

ISTITUTO SUPERIORE DI SANITÀ

Workshop

Rare Diseases and Orphan Drugs

Istituto Superiore di Sanità
Rome, November 7-8, 2007

ABSTRACT BOOK

Edited by
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Workshop. Rare Diseases and Orphan Drugs. Rome, November 7-8, 2007. Abstract book.

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2007 xv, 134 p. ISTISAN Congressi 07/C8

The international Conference on rare diseases and orphan drugs (November 5-8, 2007, Istituto Superiore di Sanità) is an annual meeting organized by the National Centre for Rare Diseases (NCRD). The aims of the Conference are to illustrate national and international activities on rare diseases and orphan drugs and to promote new Italian advancements within the European context. The main topic of the Conference is scientific research and a Workshop (November 7-8) is dedicated specifically to the preliminary results of research projects funded within the frame of the bilateral agreement between the Istituto Superiore di Sanità (Italy) and the National Institute of Health, Office for Rare Diseases (USA). These projects are intended to improve scientific knowledge on etiopathogenesis, diagnosis and treatment of specific rare diseases.

Key words: Rare Diseases, Orphan Drugs, Research

Istituto Superiore di Sanità

Workshop. Malattie Rare e Farmaci Orfani. Roma, 7-8 novembre, 2007. Riassunti.

A cura di Domenica Taruscio e Marco Salvatore

2007 xv, 134 p. ISTISAN Congressi 07/C8 (in inglese)

La Conferenza internazionale sulle malattie rare ed i farmaci orfani (5-8 novembre 2007, Istituto Superiore di Sanità) rappresenta un appuntamento annuale organizzato dal Centro Nazionale Malattie Rare (CNMR), finalizzato ad illustrare le attività nazionali ed internazionali intraprese nel settore delle malattie rare e dei farmaci orfani e a proporre possibili nuove iniziative in Italia, tenendo conto anche del più ampio contesto europeo. All'interno della Conferenza internazionale ampio spazio è dedicato alla ricerca scientifica. In questo contesto si inserisce il Workshop (7-8 novembre) dedicato alla presentazione dei progetti di ricerca finanziati nell'ambito dell'accordo bilaterale fra l'Istituto Superiore di Sanità (Italia) e il National Institute of Health, Office for Rare Diseases (USA). Questi progetti sono finalizzati ad incrementare le conoscenze scientifiche su eziopatogenesi, diagnosi e trattamento di specifiche malattie rare.

Parole Chiave: Malattie Rare, Farmaci Orfani, Ricerca

This Workshop is the presentation of research projects funded in the frame of the bilateral Italy-USA (Istituto Superiore di Sanità-National Institute of Health, Office for Rare Diseases) agreement on joint research and development of public health actions on rare diseases.

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PROGRAMME

Wednesday, November 7, 2007

- 9.00 Welcome and objectives of the Workshop
Enrico Garaci, Stephen Groft
President of the Istituto Superiore di Sanità
Director of the Office for Rare Diseases, National Institute of Health

Session I

ASPECTS OF PATHOGENESIS

Chairpersons: Filippo Belardelli, Maurizio Pocchiari

- 9.30 *β -dystrobrevin interaction with iBRAF: a new role for dystrobrevin in neuronal differentiation*
Benedetta Artegiani, Catherine Labbaye, Paola Torreri, Maria Teresa Quaranta, Carlo Ramoni, Marina Ceccarini, Tamara Corinna Petrucci, Pompeo Macioce
- 9.50 *Physical and functional interaction between the RTT Syndrome-associated factor MeCP2 and the pro-apoptotic factor HIPK2*
Giorgia Bracaglia, Barbara Conca, Simona Giglio, Fabiola Moretti, Charlotte Kilstrup-Nielsen, Nicoletta Landsberger, Silvia Soddu
- 10.10 *A novel pharmacological approach and identification of peripheral cellular biomarkers in Niemann-Pick C disease patients*
Claudio Frank, Stefano Rufini, Daniela Merlo, Giuseppe Biagini, Giovanna D'Arcangelo
- 10.30 *Pharmacological and genetic regulation of TSC2-/- cell phenotype. A novel insight for TSC and LAM*
Alfredo Gorio
- 10.50 Coffee break and parallel poster sessions
- 11.20 *Study of the genetic susceptibility and environmental factor involvement in the etiopathogenesis of autism*
Franca R. Guerini, Matteo Chiappedi, Alessandro Ghezzi, Emanuela Maggioni, Patrizia Spelta, Salvatorica Manca
- 11.40 *Does NMMHCIIA (MYH9) play a role as a transcriptional regulator?*
Carmelo Ferrai, Victor M. Diaz, Angela Bachi, Francesco Blasi, Massimo P. Crippa

- 12.00 *Molecular genetics of infantile Pompe disease in Italy*
**Silvia Dominissini, Maria Gabriela Pittis, Andrea Dardis,
 Anna Lisa Montalvo, Michela Donnarumma, Marina Stroppiano,
 Giovanni Ciana, Maja Di Rocco, Bruno Bembi, Mirella Filocamo**
- 12.20 *Thrombin generation in severe haemophiliacs with different
 clinical phenotype*
**Elena Santagostino, Maria Elisa Mancuso, Armando Tripodi,
 Veena Chantarangkul, Marigrazia Clerici, Simona Maria Siboni,
 Pier Mannuccio Mannucci**
- 12.40 *Molecular approaches for the diagnosis of genetic lymphedema*
**Andrea Caprini, Fabrizio Orsenigo, Mathias François, Peter Koopman,
 Elisabetta Dejana**
- 13.00 Lunch break
- 13.30 Parallel poster sessions

Session II

PATHOGENESIS AND DIAGNOSIS

Chairpersons: **Tamara Corinna Petrucci, Giovanna Zambruno**

- 14.30 *Clinical and genetic evaluation of a large sample of patients
 with cerebellar ataxia syndromes*
**Sara Bonato, Maria Grazia D'Angelo, Chiara Megliani, Anna Carla Turconi,
 Renato Borgatti, Claudio Zucca, Laura Villa, Alessandra Tonelli,
 Giovanni Airoidi, Claudia Crimella, Pier Luigi Baron, Gabriella Silvestri,
 Maria Teresa Bassi, Nereo Bresolin**
- 14.50 *Metachromatic leukodystrophy – gene sequencing provides further evidences
 of genotype-phenotype correlation*
**Alessandra Biffi, Martina Cesani, Francesca Fumagalli, Ubaldo del Carro,
 Cristina Baldoli, Simonetta Gerevini, Giancarlo Comi, Maria Grazia
 Roncarolo, Mirella Filocamo, Stefano Regis, Maria Sessa**
- 15.10 *Autosomal recessive spastic paraplegia with thinning of corpus callosum
 and periventricular white matter changes:
 clinical, molecular and neuroimaging studies*
**Paola S. Denora, Alessandra Tessa, Gabriella Silvestri, Enrico Bertini,
 Federico Zara, Francesco Garaci, Filippo M. Santorelli**

