
Real Time Taqman
GenomeLab SNPStream
Affymetrix

Espressione genica

Real Time PCR
Two-color
Affymetrix

Tecnologie di screening mutazionali

(DHPLC, real time HRM)

Sequenziamento

Citofluorimetria e Sorting cellulare

Proteomica

Dosaggi singoli analiti ELISA in parziale automazione

Bioplex

Western Blot

Elettroforesi Bidimensionale

Spettrometria di Massa

HPLC

Microscopia

Colture Cellulari

Modelli Animali

Ingegneria genetica

Bioinformatica

DNA EXTRACTION, QUANTITATIVE/QUALITATIVE EVALUATION, AMPLIFICATION

Freedom EVO 150 platform (Tecan Life Sciences)

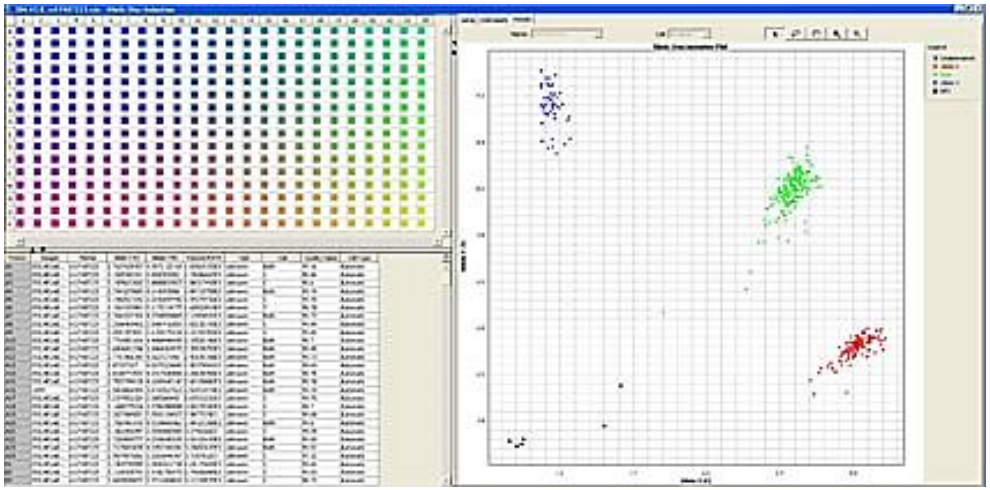
QIAsimphony 9001297 platform (QIAGEN)

Nanodrop Spectrophtometer

T100™ Thermal Cycler (BIO-RAD)

GENOTYPING OF SNPs

7900 HT Fast Real-Time PCR (Applied Biosystems)



Versatile research tool using industry-standard 96- and 384-well formats. In addition, it can also run novel 384-well TaqMan® Low Density Arrays and is equipped with a Fast 96-well block that reduces run times from 2 hours to about 30 minutes.

GENOTYPING OF SNPs

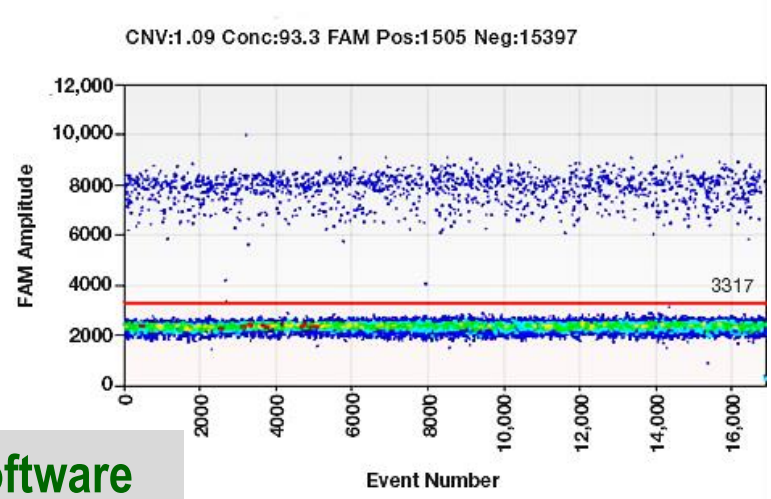
Droplet Digital™ PCR (BIO-RAD)

Reagents and consumables

Droplet reader

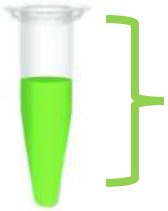
Droplet generator

Thermocycler



QuantaSoft™ Software

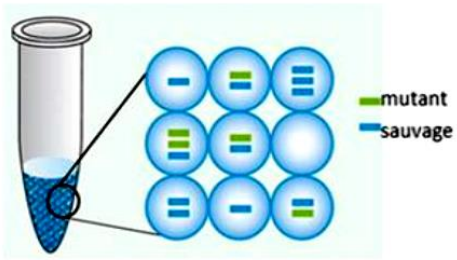
- **Nanodroplet PCR reactions are independent, single amplification events**
- **Partitioning Increases Relative Abundance of Rare Events [0.1% mutant abundance (40 mutant molecules/40,000 wildtype)]**
- **Droplet Readings Converted to a Digital Signal**



ddPCR supermix + Primers/probes + DNA or RNA sample

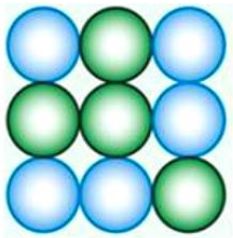
Partition

The mixture of PCR reaction is distributed randomly in ~20000 dropplets. Each constitutes an independent microreactor



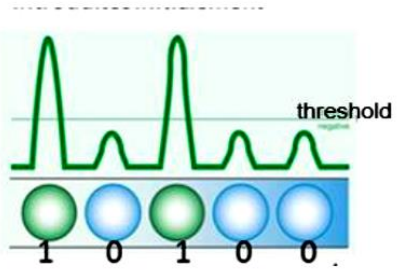
PCR anplification

as in standard PCR



Detection

All dropplets were counted. Fluorescence upper the threshold is considered as a positive PCR, whereas under it's negative.



