

*PhD Students' works*

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*Progress Report 2010*

**Oncology and Genetics**  
**Doctoral School**

DRAFT



University of Siena





*Progress Report 2010*

**Oncology and Genetics**  
**Doctoral School**

Molecular Biology Department

and

Human Pathology and Oncology Department

Information Engineering Department

Pediatrics, Obstetrics and Reproduction Medicine Department

Surgery Department

Surgery and Bioengineering Department

and

I.T.T. Istituto Toscano Tumori

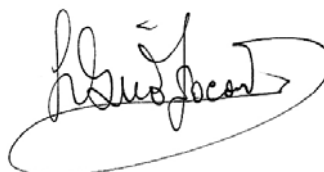
S.H.R.O. Sbarro Health Research Organization



This initiative is aimed to spread the information on the research activities of PhD students in our academic community.

The pamphlet is in English in order to promote Doctoral Schools of our University at international level, with particular attention to those foreign institutions with which we have signed international cooperation agreements. Moreover, it could also be useful to foster new agreements with foreign partners.

The Rector  
Prof. Silvano Focardi

A handwritten signature in black ink, appearing to read 'Silvano Focardi', enclosed within a large, loopy oval flourish.



This pamphlet was created to regroup and present together the research activities of the students of the Doctoral School in Oncology and Genetics in order to spread information about the work of the students and to promote the collaboration on research projects.

The first pages illustrate the activity of the “annual progress report day”. This event takes place at the end of each academic year and is dedicated to the presentation of both the research projects proposed by the new entered students and the annual progress reports of the older students. The pamphlet continues with the presentation of the research abstracts of the **35 PhD** students. Finally, the last pages are dedicated to the “thesis discussion days” and qualification of “Doctor Europaeus”.

I wish to dedicate this pamphlet to the PhD students who represent the “mainstay” of the Institution that we call University with their continuous daily work, their perseverance and motivation.

The director of the School  
Prof. Alessandra Renieri

A handwritten signature in black ink, reading "Alessandra Renieri", is centered on a light yellow rectangular background.



The Doctoral School in Oncology and Genetics is constituted of 5 sections or “education trainings”:

- 1) Medical Genetics coordinated by Alessandra Renieri
- 2) Oncological Genetics coordinated by Antonio Giordano
- 3) Colorectal and Gastroesophageal Diseases coordinated by Gabriello Tanzini
- 4) Hepatobiliopancreatic Diseases and Multitumoral Syndromes coordinated by Francesco Cetta
- 5) Bioinformatics coordinated by Monica Bianchini

In addition to the five above mentioned coordinators, the Faculty Board is composed by teachers from the University of Siena: Francesca Ariani, Alessandro Cappelli, Anton Ferdinando Carli, Maddalena Cioni, Serenella Civitelli, Paolo Frezzotti, Theodora Hadjistilianou, Marco Lorenzi, Francesca Mari, Giuseppe Marzocca, Clelia Daniela Anna Miracco, Marco Mugnaini, Roberto Ponchietti, Maria Lucia Sampoli, Franco Scarselli, Francesco Tani, Walter Testi, Paolo Toti, Luigi Verre; and by teachers from other Universities: Pier Paolo Pandolfi from the Cornell University, New York, Hans van Bokhoven from the University of Nijmegen, The Netherlands.

The following additional teachers from the University of Siena compose the Council of the School: Alfio Andronico, Maurizio Botta, Mirella Bruttini, Ilaria Cardinali, Mario Carmellini, Mario Chiarello, Concetta Gardi, Maurizio Genuardi, Marco Maggini, Carmela Marinelli, Domenico Mastrangelo, Alessandro Piccolomini, Paola Piomboni, Enrico Pinto, Franco Roviello, Edmondo Trentin, Claudia Torricelli.

On the basis of research activity the School has signed 7 International Cooperation Agreements with the following Universities:

Bilkent University, Ankara, Turkey;  
Duisburg-Essen University, Germany;  
Freiburg University, Germany;  
Greenwood Genetic Center, Greenwood, South Carolina, USA;  
Kentucky University, Lexington, USA;  
Radboud University of Nijmegen, The Netherlands;  
St. Kliment Ochriski University, Sofia, Bulgaria.

The Doctoral School in Oncology and Genetics at the University of Siena trains students to carry out research in Medical Genetics and in Clinical and Molecular Oncology over a four years program. The aim of this Doctoral School is to train researchers who will be able to plan and develop competitive research proposals. The School has a dedicated web site at the following address: [http://www.unisi.it/ricerca/dottorationweb/genetica\\_medica/](http://www.unisi.it/ricerca/dottorationweb/genetica_medica/). In this site it is possible to find general information on the School, seminar activities, research projects, and PhD students scientific “identity card”.

The School on the basis of the high quality of the education activities and the internationalization of the scientific and teaching courses has been selected by an external board as one of the PhD Schools of the University of Siena belonging to the Graduate College Santa Chiara. The Doctoral Schools of the Graduate College join in multidisciplinary and international research projects, creating a centre of high qualification for postgraduate education. The PhD students of the Graduate College are called “santachiarini” and are provided with the additional title of the Graduate College and the stay in the University residences. Residences of the Graduate College are situated in the old town. In these buildings teaching activities, conferences and interdisciplinary courses and seminars take place, but the most innovative aspect is that they are informal places for meetings where PhD students and teachers can stay and eat together.

# Index

Annual progress report day	Pag. 11
Students Project Abstracts	Pag. 15
Thesis Discussion	Pag. 53
• <b>Giovanni Abbadessa</b> A phase 1 dose escalation study of ARQ 197 in adult patients with metastatic solid tumors	Pag. 17
• <b>Mariangela Amenduni</b> Induced pluripotent stem cells as a human model to study Rett syndrome	Pag. 18
• <b>Annamaria Azzarà</b> Environmental Pollution in Metropolitan Areas and the occurrence of respiratory dysfunction and respiratory diseases	Pag. 19
• <b>Simona Benoni</b> Increased prevalence of neoplasms in subjects with progeroid syndromes: a genotype-phenotype correlation in a personal series and in patients from the literature	Pag. 20
• <b>Maria Grazia Bruccheri</b> Study of genetic susceptibility factors for Multiple Chemical Sensitivity (MCS) in sicilian patients	Pag. 21
• <b>Antonella Chessa</b> Endothelin 1 and endothelin-converting enzyme in primary colorectal cancer	Pag. 22
• <b>Filomena Cisternino</b> Genetics and molecular biology in the multidisciplinary approach to pressure ulcers and lower limb ulcers in diabetics	Pag. 23
• <b>David Colecchia</b> Multiple primary malignancies: Yeta challenge	Pag. 24
• <b>Daniele Conti</b> Identification of the regulatory mechanisms of Cdk2/CyclinA inhibition by pRb2/p130 protein	Pag. 25
• <b>Martina Cozzi</b> New small molecule inhibitors of Src as potential candidates for cancer therapy	Pag. 26
• <b>Francesca Crucianelli</b> Detection of constitutional epigenetic changes in multiple cancers by MS-MLPA analysis	Pag. 27
• <b>Roberta De Filippis</b> FOXP1 mutation leading to reduced chromatin affinity causes “Rett fruste” overlapping with EHMT1 phenotype	Pag. 28
• <b>Vittoria Disciglio</b> Role of MDM2 T309G and TP53 R72P polymorphisms in modulation of variable phenotypic expression of retinoblastoma	Pag. 29
• <b>Andrea Fontani</b> Outcome of surgical treatment of colorectal cancer in the elderly	Pag. 30
• <b>Iris Maria Forte</b> Gastric cancer and cell cycle regulation	Pag. 31
• <b>Elisa Grillo</b> European network database on Rett syndrome	Pag. 32
• <b>Valeria Guarnaccia</b> Retinoic acid and breast cancer: how to improve the therapeutic effect on the basis of molecular knowledge	Pag. 33

• <b>Valentina Guercio</b> PM chemical characterization and differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells	Pag. 34
• <b>Baharak Khadang</b> New Strategies for the Study and Treatment of Malignant Mesothelioma	Pag. 35
• <b>Raffaele La Montagna</b> Androgen receptor and PIN1 in prostate cancer	Pag. 36
• <b>Paolo Laviano</b> Study of obesity prevalence, body mass index including energy consumption measured by accelerometer and pollution related health effects in children attending primary school in Milan	Pag. 37
• <b>Gabriella Livide</b> Mutational screening in the RB1 gene	Pag. 38
• <b>Bruno Lorenzi</b> Evaluation of anorectal function after radiotherapy in patients treated for rectal cancer	Pag. 39
• <b>Giuliana Malagnino</b> Intrinsic toxicity, inflammatory potency and individual susceptibility in the occurrence of health damage from Particulate Material (PM)	Pag. 40
• <b>Elena Marcocci</b> Autosomal Alport syndrome: a new model including both dominant and recessive inheritance	Pag. 41
• <b>Monica Mischitelli</b> Prostate cancer: the influence of STAT3 and the presence of Human Polyomavirus BK in cells	Pag. 42
• <b>Mafalda Mucciolo</b> Copy number variations analysis in autism spectrum disorders	Pag. 43
• <b>Olabinjo Olayinka</b> Bioinformatics approach to the pRb pathway in cancer initiation and progression	Pag. 44
• <b>Marco Pacifici</b> Detection and role of mir146a in HIV-clinical samples	Pag. 45
• <b>Filomena Tiziana Papa</b> Diagnosis process assessment of Array-CGH analysis	Pag. 46
• <b>Veronica Parri</b> Identification and characterization of deletions and duplications by MLPA in patients with Cohen syndrome	Pag. 47
• <b>Flavio Rizzolio</b> Pin1 controls cell cycle progression through interaction with pRB	Pag. 48
• <b>Dalila Rondinella</b> QF-PCR as a tool for rapid prenatal diagnosis	Pag. 49
• <b>Ariele Spanhol Rosseto</b> Molecular work-up of patients with the congenital variant of Rett syndrome: analysis of the 14q12 region.	Pag. 50
• <b>Rosalia Zangari</b> The impact of traffic pollution on antioxidant system of two populations exposed to different levels of pollutants	Pag. 51



# **Annual Progress Report Oncology and Genetics Doctoral School September 7, 2010 Centro Didattico S Maria alle Scotte, room 13**

## **8.45 Welcome Addresses**

*Silvano Focardi*, Rector of the University of Siena  
*Alessandra Renieri*, Director of Oncology and Genetics Doctoral School

## **9.00 Progress report of the 3rd year, XXIII cycle (10 minutes for each one)**

Chairmen: Alessandra Renieri and Francesco Cetta

*Amenduni Mariangela (A. Renieri)*

Induced pluripotent stem cells as a human model to study Rett syndrome

*Azzarà Annamaria (F. Cetta)*

Environmental Pollution in Metropolitan Areas and the occurrence of respiratory dysfunction and diseases

*De Filippis Roberta (A. Renieri)*

FOXG1 mutation leading to reduced chromatin affinity causes "Rett fruste" overlapping with EHMT1 phenotype

*La Montagna Raffaele (A. Giordano)*

Androgen receptor and PIN1 in prostate cancer .

*Laviano Paolo (F. Cetta)*

Study of obesity prevalence, body mass index including energy consumption measured by accelerometer and pollution related health effects in children attending primary school in Milan

*Mischitelli Monica (A. Giordano- VA. Pietropaolo)*

Prostate cancer: the influence of STAT3 and the presence of Human Polyomavirus BK in cells

*Parri Veronica (A. Renieri)*

Identification and characterization of deletions and duplications by MLPA in patients with Cohen syndrome

*Rondinella Dalila (A. Renieri)*

QF-PCR as a tool for rapid prenatal diagnosis

## **10.30 Progress report of the 2nd year, XXIV cycle (10 minutes for each one)**

Chairman: Monica Bianchini

*Bruccheri Maria Grazia (A. Renieri)*

Study of genetic susceptibility factors for Multiple Chemical Sensitivity (MCS) in sicilian patients

*Colecchia David (M. Chiariello)*

Involvement of the Erk8 in autophagy

*Conti Daniele (A. Giordano)*

Identification of the regulatory mechanisms of Cdk2/CyclinA inhibition by pRb2/p130 protein

*Crucianelli Francesca (G. Tanzini - M. Genuardi)*

Detection of constitutional epigenetic changes in multiple cancers by MS-MLPA analysis

*Disciglio Vittoria (A. Renieri)*

Role of MDM2 T309G and TP53 R72P polymorphisms in modulation of variable phenotypic expression of retinoblastoma

*Forte Iris Maria (A. Giordano)*

Gastric cancer and cell cycle regulation

*Mucciolo Mafalda (A. Renieri)*

Copy number variations analysis in autism spectrum disorders

*Pacifici Marco (A. Giordano)*

Detection and role of mir146a in HIV-clinical samples

*Zangari Rosalia (F. Cetta)*

The impact of traffic pollution on antioxidant system of two populations exposed to different levels of pollutants

### **12.30 Finger social lunch and poster viewing**

#### **AFTERNOON SECTION**

#### **14.00 Progress report of the 1st year, XXV cycle (5 minutes for each one)**

Chairmen: Mario Chiariello e Serenella Civitelli

*Cozzi Martina (A. Giordano)*

New small molecule inhibitors of Src as potential candidates for cancer therapy

*Fontani Andrea (G. Tanzini)*

Outcome of surgical treatment of colorectal cancer in the elderly

*Grillo Elisa (A. Renieri)*

European Rett database network

*Guercio Valentina (F. Cetta)*

PM chemical characterization and differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells

*Livide Gabriella (A. Renieri)*

Mutational screening in the RB1 gene

*Olabinjo Olayinka (A. Giordano)*

Bioinformatics approach to the pRb pathway in cancer initiation and progression

#### **15.00 Presentation of the PhD students program of the XXVI cycle**

#### **16.00 Closing session and attribution of credits by the faculty board**

A copy of the minutes is available at [http://www.unisi.it/ricerca/dottorationweb/genetica\\_medica/](http://www.unisi.it/ricerca/dottorationweb/genetica_medica/) accessing the "Minutes" link.