- 15.30 *Development of new diagnostic approaches for transmissible spongiform encephalopathies*
Franco Cardone, Piero Parchi, Gianluigi Zanusso, Salvatore Monaco, Fabrizio Tagliavini, Maurizio Pocchiari
- 15.50 Coffee break and parallel poster sessions
- 16.20 *Role and prevalence of GATA4, NKX2.5, FOG2 and novel candidate genes' mutations in specific subsets of congenital heart defects*
Anna Sarkozy, Francesca Lepri, Giorgia Esposito, Rosangela Ferese, Federica Consoli, Bruno Marino, Marco Tartaglia, Maria Cristina Digilio, Bruno Dallapiccola
- 16.40 *Activating mutations in SOS1 cause a distinctive form of Noonan syndrome*
Marco Tartaglia, Len A. Pennacchio, Valentina Fodale, Francesca Lepri, Maria Lisa Dentici, Anna Sarkozy, Simone Martinelli, Cinzia Neri, Claudio Carta, Francesca Pantaleoni, Valentina Petrangeli, Viviana Cordeddu, Monia Magliozzi, Chen Zhao, Kamlesh K. Yadav, Bhaswati Pandit, Kimihiko Oishi, Wendy Schackwitz, Anna Ustaszewska, Joel Martin, James Bristow, Angelo Selicorni, Orazio Gabrielli, Francesca Faravelli, Maria Cristina Digilio, Giuseppe Zampino, Bruno Dallapiccola, Dafna Bar-Sagi, Bruce D. Gelb
- 17.00 *Tackling rare diseases yet lacking diagnosis and/or prognosis: a pilot project integrating data collection and experimental studies*
Domenica Taruscio, Antonio Antoccia, Gianluca Azzalin, Rita Devito, Alessandra Di Masi, Cinzia La Rocca, Stefano Lorenzetti, Giuseppe Macino, Armando Magrelli, Alberto Mantovani, Francesca Maranghi, Caterina Tanzarella, Fabrizio Tosto, Marco Salvatore, Sabrina Tait
- 17.20 *The TP53 NoIns-Pro haplotype is not associated with breast and ovarian cancer in hereditary cases negative for mutations in BRCA1 and BRCA2*
Paolo Peterlongo, Giovanna De Vecchi, Paolo Verderio, Sara Pizzamiglio, Siranoush Manoukian, Loris Bernard, Valeria Pensotti, Sara Volorio, Fernando Ravagnani, Marco Pierotti, Paolo Radice
- 17.40 Discussion
- 18.00 Conclusions and general remarks

Thursday, November 8, 2007

Session I

DIAGNOSIS

Chairpersons: **Stephen Groft, Lidia Larizza, Domenica Taruscio**

- 9.00 *Genomic diagnosis and classification of rare disorders with mental retardation using high throughput technologies*
Bruno Dallapiccola, Laura Bernardini, Antonio Novelli
- 9.20 *Is the “chromosomal phenotype” a clue for the diagnosis of de novo cryptic chromosomal rearrangements in people with mental retardation?*
Corrado Romano, Francesco Calì, Santina Reitano, Donatella Greco, Pinella Failla, Valeria Chiavetta, Pietro Schinocca, Ornella Galesi, Daniela Di Benedetto, Lucia Castiglia, Roberto Ciccone, Marco Fichera, Orsetta Zuffardi
- 9.40 *Genotype-phenotype correlations in the CMT neuropathies: definition of a clinical and genetic diagnostic flow-chart*
Silvia Coviello, Alessio Colombo, Sara Benedetti, Ivana Spiga, Patrizia Dacci, Raffaella Fazio, Maurizio Ferrari, Giancarlo Comi, Stefano Previtali, Alessandra Bolino, Angelo Quattrini
- 10.00 *Genotype/phenotype correlation in CdLS: Italian experience*
Angelo Selicorni, Silvia Russo, Cristina Gervasini, Donatella Milani, Francesca Menni, Marta Cerutti, Paola Castronovo, Maura Masciadri, Antonio Musio, Lidia Larizza
- 10.20 *Alteration of striatal synaptic activity in a mouse model of DYT1 dystonia*
Giuseppe Sciamanna, Paola Bonsi, Giuseppina Martella, Dario Cuomo, Paola Platania, A. Tassone, Patrizia Popoli, Giorgio Bernardi, Antonio Pisani
- 10.40 Coffee break and parallel poster sessions
- 11.10 *Genotype/phenotype analysis of neurodegenerative and aging-prone syndromes caused by mutations in the DNA damage response/repair pathway*
Domenico Delia, Pietro Pichierri, Margherita Bignami, Luciana Chessa
- 11.30 *CSB patients with unusual clinical features*
Tiziana Nardo, Roberta Oneda, Donata Orioli, Elena Botta, Heather Fawcett, Alan R. Lehmann, Maria Stefanini

- 11.50 *Clinical, genetic and morphological investigation of HRPT2-related familial hyperparathyroidism*
Giulia Masi, Luisa Barzon, Maurizio Iacobone, Giovanni Viel, Andrea Porzionato, Veronica Macchi, Raffaele De Caro, Gennaro Favia, Giorgio Palù
- 12.10 *Salivary gland tumours: comparative genomic hybridization in paraffine-embedded samples*
Giovanna Floridia, Federica Censi, Maria Pia Foschini, Vincenzo Falbo, Domenica Taruscio
- 12.30 *Classification of parathyroid tumours for optimal detection of HRPT2 mutations*
Vito Guarnieri, Alfredo Scillitani, Claudia Battista, Michelangelo Bisceglia, Raffaella Viti, Iacopo Chiodini, Michela Iacobellis, Nazzarena Malavolta, Salvatore Minisola, Lucia Anna Muscarella, Leonardo D'Agruma, Massimo Carella, David E.C. Cole, Anna Spada
- 12.50 Lunch break
- 13.30 Parallel poster sessions

Session II

TREATMENT AND CLINICAL MANAGEMENT

Chairpersons: **Giandomenico Russo, Stefano Vella**

- 14.30 *Adipose tissue-derived stem cells for the treatment of muscular dystrophy*
Ilaria Gatto, Antonietta Gentile, Stefania Straino, Antonella Mangoni, Maurizio C. Capogrossi, Giuliana Di Rocco
- 14.50 *Testing in vitro and in vivo treatments for inclusion body myositis*
Simona Saredi, Claudia Di Blasi, Pia Bernasconi, Lucia Morandi, Renato Mantegazza, Marina Mora, Cristina Sancricca, Enzo Ricci, Pietro Attilio Tonali, Massimiliano Mirabella
- 15.10 *Autoimmune pemphigus: quality of life, alternative therapeutic approaches and dynamics of autoreactive B cells*
Giuseppe Cianchini, Stefano Tabolli, Giovanni Di Zenzo, Damiano Abeni, Giovanna Zambruno, Antonio Lanzavecchia, Biagio Didona
- 15.30 *Stimulation of erythropoiesis and fetal hemoglobin reactivation induced by stem cell factor in human β -thalassemia*
Marco Gabbianelli, Ornella Morsilli, Adriana Massa, Luca Pasquini, Paolo Cianciulli, Ugo Testa, Cesare Peschle

- 15.50 Coffee break and parallel poster sessions
- 16.20 *Mesenchymal stem cells for the treatment of tibial congenital pseudarthrosis associated with type 1 neurofibromatosis*
Donatella Granchi, Valentina De Vescovi, Elisa Leonardi, Serena R. Baglio, Onofrio Donzelli, Marina Magnani, Nicola Baldini
- 16.40 *Tumour angiogenesis and inflammation as a therapeutic target of retinoblastoma and other rare ocular tumours*
Adriana Albini, Roberta Venè, Gianfranco Fassina, Massimo Nicolò, Rosaria Cammarota, Massimo Barberis, Douglas M. Noonan, Giuseppe Arena, Francesca Tosetti
- 17.00 *Novel experimental approaches for investigation on new therapies against rare human bone tumours*
Angelo De Milito, Francesco Lozupone, Rossella Canese, Maria Marino, Katia Scotlandi, Franca Podo, Stefano Fais
- 17.20 *Innovative Burkitt's lymphoma therapy*
Giovanna Cutrona, Lidia C. Boffa, Maria Rita Mariani, Serena Matis, Manlio Ferrarini
- 17.40 *Therapy-oriented large scale genomic and gene expression analysis in thymomas, mesotheliomas and lung carcinoids*
Elena Belloni, Francesca Toffalorio, Giuseppe Pelosi, Giulia Veronesi, Lorenzo Spaggiari, Tommaso De Pas, Giuliana Pelicci, Filippo De Braud
- 18.00 Conclusions and general remarks

POSTER SESSIONS

Improving diagnostic skills for inherited thrombocytopenias: identification of "novel" forms and characterization of "classical" forms to develop a DNA microchip

C.L. Balduini, C. Ambaglio, F. Faletra, A. Savoia

Gastroesophageal reflux disease in patients with Systemic Sclerosis: any relationship between reflux and pulmonary involvement?