Compatible with probes (FAM and HEX/VIC) or EvaGreen

Bio-Rad's QX200 Droplet Digital PCR system

TECHNICAL ADVANTAGES OF ddPCR

ABSOLUTE QUANTIFICATION

- Input target counting
- No relative quantification
- End-point measurement

HIGH PRECISION

- Reproducibility
- Discriminability

HIGH SENSITIVITY

- Rare events

Used for challenging applications

- Detect < 2-fold difference of DNA target between samples
- Quantitate low input concentration of DNA target
- Quantitate a rare DNA target in a large wild-type background

DETECTION OF

- COPY NUMBER VARIATIONS (CNVs)
- MOSAICISM EVENTS AT FREQUENCIES <1%
- RARE MUTATIONS EVENTS (cancer mutations, prenatal diagnosis, transplanted organs)
- microRNA
- METHYLATION
- TELOMERASE [telomere repeat amplification protocol (TRAP) assay]

PHARMACOGENETICS

Association of CYP2C19*2 loss-of-function polymorphism with occurrence of major adverse cardiovascular events (MACE) in high risk vascular patients.

Study	N Subjects	Treatment	Outcome	CYP2C19 alleles	Association
Trenk et al. [200					**No**
Si					
Colle					6.02)
Giusti et al. [2009]	772 PCI	75 mg/day maintaining	**ST (6 months)**	*2	**Yes** (OR=2.70) **Yes** (OR=3.43)
Sibbing et al. [2009]	2485 PCI+stent	600 mg LD + 75 mg/day maintaining	**ST (30 days)**	*2	**Yes** (HR=3.81)

- STATINS

- ANTIHYPERTENSIVE

DRUGS

Giusti et al., Curr Pharmacogenomics Person Med 2010

GENETIC SUSCEPTIBILITY

Characteristics	Control subjects (n = 423)	AAA (n = 423)	P value
Age, years	72.0 (41-94)	73.5 (40-94)	.651
Sex (male)	366 (86.5)	376 (88.9)	.295
Smoking habit	267 (63.1)	366 (86.5)	<.0001
Diabetes	49 (11.6)	41 (9.7)	.372
Hypertension	179 (42.3)	302 (71.4)	<.0001
Dyslipidemia	50 (11.8)	195 (46.1)	<.0001
COPD	66 (15.6)	311 (73.5)	<.0001
CAD	107 (25.3)	163 (38.5)	<.0001
CVD	38 (9.0)	111 (26.2)	<.0001
POAD	67 (15.8)	118 (27.9)	<.0001
Aortic diameter, mm	19 (12-47)	50 (31-98)	<.0001

COPD, Chronic obstructive pulmonary disease; *CAD*, coronary artery disease; *CVD*, cerebrovascular disease; *POAD*, peripheral occlusive arterial disease.
Continuous data are presented as median (range) and categoric data as number (%).

The condition of having ≥ 6 genetic risk factors determines a risk of AAA:

OR=6.32 (3.46-11.52), p<0.000000001

*Giusti B, et al. J Med Genet 2008
Saracini C et al. J Vasc Surg 2012
Galora S, et al. J Vasc Surg 2013
Galora S, et al. J Vasc Surg 2015

Polymorphisms		OR (95%CI)	p
rs4988300 LRP5	(T allele)	1.62 (1.02-2.56)	0.040
rs3781590 LRP5	(T allele)	1.83 (1.17-2.85)	0.008
rs1466535 LRP1	(T allele)	1.85 (1.20-2.84)	0.010
rs243865 MMP2	(CC genotype)	1.81 (1.17-2.94)	0.007
rs3025058 MMP3	(5A allele)	1.82 (1.04-3.12)	0.034
rs2252070 MMP13	(GG genotype)	2.14 (1.18-3.86)	0.012
rs2071307 ELN	(GG genotype)	1.56 (1.01-2.44)	0.046
rs8003379 MTHFD1	(GG genotype)	2.44 (1.54-3.85)	<0.0001
rs326118 MTRR	(GG genotype)	2.13 (1.30-3.45)	0.003

Familial Hypercholesterolemia

Dyslipidemia

Hypertriglyceridemia

LDLR, APOB, PCSK9, LDLRAP1, APOE, APOA2, APOA5, LIPI, ABCA1, EPHX2, PPP1R17, GHR, ITIH4, BTN2A1, NPC1L1, ABCG5, ABCG8, APOA1, APOC3, APOA4, LIPC, LPL, PON1, CETP, LRP1, SREBF1, SCARB1, SREBF2, GCKR, CREB3L3, APOC2, CELSR2, SLC22A1, HFE, MYLIP, ST3GAL4, NYNRIN, CH25H, INSIG2, DAB2, STAP1, GPIHBP1, LMF1, SLCO1B1, ABCG2, ABCB1, HMGCR, ANGPTL3, GPD1, LCAT, MTTP, NPC1, NPC2, OSBPL5, SAR1B

HDL deficiencies

Polygenic FH

Pharmacogenetics

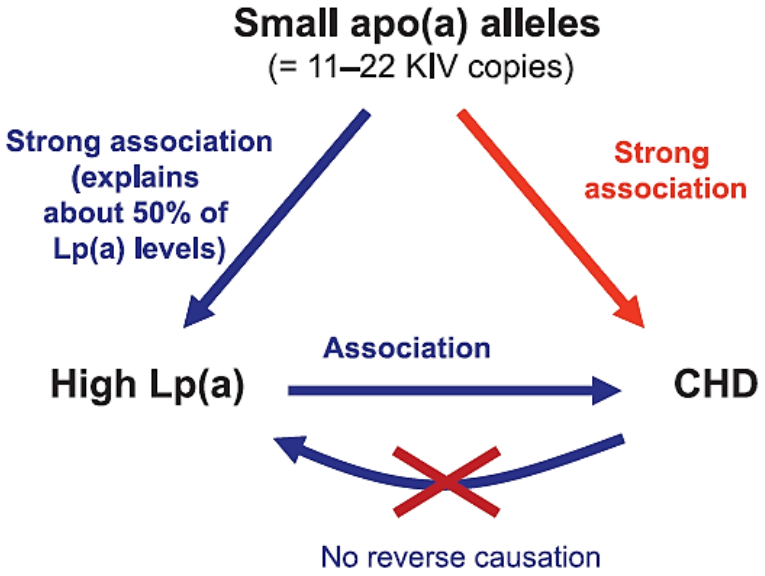
Targeted 55 genes panel (FH)

Targeted 97 genes panel (MFS and related disorders)