# Students Project Abstracts







Oncology and Genetics Doctoral School  
Oncological Genetics  
XXI cycle  
**Giovanni Abbadessa, MD**  
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Tutor A. Giordano

### **A phase 1 dose escalation study of ARQ 197 in adult patients with metastatic solid tumors**

ARQ 197, an oral selective c-MET inhibitor, and has effects on the tumour in preclinical models. Based on its pharmacologic effect, it may be a useful cancer treatment. This study was performed to determine the maximum tolerated dose (MTD) of ARQ 197 in patients with advanced, refractory metastatic or recurrent solid tumours. Dose-limiting toxicities (DLTs), safety, pharmacokinetic parameters and tumour response were also evaluated.

In this open-label, Phase I, dose escalation study ARQ 197 was administered orally initially in repeated cycles of 21 days (14 days on/7 days off treatment) and later modified to continuous dosing. Thirteen dose levels and 2 formulations were investigated: from 10 mg to 360 mg twice daily (bid). Treatment continued until unacceptable toxicity, tumour progression or death.

Seventy-four patients were treated in this trial. No MTD was determined in this study. ARQ 197 was well tolerated, with mild to moderate toxicities. DLTs consisted of Neutropenia in 1 patient and dehydration and vomiting in 1 patient, both at the 360 mg bid dose level. Sixty-one patients were evaluable for assessment of anti-tumor

activity; 3 patients with neuroendocrine, prostate, and testicular cancers achieved a partial response (PR); 38 patients had a best response of stable disease and 20 had progressive disease, the median duration of therapy being 6.5 weeks (range; 1-119 weeks). With an observed half-life of 2-4 hours, ARQ 197 showed no relevant accumulation upon multiple dosing. Increases in exposure were less than proportional to increasing dose, and all patients remained above the minimum inhibitory concentration of 0.3  $\mu\text{M}$  for 8 hours or longer. Results indicate that further clinical investigation of ARQ 197 is warranted, and suggest it could be a promising future therapy for patients with cancer. More Phase 1 and 2 studies have been conducted and are ongoing, having shown a favourable safety profile as single agent and in combination with agents such as erlotinib, sorafenib, gemcitabine, biologic activity on tumor cMET from pre- and post- therapy biopsies in patients, clinical activity in different tumor types.

This work is reported in: Mekhail T et al. J Clin Oncol 2009;27; Abstract 3548.  
This work is funded by: ArQule, Inc.



Oncology and Genetics Doctoral School  
Medical Genetics  
XXIII cycle  
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Tutor A. Renieri

### **Induced pluripotent stem cells as a human model to study Rett syndrome**

Rett syndrome (RTT) is a severe neurodevelopmental disorder representing one of the most common genetic causes of mental retardation in girls. The classic form is mainly due to MECP2 mutations, while alterations in CDKL5 and FOXP1 have been identified in RTT variants. Despite extensive efforts, the molecular mechanisms underlying the syndrome remain unclear, mainly due to the lack of satisfactory human cellular models. To overcome this obstacle, we employed the approach of genetic reprogramming that allows to generate induced pluripotent stem (iPS) cells directly from patients fibroblasts. We reprogrammed fibroblasts from 2 CDKL5 patients (a male with p.T288I and a female with p.Q347X) and obtained an iPS line with a p.R306C MECP2 mutation from the collaboration with James Ellis (Toronto) and one iPS line with a p.W255X FOXP1 mutation from the collaboration with Vania Broccoli (Milan). Pluripotency and self renewal potential of reprogrammed clones have been confirmed. We are now setting up the procedure for in vitro neuronal differentiation. The resulting neurons will offer the opportunity to study disease mechanisms directly on the primarily affected cells. In particular gene expression profile of some genes, whose expression was found altered in MECP2 mouse models (Fkbp5, Mobp, Plagl1, Ddc, Mllt2h, Eya2, S100a9) will be assessed in both MECP2, CDKL5 and FOXP1 mutated neurons, in the hypothesis of a common pathway. Particular attention will be paid to S100a9, altered also in post-mortem RTT brains. These experiments will enable disease investigation and might allow the identification of potential “druggable” targets for therapeutic approaches.

This work is reported in: Ariani et al. Induced pluripotent stem cells as a human model to study disease mechanisms in Rett syndrome. 2nd European Congress on Rett Syndrome , 7 – 10 October 2010 - Edinburgh  
This work is funded by: Telethon grants GGP09117 and GTB07001C to A.R.

Doctoral School of Oncology and Genetics  
Hepatobiliopancreatic Disease and Multitumoral Syndromes  
XXIII cycle  
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Tutor Prof. F. Cetta



### **Environmental Pollution in Metropolitan Areas and the occurrence of respiratory dysfunction and respiratory diseases**

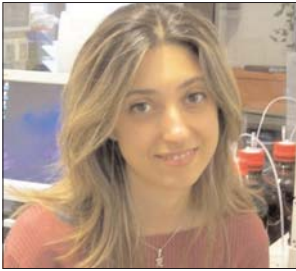
Environmental pollution, mainly due to urban traffic, is responsible for a different air quality in metropolitan areas vs remote sites and is likely to be responsible for a different incidence of respiratory complications and/or reduction of pulmonary function.

Two groups of 99 subjects were recruited (n=198), on a casual and voluntary basis: the former in Milan, Italy, i.e. a densely populated and polluted metropolitan area, the latter in Aprica, a remote alpine site (1181 m.a.s.l), with low pollution, due to traffic or other pollution sources. PM10 and PM2,5 were measured by PM detection units during two 2 week- campaigns. Each group was classified in to 2 subgroups. The former, aged 30 to 64 years, (n=72) the latter over 65 (n=27).

The following results were obtained: FEV1 (index of bronchial patency) was <80% in 20 out of 99 subjects in Milan (20,2%), whereas it was < 80% in only 8 out of 99 in Aprica (8,08%).

Evident differences were also observed in subgroups of different age, FEV1 resulting <80% in 8 out of 72 subjects under 65 in Milan (11,1%) and in 2 out of 72 in Aprica (2,8%) and in 12 out of 27 (44,4%) and 6 out of 27 (22,2%) in subjects over 65, respectively (p<0,05).

Present data, even if preliminary, suggest that, in the polluted metropolitan area of Milan, inhabitants are more prone to the occurrence of respiratory dysfunction than in the remote alpine site of Aprica (p=0,001). In particular, not only in severely symptomatic subjects with evident respiratory diseases, but also in clinically asymptomatic individuals, it is possible to detect significant differences in respiratory function because of different levels of environmental pollution.



Oncology and Genetics Doctoral School  
Hepatobiliopancreatic Diseases and Multitumoral Syndromes  
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### Increased prevalence of neoplasms in subjects with progeroid syndromes: a genotype-phenotype correlation in a personal series and in patients from the literature.

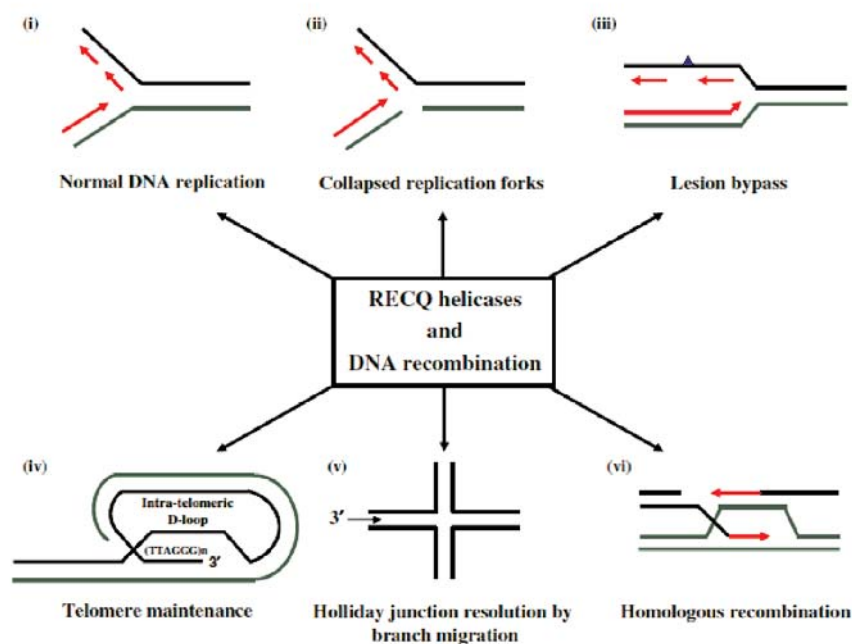
Most of diseases related to the DNA repair systems deficiency show varying degrees of "accelerated aging" or cancer. Alterations of RecQ helicase genes (WRN, BLM, RECQL4), encoding proteins involved in various types of DNA repair, determine the onset of progeroid syndromes, autosomic recessive diseases, characterized by chromosomal abnormalities, premature aging and high incidence of rare or multiple neoplasms. It is presumed that the loss of these proteins leads to limited replicative capacity, telomere instability, so to premature cellular senescence and predisposition to cancer.

The aim of this study has been to assess the number and types of cancers concomitantly present in individuals with germ-line mutations in RecQ genes, and to detect possible genotype-phenotype correlations in this cohort of patients.

It included an exhaustive review of the literature, the observation of 2 additional Werner patients in Siena and a cross-sectional approach in a geriatric hospital in Milan, to find similar cases.

No new case of Werner syndrome with associated neoplasms has been detected in our series.

However, at least 17 subjects with mutated WRN gene and with different types of cancer have been found in the literature. The most frequent associated resulted osteosarcomas, soft-tissue sarcomas and melanomas. It is likely that the accelerated aging process in WS patients contributes to the higher incidence of rare tumours, like as soft-tissue sarcomas and other non epithelial malignancies.



Oncology and Genetics Doctoral School  
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Tutor A. Renieri



### **Study of genetic susceptibility factors for Multiple Chemical Sensitivity (MCS) in sicilian patients**

Multiple chemical sensitivity (MCS) is a "chronic, recurring disease caused by a person's inability to tolerate an environmental chemical or class of foreign chemicals". Various theories have been proposed as a cause of the MCS syndrome including immunologic, genetic, toxicologic, psychologic and sociologic theories. MCS causes negative health effects in multiple organ systems, like respiratory distress, recurrent infections, seizures, cognitive dysfunction, heart arrhythmia, nausea, headache, and fatigue. The purpose of this study was to investigate genetic and immunological mechanism, in particular we studied genetic susceptibility, especially genes of importance to the metabolism of xenobiotic compounds. MCS patients were genotyped for polymorphism in the genes encoding cytochrome P450 (CYP2C9, CYP2C19, CYP2D6) in order to explain the adverse reactions to drug observed in our patients. We found an apparent association between number of active cytochrome P450 alleles and MCS status but we could not extend this analysis to healthy controls to confirm the susceptibility. So we can conclude that polymorphisms in several genes contribute to interindividual differences in the metabolism of xenobiotics, and may lead to toxicity and disease. The balance between activation and/or detoxification processes may influence an individual's susceptibility to diseases. Polymorphisms cytochrome P450 are not sufficient to cause the MCS phenotype, but further data is needed before reaching a definitive conclusion.

This work is reported in:

- M. G. Bruccheri et al. Update on Multiple Chemical Sensitivity: from clinical findings to genes using clinical, biochemical, immunological and genetic approaches. I National congress On MCS, Acireale, 17 April 2010 ( as platform presentations )
- M. G. Bruccheri et al . Analysis of Oxidative Burst activity in 20 patients affected by Multiple Chemical. 42° National congress of SIBIOC (Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica). Roma , 5-8 October 2010 (as poster)

This work is funded by:

IRMA (Istituto di Ricerca Medica e Ambientale)



Oncology and Genetics Doctoral School  
Colorectal and Gastroesophageal Diseases  
XXI cycle  
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Tutor Prof. G. Tanzini

## **Endothelin 1 and endothelin-converting enzyme in primary colorectal cancer**

Endothelin 1 ( ET1) is a peptide produced by different human cancer cell lines. HT29 and COLO320 are colon carcinoma cell lines producing endothelin 1. Elevated plasma levels of ET1 have been demonstrated in patients with primary colorectal cancer (CRC) with and without liver metastases compared to controls. There is increasing evidence that ET1 may play a role in the development of CRC both locally and systemically. Mature ET1, produced mainly by specific endothelin converting enzyme (ECE1) exerts its biological effect via ETA and ETB receptors. Recent results demonstrated that ETA receptors are overexpressed while ETB are underexpressed in CRC tissue compared to normal colon. In addition application of ETA antagonist via portal vein at the time of tumor implantation reduces subsequent hepatic involvement in the rat model of colorectal liver metastases. Therefore we investigated the expression and distribution of proteins and mRNAs of all components of the ET1 system in CRC. Preliminary results by immunohistochemistry show strong expression of ET1 in stroma and blood vessels of CRC specimens and weak positivity in the normal colon. Intracellular staining for ECE1 was located in the perinuclear region, probably the Golgi of colonic epithelial cells with essentially the same distribution in neoplastic and normal tissues. ECE1 inhibitors and endothelin receptors antagonists are indicated as potential anti-cancer agents. Thus knowledge of ECE1 precise subcellular localization is crucial for effective drug design and delivery.

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Tutor F. Cetta



### **Genetics and molecular biology in the multidisciplinary approach to pressure ulcers and lower limb ulcers in diabetics**

Pressure ulcers in the sacral region and ulcers of lower limbs in subjects affected by diabetes are frequent (up to 15% of diabetic subjects) in particular in old, frail subjects, with multidistrict imbalance and /or prolonged bed stay.

Recent advance in genetics and molecular biology has shown that hyperexpression of c-myc and endonuclear localization of Beta- catenin, together with abnormal localization of EGFR are typical findings of non healing ulcers. Analysis using microarray technology of specimens from non healing ulcers have shown significant differences in comparison to specimens that undergo easy healing.

Thanks to a cooperation with the Dept of Special Surgery of the N:Y University (Prof. H. Brem), a multidisciplinary approach to pressure ulcers has been designed, including systematic biopsy of non healing ulcers coupled with genetic analysis of ulcer borders.