R. Barbera, C. Gambaro, L. Belloli, B. Marasini, A. Malesci

Systematic diagnosis of rare erythroenzymopathies: generation of guidelines and study of the genotype/phenotype correlation

W. Barcellini, P. Bianchi, E. Fermo, G. Valentini, A. Zanella

Preliminary results on knowledge about reproductive risks in families with a child affected by Cornelia de Lange syndrome

M.F. Bedeschi, V. Bianchi, F. Lalatta, D. Milani, F. Menni, M. Cerutti, A. Selicorni

Trauma and risk of amyotrophic lateral sclerosis: a population-based case-control study

E. Beghi, A. Millul, A. Chiò, G. Logroscino

Preclinical studies aimed to develop target genes-based therapies for the treatment of amyotrophic lateral sclerosis

C. Bendotti, M. Peviani, T. Borsello, R. Piva

Dysbindin, the product of the DTNBP1 gene associated to schizophrenia susceptibility and mutated in HPS7 syndrome, interacts with dystrobrevin: possible involvement in intracellular trafficking and signalling

S. Benvegnù, C. Bucalossi, P. Torreri, G. Macchia, P. Macioce, T.C. Petrucci, M. Ceccarini

Type 1 Neurofibromatosis: advanced diagnostics

D. Bianchessi, F. Orzan, M.R. Balestrini, S. Guzzetti, D. Riva, F. Nataci, F. Lalatta, G. Finocchiaro

Natural history of CMT1A including QoL within 2 year

P. Caliandro, C. Pazzaglia, D. Pareyson, I. Aprile, T. Cavallaro, A. Quattrone, N. Rizzuto, G. Vita, P.A. Tonali, A. Schenone, L. Padua, Italian CMT QoL Study Group

Variables influencing quality of life and disability in CMT patients: Italian multicenter study

P. Caliandro, C. Pazzaglia, D. Pareyson, I. Aprile, T. Cavallaro, A. Quattrone, N. Rizzuto, G. Vita, P.A. Tonali, A. Schenone, L. Padua, Italian CMT QoL Study Group

Efficient molecular diagnostics and guidelines for rare genodermatoses affecting epithelial adhesion

D. Castiglia, M. Castori, G. Floriddia, M. D'Alessio, M. El Hachem, M. Colombi, D. Taruscio, P. Salerno, G. Zambruno

Development of an epidemiological and molecular integrated approach for the prevention of congenital hypothyroidism: a model for other rare diseases

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Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in the treatment of diffuse malignant peritoneal mesothelioma

M. Deraco, S. Kusamura, D. Baratti, A.D. Cabras, N. Zaffaroni

Characterization of a novel splicing mutation causing glycogen storage disease type II

S. Dominissini, A.L. Montalvo, E. Buratti, M.G. Pittis, B. Bembi, A. Dardis

The Italian external quality assessment in genetic tests: development of a web-based system

V. Falbo, G. Floridia, M. Salvatore, F. Tosto, F. Censi, D. Taruscio

Rare diseases: infant botulism

L. Fenicia, F. Anniballi, D. De Medici, E. Delibato, D. Lonati, C. Locatelli

Reliability and efficacy of the current diagnostic approach in narcolepsy and search for new genetic markers

R. Ferri, P. Bosco, O. Bruni, L. Ferini-Strambi, G. Plazzi

Inhibition of PDGFR phosphorylation: a pathogenetic treatment of Systemic Sclerosis

A. Gabrielli, G. Pomponio, P. Fraticelli, M. Luchetti, S. Svegliati, G. Moroncini, R. Giacomelli, P. Cipriani, A. Marrelli, V. Liakouli, E. Pingiotti, V. Dolo, D. Millimaggi, S. D'Ascenzo, I. Giusti, S. Guiducci, M. Matucci-Cerinic, S. Generini, G. Ferraccioli, B. Tolusso, M. De Sanctis, W. Malorni, A.M. Giammarioli, E. Straface, M. Pierdominici, A. Maselli, L. Somma, S. Vettori, G. Abignano, G. Valentini, P. Rovere-Querini, A.A. Manfredi, M.G. Sabbadini

Surveillance of rare cancers in Europe

G. Gatta, R. Capocaccia, L. Ciccolallo, S. Sowe

Neural tube defects and Folic Acid: an integrated, evidence-based approach to primary prevention in the Italian context

M. Grandolfo, F. Baldi, S. Brescianini, A. Mantovani, D. Taruscio

New genetic syndromes with aortic tortuosity and dissection

M. Grasso, N. Marziliano, E. Disabella, M. Diegoli, E. Porcu, A. Pilotto, M. Tagliani, M. Concardi, M. Agozzino, F. Inzani, M. Pasotti, A. Serio, F. Gambarin, S. Mannarino, A. Brega, E. Arbustini

Clinical and diagnostic course for patients affected by Prader-Willi syndrome

T. Greggi, L. Sangiorgi, E. Pedrini, P. Parisini

A family-based linkage analysis of HLA and 5-HTTLPR gene polymorphisms in Sardinian children with autism spectrum disorder

F.R. Guerini, S. Manca, S. Sotgiu, S. Tremolada, M. Zanzottera, C. Agliardi, L. Zanetta, M. Saresella, R. Mancuso, A. De Silvestri, M.L. Fois, G. Arru, P. Ferrante

Is there a role of oxidative DNA damage in Cockayne Syndrome?

T. Lemma, P. Degan, A. Calcagnile, L. Narciso, M. Stefanini, M. D'Errico, E. Dogliotti

Proposal for an integrated approach to rare diseases: a study between basic laboratory models and clinical epidemiology in Amyotrophic Lateral Sclerosis (ALS)

A. Loizzo, D. Taruscio, L. Nisticò, M. Vichi, E. Beghi, A. Chiò, G. Campana, C. Campanella, S. Conti, L. Costa, R. Cotichini, S. D'Alfonso, M. Leone, G. Logroscino, S. Loizzo, A. Fortuna, M. Masocco, L. Mazzini, N. Nasuelli, C. Petrini, Paolo Salerno, S. Spampinato, V. Toccaceli, N. Vanacore

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Biochemical and cellular real-time biomarkers of diagnostic and prognostic value in the management of Kawasaki's and Henoch-Schonlein Purpura diseases

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The Bladder Extrophy-Epispadias Complex and exogenous risk factors: the blade project

A. Mantovani, C. La Rocca, M. Luconi, E.M. Faustman, S. Lorenzetti, M. Maggi, S. Tait

Callosal agenesis: a brain malformation with polygenic origin. Identification of candidate genes and loci through a multidisciplinary approach of clinical, cytogenetic and molecular studies of a large set of patient with corpus callosum anomalies

S. Marelli, R. Grasso, C. Bonaglia, R. Giorda, M.T. Bassi, R. Borgatti

Preliminary data of a Tissue Microarray (TMA)-based multicenter study of thymic epithelial cell tumors with clinical implications

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S. Martinelli, P. Torreri, M. Tinti, L. Stella, G. Bocchinfuso, E. Flex, A. Grottesi, A. Palleschi, G. Cesareni, B.D. Gelb, L. Castagnoli, T.C. Petrucci, M. Tartaglia

Usefulness of MLPA in the molecular diagnosis of lissencephalies and neuronal migration disorders: high diagnostic yield in p>a lissencephaly