COL11A1; MTHFR; PLOD1; ADAMTSL4; MFAP2; PTGS2; SKI; TGFB2; CAPN2; AGT; MTR; TGFBR3; FGF8; RET; ACTA2; B3GAT3; LTBP3; EFEMP2/fbln4; LRP5; CCND1; LRP6; COL2A1; LRP1; DCN; LTBP2; TGFB3; FBLN5; ADAMTS17; CHST14; FBN1; SMAD3; MYH11; ABCC6; MAPK3; PDIA2; AXIN1; MMP2; CRYBA1; COL1A1; ACE; KCNJ2; EMILIN2; SMAD2; SMAD4; LTBP4; TGFB1; ADAMTS10; MMADHC; ACVR1; COL3A1; COL5A2; FN1; COL6A3; EMILIN1; LTBP1; JAG1; EMILIN3; MMP9; SLC2A10; GATA5; CBS; COL6A1; COL6A2; UFD1L; MAPK1; VHL; ZPLD1; MYLK; AGTR1; PDCD10; TGFBR2; FBN2; NKX2-5; B4GALT7; ADAMTS2; AGGF1; MTRR; NOS3; HOXA1; CCM2; KRIT1; COL1A2; TGFBR1; PTGS1; ENG; COL5A1; NOTCH1; GNAQ; AGTR2; FLNA; ELN; COL9A1; COL11A2; TNXB

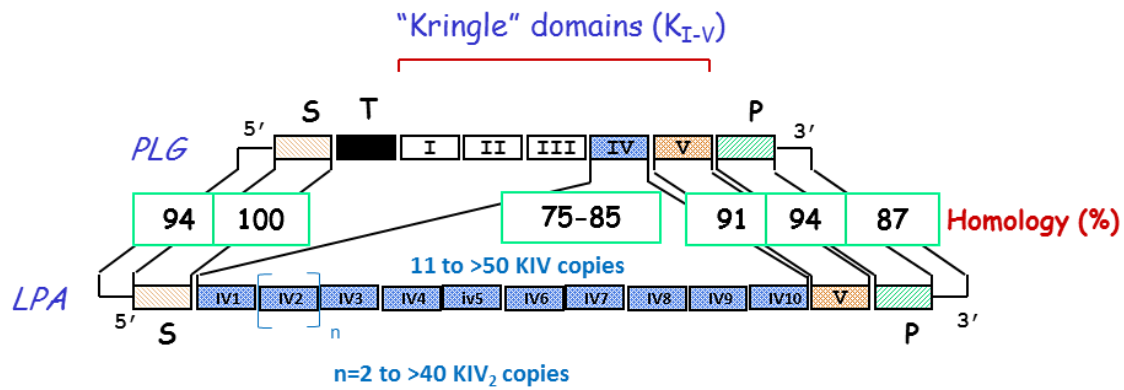
Lipoprotein(a): resurrected by genetics

- F. Kronenberg[1] & G. Utermann[2]



Kringle IV: classes 1-10
Each class is present as a single copy, except kringle IV class 2 that is present in multiple copies (from 2 to >40 in one allele)

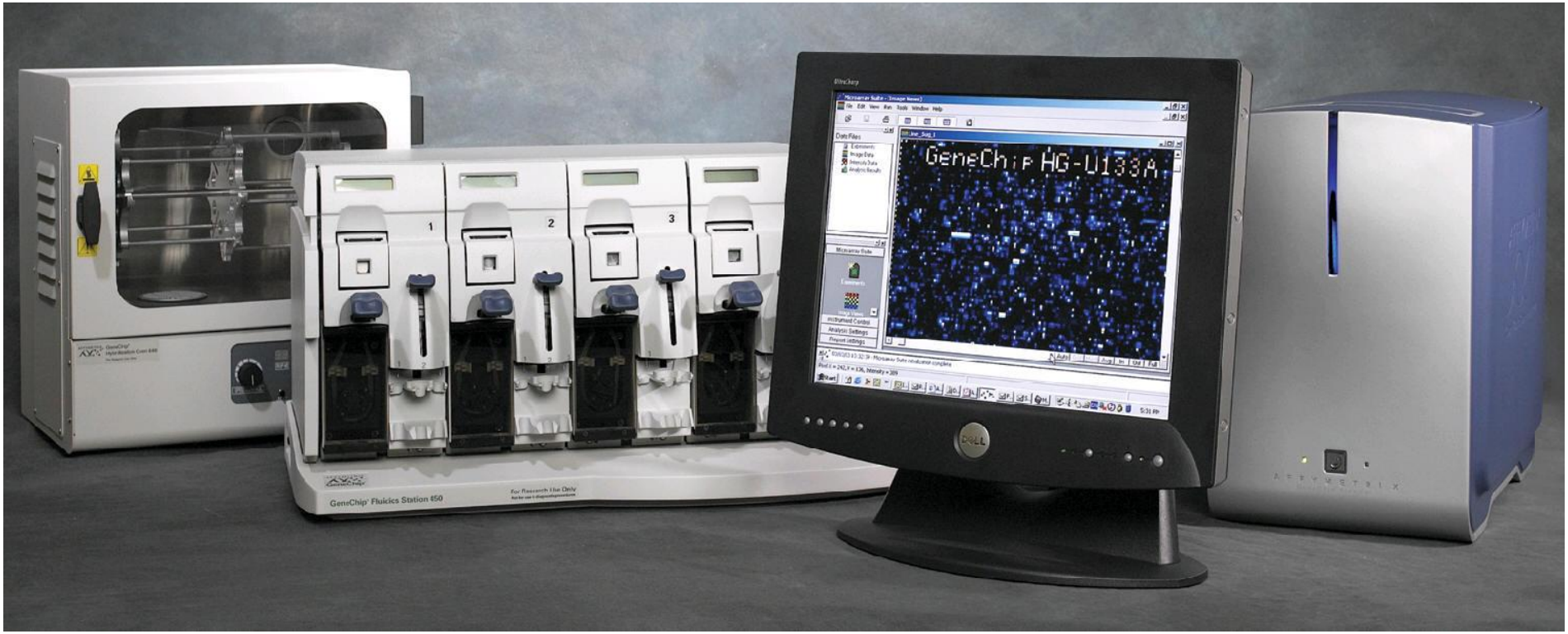
Apolipoprotein (a) gene (*LPA*)