At the Pio Albergo Tribuzio, (PAT) which is the largest Geriatric Institute in Milan, the prevalence of pressure ulcers during years 2004-2008 varied from 9% to 14%, i.e. 110 out of 705 subjects (29F, 49M), whereas ulcers of lower limbs were found in 35 (17M, 18F). In particular, in March 2009, in 473 subjects who were admitted to the PAT-RSA, there were 42 subjects with pressure ulcers stage IV. There were 28 F and 14M. Diabetes requiring treatment was found in 7 of 42 (insulin administration in 4 cases). Data from molecular biology were found of particular help to guide complete removal of the border showing hyperexpression of c-myc and  $\beta$ -catenin, so facilitating early recovery using a multidisciplinary approach, that involves surgeons, diabetologists, dermatologists, orthopedicians, angiologists, geneticians and pathologists.



Medical Genetic Doctoral School  
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XXIV cycle  
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Tutor A. Renieri, M. Chiariello

### Multiple primary malignancies: Yeta challenge

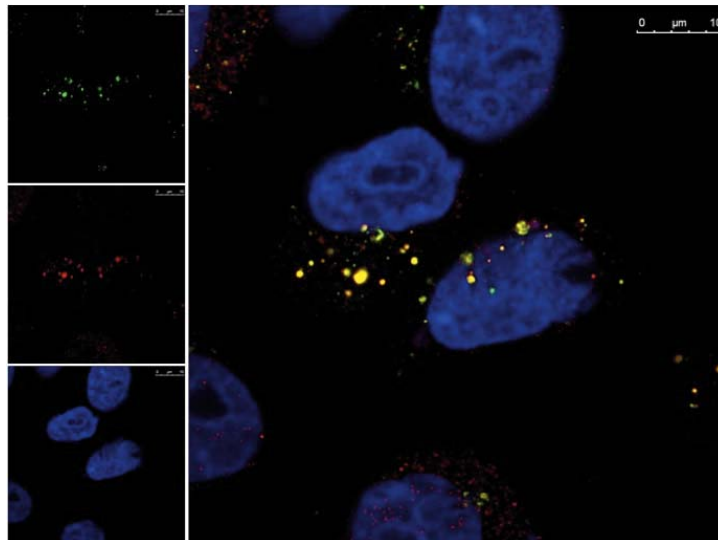
We analyzed databases on 815 subjects who underwent surgery for colorectal cancer from 1985 to 2005 periodically followed up by clinical and instrumental examinations.

The aim of our follow up program is the detection of the first cancer representation and the prevention of metachrone colic or extracolonic malignancies. We detected MPM (Multiple Primary Malignancies) in 120 out of 815 patients (14.72%). Metachronous malignancies are more frequent than synchronous ones (75 vs 45). The mid time between two neoplasms is 40 months

Among metachronous neoplasms, extracolonic ones are more frequent. In subjects that developed a colorectal cancer after a first colorectal cancer, the last pancolonoscopy was performed 22.5 months before. Three metachronous tumors found six months after first surgery have been considered misunderstood synchronous malignancies. The colic neoplasia localization, proximal or distal to the right flexure, is similar in patient with unic colorectal cancer and in patient affected by MPM.

In 15 MPM patients suggestive criteria for hereditary colorectal cancer are present and these subjects have been invited to genetical counselling and we are studying results.

Skin, breast and colonrectum are in this order the most frequent sites interested by multiple primary malignancies. Colonrectum is involved by MPM in a percentage from 30 % to 50 % according to Literature.



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## Identification of the regulatory mechanisms of Cdk2/CyclinA inhibition by pRb2/p130 protein

Over the past decades, cancer research has been mainly aimed at identifying the molecular alterations underlying cancer development, in order to design new drugs for targeted therapy.

Retinoblastoma (RB) family proteins pRb, p107 and pRb2/p130 are important cellular factors which play a well-recognized role as tumor and growth suppressors. These proteins are actively involved in the negative control of the cell cycle and their function is modulated via complex homeostatic processes, most of them involving post-translational regulation of their phosphorylation status. Interestingly, the family members p107 and pRb2/p130 share the ability to physically interact and inhibit the kinase activity of the Cdk2/Cyclin A and Cdk2/Cyclin E complexes. Regarding pRb2/p130, its inhibitory effect on Cdk2/Cyclin A activity has been attributed to the "spacer" region, in particular to a 39 aa-long pRb2/p130 spacer-derived peptide (Spa310, aa 641-679) was selected as the sequence responsible for Cdk2/CyclinA inhibition.

We used a computational chemistry approach to select a pool of small molecules that mimic Spa310 activity. The analysis of the CDK2-CyclinA crystal structure allowed us to select five hypothetical CDK2-CyclinA inhibitors from chemical libraries. We tested the antiproliferative effects of these five small molecules on cell lines of different tumor types (lung and prostate cancer, osteosarcoma, mesothelioma and medulloblastoma) by the MTS cytotoxicity assay. We observed a significant reduction in the growth rate of these tumor cells and we focused our further analyses on the two most effective compounds. In order to rule out the potential cytotoxic effect on normal cells, we tested these molecules also on non-neoplastic cell lines. We found that they have a significant minor effect on normal cells with respect to their tumoral counterpart. Preliminary FACS analyses show that both the selected small molecules can induce apoptosis in lung cancer cell lines. Additionally combinatory tests with cisplatin show that the use of our small molecules is able to decrease the necessary concentration of the chemotherapeutic to reach the same cellular mortality *in vitro*.

To dissect the molecular mechanisms of these small-molecules-induced apoptosis we will also analyze by western blotting and real-time qRT-PCR the expression of proteins involved in the regulation of cell cycle and apoptosis. As a future objective, we intend to test these small molecules in mouse tumor xenografts in order to evaluate their ability to inhibit tumor growth also *in vivo*.

This work is reported in:

Conti D et al. Small molecules mimicking the spa310 peptide from the spacer region of pRB2/p130 as potential anticancer agents. Fifth Annual Scientific Conference – Istituto Toscano Tumori (ITT), Il Borro, San Giustino Valdarno (Arezzo), July 1, 2010.

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### **New small molecule inhibitors of Src as potential candidates for cancer therapy**

Accumulating data show that alterations in the activity of the tyrosine kinase Src play a key role in the development and progression of several human cancers. Src has been shown to be an important molecular target in cancer therapy.

This study aims at investigating the effects of new pyrimidine derivative Src inhibitors in a panel of tumors that show a high Src kinase activity. Given the central role of Src in regulating several key processes in tumor development we plan to analyze the effects of the Src inhibitors on these processes.

We have recently studied the effects of these molecules in medulloblastoma, the most common cerebellar tumor of the central nervous system in childhood. Therapeutic approaches for medulloblastoma are currently based on the combination of surgery, radiotherapy, and chemotherapy. Despite improvements in the overall survival rate following the multimodality treatment, about one third of patients will have a recurrent disease and current treatments cause neurocognitive sequelae. Therefore, there is a great need to develop new therapies that minimize adverse effects. Substantial progress has been made in understanding the molecular mechanisms underlying medulloblastoma and in offering new targets for the development of more effective and specific therapies. One possible target for medulloblastoma therapy is Src. A high Src activity was identified in medulloblastoma, suggesting that Src could have a key role in the development of this tumor. We have examined the effects of the Src inhibitors in human medulloblastoma cells (Daoy and D283). We showed that the pyrimidine derivatives greatly reduce the growth rate of medulloblastoma cells compared with a non-neoplastic nerve cell line (HT22). These compounds halt cells in the G2/M phase, and this effect likely occurs through the regulation of cdc2 and CDC25C. Moreover, the exposure to pyrimidine derivatives induces apoptosis through modulation of the apoptotic proteins Bax and Bcl2, and inhibits tumor growth in a xenograft mouse model. Notably, the small molecules show major inhibitory effects on medulloblastoma cell growth compared with the chemotherapeutic agents cisplatin and etoposide. In conclusion, our results suggest that Src inhibitors could be novel attractive candidates for the treatment of medulloblastoma or tumors characterized by high Src activity.

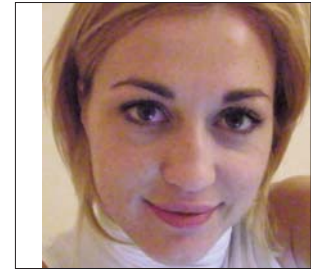
This work is published in:

New pyrazolo-[3,4-d]-pyrimidine derivative Src kinase inhibitors lead to cell cycle arrest and tumor growth reduction of human medulloblastoma cells. Rossi A, et al. FASEB J. 2010 Apr 29.

This work is funded by:

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## Detection of constitutional epigenetic changes in multiple cancers by MS-MLPA analysis

Aberrant methylation of CpG-islands has been shown to be associated with transcriptional inactivation of tumor suppressor genes in a wide spectrum of human cancers. Recently, evidence has been accrued showing that constitutional epigenetic silencing abolishes gene expression.

This project focuses on the investigation of aberrant constitutional DNA methylation in mutation-negative patients with multiple primary tumors.

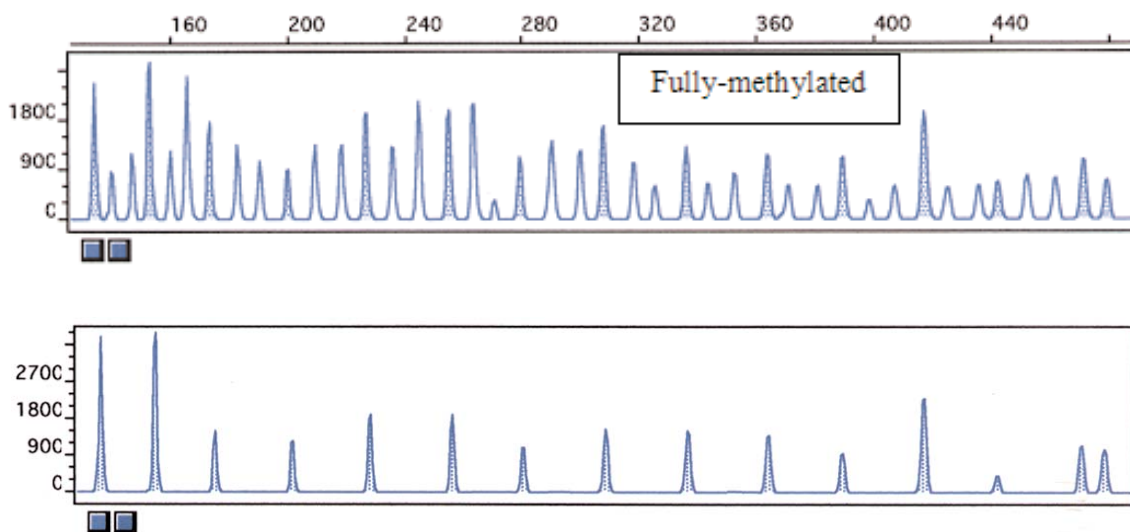
The search has been performed on non cancerous tissues. In addition, we have analyzed tumor tissue, when available.

Methylation analysis has been performed by a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay that allows detection of CpG methylation in 24 different tumor suppressor genes. In a first phase, purification of DNA from formalin-fixed, paraffin-embedded (FFPE) tumor tissues has been performed with QIAamp DNA Mini Kit followed by preamplification of total DNA. Subsequently, to improve yield as well as DNA performance in MS-MLPA, the QIAamp DNA FFPE Tissue Kit has been used.

Constitutional methylation is then confirmed by methylation-sensitive high-resolution melting (MS-HRM) and/or pyrosequencing.

So far, DNA samples from peripheral blood of 43 patients, have been investigated. For two of these, tumor samples were available to compare methylation patterns.

No alteration of MS-MLPA peak ratios has been observed in this small subset.

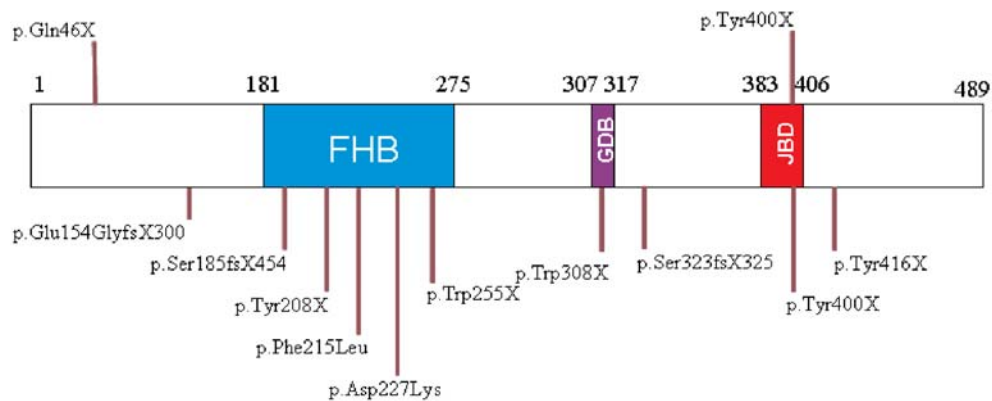




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## FOXG1 mutation leading to reduced chromatin affinity causes “Rett fruste” overlapping with EHMT1 phenotype

The Forkhead box G1 (FOXG1) gene, a brain specific transcriptional factor essential for the early development of telencephalon, located in 14q12, has been recently implicated in the congenital variant of Rett Syndrome (RTT). Until now only 10 patients with FOXG1 point mutations have been reported with a quite homogeneous phenotype including a severe neurological impairment in accordance with a clinical diagnosis of congenital variant RTT. These patients do not show peculiar facial features in contrast with those with the 14q12 microdeletion syndrome. Here we report on two unrelated patients with a de novo FOXG1 point mutation, p.Gln46X and p.Tyr400X respectively, having a milder RTT phenotype according with RTT “forme fruste” and sharing strikingly similar facial features resembling the Kleefstra syndrome due to EHMT1 gene. Although FoxG1 action as well as EHMT1 depends critically on its binding to chromatin, very little is known about the dynamics of this process. Here we apply photobleaching strategies within the nucleus comparing the wild type GFP tagged FoxG1 with the protein carrying pathological mutations. We report for the first time that most of the FoxG1 fusion protein is transiently associated with chromatin in vivo and that mutations caused a mislocalization of FoxG1 and a dramatic alteration in chromatin affinity which is particularly high in the two mutations reported here. Interestingly both FoxG1 and EHMT1 proteins interact with members of JARID1 family and are involved in modulation of the chromatin structure. In this perspective, the overlapping phenotype described in this paper could not be completely unexpected.