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From proteomic to structural biology: a comprehensive approach to amyloid diseases

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C. Mosci, B. Carnemolla, L. Borsi, A. Rubartelli, S. Ferrini

Genetic abnormalities of complement regulatory molecules in hemolytic uremic syndrome N. 526D/9

C. Mossali, G. Pianetti, F. Castelletti, J. Caprioli, E. Bresin, G. Monteferrante, G. Remuzzi, M. Noris

Characterization of the molecular and cellular mechanisms underlying the liver pathogenesis in Hemophagocytic Syndromes (HS)

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Gain-of-function RAF1 mutations cause Noonan and Leopard syndromes with hypertrophic cardiomyopathy

B. Pandit, A. Sarkozy, L.A. Pennacchio, C. Carta, K. Oishi, S. Martinelli, G. Esposito, E.A. Pogna, F. Lepri, R. Ferese, F. Consoli, F. Pantaleoni, V. Petrangeli, E. Flex, W. Schackwitz, A. Ustaszewska, A. Landstrom, J.M. Bos, S.R. Ommen, C. Faul, P. Mundel, J.P. López Siguero, R. Tenconi, A. Selicorni, C. Rossi, L. Mazzanti, I. Torrente, B. Marino, F. Stanzial, L. Memo, M.C. Digilio, G. Zampino, M.J. Ackerman, B. Dallapiccola, M. Tartaglia, B.D. Gelb

Diversity, parental germline origin and phenotypic spectrum of de novo HRAS missense change in Costello syndrome

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Elaboration of novel vaccines for the treatment of selected soft-tissue sarcoma histotypes
R. Perris, M.S. Benassi, S. Cattaruzza, P.A. Nicolosi, K. Lacrima, N. Bertani, L. Pazzaglia, P. Braghetta, P. Bonaldo, P.G. Natali, M. Maio, P. Picci

Role of the dystrophin-associated glycoprotein complex in Limb-Girdle and congenital muscular dystrophies: from molecular pathophysiology to potential therapy (7DR1)
T.C. Petrucci, E. Ricci, A. Brancaccio, P. Macioce, M. Ceccarini

Establishment of a European network of Rare Bleeding Disorders
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A genome wide non-synonymous SNP scan of Amyotrophic Lateral Sclerosis
J. Powell, I. Fogh, A. Ratti, C. Gellera, F. Squitieri, V. Silani

Effective communication and counselling: improving listening in rare diseases
S. Pulciani, A.M. Luzzi, A. Colucci, B. De Mei, P. Gallo, C. Cattaneo, R. Petrigliano, Italian Patient's Associations for Rare Diseases, D. Taruscio

Molecular characterisation of Italian patients affected by metachromatic leukodystrophy
S. Regis, A. Biffi, S. Grossi, R. Biancheri, M. Di Rocco, M. Sessa, M. Filocamo

X-linked or autosomal rare mental retardation syndromes: phenotypic analysis in transgenic mouse models
L. Ricceri, B. De Filippis, C. Perrone Capano, M.G. Miano, G. Laviola

Oxidatively damaged DNA repair defect in Cockayne syndrome and its complementation by heterologous repair proteins
M. Ropolo, P. Degan, M. Foresta, M. D'Errico, D. Lasigliè, E. Dogliotti, G. Casartelli, S. Zupo, A. Poggi, G. Frosina

Genetic and clinical aspects of rare lymphomas
G. Russo, G. Baliva, F. Sampogna, D. Taruscio, M. Fiorilli

Investigation of genetic and epigenetic mechanisms underlying Beckwith-Wiedemann Syndrome (BWS) on a large cohort of Italian patients
S. Russo, F. Cerrato, S. Ferraiuolo, A. Sparago, F. Bedeschi, F. Lalatta, D. Milani, A. Selicorni, L. Fedele, A. Riccio, L. Larizza

Analysis of dystroglycan in human patients affected by neuromuscular disorders
F. Sciandra, M. Bozzi, E. Pavoni, A. Brancaccio, E. Ricci

Immunobiologic and clinical activity of DNA hypomethylating agents in human sarcomas
L. Sigalotti, M. Maio

Genetic analysis of arrhythmogenic inherited diseases

E. Sommariva, S. Benedetti, F. Sacco, S. Sala, D. Zeni, M. Ferrari, C. Pappone

Evaluation and rehabilitation of swallowing dysfunction in patients with rare neurological disorders and movement disorders

F. Stocchi, D. Tufarelli, E. Mercuri

Functional characterization of different connexin 26 mutants identified in kid syndrome patients: impairment of membrane trafficking and connexon assembly

A. Terrinoni, A. Codispoti, M. Paradisi, B. Didona, L. Zocchi, G. Melino

Phenotype correction of ADAMTS13 deficiency and protection from the development of thrombotic thrombocytopenic purpura through intravascular and skeletal muscle ADAMTS13 gene delivery in mice

P. Trionfini, S. Tomasoni, M. Galbusera, R. Donadelli, D. Corna, L. Zentilin, D. Motto, M. Giacca, G. Remuzzi, A. Benigni

Telomerase activity is largely expressed and significantly affects clinical outcome in diffuse malignant peritoneal mesothelioma

N. Zaffaroni, M.G. Daidone, R. Villa, D. Baratti, S. Kusamura, M. Deraco

NOTES FOR READERS

This abstract book presents the oral and poster presentations illustrated during the two days Workshop (7-8 November) organized in the frame of the annual international Conference on rare diseases and orphan drugs (Istituto Superiore di Sanità, 5-8 November, 2007).

The abstracts are listed in alphabetical order based on the first author of the contribution (posters are preceded by the letter "P."); all authors of the abstracts are listed at the end of the volume, in the specific "author index".

The programme of the Workshop is included; all abstracts are presented as oral or poster presentations.

In fact, 36 abstracts have been selected for a short oral communication within the 4 sessions of the workshop (*aspects of pathogenesis; pathogenesis and diagnosis; diagnosis; treatment and clinical management*); the other abstracts (56) have been illustrated as poster presentation in specific sessions during the Workshop.

Communications and Posters

TUMOUR ANGIOGENESIS AND INFLAMMATION AS A THERAPEUTIC TARGET OF RETINOBLASTOMA AND OTHER RARE OCULAR TUMOURS

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Ocular tumours, in particular retinoblastoma and uveal melanoma, are highly vascular and appear to be readily targeted by anti-angiogenic agents such as fenretinide. The synthetic retinoid N-(4-hydroxyphenyl)retinamide (4HPR) also directly kills neurogenic tumour cells through a reactive oxygen species-mediated mechanism. We have previously shown that insulin-like growth factor I (IGF-I), which sustains the survival of human retinoblastoma cells, is unable to prevent Y79 retinoblastoma cell death induced by 4HPR and that 4HPR decreases IGF-1-stimulated AKT activating phosphorylation. Here we show that in Y79 cells treated with 4HPR where IGF-I-induced AKT phosphorylation is repressed, phosphorylation at Ser 9 of the multifunctional kinase and AKT substrate Glycogen Synthase Kinase 3 β (GSK3 β) is sustained. Further, 4HPR itself was able to induce GSK3 β phosphorylation in unstimulated cells. GSK3 β phosphorylation was concomitant with DNA fragmentation, PARP cleavage and ATP depletion induced by 4HPR. Pharmacological inhibition of GSK3 β by lithium chloride enhanced 4HPR cytotoxicity, conversely, decreasing expression of GSK3 β by siRNA gene silencing did not mimic the effects of lithium, indicating that Ser 9 phosphorylation is an important event implicated in 4HPR action. The antioxidants N-acetyl cysteine, Tiron, ebselen and neuro-protective doses of cycloheximide decreased GSK3 β Ser 9 phosphorylation and PARP cleavage induced by 4HPR. These data suggest that the elevation of reactive oxygen species induced by 4HPR correlates with sustained growth factor-independent Ser 9 phosphorylation of GSK3 β and this mechanism is involved in the cell death pathway engaged by 4HPR in retinoblastoma cells.