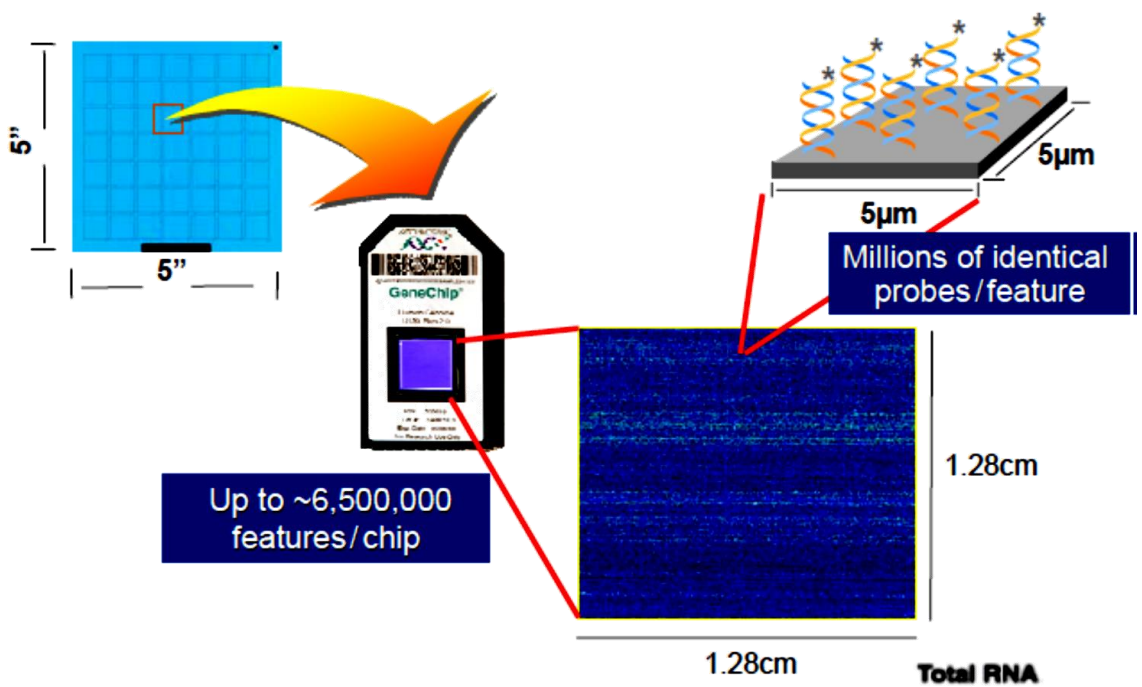


many isoforms of apo(a) (from 300 to 800 KD)

LMW= Higher Lp(a) levels / **HMW**= Lower Lp(a) levels

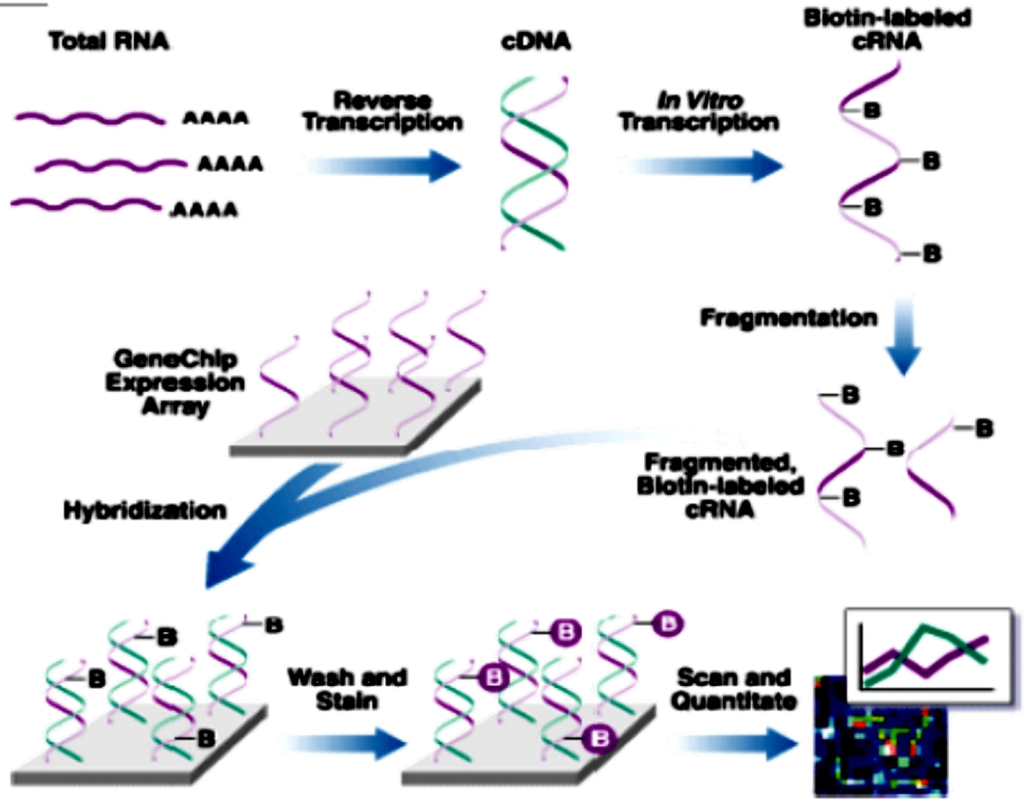
Tecnologia Affymetrix:
Stazione fluidica per applicazione del campione e lavaggi del GeneChip, Incubatore e Analizzatore d'immagine





- A probes is a 25 base pair (25-mer) length piece of DNA that is attached to the chip
- Each feature has millions of identical probes attached to them
- The probes are used to "probe" the sample for certain DNA or RNA segments