This work has been submitted to the Journal of Medical Genetics:  
“FOXG1 mutation leading to reduced chromatin affinity causes “Rett fruste” overlapping with EHMT1 phenotype”  
De Filippis R et al.

This work is reported in: “Mutazioni in FOXG1 che causano ridotta affinità per la cromatina, sono responsabili della sindrome di Rett nella variante “Rett fruste” con fenotipo sovrapponibile a quello da Mutazione in Ehmt 1” De Filippis et al. SIGU 2010.

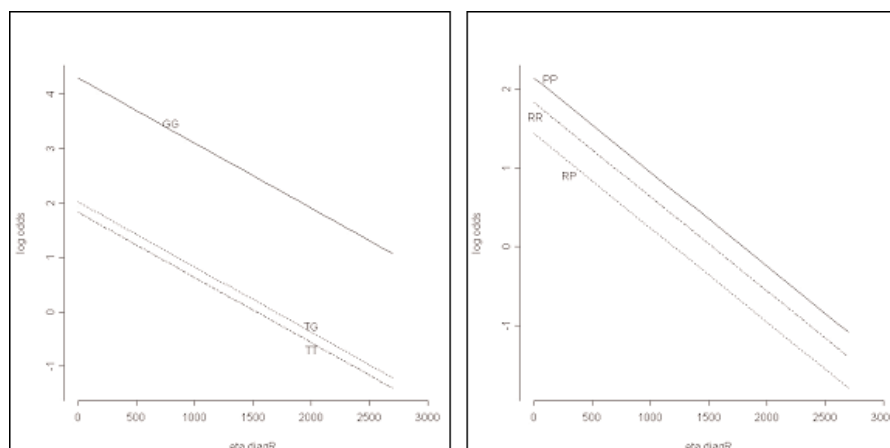
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## Role of MDM2 T309G and TP53 R72P polymorphisms in modulation of variable phenotypic expression of retinoblastoma

Current evidence support the role of DNA repair and apoptosis gene polymorphisms as cancer modifiers. Two common SNPs TP53 R72P and MDM2 SNP309 with known functional effects have been studied with contrasting findings in both sporadic cancer (gastric, lung, childhood ALL) and the inherited Lynch syndrome, and in Li-Fraumeni syndrome a significant interaction between the germline TP53 mutation and the MDM2 SNP has been shown. To investigate their role in hereditary retinoblastoma we genotyped the two SNPs by Pyrosequencing® assays on blood DNA of 90 patients with known germinal RB1 mutation, 34 familiar. A descriptive analysis showed an earlier age at diagnosis in patients with bilateral retinoblastoma than in those with unilateral retinoblastoma (median age: 0.57 yrs vs 1.49 yrs, respectively,  $p < 0.001$ ). Since age of onset is often not exactly known, we considered bilaterality as a more robust measure of the variable genetic risk. A multivariate logistic regression model adjusted for age and gender showed the risk of bilateral disease to be: i) as for the type of RB1 mutation higher for splicing and missense mutations than for deletions, duplications, nonsense and frameshift mutations but not significantly so (OR=1.33; 95% CI 0.22 – 8.22); ii) as for the MDM2 SNP309, significantly higher for the GG genotype than TT (OR=11.78, 95% CI 2.18 – 63.65) but not significantly for TG; iii) as for the TP53 R72P SNP not significantly for the PP genotype. Our results suggest for the first time that MDM2 may be modifier gene of Retinoblastoma as well.



This work is reported in:

Disciglio V. et al. MDM2 and TP53 are modifier genes of retinoblastoma. European Congress of Human Genetics, Gothenburg, Sweden - June 12 - 15, 2010. Poster

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### **Outcome of surgical treatment of colorectal cancer in the elderly**

Aim of this study is to compare the clinical features and the perioperative and long term outcomes after primary surgery for colorectal cancer in the elderly population with those observed in younger patients.

All the patients over the age of 55 who underwent primary surgery for colorectal cancer in our clinic from 1988 to 2008 were included in this study and divided into two age groups: 55-75 years and >75 years considering the age of diagnosis.

914 consecutive patients were enrolled in the study (352 >75 years). In the elderly group tumours were predominantly right sided and the overall number of comorbidities was statistical more frequent.

Elderly patients underwent emergency surgery more than the control group ( $p=0.0008$ ). There were no significant differences between the two group in terms of curative and palliative resections.

The overall operative mortality rate was 5,9% in the Study group compared with 2,1% in the Control Study( $p=0,0033$ ).

The overall 3-year, 5-year and 10-year survival rates were respectively 37%, 16,2% and 5,1% in the Study group, compared with 52,3%, 35,1% and 24,7% in the Control group. ( $p=0,022$ ,  $p=0,0001$  and  $p=0,0001$  respectively).

More patients were lost during the follow-up in the elderly group ( $p=0,0003$ ) and more deaths unrelated to cancer were found in the study group compared with the control group ( $p=0,0005$ ).

The cancer specific mortality was similar between the two groups.

Elderly patients that underwent major colorectal resection have an acceptable perioperative morbidity, mortality and survival rate compared to younger patients.

Age alone should not be considered a reason to deny surgery to these patients.

This work is reported in:

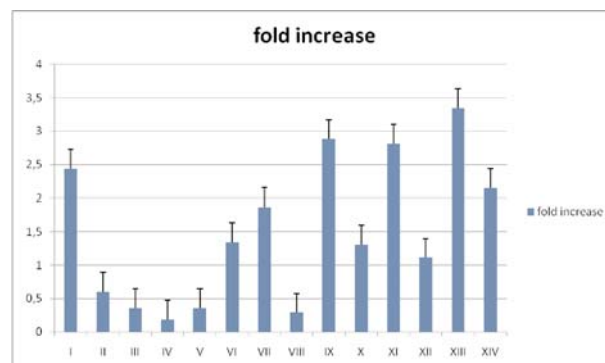
Fontani A et al. Outcome of surgical treatment of colorectal cancer in the elderly. ESCP, Sorrento, 22-25 Settembre 2010.

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## Gastric cancer and cell cycle regulation

Gastric cancer is one of the most diffuse neoplastic pathologies in the world whose environmental and molecular causes, although deeply investigated, have not been completely clarified. Gastric cancer aetiology is related to *Helicobacter pylori* infection, high intake of smoked and salted food and genetic alteration of E-cadherin gene, CDH1, a calcium-dependent cell adhesion glycoprotein, the loss of which contributes to cancer progression by increasing proliferation, invasion and metastasis. Two histotypes of gastric cancer are recognized: the diffuse histotype, characterized by poorly differentiated secreting mucus cells, often associated to CDH1 germline mutation; and the intestinal histotype, characterized by a series of multi-defined steps beginning with atrophic gastritis and finally developing into carcinoma and diffuse metastasis. Since molecular bases of gastric cancer are so far not clear, the aim of our work is trying to define the role of cell cycle regulatory genes in the pathogenesis of the gastric cancer, focusing on the Rb family proteins. The pRb family proteins (including pRb/p105, pRb2/p130 and p107) are involved in cell cycle regulation and their function and/or expression is often lost in various kind of tumors. They also regulate growth processes and apoptosis above all by interacting with E2Fs transcription factors. Only few studies have been performed about the role of Rb family proteins in gastric cancer. So far we have collected 14 tissue specimens of gastric cancer with their corresponding normal gastric tissue and we studied the expression level of both protein and mRNA of pRb2/p130 both by immunohistochemistry and by real-time PCR in all samples. Until now we found that pRb2/p130 shows gene downregulation in all samples while the protein expression of pRb2/p130 does not. We obtained the same results studying gene and protein expression of pRb2/p130 in 5 cell lines. However, our statistical data do not show a direct correlation between pRb2/p130 gene level expression and grade of tumor. At the present we are studying the expression of cyclin A2 in all tissues samples. In fact, Cyclin A2 is a target of E2F4 transcription factor which is released after pRb2/p130 phosphorylation. So far we found that Cyclin A2 shows a gene upregulation, probably caused by hyperphosphorylation or mutation of pRb2/p130. Our future purpose is investigating on possible altered function of this protein and sequencing of its related gene to detect possible mutations.



This topic is reviewed in:

Rb family proteins in gastric cancer Cito L., et al. *Oncology reports* (Accepted 05/05/2010)

This work is funded by:

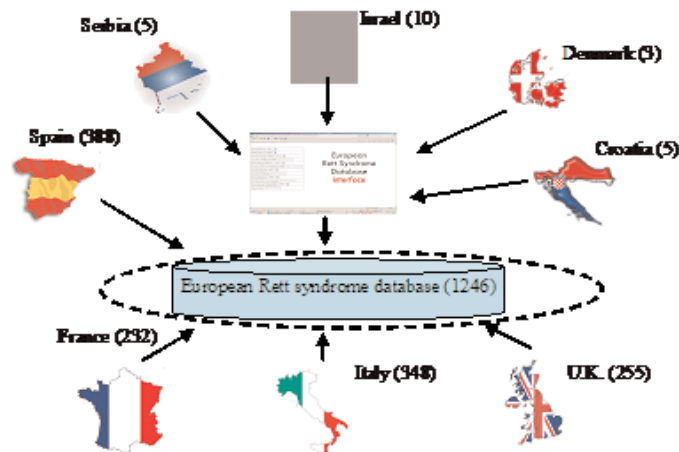
Centro Ricerche Oncologiche Mercogliano, CROM.



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## European Rett Database Network

Rett database network project started in 2008 in order to connect the already existing databases and to create a unified repository following an "adaptor approach". In addition, this network allows data storage for users who do not have a local computerized data management system. Presently, the network contains data of 1246 patients. The whole Italian (Italian Rett database and biobank), French (SYRENE), British (British Isles Rett Syndrome Survey) and Spanish (Barcelona Rett database) cohort have been converted and patients inserted from the pre-existing databases. In addition, patients from Israel, Denmark, Croatia and Serbia have been inserted directly in the network web site. The access is now open worldwide to whom that want to join (email contacts renieri@unisi.it and rossano@3wnet.net). For those who do not have a pre-existing database there are two options: i) insert directly in the main archive (geographical and institution provenience will be displayed); ii) construct a local or national archive connected with the main one. The web site is now moved to the definitive address (<https://www.rettdatabasenetwork.org>) and it has been implemented with the following 5 general pages (accessible by clicking on the top of the home page): i) "about this project"; ii) "how to join"; iii) "access rules"; iv) "guidelines"; v) "consent form". During the next year we are planning to improve the database and to develop a data mining system, which can manipulate large scientific databases. This international effort will be of great value in order to perform genotype-phenotype correlations, to study modifier genes, and to select subgroups of patients for clinical trials. The data are accessible to the participants and to the scientific community according to rules that assure transparency and equity (published in the web site).



This work is reported in:

A. Renieri et al. "Rett database network project". IRSF's 11th Annual Rett syndrome Symposium, Leesburg, Virginia, U.S.A., June 27 - 29, 2010. Poster

This work is founded by: IRSF(International Rett Syndrome Foundation), E-RARE (European Network on Rett Syndrome)

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### **Retinoic acid and breast cancer: how to improve the therapeutic effect on the basis of molecular knowledge**

Breast cancer is the second leading cause of cancer deaths in women. At least five distinct subtypes have been described on the basis of gene expression profiling, with the most important determinants of these subtypes being the presence or absence of the estrogen receptor alpha (ER) or the amplification/over expression of the Her2/ERBB2 locus. Breast carcinoma is a paradigmatic example of heterogeneity in the responses to retinoids. Clinical data indicate that only pre-menopausal women benefit from the use of the synthetic retinoid, fenretidine, when the drug is used in an adjuvant setting. The major aim of the research program of my PhD is to characterize the retinoid sensitivity/resistance in breast carcinoma and to define its molecular mechanism across the ER alpha and Her2 pathway. Retinoids exert their pleiotropic effects by binding to specific nuclear receptors (RARs and RXRs) acting as nuclear transcription factors. Generally, breast carcinoma cells expressing ER alpha are described as sensitive to, while the ER alpha-negative counterparts are refractory to the anti-proliferative activity of retinoids. On this basis, we have taken advantage of paradigms of ER alpha-positive (MCF7) and ER alpha-negative (MDA-MB231) breast cancer cells to assess the role of ER alpha signaling in determining ATRA sensitivity. After evaluation of the sensitivity of the cell lines and their stable transfectants restoring or silencing ER alpha, we performed gene expression and miRNA analysis for the dissection of the molecular mechanisms underlying the cross-talk between ER alpha and the retinoid signaling pathways. Interestingly, we found that the mir-21 is selectively induced by ATRA in ER alpha-positive cells but not in a panel of ER alpha-negative cell lines. This induction is the result of a transcriptional effect exerted by ATRA on the MIR21 gene. RNA silencing and over-expression experiments indicates that mir-21 could counteract the anti-proliferative effect of ATRA possibly acting in a negative feed-back loop. As far as Her2 is concerned, we considered that a significant fraction of Her2/Neu+ breast carcinomas are ER-, hence this type of cancer is predicted to be refractory to retinoids. Her2/Neu+ is often the result of an amplification of the corresponding gene (ERBB2) which maps to chr17, 650 kb upstream of RAR $\alpha$  (RARA locus). For this reason we evaluated whether a proportion of Her2/Neu+ and ER- breast cancer cells are characterized by co-amplification of ERBB2 and RARA, and hopefully could represent a new therapeutic target for retinoids. Using real time PCR on genomic DNA from 76 patients positive for Her2 amplification, we found that at least 20% harbours RARA amplification. To evaluate whether RARA co-amplification and consequent RAR $\alpha$  over-expression was associated with retinoid sensitivity, we turned to a panel of representative breast carcinoma cell lines. We found that Her2-RAR $\alpha$  co-amplified cell lines are particularly sensitive to retinoic acid. Moreover we found in SKBR3 cells that ATRA strongly synergize with lapatinib, a small tyrosine kinase inhibitor, used in the treatment of Her2 amplified breast carcinomas thus opening the doors for a novel therapeutic approach.

Part of this work has been submitted to Oncogene. Terao et al. Induction of miR-21 by retinoic acid in estrogen-receptor-positive breast carcinoma cells: biological correlates and molecular targets.