To further examine the potential of targeting the microenvironment as a strategy to control tumour growth, we adopted a gene transduction approach using a potent TH1 cytokine endowed with strong anti-angiogenic activity, Interleukin-12 (IL-12).

Gene transfer into murine 99e1 ocular tumour cells, while having no effects on growth *in vitro*, essentially blocked growth of vascular tumours *in vivo* without evident signs of toxicity. Orthotopic intraocular injection resulted in invasive tumours that destroyed ocular

architecture by the control cells while the IL-12 transduced cells rarely formed tumours. histological analysis revealed highly invasive and angiogenic tumour growth in the controls and poorly vascularised tumours in the presence of IL-12. The tumour repression effect could be reproduced by a systemic anti-angiogenic effect, where controlateral injection of IL-12 expressing cells strongly repressed growth in tumours formed by parental 99e1 cells. This was associated with significantly lowered tumour vessel densities, a trend towards lower vegf levels in the lesion, and significantly decreased nk cells in the parental tumours exposed to systemic IL-12. Taken together, these data suggest that IL-12 gene transfer can provide anti-angiogenic effects without toxicity and may be particularly suited for therapy of vascularised ocular tumours.

β-DYSTROBREVIN INTERACTION WITH IBRAF: A NEW ROLE FOR DYSTROBREVIN IN NEURONAL DIFFERENTIATION

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α and β dystrobrevins are cytoplasmic components of the Dystrophin-associated Protein Complex (DPC), which directly links the cytoskeleton to the extra-cellular matrix. It is now thought that dystrobrevins also play a role as scaffold proteins in intracellular transport and signal transduction. In the search of new insights into the functions of β -dystrobrevin, the isoform restricted to non-muscle tissues, we performed a two-hybrid screen of a mouse cDNA library to look for interacting proteins. Among the positive clones, one encodes iBRAF/HMG20a, a member of the HMG (*High-Mobility Group*) proteins that bind to DNA and induce structural changes in chromatin.

iBRAF (*inhibitor of BRAF35*) is an HMG-domain-containing protein with close sequence and structural homology to BRAF35/HMG20b, a component of the BHC complex that interacts with REST (*RE-1 Silencing Transcription factor*) to repress neural specific genes in neuronal progenitors and in non-neuronal tissues. In contrast to BRAF35, iBRAF expression leads to the abrogation of REST-mediated transcriptional repression and the resultant activation of neuronal-specific genes.

We confirmed the interaction by *in vitro* and *in vivo* association assays, and localized the binding region of one protein to the other. We also assessed the kinetics of the β -dystrobrevin-iBRAF interaction by *Surface Plasmon Resonance* (SPR) analysis, and found that β -dystrobrevin binds to iBRAF with high affinity. In a previous paper we reported that β -dystrobrevin could interact with the extra-cellular matrix constituents pancortins, which are thought to support neuronal differentiation and survival. The interaction of β -dystrobrevin and the HMG-domain protein iBRAF implies a new role for β -dystrobrevin during neuronal differentiation. The association we report here suggests that β -dystrobrevin may be involved in regulating chromatin dynamics, thus contributing to the activation of neuronal-specific genes.

Our results reveal an emerging role of dystrobrevin as a multifunctional scaffold protein, with exciting new functions in brain development as well as in intracellular transport and cell signaling.

IMPROVING DIAGNOSTIC SKILLS FOR INHERITED THROMBOCYTOPENIAS: IDENTIFICATION OF "NOVEL" FORMS AND CHARACTERIZATION OF "CLASSICAL" FORMS TO DEVELOP A DNA MICROCHIP

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A definite diagnosis for patients with inherited thrombocytopenias is essential to identify prognosis and choose the most appropriate therapeutic intervention. However, making diagnosis presents two major problems: a) recognition of "classical" disorders requires a number of complex analyses that are available only in few specialistic centres; b) nearly 50% of patients do not fit the criteria for any known disorder since they are affected by "new" forms. Moreover, also when diagnosis is possible, prognosis may remain undefined since many genetic thrombocytopenias are at risk of developing additional, and often severe, defects.

This project is aimed at overcoming these difficulties by improving knowledge on inherited thrombocytopenias. In details, the main objectives of the project are the following:

- identification and etiologic characterization of inherited thrombocytopenias not yet described;
- identification of genes responsible for inherited thrombocytopenias that have been described previously but whose etiology is still unknown;
- characterization of the mutations causing inherited thrombocytopenias in Italy;
- identification of genotype/phenotype correlations in patients affected by diseases with known etiology and wide phenotypic variability: mutations will thus assume even a prognostic significance.

To achieve these aims, we will enroll Italian patients and will perform their clinical evaluation according to the diagnostic algorithm proposed by the Italian Platelet Study Group. Subjects whose features do not match with any known form will be studied in greater detail in order to define the "new" diseases. All patients, together with their family members, will be included in our database that already includes 220 families, from which we will get information and biological samples for the genetic studies. In patients with disorders of known etiology we will identify the mutations, whereas in families with disorders of unknown etiology we will carry out a linkage analysis to identify the genes by positional cloning.

The better knowledge of inherited thrombocytopenias obtained by this research, which is based on the collaborations between two research units whose competencies are complementary in the fields of clinical medicine, cell biology and genetics, will be immediately translated into the clinical practice by updating the diagnostic algorithm to

comprehend the "new" disorders. Moreover, identification of all the genetic defects responsible in Italy for these diseases will represent the starting point for the development of a diagnostic platform that, by the use of nanotechnologies, enables comprehensive and simultaneous mutation detection in a number of genes linked to specific disorders.

P GASTROESOPHAGEAL REFLUX DISEASE IN PATIENTS WITH SYSTEMIC SCLEROSIS: ANY RELATIONSHIP BETWEEN REFLUX AND PULMONARY INVOLVEMENT?

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In Systemic Sclerosis (SSc), gastrointestinal involvement is predominantly esophageal, including impaired motility and gastroesophageal reflux. Acid reflux has been associated with a number of respiratory diseases, including interstitial lung disease, but its role as pathogenetic factor is far from clear. Many SSc patients with pulmonary involvement have esophagitis, although on antisecretory therapy. Lung involvement is currently the main cause of death in SSc, thus, the hypothesis of a lung disease due to reflux is a major challenge. Currently oesophageal pH monitoring is the gold standard in the diagnosis of reflux, although detect only acid events, pH-multichannel intraluminal impedance/ 24h (pH-MII) has been recently introduced as a new diagnostic tool for the evaluation of gastroesophageal disease, detecting the nature (liquid, gas or mixed) and pH of the refluxate (acid, weakly acid, no acid), as well as the proximal extent of the reflux events. The objectives of this study are to characterize with pH-MMI/24h the physical and pH properties of the gastroesophageal refluxate in patients with SSc, analyzing whether a relationship exist between type of reflux and pulmonary involvement. If this is the case, we aim to identify patients at increased risk for interstitial lung disease, to prevent them with a better target therapy. Patients fulfilling the American College of Rheumatology criteria for SSc will enter the study.

All patients, off of antisecretory therapy by at least 15 days, will undergo to a esophagogastroduodenoscopy followed by a combined oesophageal 24 h pH-impedance monitoring (MII). Pulmonary involvement will be assessed by pulmonary function tests, including spirometry and diffusing capacity for carbon monoxide (DLCO), and High Resolution chest Computed Tomography (HRCT).