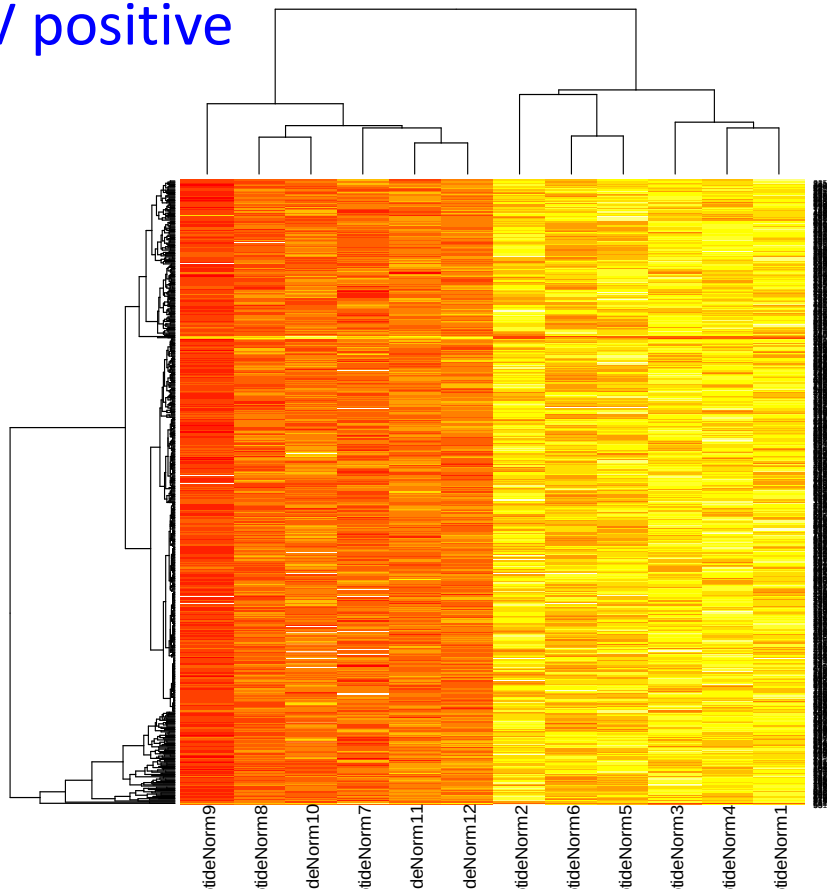
- Estraction and retro/transcription of total RNA
- In-vitro incorporation of biotilinated UTPs and CTPs in cRNA
- Fragmentation
- Hybridization
- Phycoerythrin washing
- Software analysis



Gene expression profiling of carotid biopsies of HCV- and HCV+ patients by Affymetrix GeneChip technology (Human Genome U133 Plus 2.0 Array, 47,000 transcripts)

HCV negative

HCV positive



Patients 1 2 3 4 5 6 7 8 9 10 11 12

278 transcripts differentially up- (n=225) or down-regulated (n=53) in HCV+ vs HCV- patients with carotid artery stenosis

Total RNA isolated from carotid artery wall of patients obtained during surgery.

HCV- and HCV+ patients were age and sex matched.

We applied unsupervised hierarchical clustering to both sample and genes, with complete method using euclidean distance as similarity measure.

Affymetrix Gene Expression Profiling of exercise-trained and control rats, effect on cardioprotection (GeneChip Rat Genome 230 v2.0 31,000 transcripts)

Gene Name (Gene Symbol)	Gene ID	GenBank	Probe name	D	r
similar to C11orf17 protein (RGD1306959)	361624	BF561368	1392938_s_at	5.039	1.5
caveolin 3 (Cav3)	29161	NM_019155	1387814_at	4.216	1.4
similar to RIKEN cDNA 1700012G19 gene (RGD1307773)	287115	BG380656	1388881_at	4.206	1.2
similar to C11orf17 protein (RGD1306959)	361624	AA799992	1385458_a_at	4.168	16
enolase 3, beta (Eno3)	25438	NM_012949	1386907_at	4.135	1.5
similar to C11orf17 protein (RGD1306959)	361624	BF561368	1383175_a_at	3.947	1.5
cytochrome P450, family 27, subfamily a, polypeptide 1 (Cyp27a1)	301517	M73231	1387914_at	3.927	1.4
similar to RIKEN cDNA 2700002I20 (RGD1307279)	307210	AI171367	1373074_at	3.783	1.3
EGL nine homolog 1 (Egln1)	308913	BI282122	1389207_at	3.652	1.2
Unknown	NA	BI295165	1373167_at	3.594	1.2
cystatin C (Cst3)	25307	BG666933	1370855_at	3.457	1.3
tumor necrosis factor, alpha-induced protein 1 (endothelial) (Tnfaip1)	287543	BM390023	1371911_at	3.433	1.2

Probe name = Affymetrix number of the probe set that recognized the specific transcript; d = significance analysis of microarrays (SAM) t-statistic; r = fold change according to SAM [mean(TRA intensities)/mean(CTR intensities)].

3 genes characterize the gene expression profile of the rat left ventricle (LV), when examined 48 hrs since the last training section and that mild exercise training determines cardioprotection without the induction of hypertrophy

RISK STUDY

Reperfusion Injury in StroKe (RISK) Study

Azienda Ospedaliero Universitaria Careggi

Accesso riservato

User name

Password

OK

Principal investigator: D. Inzitari

Referenti:

- S. Mangiafico (Neuroradiologia interventistica)
- R. Abbate (Markers Biologici
- M. Moretti (Markers neuroradiologici
- M. Nesi (Coordinatore clinico

Clinici:

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- F. Arba
- P. Nencini
- S. Nannoni
- M. Lamassa
- L. Pantoni
- F. Pescini
- A. Poggesi
- C. Sarti

Students:

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- A. Giannini
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- S. Vanni

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- A.M. Gori

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- P. Pezzati

Data manager:

- G. Pracucci

Neuroradiologi:

- P. Simonelli
- D. Gadda

Neuroradiologi interventisti:

- A. Consoli
- N. Limbucci
- S. Nappini

Reperfusion Injury in StroKe Study

(RISKS)

Coordinatore: **Prof. Domenico Inzitari**
Dipartimento NEUROFARBA, Università degli Studi di Firenze

Highlights dalla letteratura

Reperfusion Injury in Stroke

L'ictus cerebrale rappresenta la terza causa di morte e la prima di disabilità nella popolazione adulta. I trattamenti di fase acuta possono consentire la ricanalizzazione del vaso occluso, salvando il tessuto dal danno ischemico. La ricanalizzazione, tuttavia, non è sempre accompagnata da un miglioramento clinico poiché può verificarsi il cosi detto "danno da riperfusione". Studi preliminari mostrano che la rottura della barriera emato-encefalica (BEE), espressione del danno da riperfusione, può essere misurata in vivo con TC di perfusione (TCP). Evidenze sperimentali e cliniche hanno dimostrato che alcuni biomarcatori circolanti (come le metalloproteinasi) possono essere considerati potenziali marcatori biologici di danno da riperfusione. Lo studio dell'associazione fra biomarcatori circolanti e marcatori neuroradiologici di danno della BEE in relazione alle misure di esito clinico potrebbe fornire informazioni importanti per contrastare il danno da riperfusione. In una serie consecutiva di pazienti con ictus ischemico acuto candidati a trombolisi sistemica o a trattamento endovascolare, verranno misurati livelli di una serie di biomarcatori circolanti di rottura della BEE/danno da riperfusione (fattori pro-, anti-infiammatori, fattori immunomodulatori, induttori/inibitori di MMP, fattori di disfunzione endoteliale, fattori di resistenza alla fibrinolisi), in relazione al grado di permeabilità della BEE. Utilizzando un protocollo di studio definito, un disegno prospettico, ed una numerosità del campione tale da garantire un'adeguata potenza statistica, i riaultati di questo studio potrebbero integrare ed espandere le informazioni relative ai fattori biologici o ai marcatori surrogati coinvolti nel danno da riperfusione nell'ictus acuto. I dati ottenuti potrebbero supportare il disegno di trial randomizzati e controllati dove vengano testate farmaci potenzialmente efficaci nel contrastare il danno della BEE/danno da riperfusione.