Part of this work is reported in: Garattini et al. "Combinations of retinoids and lapatinib in the treatment of Her2/Neu-positive breast carcinomas with co-amplification of the ERBB2 and RARA genes". AACR 101st ANNUAL MEETING 2010, April 17-21,2010, Washington.



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### **PM chemical characterization and differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells**

Air pollution in Milan causes health concern due to the high concentrations of particulate matter (PM<sub>10</sub> and PM<sub>2.5</sub>). The aim of my study was to investigate possible seasonal differences in PM<sub>10</sub> and PM<sub>2.5</sub> chemical composition and their biological effects. The PM was sampled during winter and summer seasons. The winter PMs had higher levels of PAHs than the summer samples which contained a greater amount of mineral dust elements. The PM toxicity was tested in the human pulmonary epithelial cell lines BEAS-2B and A549. It was found that, whereas A549 cell viability was not significantly reduced after summer and winter PM exposure, summer PM had no significant effects on BEAS-2B viability, whereas winter PM treatment induced a decrease in cell viability. In addition, whereas both winter and summer PM<sub>2.5</sub> produced only a slight increase in IL-8 release, winter PM<sub>10</sub> induced a 5-fold increase in IL-8 release in treated cells, and summer PM<sub>10</sub> induced a 20-fold increase ( $p < 0,05$ ) in IL-8 expression. In particular, BEAS-2B resulted more responsive to PM treatment than A549. Winter PMs were more cytotoxic than summer PMs; Summer PM<sub>10</sub> had a higher proinflammatory potential, which could be partly due to biological components (LPS). These results underline that the in vitro responsiveness to PM may be cell line dependent and suggest that the PM different properties may trigger different endpoints such as inflammation, perturbation of cell cycle and cell death.

This work is reported in:

Cetta, F. et al. A comparative study on the outcomes between in vitro results of PM incubation with cell-lines and clinical effects in children. The Milan Prolife Project. 1048 "International Aerosol Conference (IAC 2010) Helsinki, Finland 29 August – 3 September 2010.

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## **New Strategies for the Study and Treatment of Malignant Mesothelioma**

Malignant mesothelioma (MM) is a rare highly aggressive tumor whose principal risk factor is exposure to asbestos. The extensive use of asbestos during the twentieth century, together with the long-latency time (30-40 years after the first exposure), determined an increase in incidence, which is expected to reach a peak over the period 2020-2050. The prognosis is extremely poor, with a median survival between 10 and 17 months from clinical presentation. At present there is no known curative modality for MM. Understanding the biological mechanisms underlying mesothelioma development, progression and resistance to therapy is essential to identify new targets for the development of more effective and specific therapies and to identify new diagnostic, prognostic and predictive markers. Given its possible role in MM pathogenesis, one potential therapeutic target could be the Src kinase. We recently showed that new pyrimidine derivative Src inhibitors, which bind the ATP pocket of the Src kinase, have antiproliferative and proapoptotic properties in several tumor types. We evaluated the effect of these new Src inhibitors on a panel of human MM cell lines expressing the active form of Src (phospho-Src Y419) and also on a normal mesothelial cell line not expressing the active form of Src. Our results showed that these Src inhibitors exert a significant proapoptotic effect on MM cell lines, without affecting the normal mesothelial cells. Interestingly, we found that Src inhibition leads to nuclear stabilization of the cyclin-dependent kinase inhibitor p27. This finding is remarkable considering that loss of nuclear p27 expression is a well-established adverse prognostic factor in MM. Therefore, our observations provide a new rationale for the use of Src inhibitors in MM therapy.

Because miRNAs, a new family of gene expression regulators, have a crucial role in cancer development, we analysed the miRNA expression signature in patients with MM compared to normal pleural samples in order to identify new possible diagnostic, prognostic and predictive markers. We identified several miRNAs that are downregulated or upregulated in MM tumor samples compared to normal pleura. So far we have confirmed by real-time qRT-PCR the differences observed by microarray for 10% of the miRNAs that resulted more significantly deregulated in tumors. We focused our further analysis on miR-145, because it has proved to be a putative tumor-suppressive miRNA in several tumor types and it is markedly downregulated in the patient's group with respect to normal controls. We observed a significant downregulation of miR-145 in biphasic and sarcomatoid samples with respect to epithelioid specimens indicating that loss of miR-145 expression is a feature of the more aggressive tumor types. Consistently, we also observed a correlation between low miR-145 expression and a high proliferation index, measured by Ki67 immunohistochemical analysis. Finally, ROC curve analysis demonstrated that miR-145 could be a good prognostic and diagnostic marker for malignant mesothelioma.



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### Androgen receptor and PIN1 in prostate cancer

Prostatic adenocarcinoma is the most frequently diagnosed malignancy and second leading cause of cancer death amongst men in the United States. Among different players, many lines of evidence indicate that the androgen receptor (AR) functions as a positive regulator of cell proliferation in Prostate Cancer, and androgen deprivation therapy is still the standard treatment for metastatic disease. AR is a member of the steroid hormone receptor subfamily of ligand-regulated nuclear receptors, and its natural ligands are testosterone and 5 $\alpha$ -dihydrotestosterone (DHT). As other steroid receptors, AR is a modular protein that contains an N-terminal transactivation domain, a conserved DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD). Mechanistic investigation has revealed that AR acts as a master regulator of G1-S phase progression, regulating the activity of G1 cyclin-dependent kinase (CDK) proteins and induce phosphorylation/inactivation of the retinoblastoma tumor suppressor (RB).

Many evidences suggest that Pin1 regulate the activity of AR. Pin1 is an isomerase specific of pSer/Thr-Pro motifs that catalyzed the conformational switch from cis to trans, which is especially important because Pro-directed kinases and phosphatases are conformation-specific and act only on the trans conformation. Androgen receptor interacts with  $\beta$ -catenin and can suppress its coactivation of T cell factor 4 (Tcf4) in prostate cancer cells. Pin1 abrogated the ability of AR to antagonize  $\beta$ -catenin/Tcf4 binding and transcriptional activity. Abrogation of this interaction can enhance  $\beta$ -catenin/Tcf4 signaling and contribute to aggressive biological behavior in Prostate Cancer. In addition, higher Pin1 expression in primary Prostate Cancer cells is correlated with disease recurrence, and Pin1 expression was found markedly increased in metastatic Prostate Cancer. Our preliminary data showed that Pin1 can form a protein complex with AR. We are currently investigating which domains are involved in the Pin1/AR interaction. We are modulating the expression of Pin1 by shRNA and ectopic expression to understand how the AR activity is affected.

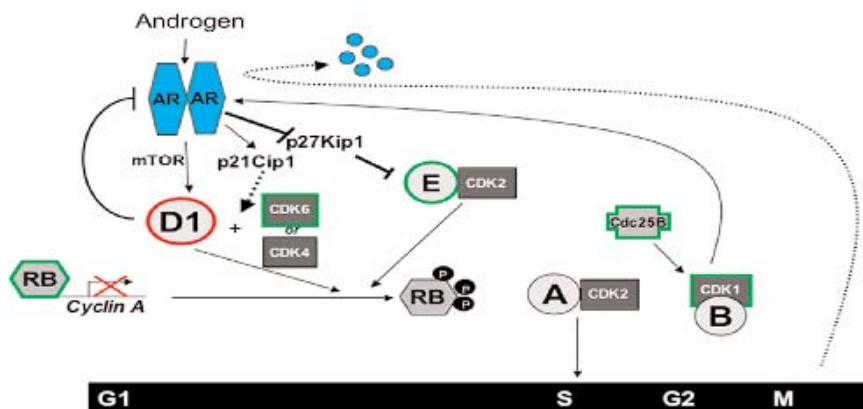


Fig. 1: AR-cell cycle crosstalk

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### **Study of obesity prevalence, body mass index including energy consumption measured by accelerometer and pollution related health effects in children attending primary school in Milan**

Traffic related pollution adversely affects lung function development. In addition, obesity and reduction of physical activity have been suggested as possible co-factors, determining the occurrence of functional alterations, or early changes in individual susceptibility to the “pollution diseases”, which can be responsible for cardiovascular diseases later in life.

228 children ( $\pm 8$  years) were enrolled from 2 primary schools in different sites of Milan with a different traffic related exposure: School1 near a large park, School2 close to main crossroads. Daily levels of PM10 and PM2,5 were measured both outdoor and indoor, during two 2-week different campaigns. Children underwent skin prick testing for inhaled allergens, analysis of Fractional Exhaled Nitric Oxide (FeNO) and spirometry.

In particular, in 188 children (62M, 126F), in addition to anthropometric data concerning parents, a questionnaire concerning dietary habits and timing and numbers of meals, physical activity of children was analyzed and quantitatively measured using a portable accelerometer (Lifecorder PLUS, KENZ), to be used for at least 3 days (2 working days +1 week-end) for at least 8 hours for day (N= 180).

30 children (16%) made a regular physical activity for less than 30 minutes; 127 (67,6%) between 30 and 60 minutes, whereas 21 (11,2%) for more than 1 hour. The percentage of children with FeNO values  $< 5$ ppb in School1 was higher (almost double) than in School2 ( $p=0,02$ ). In 73% of children attending the School2 FeNO was between 5 and 20ppb. The percentage of asthma exacerbations in the previous 12 months was higher in children from School2 ( $p=0.05$ ). On the contrary, the prevalence of persistent allergic rhinitis in children allergic to grass pollen was higher in School1 ( $p=0.03$ ). In particular, the latter children also had a greater activity limitation, due to rhinitis and concomitant conjunctivitis ( $p=0.03$ ).

Continuous on field monitoring of the various types of PM and analysis of clinical outcomes and hospital admission shows that: -Short durations PM10 peaks, even reaching high concentrations (up to 1000  $\mu\text{g}/\text{m}^3$ ) have no evident consequences on children health and are usually induced by trivial causes (resuspension of crustal components).- There is an enormous daily variability in PM10 concentration, and a wide range of clinical outcomes, showing seasonal variability, likely related to different seasonal composition of PM.

Traffic could be responsible at least in part for the different air quality, but individual susceptibility and seasonal changes are also major determinants of clinical outcomes. Preliminary data concerning concomitant obesity and overweight, because of the too short observation period, resulted insufficient to detect a facilitating role of overweight, dietary habits and reduction of physical activity, in the occurrence of more severe clinical outcomes.

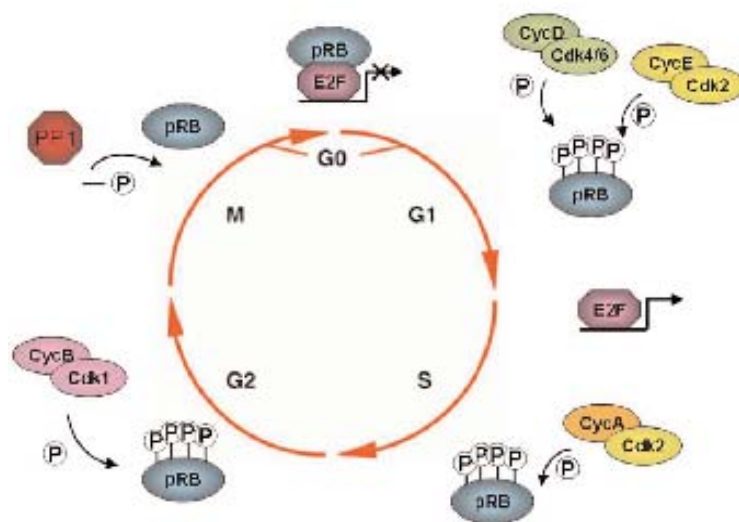
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### Mutational screening in the RB1 gene

The RB1 gene (Gene Bank accession number L11910) consists of 27 exons and of a promoter region containing binding motifs for transcription factors such as Sp1, ATF and E2F. It encodes for a 928 amino acids phosphoprotein that is part of a small family of nuclear proteins, acting on cell cycle regulation. The pRB pathway plays an important role in controlling proliferation and is frequently perturbed in human tumors. Retinoblastoma is a childhood tumor of the developing retina, occurring either in a sporadic or in a hereditary form. Sporadic tumors are unilateral, whereas hereditary tumors are often bilateral and multifocal; the latter show an earlier age of onset, and are transmitted in a dominant mode with a penetrance of about 90%. The first model to explain tumor development was proposed by Alfred Knudson in 1971; according to this model, two mutational events (M1–M2) in RB1 gene represent the first rate-limiting step in tumor development. Next high resolution studies demonstrated that these mutational events are followed by further genomic rearrangements (M3–Mn) that involve oncogene/oncosuppressor, necessary for malignant transformation of retinoblastoma. The aim of my study was to detect the loss/alteration of pRB function, through the application of MLPA, DHPLC and Automatic Sequencing techniques. I studied a cohort of 27 patients: 13 unilateral case (12 sporadic, 1 familial) and 14 bilateral cases (12 sporadic, 2 familial). Among bilateral cases I found 13 mutations: 8 non sense mutations, 3 splice mutations, a deletion involving exon 20, and a partial gene deletion involving exons 1-18; among unilateral cases we found a nucleotide substitution involving the promoter and a non sense mutation. The consequence of the nucleotide substitution involving the promoter region is that the nuclear factors do not bind to the mutant sequences; these nuclear factors are necessary for the expression of the Rb gene and the suppression of cancer.



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## **Evaluation of anorectal function after radiotherapy in patients treated for rectal cancer**

Radiotherapy improves local control in rectal cancer treatment and it is currently recommended in an increasing number of patients. However, there are few reports on the influence of radiotherapy on anorectal function.

In this study, we first investigated the functional changes of the internal anal sphincter (IAS) following radiotherapy in vitro. We collected IAS strips from patients undergoing abdominoperineal resection or proctectomy and monitored the responses to electrical field stimulation (EFS) and different drugs. Five patients were treated by surgery alone, and six received pre-operative radiotherapy. There were significant differences in the responses of control and irradiated strips to EFS ( $p < 0.01$ ), N $\omega$ -nitro-L-arginine ( $p < 0.01$ ), carbachol ( $p < 0.05$ ) and phenylephrine ( $p < 0.05$ ).

Then, we evaluated the anorectal function and clinical outcome of patients undergoing anterior resection for rectal cancer who received preoperative radiotherapy, and compared with the results of patients treated with surgery alone. Twenty-one patients had received preoperative radiotherapy and 13 patients had been treated with surgery alone.