Three patients completed the study (all females, mean age 61 yrs, mean disease duration since diagnosis 10 yrs). Two patients had distal esophagitis at EGDS and pathological pH-MII results, with acid and non acid reflux. In both DLCO was abnormal (51% and 59% respectively; vn 80%). In the other patient, no esophagitis was detected and a non acid refluxate at the pH-MMI. Normal was the pulmonary function (DLCO=81%).

A better definition of gastroesophageal reflux with pH-MMI might be helpful in SSc patients, tailoring antireflux therapy for preventing severe oesophageal and extraesophageal complications.

P SYSTEMATIC DIAGNOSIS OF RARE ERYTHROENZYMOPATHIES: GENERATION OF GUIDELINES AND STUDY OF THE GENOTYPE/PHENOTYPE CORRELATION

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Over the past few years the inherited disorders of erythrocyte metabolism have been the object of intensive research which has resulted in a better understanding of their molecular basis; however red cell metabolism disorders remain a rare group of diseases of difficult diagnosis. The phenotypic heterogeneity of these diseases pinpoints to the need to correlate the molecular and the clinical phenotype. The most frequent red cell enzymopathies associated with chronic hemolytic anemia are Pyruvate Kinase (PK) (about 400 cases described), pyrimidine-5' nucleotidase (Pyr5'N) (about 100 cases), Glucosephosphate Isomerase (GPI) (50 cases), Hexokinase (Hx), Phosphoglycerate Kinase (PGK), Phosphofructokinase (PFK), Adenylate Kinase (AK) and Triose Phosphate Isomerase (TPI) deficiency.

At Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli, Regina Elena there is the unique opportunity to study these diseases because of the presence of renowned experts interested in prevention, molecular and prenatal diagnosis, and clinical care of these diseases during childhood and adult life.

In particular, at the Division of Hematology more than 100 cases of disorders of red cell metabolism coming from Italy and other countries have been diagnosed and characterized at molecular level in the past 30 years. Most of them are in regular follow-up. In the most severe cases prenatal diagnosis has been offered. Patho-physiological mechanisms and biochemical characteristics of the defective enzymes have also been studied.

The project is divided in two distinct objectives:

- to study the genotype/phenotype relationship to be used in prognosis and genetic counseling;
- to elaborate guidelines for diagnostic and therapeutic approach to rare chronic hemolytic anemias due to defects of red cell metabolism.

In the first part of the project we focused on the characterization of the molecular variant of red cell enzyme deficiency diagnosed at Division of Hematology.

In particular, we investigated PK deficiency and performed an in-depth functional analysis of the highly purified mutant enzymes, comparing their stability, molecular, structural, and catalytic properties with those of corresponding wild-type proteins.

PK mutants have been generated by means of heterologous expression systems and site-directed mutagenesis techniques, overcoming the difficulties of obtaining the pathological variants from blood.

The information obtained on mutant enzymes may serve as a valuable tool to make a definitive diagnosis of erythroenzymopathies. The knowledge of new molecular variants and the compared analysis with the corresponding clinical pattern will be useful for prognosis and genetic counselling.

P PRELIMINARY RESULTS ON KNOWLEDGE ABOUT REPRODUCTIVE RISKS IN FAMILIES WITH A CHILD AFFECTED BY CORNELIA DE LANGE SYNDROME

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Cornelia de Lange Syndrome (CdLS [OMIM 122470]), is a rare multiple congenital anomalies/ mental retardation syndrome (MCA/MR) with high phenotypic variability. The prevalence of CdLS is 1/10000 and most cases appear sporadic.

In 2004 the Nipped B Like (NIPBL) gene was identified as the candidate in Cornelia de Lange syndrome. Mutational screening carried on patients affected by Cornelia de Lange syndrome showed the occurrence of frameshift, nonsense, missense and intron splicing mutations at a frequency ranging from 35% to 56%.

Very recently mutation of a new gene, SMC1L1, localized on the short arm of chromosome X, has been demonstrated in 4 CdLS patients within a group of 33 NIPBL-negative affected subjects, revealing a genetic heterogeneity.

This discovery immediately produced the need to inform families (parents, brother and sisters of affected individuals) about a possible reproductive risk in case of X-linked transmission.

The need of genetic counselling both in prenatal and preconceptional period for couples with positive familial history for Mental Retardation (MR) and Cornelia de Lange phenotype has never been extensively evaluated in the past ten years due to the unsuspected genetic heterogeneity.

In our Institution a large cohort of CdLS patients is followed since the first diagnosis up to adulthood. Families are invariably put in contact with the Association which integrates the clinical activities and since 2007 are being offered a genetic counselling session to discuss issues related to genetic testing, reproductive risk and prenatal diagnosis.

In order to collect more data about the specific needs of these families, we decided to prepare a questionnaire of 24 items. The questionnaire has the following sections: data about parents, data about severity of the clinical picture of the proband, understanding of genetic basis of the syndrome, knowledge and access to genetic testing, significance of test result according to reproductive decisions.

We are presenting the preliminary results of the different issues which demonstrate a lack of knowledge about the genes which are causative of the syndrome and the desire to be more informed about reproductive issues for the different family members.

P TRAUMA AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS: A POPULATION-BASED CASE-CONTROL STUDY

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There is no valuable evidence on the role of environmental factors as putative risk factors in Amyotrophic Lateral Sclerosis (ALS). Several studies showed that traumatic events are more common in the history of patients with ALS compared to the general population. Biological mechanisms have been implicated to explain the causative role of trauma in patients with ALS.

The proponents of the trauma/ALS connection base their assumption on anecdotal reports and observations that the disease started after the traumatic event. However, a cause-effect relationship between trauma and ALS is still unproven as the results of these studies are contrasted by other, negative reports and by the methodological shortcomings in studies purporting to show such a connection. These include, among others, referral patients, small study populations, recall bias, and the possibility that the disease was already active at the time of occurrence of traumatic events. For these reasons, a large population-based case-control study has been undertaken in three Italian regions (Lombardia, Piemonte, Puglia: total population 13,266,799). The hypotheses tested are: 1) whether or not patients with ALS were at increased risk of trauma, repeated trauma, and/or severe trauma; 2) whether the site of the (major) traumatic event coincides with the site of onset of symptoms. Starting in 1995, population-based registries were activated in these regions. Given the incidence of ALS in these areas (1.5-2.5 cases per 100,000 per year), a total of 400-600 registered patients are expected over a two-year period. Cases are patients with ALS classified according to the El-Escorial criteria and firstly diagnosed starting on January 1 2007. For each case, two sex and age-matched (± 5 years) controls are identified among patients admitted into the same hospital.

A neurological and a non-neurological control are selected, the former with a clinical condition other than a neurodegenerative disorder and the latter from non-orthopaedic surgical Departments. After direct interview of each case and matched controls, the following data are collected: main demographic findings, family history of neurodegenerative disorders, occupation and sports (type and number of years spent), physical activity (type and intensity), exposure to toxic agents.

These data are recorded to assess the role of trauma after controlling for the most relevant confounders. History of traumatic events (occurred up to 10 years prior to the onset of symptoms) are then collected with type, site, severity, circumstances, and complications. In a pilot study including 44 cases and 82 controls, blue collar workers were 40.9% (cases) vs 19.5% (controls) (OR 2.9; 95% CI 1.3-6.4). The mean number of years spent with any

occupation was 25.1 in ALS patients and 17.0 in controls ($p<0.05$). The mean number of years spent doing any sport was 7.9 in cases and 3.3 in controls ($p<0.01$). ALS patients practiced strenuous physical exercise during work more than controls (15.9 vs 2.4; OR 7.6; 95% CI 1.5-38.2). Traumatic events and drug exposure were no different in cases and controls.