Reperfusion Injury in Stroke

Ischemic stroke is a major cause of death and disability. Revascularization techniques are able to re-open the occluded vessel, salvaging the ischemic tissue from death. However recanalization may cause further injury due to activation of detrimental molecular pathways, leading to "reperfusion injury". Preliminary data show that blood brain barrier (BBB) disruption and reperfusion injury can be traced in vivo by perfusion CT (TCP). Both experimental and clinical evidence indicate a number of circulating molecular factors (like metalloproteinases) as potential biological markers of the same injury. Investigating interactions between circulating factors and BBB permeability, in relation to stroke outcomes may be of help aiming to contrast the problem of reperfusion injury. In a consecutive series patients with acute ischemic stroke candidates to intravenous thrombolysis or to endovascular treatment, circulating levels of diverse molecular markers of BBB disruption/ reperfusion injury (pro-, anti-inflammatory, and immunomodulatory factors, matrix MMPs and their inductors/inhibitors, factors of endothelial dysfunction, factors of fibrin resistance to lysis), will be determined, and analysed in relation to in vivo measurement of BBB permeability. Using a definite protocol, a prospective collection of data, and an adequate number of patients assuring statistically powered data, this study can expand and integrate substantially scanty clinical information about biological factors or surrogate markers involved in the damage of brain tissue after reperfusion in acute cerebral ischemia. Data obtained may eventually support designing randomized control trials where therapeutic strategies potentially effective in contrasting BBB disruption/reperfusion damage are tested.

Highlights dalla letteratura

- *Front Neurol.* 2015 May 27;6:121. doi: 10.3389/fneur.2015.00121. eCollection 2015.
  Unbalanced Metalloproteinase-9 and Tissue Inhibitors of Metalloproteinases Ratios Predict Hemorrhagic Transformation of Lesion in Ischemic Stroke Patients Treated with Thrombolysis: Results from the MAGIC Study.
  Piccardi B, Palumbo V, Nesi M, Nencini P, Gori AM, Giusti B, Pracucci G, Tonelli P, Innocenti E, Sereni A, Stiochi E, Toni D, Bovi P, Guidotti M, Tola MR, Consoli D, Micieli G, Tassi R, Orlandi G, Perini F, Marcello N, Nucera A, Massaro F, DeLodovici ML, Bono G, Sessa M, Abbate R, Inzitari D.
  BACKGROUND: Experimentally, metalloproteinases (MMPs) play a detrimental role related to the severity of ischemic brain lesions. Both MMPs activity and function in tissues reflect the balance between MMPs and tissue inhibitors of metalloproteinases (TIMPs). We aimed to evaluate the role of MMPs/TIMPs balance in the setting of rtPA-treated stroke patients.
  METHODS: Blood was taken before and 24-h after rtPA from 327 patients (mean age 68 years, median NIHSS 11) with acute ischemic stroke. Delta median values of each MMP/TIMP ratio [(post rtPA MMP/TIMP-baseline MMP/TIMP)/(baseline MMP/TIMP)] were analyzed related to symptomatic intracranial hemorrhage (sICH) according to NINDS criteria, relevant hemorrhagic transformation (HT) defined as confluent petechiae within the infarcted area or any parenchymal hemorrhage, stroke subtypes (according to Oxfordshire Community Stroke Project) and 3-month death. The net effect of each MMP/TIMP ratio was estimated by a logistic regression model including major clinical determinants of outcomes.
  RESULTS: Adjusting for major clinical determinants, only increase in MMP9/TIMP1 and MMP9/TIMP2 ratios remained significantly associated with sICH (odds ratio [95% confidence interval], 1.67 [1.17-2.38], p=0.005; 1.74 [1.21-2.49], p=0.003, respectively). Only relative increase in MMP9/TIMP1 ratio proved significantly associated with relevant HT (odds ratio [95% confidence interval], 1.74 [1.17-2.57], p=0.006) with a trend toward significance for MMP9/TIMP2 ratio (p=0.007).
  DISCUSSION: Our data add substantial clinical evidence about the role of MMPs/TIMPs balance in rtPA-treated stroke patients. These results may serve to generate hypotheses on MMPs inhibitors to be administered together with rtPA in order to counteract its deleterious effect.
  PubMed
- *Stroke.* 2013 Oct;44(10):2901-3. doi: 10.1161/STROKEAHA.113.002274. Epub 2013 Aug
  MMP9 variation after thrombolysis is associated with hemorrhagic transformation of lesion and death.
  Inzitari D, Giusti B, Nencini P, Gori AM, Nesi M, Palumbo V, Piccardi B, Armillis A, Pracucci G, Bono G, Bovi P, Consoli D, Guidotti M, Nucera A, Massaro F, Micieli G, Orlandi G, Perini F, Tassi R, Tola MR, Sessa M, Toni D, Abbate R; MAGIC Study Group.
  BACKGROUND AND PURPOSE: Experimentally, matrix metalloproteinases (MMPs) play a detrimental role related to hemorrhagic transformation and severity of an ischemic brain lesion. Tissue-type plasminogen activator (tPA) enhances such effects. This study aimed to expand clinical evidence in this connection.
  METHODS: We measured MMPs 1, 2, 3, 7, 8, 9, and tissue inhibitors of metalloproteinases 1, 2, 4 circulating level in blood taken before and 24 hours after tPA from 327 patients (mean age, 68.9±12.1 years; median National Institutes of Health Stroke Scale, 11) with acute ischemic stroke. Delta median values ([24 hours post tPA-pre tPA]/pre tPA) of each MMP or tissue inhibitors of metalloproteinase were analyzed across subgroups of patients undergoing symptomatic intracerebral hemorrhage, 3-month death, or 3-month modified Rankin Scale score 3 to 6.
  RESULTS: Adjusting for major clinical determinants, only matrix metalloproteinase-9 variation proved independently associated with death (odds ratio [95% confidence interval], 1.58 [1.11-2.26]; P=0.045) or symptomatic intracerebral hemorrhage (odds ratio [95% confidence interval], 1.40 [1.02-1.92]; P=0.049). Both matrix metalloproteinase-9 and tissue inhibitors of metalloproteinase-4 changes were correlated with baseline, 24 hours, and 7 days National Institutes of Health Stroke Scale (Spearman P from <0.001 to 0.040).
  CONCLUSIONS: Our clinical evidence corroborates the detrimental role of matrix metalloproteinase-9 during ischemic stroke treated with thrombolysis, and prompts clinical trials testing agents antagonizing its effects.
  PubMed
- *Stroke.* 2010 Mar;41(3):e123-8. doi: 10.1161/STROKEAHA.109.570515. Epub 2009 Dec 24.
  Blood-brain barrier disruption in humans is independently associated with increased matrix metalloproteinase-9.
  Barr TL, Latour LL, Lee KY, Schaewe TJ, Luby M, Chang GS, El-Zammar Z, Alam S, Hallenbeck JM, Kidwell CS, Warach S.
  BACKGROUND AND PURPOSE: Matrix metalloproteinases (MMP) may play a role in blood-brain barrier (BBB) disruption after ischemic stroke. We hypothesized that plasma concentrations of MMP-9 are associated with a marker of BBB disruption in patients evaluated for acute stroke.
  METHODS: Patients underwent MRI on presentation and approximately 24 hours later. The MRI marker, termed hyperintense acute reperfusion injury marker (HARM), is gadolinium enhancement of cerebrospinal fluid on fluid-attenuated inversion recovery MRI. Plasma MMP-9 and tissue inhibitor of matrix metalloproteinase-1 were