Anorectal function was evaluated using questionnaires, anorectal manometry and endoanal ultrasound.

Functional outcome was disappointing in both groups comparing with preoperative results. In particular, rectal compliance was significantly reduced after surgery. However, irradiated patients had significantly more symptoms of faecal incontinence (57 vs. 26 percent,  $p < 0.01$ ), soiling (38 vs. 16 percent,  $p < 0.05$ ) and bowel movements (20 vs. 10,  $p < 0.05$ ) compared to controls. At anorectal manometry, irradiated patients had significantly lower resting (35 mmHg vs. 62 mmHg,  $p < 0.01$ ) and squeeze pressures (104 mmHg vs. 143 mmHg,  $p < 0.05$ ), and showed more scarring of the anal sphincters at endoanal ultrasound (33 vs. 13 percent,  $p < 0.05$ ). In conclusion, anterior resection alone adversely affects the functional outcome of patients treated for rectal cancer, resulting in increased evacuation disorders and symptoms of faecal incontinence. In particular, anorectal function after rectal surgery with or without radiotherapy is hampered because of a decreased rectal compliance. Radiotherapy further worsens anal continence mainly by impairing IAS function.



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### **Intrinsic toxicity, inflammatory potency and individual susceptibility in the occurrence of health damage from Particulate Material (PM)**

Reactive oxygen species (ROS)—which are considered one of the main cellular stressors generated by PM exposure—may produce genomic hypomethylation and increased expression and activity of inducible nitric oxide synthase (iNOS) not only in vitro, but in humans exposed to particulate matter (PM). Although this finding is expected, it is a step forward, based on DNA adduct generation produced by polycyclic aromatic hydrocarbons (PAHs) and other PM components, namely transition metals. Alterations of DNA methylation of the promoter is a common finding in environmental-related chronic or cancerous diseases.

The occurrence of DNA adducts or mutations in some cells or tissues due to exposure to PAHs or diesel exhaust does not necessarily induce clinically evident outcomes in the future, because each individual is endowed with a wide variety of natural defenses and repair mechanisms that usually overcome every type of DNA damage. Chronic exposure to toxic or carcinogenic environmental substances does not elicit the same results in all individuals. The final clinical outcome (cancer, pulmonary fibrosis) seems to be less dependent on the toxic potency of the pollutant or of the exposure dose and more on the individual susceptibility of the host. This approach should facilitate a better understanding of the < 5% incidence of mesotheliomas in subjects with the same chronic exposure to asbestos, or the absence of health effects in husbands with chronic occupational exposure to asbestos but the occurrence of mesotheliomas in wives with minor indirect exposure from their husbands.

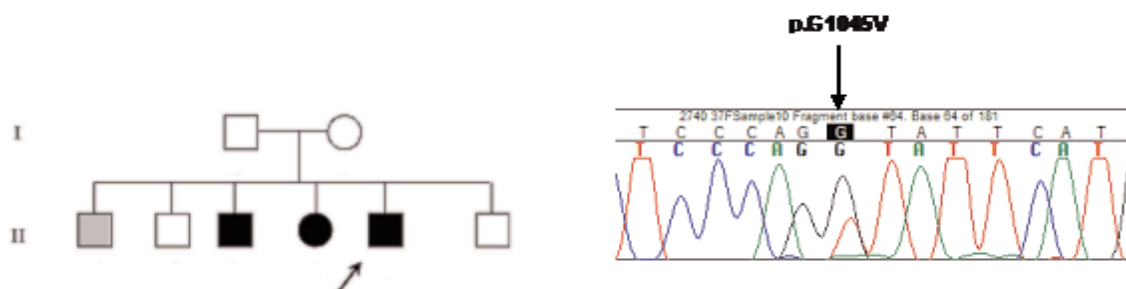
The final clinical outcome (e.g. cancer) does not depend on the first DNA adduct formed or a genetic mutation produced by xenobiotics but is greatly influenced by individual susceptibility or resistance.

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### Autosomal Alport syndrome: a new model including both dominant and recessive inheritance

Alport syndrome is a clinically and genetically heterogeneous nephropathy characterized by irregular thinning, thickening, and splitting of the glomerular basement membrane often associated with hearing loss and ocular symptoms. The majority of cases, about 85%, are transmitted as an X-linked form due to COL4A5 mutations. Less than 15% of cases are autosomal recessive and dominant forms due to mutations in either COL4A3 or COL4A4 genes. My research project focus on the analysis of the COL4A4 and COL4A3 genes in a large cohort of patients by DHPLC (Denaturing High Performance Liquid Chromatography) and automated sequencing. Molecular analysis revealed in COL4A4 or COL4A3 gene 24 different mutations: 18 autosomal dominant forms (from 20 different families) and 6 autosomal recessive forms (from 4 different families). The single base deletion p.P629fsX652 was found in two different unrelated families coming from Marsala, while the missense mutation p.G1045V was found in three different unrelated families coming from Castelfranco Veneto. This could lead us to think of a possible founder effect for this two mutations. In one of the three families with p.G1045V mutation, an initial clinical analysis of the proband, of the brother and the sister showed that they developed renal disease at the age of 31, 26 and 19 years respectively, suggesting the hypothesis of a recessive Alport syndrome. This hypothesis is confirmed by the fact that the proband's father present a mutation with a normal clinical profile. However, the older brother, aged 44, showed only microscopic hematuria and proteinuria suggesting an autosomal dominant form. This suggest a new model including both dominant and recessive inheritance. In conclusion, it is very difficult to predict the prognosis in a patient with an heterozygous mutation in either the COL4A3 or the COL4A4 gene. A correct diagnosis and prognosis is based on a combination of a comprehensive clinical investigation of all family members, including examination of renal and extra-renal signs of Alport syndrome in older members, associated with a broadly formal genetic analysis of the pedigree.



Part of this work is published in:

Marcocci et al. Autosomal Dominant Alport syndrome: molecular analysis of the COL4A4 gene and clinical outcome. Nephrol Dial Transplant. 2009 May; 24(5):1464-71.



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### **Prostate cancer: the influence of STAT3 and the presence of Human Polyomavirus BK in cells**

Prostate cancer (PC) is a common cause of death and is incurable in the androgen-refractory phase. Multiple factors contribute to PC development, especially, signal messengers and transcription factors such as the Signal Transducer and Activator of Transcription 3 (STAT3). STAT3 is abnormal activated in PC cells where it changes gene expression leading to transformation.

Oncogenic infectious agents could also play a role in PC. The Human Polyomavirus BK (BKV) could be a candidate since it is an ureteliotropic virus, almost 90% of adults are seroconvert and the in vitro oncogenesis is proven. In fact, BKV large T-Antigen (TAg) could deregulate cell's cycle by sequestering tumor suppressors as p53. In this study, fresh biopsies of 15 patients (median age of 60) with clinically pT3a androgen-refractory PC, were analysed. STAT3 target genes, in PC gene profile, were detected using ONCOMINE database and their expression was investigated using RetroTranscriptional Quantitative PCR (RT-QPCR). BKV DNA and AgT RNA were searched using Quantitative PCR (QPCR) and RT-QPCR. As controls for BKV, non tumour biopsies of the same patients were analyzed. Results showed that STAT3 target oncogenes such as c-myc, cdc25A, survivin and Erp57 was overexpressed, BKV DNA was found in 3/15 patients and TAg mRNA was not detected. About controls, BKV was detected only in one. Data underline that STAT3 has a causal role in oncogenesis and is a target for cancer drug discovery and therapies. About BKV, DNA presence doesn't exclude viral pressure on cell transformation. Nevertheless, more investigations are required to elucidate how STAT3 influence gene expression, how cytokines could influence STAT3 response and finally if really BKV could operate on PC susceptibility.

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## Copy Number Variations analysis in Autism Spectrum Disorders

Autism Spectrum Disorders (ASDs) have a complex and heterogeneous aetiology with a strong evidence of a genetic involvement. To assess the frequency and type of copy number variations (CNVs) in ASD, a cohort of 95 patients has been selected and analyzed by oligo array-CGH with a functional resolution of nearly 100 kb. Array-CGH resulted negative in 60 patients while in 35 at least one rearrangement was identified. A total of 49 rearrangements was identified: 22 deletions and 27 duplications. Among the 35 patients with CNVs the M:F ratio is higher than expected resulting in 6:1. Seven CNVs turned to be pathogenic: two de novo (del16p11.2; del Xq12) and 5 located in known autism susceptibility regions (dup15q13.3, dup16p13.1, del15q11.2, del11p12, dup17q12). The 7 patients with pathogenic CNVs were all males with intellectual disability (ID). The majority presented with congenital anomalies (MCA) and dysmorphisms (57.1%); none suffered from epilepsy. Among the cohort of 60 patients without CNVs the M:F ratio resulted in 5.6:1, as currently reported in ASDs. ID was present in 96.5%; MCA and dysmorphisms were present respectively in 21% and 63.2%. Epilepsy rate was 19.3%. The detection rate of CNVs in our series of patients is 7.4%. Patients with pathogenic CNVs differed from the cohort without any CNVs for the presence of congenital anomalies, more frequent in the first group. Furthermore, the rate of epilepsy found in the all group was 19.3%, while in the subgroup of patients with pathogenic CNVs (n=7) epilepsy was not detected. Finally, three patients presented two inherited rearrangements each, one inherited by the mother and the other inherited by the father, leading to hypothesize the possibility of a digenic or multi-genic inheritance.

Sample ID	Gender	Chromosome	Rearrangement	Inheritance	Size	Comments
<b>De novo rearrangements</b>						
#518	M	16p11.2	Loss	De novo	450Kb	susceptibility region to ASD
#142	M	Xq12	Loss	De novo	80Kb	It contains the EDA2R gene
<b>Rearrangements in known susceptibility regions</b>						
#1537	M	16p13.11	Gain	maternal	1.1Mb	susceptibility region to ASD
#221	M	15q13.3	Gain	unknown	500Kb	susceptibility region to ASD
#1	M	17q12	Gain	paternal	1.8Mb	susceptibility to cognitive impairment and behavioral abnormalities
#1475	M	3q29 11p12	Gain Loss	maternal maternal	230Kb 1.1Mb	Recurrent and overlapping locus in ASD (Marshall et al, 2008)
#746	M	15q11.2	Loss	unknown	17Kb 159Kb	included in susceptibility region to developmental delay, behavioural problems and dysmorphisms

Part of this work is reported in:

Mucciolo et al. Autism Spectrum Disorders and Copy Number Variations. 6th International DECIPHER Symposium, Hinxton (UK), May 19-21, 2010. Oral presentation

Mari et al. Array-CGH analysis in Autism Spectrum Disorders. 6th International meeting on cryptic chromosomal rearrangements and genes in mental retardation and autism, Troina, April 22-24, 2010. Oral presentation

Mucciolo et al. Autism Spectrum Disorders: emerging data from Copy Number Variations analysis. European Congress of Human Genetics, Goteborg, June 14-16, 2010. Poster

Canitano et al. CNVs in Autism Spectrum Disorders. American Academy of Child & Adolescent Psychiatry, New York City, NY, October 27-31, 2010. Poster



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### Bioinformatics Approach to The pRb pathway in cancer initiation and progression

pRb is the key gene in a rare pediatric eye neoplasm (sporadic and hereditary) arising from retinal cells that harbor either a deletion or mutation inactivation of both pRb alleles. pRb is considered a bona fide tumor suppressor gene. Its mutation and deletion is shared by several other malignancies. In a succinct word, pRb is considered one of the hallmarks of human malignancies. pRb prevents the cell from replicating damaged DNA by preventing its progression along the cell cycle through G1 (first gap phase) into S (synthesis phase). pRb binds and inhibits transcription factors of the E2F family, which are composed of dimers of an E2F protein and a DP protein. The transcription activating complexes of E2 promoter-binding–protein-dimerization partners (E2F-DP) can allow the cell into S phase. As long as E2F-DP is inactivated, the cell remains stalled in the G1 phase. When pRb is bound to E2F, the complex acts as a growth suppressor and prevents progression through the cell cycle. The pRb-E2F/DP complex also interacts with histone deacetylase (HDAC) protein to the chromatin, reducing transcription of S phase promoting factors, further suppressing DNA synthesis

We are interested in finding pRb pathway in relation to other key players using bioinformatics approach. Deregulations and mutations in this pathway are observed in most human cancer and the data analysis of this pathway is still on the developmental stage and more complex structural analysis of the pRb pathway will be undertaken. On the final analysis, a more thorough experimentation using CellDesigner software that will be translated into BioPAX format will be performed and the result will be tested with the existing pathway databases. The construction of this pathway will serve among other purposes, to provide a map of pRb and other related proteins that not only serve as a reference and a tool to understand the pathway but we will be able to predict its behavior in response to different types of deregulations. As a long-term goal, we will connect the pRb pathway with other pathways involved in the tumorigenesis with the scope to design new specific molecules for a multi-drug approach.

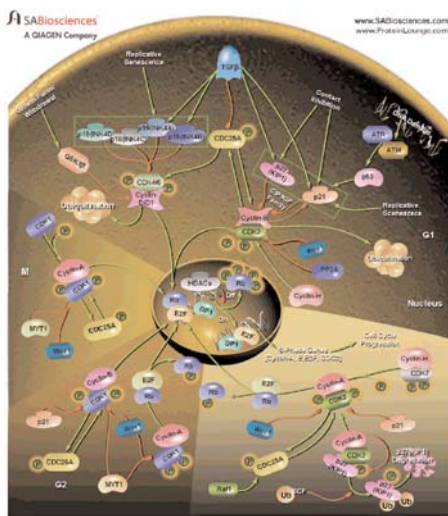


Diagram: Comprehensive cyclins and cell cycle regulation showing RB pathways - courtesy of SABiosciences.

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### Detection and role of mir146a in HIV-clinical samples

MicroRNA-mediated regulation of gene expression appears to be involved in a variety of cellular processes, including development, differentiation, proliferation and apoptosis.

Interestingly, miRNAs are secreted actively as free miRNAs or through microvesicles (MVs), suggesting that these miRNAs may function outside the cells in which they are produced.