THERAPY-ORIENTED LARGE SCALE GENOMIC AND GENE EXPRESSION ANALYSIS IN THYMOMAS, MESOTHELIOMAS AND LUNG CARCINOIDS

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Rare tumours are rare diseases and common strategies for their cure are still missing. The need for a deeper knowledge of the mechanisms underlying tumourgenesis and the definition of tumour-specific signatures become essential as the basis for novel therapeutic approaches. The role of large scale genomic and gene expression studies has been proven decisive in enhancing the understanding of human diseases, with unquestioned breakthrough concerning diagnosis, prognosis and treatment.

At the European Institute of Oncology a collection of rare tumour samples has started, with the aim to gather all the available cases and create a rare tumours tissue bank. In particular, a big effort is being directed towards the collection of thymoma, mesothelioma, and lung carcinoid samples, to be studied from the genomic and gene expression point of view. These tumours are mostly characterized by high aggressiveness and resistance to conventional treatments with radio-chemotherapy. It is therefore necessary to find novel strategies for their cure. We have started a genetic approach having as final goal the identification of specific genetic anomalies or gene expression patterns, which might help both in acquiring new insights in the tumourgenic processes underlying these tumours and in developing new specific antitumoural drugs.

In this context, the gene expression microarray analysis will intend to identify, within the different tumour types, new categories with specific gene signatures, which are expected to greatly improve the knowledge of the tumours, mainly in terms of disease development and progression (aggressiveness). At the same time, the definition of the genomic status of the different tumour samples, macroscopically (diploidy or aneuploidy) and microscopically (chromosome-specific amplifications or deletions), will highlight tumour-type or tumour-category specific genetic defects. Altogether, this information will result in a more complete knowledge of thymomas, mesotheliomas and lung carcinoids, allowing the choice of the best existing therapeutic treatment, on one side, and the development of new effective approaches, on the others.

We are in the process of analyzing the gene expression profiling of a first group of lung carcinoids, constituted of 5 typical and 5 atypical tumours, each with its normal counterparts.

P PRECLINICAL STUDIES AIMED TO DEVELOP TARGET GENES-BASED THERAPIES FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the selective and progressive loss of motoneurons, which affect about 4000 people in Italy. This leads to muscular atrophy and progressive paralysis culminating in death of patients within 2-5 years after diagnosis, due to respiratory failure. ALS is still orphan of a truly effective therapy that may halt and reverse the course of the disease. Our overall goal is to find novel strategies that may target the selective molecular mechanisms contributing to motoneuron degeneration.

Recently, we and other groups have demonstrated that in the motoneurons of SOD1 mutant mice, an animal model of familial ALS, and in patients with sporadic ALS there is a remarkable activation of the p38 Mitogen Activated Protein Kinase (p38MAPK) pathway. In contrast, the mechanisms specifically involved in the modulation of cell survival like the PI3K/Akt pathway remained unaltered in the motoneurons of SOD1 mutant mice and ALS patients suggesting an impairment in this neuroprotective signal transduction cascade.

The specific aim of this project was therefore focused on: 1) to counteract the activation of the detrimental p38MAPK pathway signaling by using specific small interfering RNAs aimed at silencing different genes of the pathway or inhibitor peptides aimed to interfere with the activation of p38MAPK delivered through viral vectors or through the TAT protein sequence of HIV permeable to the brain blood barrier permeable; 2) to activate the pro-survival mechanism in the motoneurons by using viral vectors expressing the active form of Akt delivered specifically to the motoneurons.

In the first six months of the project we have successfully developed the recombinant lentiviral vectors pseudotyped with VSVG or rabies G proteins expressing the siRNAs targeting the p38 α and selected the most functional candidates in NIH3T3 cell lines. We then tested these siRNAs in primary astrocytes cultures stimulated by TNF α and have found a specific and selective inhibition of p38MAPK and its phosphorylated (activated) form (P-p38). We are now testing the effect of siRNA on the levels of P-p38 and cell death in spinal motoneurons and cortical neurons under excitotoxic stimuli. Moreover, we are testing the effect of p-38 peptide inhibitors associated with TAT protein in the same experimental paradigms.

Meanwhile, we have also develop the method for the delivery of viral vectors expressing the siRNAs intrathecal, in the muscles or directly into the nerves in the mice. The results of these experiments are under analysis.

P DYSBINDIN, THE PRODUCT OF THE DTNBP1 GENE ASSOCIATED TO SCHIZOPHRENIA SUSCEPTIBILITY AND MUTATED IN HPS7 SYNDROME, INTERACTS WITH DYSTROBREVIN: POSSIBLE INVOLVEMENT IN INTRACELLULAR TRAFFICKING AND SIGNALLING

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Dystrobrevins are a family of cytoplasmic components of the Dystrophin-associated Protein Complex (DPC), whose function in muscle is to link the actin cytoskeleton to the extracellular matrix. Moreover, the DPC is now thought as a functional scaffold, which recruits proteins involved in signal transduction. Beta-dystrobrevin, a member of this family, is not found in muscle, but it is expressed in several tissues, among which the brain, where it localizes in neurons, particularly at post-synaptic densities.

Beta-dystrobrevin binds to several proteins in the DPC, among which the dystrophin itself, and it has been recently shown that dysbindin, a protein encoded by the human gene DTNBP1, is a new beta-dystrobrevin binding partner. Haplotypes at the DTNBP1 gene locus have been found to mediate risk to develop schizophrenia. Additionally, a non-sense mutation in the DTNBP1 gene has been linked to the pathogenesis of Hermansky-Pudlak Syndrome (HPS, type 7), a disorder characterized by prolonged bleeding time and oculocutaneous albinism due to deficiencies in lysosome-related organelles. Dysbindin has been found to be a stable component of the Biogenesis of Lysosome-related Organelles Complex 1 (BLOC-1), which regulates synthesis and trafficking of lysosome-related organelles and whose components are linked to HPS.

The functional significance of the interaction between beta-dystrobrevin and dysbindin is still unclear, and it may play a role in different cellular activities, such as intracellular signalling and vesicular trafficking. Recently our group demonstrated a direct interaction between beta-dystrobrevin and the heavy chain of the motor protein kinesin. This finding corroborates the hypothesis of a functional role of beta-dystrobrevin in vesicular trafficking.

In this work we investigated the interactions between beta-dystrobrevin and dysbindin, in order to shed light on its functional role. By Surface Plasmon Resonance (SPR) assay we studied the kinetics of this interaction and found a high-affinity binding between the two proteins. We have characterized the reciprocal binding sites within the two proteins by pull-down and SPR experiments. The formation of a trimolecular complex between dysbindin, dystrobrevin and the kinesin-heavy-chain is highly suggestive of a role of these proteins in intracellular trafficking. The possible role of dysbindin in intracellular signalling will be discussed, on the basis of our data on a PKA-dependent phosphorylation of dysbindin itself.

P TYPE 1 NEUROFIBROMATOSIS: ADVANCED DIAGNOSTICS

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Neurofibromatosis type 1 (NF1) affects approximately 1 in 3,500 individuals worldwide. The most common features of NF1 are pigmentary abnormalities, such as *café-au-lait* spots, skinfold freckling, and Lisch nodules. Individuals with NF1 may also develop a wide variety of nervous system abnormalities. Children with NF1 frequently exhibit specific learning disabilities, attention deficit disorder, and hyperintense lesions on T2-weighted brain magnetic resonance imaging. Both children and adults with NF1 can develop benign and malignant tumours involving the brain and peripheral nerves.

The NF1 gene is a large gene encoding the protein neurofibromin, composed of 2818 amino acids. Sequence analysis suggest that NF1 protein may work as a negative regulator of Ras Guanosine Triphosphate (GTP)ase proteins.

The main goal of this project is the improvement of the molecular diagnosis of NF1 patients in order to enhance mutational detection rate. We combined the already validated PCR-DHPLC-Sequencing technique with

Using PCR-DHPLC-Sequencing we analyzed, 52 unrelated patients: 34 presenting at least two major clinical features of NF1 (8 children and 26 adult), and 18 presenting some clinical features of NF1, but without clear clinical diagnosis (7 children and 11 adult).