EXPERIMENTAL DESIGN

388 patients, 168 underwent thromboectomy

PATIENT

STROKE

Basal blood withdrawal

Serum and Plasma + Blood stabilized in PAX tubes for global gene expression

rtPA

end/or

THROMBECTOMY

24h blood withdrawal

Serum and Plasma + Blood stabilized in PAX tubes for global gene expression

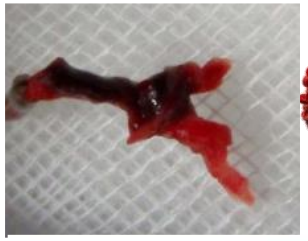
FOLLOW-UP at least 3 months

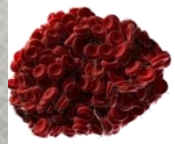
INCLUSION AND EXCLUSION CRITERIA

- Patients with estimated clinical severity NIHSS> 7 eligible for systemic thrombolysis with rtPA and no upper age limit;
- Patients eligible for endovascular thrombolysis and / or thrombectomy;
- Patients without contraindications to the iodinated contrast medium.

Thrombus aspirated during thrombectomy stored in RNA Later

RNA extraction and global gene expression study

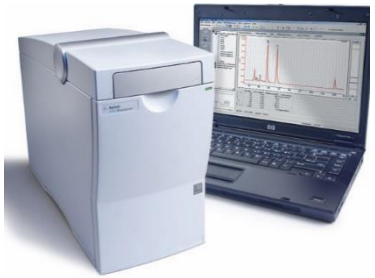


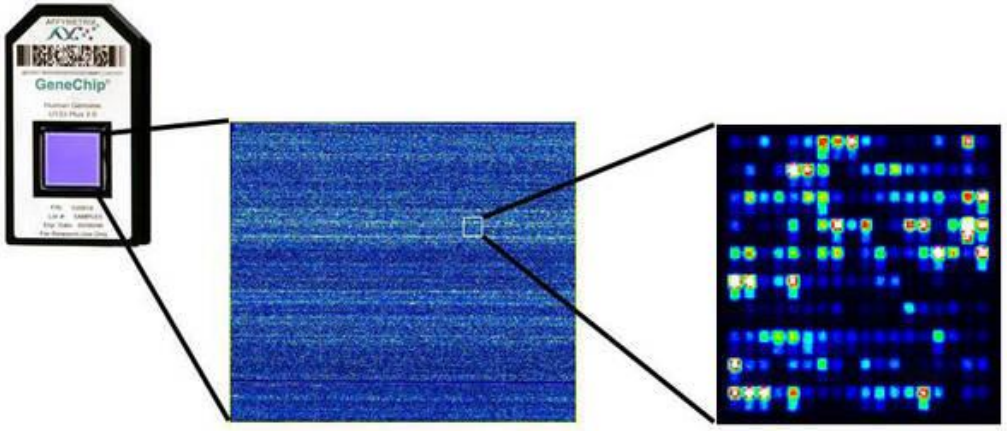


✓ **Thrombus stabilized in RNA Later**

✓ **RNA extraction by PaxGene miRNA kit**

✓ **Quantitative and qualitative analysis by Bioanalyzer *Agilent 2100***





GeneChip Human Transcriptome HTA 2.0 Array (Affymetrix)

Array protein coding content	No.
Genes (transcript clusters)	44,699
Transcripts	245,349
Exons	560,472
Exon clusters	296,058

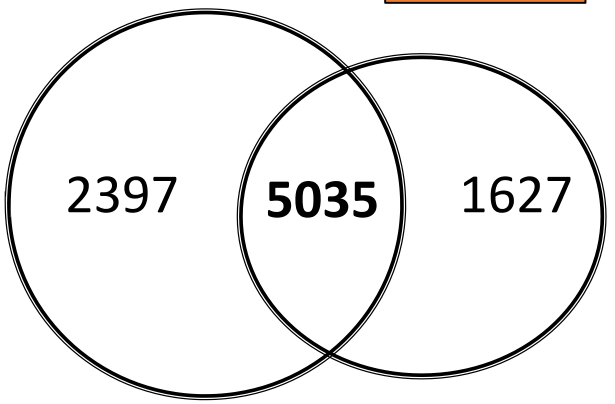
Array non-protein coding content	No.
Genes (transcript clusters)	22,829
Transcripts	40,914
Exons	109,930
Exon clusters	82,444