Mir146a is thought to be involved in the regulation of the innate immune response, and its expression is increased in tissues associated with chronic inflammation.

In our previous study, we have analyzed changes in the expression of mir146a in primary human fetal microglial cells upon infection with HIV and we have found increased expression of mir146a.

Furthermore, we have showed that CCL8/MCP-2, a ligand for the CCR5 chemokine receptor and a potent inhibitor of CD4/CCR5-mediated HIV-1 entry and replication, is a target for mir146a in HIV-1 infected microglial cells.

Accordingly, overexpression of mir146a prevented HIV-induced secretion of MCP-2 chemokine, suggesting a role for mir146a in the maintenance of HIV-mediated chronic inflammation of the brain.

Presently, we are focusing on the role of free and MVs-released mir-146a in the settings of HIV, in particular on the capability of mir146a to be uptaken by myeloid dendritic cells (DC) and, eventually, on the modulation of different cellular events, including the innate immune response.

This work is published in:

CCL8/MCP-2 is a target for mir-146a in HIV-1-infected human microglial cells. Rom S. et al. *Faseb J.* 24, 000-000 (2010)

This work is funded by:

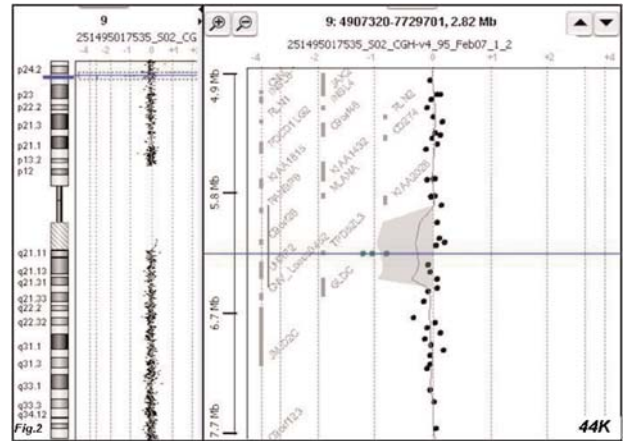
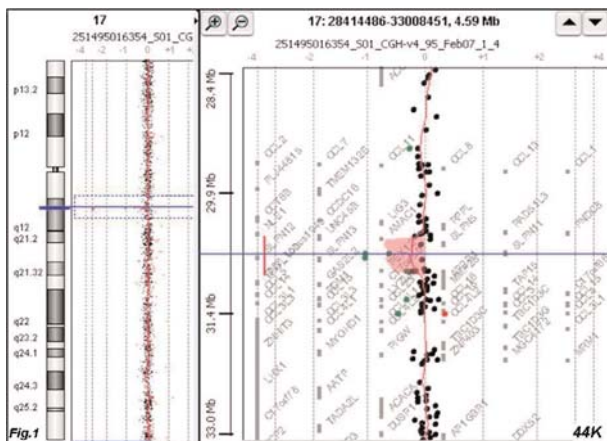
Francesca Peruzzi NIH Grants



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## Diagnosis process assessment of Array-CGH analysis

The use array-CGH (comparative genomic hybridization) has had a great impact on the practice of medical genetics. This technology has been used to expand the phenotypes of well recognized clinical entities, determine the genomic lesions in known conditions, discover new syndromes, assess the prevalence of mosaicism, and ascertain the unexpected frequency of copy number variants (CNVs) across the genome. We performed oligo array-CGH analysis in DNA extracted from 330 patients with mild to severe mental retardation associated with facial dysmorphisms and/or congenital anomalies. We identified 61 CNVs, ranging from 0.06 to 2.9 Mb, that were not previously described in the Database of Genomic Variants. In all cases, the same rearrangement was inherited from a healthy parent. Fifty nine of the rearrangements were private, while del17q12 (Fig. 1) was found in 3 patients and a del9p24.1 (Fig. 2) was found in 2 patients. Fourteen of the rearranged regions contain disease genes. It is important to note that the presence of CNVs may be of difficult interpretation for the geneticist since some CNV may comprise benign genomic variants along with pathogenic rearrangements. Furthermore, concurrent variations in the other alleles or in another chromosome may influence the phenotype. In order to firmly establish the clinical phenotype associated with a rearrangement, it is of critical importance to examine additional patients with the same rearrangements, preferably through collaborative efforts among several genetics units, and to update frequently the database and literature.



This work is published in:  
 Mencarelli MA et al. Private inherited microdeletion/microduplications: implications in clinical practice.  
 Eur J Med Genet. 2008 Sep-Oct;51(5):409-16.

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### Identification and characterization of deletions and duplications by MLPA in patients with Cohen syndrome

Cohen syndrome is a rare, clinically variable autosomal recessive disorder associated to mutations in COH1 gene and characterized by mental retardation, postnatal microcephaly, facial dysmorphisms, ocular abnormalities and intermittent neutropenia. About 100 mutations of COH1 have been identified so far, most of which are point mutations identified by automatic sequencing. Recently, MLPA was used to screen for COH1 large rearrangements instead of quantitative PCR, that is prone to miss a high fraction of mutated alleles. In 14 patients MLPA allowed us to disclose 11 deleted and 4 duplicated COH1 alleles. Three Italian families shared the same deletion, spanning exons 6 to 16, already reported in a large Greek consanguineous family. Haplotype analysis suggested that the recurrent deletion is due to an ancestral founder effect in the Mediterranean area. Two other deletions, spanning exons 4–16 and 40–43, have been already reported in the Northern European population. In this case, these could be independent mutations favored by the presence of repeated elements located at the breakpoints. MLPA also identified three duplications spanning exons 4–13, 20–30 and 57–60. Until now, COH1 intragenic duplications have never been reported in Cohen syndrome. These rearrangements probably led to a frameshift and a premature truncation of the protein, as demonstrated by sequencing the breakpoints of one of them. In conclusion, our study confirms that COH1 copy number variations are a frequent cause of Cohen syndrome and consist of intragenic deletions as well as duplications. Therefore, the use of MLPA for molecular analysis of COH1 gene is mandatory in the molecular diagnosis of Cohen syndrome.

Marker	Position (Mb)	C37 (Greek)		Case 5 (Italian)		Case 9A (Italian)		Case 8 (Italian)	
D8S1018	97,598	315	319	315	315	319	323	319	315
D8S257	99,451	109	109	109	109	109	-	109	113
8-23TC	99,924	214	214	214	214	214	218	214	204
8-25GT	100,056	353	353	353	353	353	379	353	351
8-20TG	100,601	169	169	169	169	169	173	169	173
VPS13B	-	del6_16	del6_16	del6_16	del6_16	del6_16	del46_49	del6_16	Y3855fsX22
D8S1789*	100,738	255	255	255	255	255	255	255	255
D8S470*	100,743	226	226	226	226	226	226	226	226
D8S300	100,987	485	485	485	485	485	499	485	499
8-18AC	101,066	95	95	95	95	95	97	95	97
D8S398	101,588	141	141	141	141	137	141	137	141

\* intragenic markers

grey columns: haplotype co-segregating with the deletion

This work is published in:

Parri V. et al. High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome. Eur J Hum Genet. 2010 May 12.

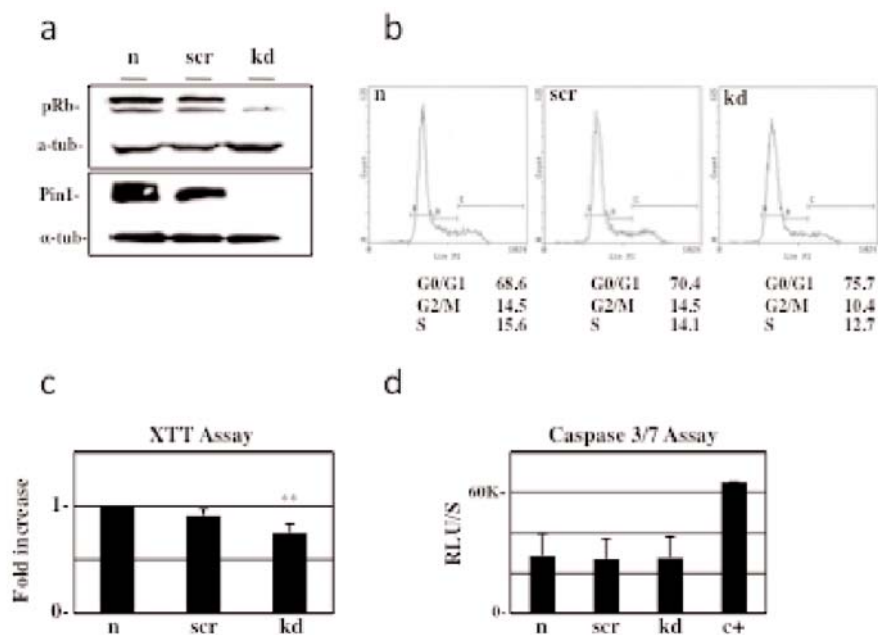


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### Pin1 controls cell cycle progression through interaction with pRB

Normal cells became tumor cells through deregulation of multiple pathways. Much evidence suggests that each type of tumors involves different proteins so that each type of cancer cells is different from the others. Nevertheless, there are some pathways that are altered in many tumors and RB pathway is one of the most important. pRB controls the cell cycle through the interaction with E2F transcription factors. These interactions are regulated during cell cycle by a phosphorylation mechanism. Ser or Thr followed by Pro are major phosphorylation motifs in the cells but their significance was obscure until the discovery of the PIN1 protein (protein interacting with NIMA (never in mitosis A)-1).

Pin1 is an isomerase specific of pSer/Thr-Pro motifs that catalyzed the conformational switch from cis to trans, which is especially important because Pro-directed kinases and phosphatases are conformation-specific and act only on the trans conformation. In vivo and in vitro data have demonstrated that Pin1 is involved in many aspects of cell cycle control. PIN1 was originally identified and defined as a protein that function in mitosis. Since then, a plethora of protein targets have now been discovered many of which are involved in the G0, G1/S control. We have demonstrated that Pin1 control cell proliferation through direct interaction with pRB protein. The interaction is phosphorylation-dependent and it is also necessary for pRB phosphorylation. An increase number of cells in G1 is observed in Pin1 knockdown cells. No apoptosis or senescence is detected. These results suggest that Pin1 can function as oncogene and Pin1 represents a excellent “druggable” target since it appear a not essential gene in normal cells.



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## QF-PCR as a tool for rapid prenatal diagnosis

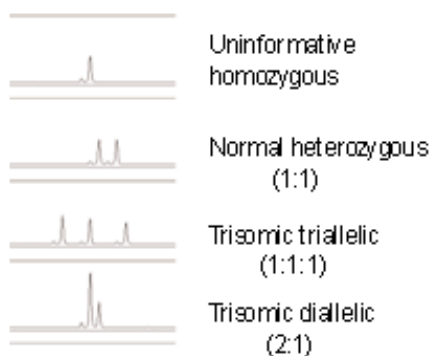
Rapid prenatal diagnosis of major chromosome abnormalities can be performed using Short Tandem Repeats (STRs) amplified by the Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR).

The Aneufast QF-PCR Kit allows the detection of aneuploidies involving chromosomes 13, 18, 21, X and Y with 100% sensitivity and specificity, reporting results in 24-72h. This kit contains a total of 29 markers in six mixes ready to use that amplify selected STRs and the gender determining sequences Amelogenin-SRY.

From May 2008 until today, 976 prenatal samples arrived and we have analysed by QF-PCR 386 samples: 232 amniotic fluids and 148 chorionic villus. The most common indications for prenatal diagnosis in our cohort were advanced maternal age (86,4%), ecographic anomalies (5,5%), and biochemical risk (8,1%). QF-PCR assays detected two cases of trisomy 13, five cases of trisomy 18, nine cases of trisomy 21 and one case of triploidy which were confirmed by traditional karyotyping.

We have also a small casistic of abort samples: 23 cases analysed by QF-PCR that detected six cases of trisomy 21 and a case of X monosomy.

Our results are in accordance with recent studies, that following the analysis of several thousand samples, have shown that this rapid approach has a very high rate of success and it could reduce the need for cytogenetic investigations. The main advantages of the QF-PCR are its accuracy, speed, automation, and low cost that allows very large number of samples to be analyzed by few operators. For these reasons, in a near future, this kind of assay could replace cytogenetic analyses.



ANEUPLOIDIE	N° CASI
<u>Trisomy 13</u>	2
<u>Trisomy 18</u>	5
<u>Trisomy 21</u>	15
<u>Monosomy X</u>	1

POLIPLOIDIE	N° CASI
<u>Triploidy</u>	1



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Tutor A. Renieri

### **Molecular work-up of patients with the congenital variant of Rett syndrome: analysis of the 14q12 region.**

Rett syndrome is a severe neurodevelopment disorder and it is the second most common cause of mental retardation in females. Beside the most common classic form, due to MECP2 mutations, a number of variants have been observed such as the congenital variant. During my Ph.D program, I work in a project to study the molecular cases of RETT Syndrome and By “candidate gene approach”, we have recently identified the new gene FOXG1 (MIM 164874) as responsible for the congenital variant of RTT. FOXG1 gene encodes for a brain-specific transcriptional repressor that bears three main functional domains: the fork-head domain (FHD), the Groucho-binding domain (GTBD), and the JARID1B binding domain (JBD). After our first study, mutations in the FOXG1 gene, located in 14q12 have been identified as the cause of this variant. Nineteen cases have been reported to date with a FOXG1 loss of function alteration: seven deletions one duplication, one inversion and ten point mutations. In collaboration with university of Freiburg we identified an additional patient affected by the congenital variant of Rett having a whole FOXG1 deletion. We compared her clinical features with the characteristics of patients already reported in the literature. Even though the reported cases are still few and genotype phenotype correlation cannot be performed, there seems not to be a difference in the clinical picture between deleted patients and patients with point mutations. This thesis work underlines the importance to perform FOXG1 mutation analysis for both deletions and point mutations in patients with a phenotype resembling the congenital variant of Rett, prior the analysis of the MECP2 gene.

This work is reported in:

Ariani et al. FOXG1 is responsible for the congenital variant of Rett syndrome. *A. Am J Hum Genet.* 2008 Jul;83(1):89-93.

Mencarelli et al. Novel FOXG1 mutations associated with the congenital variant of Rett syndrome. *J Med Genet* 2010;47:49e53. doi:10.1136/jmg.2009.067884

This work is funded by:

EMBO Fellowship Programme ASTF number 322-2010 and the University of Siena.

Oncology and Genetics Doctoral School  
Hepatobiliopancreatic Disease and Multitumoral syndromes  
XXIV cycle  
**Rosalia Zangari, BS**  
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Tutor F. Cetta



### **The impact of traffic pollution on antioxidant system of two populations exposed to different levels of pollutants**

Aim of this study was to investigate whether the traffic pollution is related with alteration of thiol, redox and energetic status, for the early recognition of at risk-subjects in a population exposed to environmental pollution, in a heavy polluted city, such as Milan, Italy.

We compared thiol levels, both in blood and in plasma, and erythrocyte redox (NADH, NAD<sup>+</sup>, NADPH AND NADP<sup>+</sup>) and energetic (ATP, ADP and AMP) status in a group of subjects living downtown compared to a control population.

All analytes were assayed by HPLC. Thiols were measured both in reduced and total forms. Daily levels of outdoor and indoor PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>1</sub> concentrations were conducted using an optical particle counter (OPC Grimm).

The concentrations within the building were heavily dependent on external concentration. During the day, indoor PM concentration was higher than outdoor, but PM<sub>10</sub> was the highest of all. This phenomenon is due to a coarse particle resuspension by internal domestic activity.

There was no evident difference in the GSH levels between the two populations. In fact, GSH alteration is usually a late event, occurring in severe imbalances and no subject in both groups had clinically evident diseases. Inhabitants of areas with different traffic volumes show significantly different Cys CG and Hcy levels, even if GSH remains unchanged, suggesting a greater- pro-oxidant effect in more exposed populations, affecting Cys CG and Hcy levels, before GSH alterations. A lower NADH level and a higher plasma total Hcy level in more exposed populations is able to distinguish between more and less exposed subjects and could be a useful diagnostic tool for early detection of subjects at higher risk of health effects from environmental pollution.

This work is reported in:

Zangari R. et al. The impact of traffic pollution on antioxidant system of two populations exposed to different levels of pollutants: estimation of redox status of thiols. 1286 "International Aerosol Conference (IAC 2010) Helsinki, Finland 29 August – 3 September 2010.

This work is funded by:

CARIPLO Grant to POLARIS and by the PROLIFE-Project, City of Milan, Italy.



## **Thesis discussion Doctorate in Medical Genetics February 5, 2010 Centro Didattico S Maria alle Scotte, room 15**

### **10.00 Entering of the PhD dissertation board composed by:**

- Prof. Maurizio Genuardi (President)  
Professor of Medical Genetics, University of Firenze, Italy
- Prof. Emma D'Andrea (Member)  
Professor of Pathology, University of Padova, Italy
- Prof. Giovanna Bianchi-Scarrà (Secretary)  
Professor of Biology, University of Genova, Italy

### **Thesis discussion in English language:**

- "Comprehensive analysis of the CDKN2A gene mutations in familial and multiple primary cutaneous melanoma" , Marina Vignoli, XXI cycle

### **10.45 Awarding of the PhD degree in Medical Genetics**



From left to right:  
Prof. Giovanna Bianchi-Scarrà,  
Prof. Maurizio Genuardi,  
Marina Vignoli,  
Prof. Emma D'Andrea.

**Thesis discussion  
Doctorate in  
October 1, 2010 Centro Didattico S Maria alle Scotte, room 15**

**MORNING SECTION**

**9.00 Entering of the PhD dissertation board composed by:**

- Prof. Gabriello Tanzini (President)  
Professor of Medical Surgery, University of Siena, Italy
- Prof. Pier Francesco Tassone (Member)  
Professor of Medical Oncology, University of Catanzaro, Italy
- Prof. Generoso Bevilacqua (Member)  
Professor of Human Pathology, University of Pisa, Italy
- Prof. Paola Mandich (Secretary)  
Professor of Medical Genetics, University of Genova, Italy

**Thesis discussion in English language:**

- “Endothelin and colorectal cancer: information for prognosis and treatment” , Antonella Chessa, XXI cycle

9.45 Awarding of the PhD degree in Colorectal and Gastroesophageal Diseases

**Thesis discussion  
Doctorate in  
October 1, 2010 Centro Didattico S Maria alle Scotte, room 15**

**10.00 Entering of the PhD dissertation board composed by:**

- Prof. Alessandra Renieri (President)  
Professor of Medical Genetics, University of Siena, Italy
- Prof. Paola Mandich (Member)  
Professor of Medical Genetics, University of Genova, Italy
- Prof. Vaiditus Kucinskas (Member)  
Human and Medical Genetics, Vilnius, Lithuania
- Prof. Mario Tecce (Secretary)  
Professor of Biochemistry, University of Salerno, Italy

**Thesis discussion in English language**

- “Molecular work-up of patients with the congenital variant of Rett syndrome: analysis of the 14q12 region”,  
Ariele Spanhol-Rosseto, XXII cycle

10.45 Awarding of the PhD degree in Medical Genetics and qualification of Doctor Europaeus

**Thesis discussion  
Doctorate in  
October 1, 2010 Centro Didattico S Maria alle Scotte, room 15**

**11.00 Entering of the PhD dissertation board composed by:**

- Prof. Alessandra Renieri (President)  
Professor of Medical Genetics, University of Siena, Italy
- Prof. Paola Mandich (Member)  
Professor of Medical Genetics, University of Genova, Italy
- Prof. Pier Francesco Tassone (Secretary)  
Professor of Medical Oncology, University of Catanzaro, Italy

**Thesis discussion in English language:**

- “Autosomal Alport syndrome: a new model including both dominant and recessive inheritance” , Elena Marocci, XXII cycle

11.45 Awarding of the PhD degree in Medical Genetics

**Thesis discussion  
Doctorate in  
October 1, 2010 Centro Didattico S Maria alle Scotte, room 15**

**12.00 Entering of the PhD dissertation board composed by:**

- Prof. Alessandra Renieri (President)  
Professor of Medical Genetics, University of Siena, Italy
- Prof. Mario Tecce (Member)  
Professor of Biochemistry, University of Salerno, Italy
- Prof. Generoso Bevilacqua (Member)  
Professor of Human Pathology, University of Pisa, Italy
- Prof. Paola Mandich (Secretary)  
Professor of Medical Genetics, University of Genova, Italy

**Thesis discussion in English language:**

- “Retinoic acid and breast cancer: how to improve the therapeutic effect on the basis of molecular knowledge”, Valeria Guarnaccia, XXII cycle

12.45 Awarding of the PhD degree in Medical Genetics

**Thesis discussion  
Doctorate in  
October 1, 2010 Centro Didattico S Maria alle Scotte, room 15**

**AFTERNOON SECTION**

**14.00 Entering of the PhD dissertation board composed by:**

- Prof. Antonio Giordano (President)  
Professor of Human Pathology, University of Siena, Italy
- Prof. Pier Francesco Tassone (Member)  
Professor of Medical Oncology, University of Catanzaro, Italy
- Prof. Generoso Bevilacqua (Member)  
Professor of Human Pathology, University of Pisa, Italy
- Prof. Wolfgang Bohn (Member)  
Heinrich-Pette-Institute for Experimental Virology and Immunology, University of Hamburg, Germany
- Prof. Mario Tecce (Secretary)  
Professor of Biochemistry, University of Salerno, Italy

**Thesis discussion in English language**

- "A Phase 1 Dose Escalation Study of ARQ 197 in Adult Patients with Metastatic Solid Tumors" ,  
Abbadessa Giovanni, XXII cycle

14.45 Awarding of the PhD degree in Oncological Genetics and qualification of Doctor Europaeus

**Thesis discussion  
Doctorate in  
October 1, 2010 Centro Didattico S Maria alle Scotte, room 15**

**15.00 Entering of the PhD dissertation board composed by:**

- Prof. Antonio Giordano (President)  
Professor of Human Pathology, University of Siena, Italy
- Prof. Pier Francesco Tassone (Member)  
Professor of Medical Oncology, University of Catanzaro, Italy
- Prof. Generoso Bevilacqua (Member)  
Professor of Human Pathology, University of Pisa, Italy
- Prof. Wolfgang Bohn (Member)  
Heinrich-Pette-Institute for Experimental Virology and Immunology, University of Hamburg, Germany
- Prof. Mario Tecce (Secretary)  
Professor of Biochemistry, University of Salerno, Italy

**Thesis discussion in English language:**

- “miRNA expression profiling in malignant mesothelioma” , Khadang Baharak, XXII cycle

15.45 Awarding of the PhD degree in Oncological Genetics and qualification of Doctor Europaeus

**Thesis discussion  
Doctorate in  
October 1, 2010 Centro Didattico S Maria alle Scotte, room 15**

**16.00 Entering of the PhD dissertation board composed by:**

- Prof. Antonio Giordano (President)  
Professor of Human Pathology, University of Siena, Italy
- Prof. Pier Francesco Tassone (Member)  
Professor of Medical Oncology, University of Catanzaro, Italy
- Prof. Generoso Bevilacqua (Member)  
Professor of Human Pathology, University of Pisa, Italy
- Prof. Wolfgang Bohn (Member)  
Heinrich-Pette-Institute for Experimental Virology and Immunology, University of Hamburg, Germany
- Prof. Mario Tecce (Secretary)  
Professor of Biochemistry, University of Salerno, Italy

**Thesis discussion in English language:**

- "PIN1, the cell cycle control and cancer: a new player in the RB pathway", Flavio Rizzolio, XXII cycle

16.45 Awarding of the PhD degree in Oncological Genetics and qualification of Doctor Europaeus

**Thesis discussion  
Doctorate in  
December 14, 2010 Centro Didattico S Maria alle Scotte, room 15**

**MORNING SECTION**

**9.00 Entering of the PhD dissertation board composed by:**

- Prof. Alessandra Renieri (President)  
Professor of Medical Genetics, University of Siena, Italy
- Prof. Mario De Marchi (Member)  
Professor of Medical Genetics, University of Torino, Italy
- Prof. Francesca Gensini (Secretary)  
Professor of Medical Genetics, University of Firenze, Italy

**Thesis discussion in English language:**

- "Diagnosis process assessment of Array-CGH analysis" , Filomena Tiziana Papa, XXII cycle

9.45 Awarding of the PhD degree in Medical Genetics

**Thesis discussion  
Doctorate in  
December 14, 2010 Centro Didattico S Maria alle Scotte, room 15**

**10.00 Entering of the PhD dissertation board composed by:**

- Prof. Francesco Cetta (President)  
Professor of Medical Surgery, University of Siena, Italy
- Prof. Mario De Marchi (Member)  
Professor of Medical Genetics, University of Torino, Italy
- Prof. Francesca Gensini (Secretary)  
Professor of Medical Genetics, University of Firenze, Italy

**Thesis discussion in English language:**

- “Correlation between aging and tumorigenesis: the role of genetic factors in Werner syndrome and related syndromes” , Simona Benoni, XXII cycle

10.45 Awarding of the PhD degree in Hepatobiliopancreatic Diseases and Multitumoral Syndromes

**Thesis discussion  
Doctorate in  
December 14, 2010 Centro Didattico S Maria alle Scotte, room 15**

**11.00 Entering of the PhD dissertation board composed by:**

- Prof. Francesco Cetta (President)  
Professor of Medical Surgery, University of Siena, Italy
- Prof. Mario De Marchi (Member)  
Professor of Medical Genetics, University of Torino, Italy
- Prof. Francesca Gensini (Secretary)  
Professor of Medical Genetics, University of Firenze, Italy

**Thesis discussion in English language:**

- “Role of diet and gender in the onset and/or protection in tumors” , Giuliana Malagnino, XXI cycle
- 11.45 Awarding of the PhD degree in Hepatobiliopancreatic Diseases and Multitumoral Syndromes

**Thesis discussion  
Doctorate in  
December 14, 2010 Centro Didattico S Maria alle Scotte, room 15**

**12.00 Entering of the PhD dissertation board composed by:**

- Prof. Francesco Cetta (President)  
Professor of Medical Surgery, University of Siena, Italy
- Prof. Mario De Marchi (Member)  
Professor of Medical Genetics, University of Torino, Italy
- Prof. Francesca Gensini (Secretary)  
Professor of Medical Genetics, University of Firenze, Italy

**Thesis discussion in English language:**

- “Genetics and molecular biology in the multidisciplinary approach to pressure ulcers and lower limb ulcers in diabetics” , Cisternino Filomena, XXII cycle

12.45 Awarding of the PhD degree in Hepatobiliopancreatic Diseases and Multitumoral Syndromes

**Thesis discussion  
Doctorate in  
December 14, 2010 Centro Didattico S Maria alle Scotte, room 15**

**AFTERNOON SECTION**

**14.00 Entering of the PhD dissertation board composed by:**

- Prof. Francesco Cetta (President)  
Professor of Medical Surgery, University of Siena, Italy
- Prof. Mario De Marchi (Member)  
Professor of Medical Genetics, University of Torino, Italy
- Prof. Francesca Gensini (Secretary)  
Professor of Medical Genetics, University of Firenze, Italy

**Thesis discussion in English language:**

- “Papillary carcinoma of thyroid associated to familial adenomatosis” , Barellini Leonardo , XX cycle
- 14.45 Awarding of the PhD degree in Hepatobiliopancreatic Diseases and Multitumoral Syndromes

**Thesis discussion  
Doctorate in  
December 14, 2010 Centro Didattico S Maria alle Scotte, room 15**

**15.00 Entering of the PhD dissertation board composed by:**

- Prof. Francesco Cetta (President)  
Professor of Medical Surgery, University of Siena, Italy
- Prof. Mario De Marchi (Member)  
Professor of Medical Genetics, University of Torino, Italy
- Prof. Francesca Gensini (Secretary)  
Professor of Medical Genetics, University of Firenze, Italy

**Thesis discussion in English language:**

- “Predictors of invasivity in Intraductal Pancreatic Mucinous Neoplasms” , Mariani Federico , XIX cycle
- 15.45 Awarding of the PhD degree in Hepatobiliopancreatic Diseases and Multitumoral Syndromes