Controls	
ERCC probe set[1,2]	63
Background probes	Antigenomic set
Poly-A controls[2]	*dap, lys, phe, thr*
Hybridization controls	*bioB, bioc, bioD, creX*

GENETIC EXPRESSION PROFILES ANALYSIS

	PROBE SET	PROBE SET ANNOTTATE	microRNA (mirRNA)	Long intergenic non-protein coding RNA (lncRNA)	GENI
	TROMBI				
MEDIANA	14584	8850,5	285,5	117,5	6986
RANGE	(11619-15496)	(7653-9277)	(226-312)	(62-173)	(6111-7370)
	PROBE SET	PROBE SET ANNOTTATE	microRNA (mirRNA)	Long intergenic non-protein coding RNA (lncRNA)	GENI
	SANGUE VENOSO PERIFERICO				
MEDIANA	12043	7965,5	224	67	6369,5
RANGE	(11389-13031)	(7630-8468)	(194-255)	(53-78)	(6089-6672)

CLOT **MIC**



Aree Laboratorio Integrato

Biologia Molecolare

Genomica e Trascrittomica

Estrazione Acidi Nucleici (DNA, RNA, miRNA)

manuale, automazione piccoli e grandi volumi

Analisi quantitativa e qualitativa acidi nucleici

Genotipizzazione

RFLP in parziale automazione
Real Time Taqman
GenomeLab SNPStream
Affymetrix

Espressione genica

Real Time PCR
Two-color
Affymetrix

Tecnologie di screening mutazionali

(DHPLC, real time HRM)

Sequenziamento

Citofluorimetria e Sorting cellulare

Proteomica

Dosaggi singoli analiti ELISA in parziale automazione

Bioplex

Western Blot

Elettroforesi Bidimensionale

Spettrometria di Massa

HPLC

Microscopia

Colture Cellulari

Modelli Animali

Ingegneria genetica

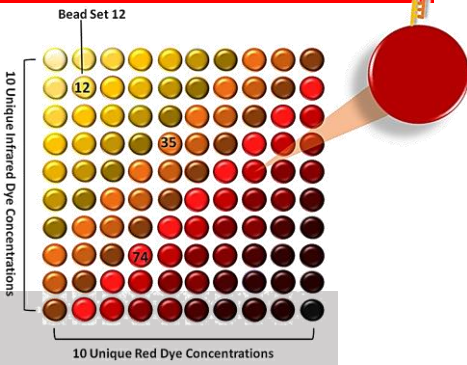
Bioinformatica

IDENTIFICATION OF DIAGNOSTIC BIOMARKERS

BioPlex 200 (BIO-RAD)

Flow-based dual-laser system for simultaneous identification and quantification up to 100 differents analytes in a single biomolecular assay

Fully integrated array reader and microplate platform. Optional HTF (High-Throughput Fluidics) sheath delivery module



- Human cytokines and chemokines
- Antibodies
- Antigens
- Bacterial Protein Toxins
- DNA
- Receptors

- HUMAN
Acute Phase Proteins
Apolipoproteins
Apoptosis Pathway
Chemokines
Cytokines and Growth Factors
Diabetes Markers
Inflammation Markers
Immunotherapy Panel
Isotyping
Kidney Toxicity Markers
Th17 Cytokines

- MOUSE
Chemokines
Cytokines and Growth Factors
Diabetes Markers
Th17 Cytokines

- RAT
Cytokines and Growth Factors
Diabetes Markers
Kidney Toxicity Markers

- MULTI-SPECIES
TGF-Beta Assays
Cell Signaling Assays

MAGIC

MARKERS BIOLOGICI ASSOCIATI ALL'ICTUS CEREBRALE ACUTO

***Ministero della Salute* :**

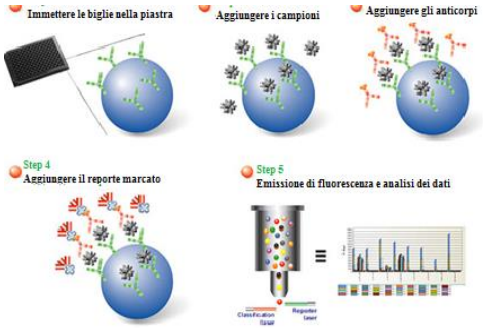
Programma Strategico 2006:

"Nuove conoscenze e problematiche assistenziali nell'ictus cerebrale"

327 pazienti arruolati

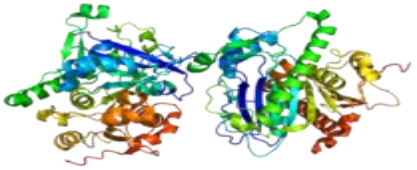
prelievo: eseguito prima e **24h** dopo trombolisi

- ✓ Tecnologia multiplex (BioPlex 200 System) per la misurazione dei livelli dei seguenti



Marcatori: IL-1, TNF-α, IL-6, IL-1β, IL-4, IL-10, IL-1Ra, IL-8, IL-12, IFN-ɣ, IP-10, α2M, PCR

Endopeptidasi e loro inibitori: MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, TIMP-1, TIMP-2;



- Reperfusion Injury after ischemic Stroke Study (RISKS): single-centre (Florence, Italy), prospective observational protocol study (BMJ Open. 2018)

- Low-Calorie Vegetarian Versus Mediterranean Diets for Reducing Body Weight and Improving Cardiovascular Risk Profile: CARDIVEG Study (Cardiovascular Prevention With Vegetarian Diet) (Circulation, 2018)

- Circulating Biomarkers in Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy Patients.(J Stroke Cerebrovasc Dis., 2017) ……..

DIAGNOSTIC OF THE PLATELET FUNCTION

VerifyNow (Werfen)

Multiplate (Roche)

- High productivity: 30 tests / hour
- Sample volume: 300 µL of whole blood
- 5 different positions for the simultaneous measurement of different samples/agonists
- Fast response times: 10 minutes/test

Fluoroskan Ascent™ Microplate Fluorometer (Thermo Fisher)

- General Screening and Anticoagulant Monitoring/Testing
- D-Dimer
- Heparin-Induced Thrombocytopenia
- Antiphospholipid Syndrome
- Coagulation Factors
- Fibrinolysis
- Thrombophilia
- von Willebrand Disease

ACL-TOP 750 series (Werfen)

Aknowledgments

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Dott.ssa Silvia Galora
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Grazie per l'attenzione!