Among clinically diagnosed cases we found 17 mutations: 7 known mutation and 10 novel mutations, resulting in a 50% detection rate. We also found 3 mutations located at splice-sites: their role in Nf1 inactivation will be confirmed by protein truncation test. We also found 3 mutations in the other cohort of 18 patients (2 adults and 1 child), allowing the formulation of the diagnosis. In order to test Conformation-Sensitive Capillary Electrophoresis (CSCE) as an alternative screening method of NF1 mutations we are presently investigating 4 NF1 exons (ex3, ex4b, ex6, ex8) in patients carrying either mutations or polymorphisms in these regions previously detected by DHPLC. Preliminary data, obtained on an ABI PRISM 3130 Genetic Analyzer (PE Applied Biosystems, Foster City, CA), allowed to confirm sequence variation in exons 3 and 4b. For the other exons new experimental conditions will be considered. We also plan to evaluate as a new screening test of NF1 mutations the High Resolution MeltingTM (HRMTM) technique.

These genetic data will be integrated with accurate clinical and instrumental protocols for the follow-up of paediatric and adult patients focussing on neuro-psychological aspects of the disease and working on improvement of the quality of genetic counselling that can offered to patients and families.

METACHROMATIC LEUKODYSTROPHY – GENE SEQUENCING PROVIDES FURTHER EVIDENCES OF GENOTYPE-PHENOTYPE CORRELATION

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Metachromatic Leukodystrophy (MLD) is a rare and fatal demyelinating lysosomal storage disorder, characterized by an unrelenting involvement of central and peripheral nervous system. Even if no effective treatments are available at present, new therapeutic strategies, such as enzyme replacement therapy and gene therapy, are at the horizon. However, because of the rapid progression of the disease observed in the majority of MLD patients, it is likely that any of these approaches need to be applied in a pre-symptomatic stage or in a very early phase of the disease to exert their potential clinical benefits. The identification of reliable early prognostic markers, besides age at symptom onset, is instrumental for accurate patients' identification and selection for new therapeutic protocols. We characterized a large cohort of 28 MLD patients by complete sequencing of the coding region of the ARSA gene and functional analysis of unknown mutations. Progression of the disease was monitored and quantified by means of motor (Gross Motor Function Measure) and neuropsychological scales, and through appropriate scoring of ElectroNeuroGraphic recordings (NCV Index) and brain MRs (adapted Loes' scale). A precise correlation exists between patients' phenotype and mutations in the ARSA gene. We show that this correlation, which has been already demonstrated for common mutations, similarly applies to rare ones. Patients grouped according to their genotype show different pattern of disease progression, as quantified by the use of Gross Motor Function Measure, NCV Index and adapted Loes' scale. Moreover, the involvement of the peripheral nervous system since disease onset constitutes an additional sensitive prognostic marker for the prediction of a severe disease progression. This work demonstrates the value of gene sequencing and rare mutation analysis and the relevance of peripheral nervous system evaluation at disease onset in order to elaborate an accurate prognosis. Moreover, it shows that Gross Motor Function Measure, ElectroNeuroGraphic recordings and brain Magnetic Resonance represent reliable and sensitive tools to quantify and monitor the progression of neurological involvement. Further studies on a larger number of patients are necessary to validate these preliminary observations.

CLINICAL AND GENETIC EVALUATION OF A LARGE SAMPLE OF PATIENTS WITH CEREBELLAR ATAXIA SYNDROMES

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Inherited ataxias are a heterogeneous group of neurodegenerative disorders characterized by the progressive degeneration of cerebellum, brainstem and spinocerebellar tracts with a variable combination of signs of central and peripheral nervous system involvement. Hereditary ataxias are classified as AD, AR and X-linked. Mitochondrial inheritance is also involved.

The onset of recessive ataxias is generally in early childhood or infancy and presents both neurological and multisystem involvement. The pathogenesis of recessive forms is associated with a “loss of function” of specific cellular proteins involved in metabolic homeostasis or DNA repair. Dominant ataxias such as SCA 1, 2, 3, 6, 7 and 17 share instead an expanded repeat of polyQ sequences in the corresponding proteins.

The first aim of our study was the characterization of a large sample of ataxic patients in order to create defined subgroups of patients in which further clinical, functional, biochemical and pharmacological studies could be carried out. Up to date 20 ataxic patients presenting autosomal dominant, 15 recessive and 25 sporadic forms were enrolled.

The applied diagnostic protocol includes an history collection, neurological, neuroradiological and neurophysiological evaluations and biochemical screenings. ICARS and SARA scales are used to estimate the disease severity. Analysis of SCA 1-3,6,7,17, APTX1, SETX, and FA genes is currently performed.

We identified 6 families with ataxia and oculomotor apraxia for whom SETX and APTX genes analysis is underway. One family with two affected siblings presenting cerebellar ataxia, slurred speech, nystagmus, peripheral neuropathy, head and limb tremor,

without oculomotor apraxia was also characterized. Patients in this family carry a novel mutation in SETX. Some very rare forms of recessive ataxias we also studied such as a family of gypsy origin with two children affected by ataxia, congenital cataract, facial dysmorphism and neuropathy the CCFDN syndrome. Our patient as all reported with the same ethnic origin carries an homozygous mutations in CTDPI gene, a base change falling within an intronic ALU sequence that generates an aberrant splicing of the transcript with insertion of the ALU in the transcript.

Among the putative sporadic forms we identified a 3-year-old child affected by early-onset ataxia, absence of succedaneous teeth, hypomyelination and cerebellar atrophy. Proton MR spectroscopy showed elevated white matter myo-inositol. Her clinical and radiological picture strikingly overlaps to the reported ADDH, (Ataxia, Delayed Dentition and Hypomyelination) syndrome for which no genetic defect has been identified yet. Mutation analysis in several candidate genes is in progress.

PHYSICAL AND FUNCTIONAL INTERACTION BETWEEN THE RTT SYNDROME-ASSOCIATED FACTOR MECP2 AND THE PRO-APOPTOTIC FACTOR HIPK2

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Rett Syndrome (RTT; OMIM312750) is an X-linked neuronal progressive disorder causing mental retardation mostly in females. The pathogenesis of the neurological abnormalities is still unclear. MeCP2, a methyl-DNA-binding protein that represses transcription by modulating chromatin structure, is mutated in about 80% of RTT-patients; however, several studies have indicated that there is no apparent genotype-phenotype correlation; indeed, the same MeCP2 mutations can cause a wide spectrum of problems suggesting that other genes may influence the clinical severity. Based on these considerations, we reasoned that the identification of novel MeCP2 interacting factors might help to refine the comprehension of the molecular mechanism(s) involved in the RTT syndrome. Thus, we performed a yeast two-hybrid screening using MeCP2 as a bait. We identified the Homeodomain Interacting Protein Kinase 2 (HIPK2) as a new MeCP2 interactor. HIPK2 belongs to the nuclear serine/threonine kinases of the HIPK family that have recently been identified among the enzymes able to regulate gene transcription. These kinases can either interact with homeobox proteins and act as co-repressors, or with other types of transcription factors and act as co-activators or co-repressors, depending on the promoters and/or the cellular contexts. HIPK2 binds to several proteins involved in the regulation of cell survival and proliferation, including the oncosuppressor p53, that is specifically phosphorylated by HIPK2, and the p53-inhibitor MDM2, that ubiquitylates HIPK2 and promotes its degradation. In the present study, we concentrated our attention on three different aspects: 1) the biochemical and molecular analyses of MeCP2/HIPK2 interaction *in vitro* and *in vivo*; 2) the functional analysis of the MeCP2/HIPK2 interaction *in vivo* in cell lines, in cultured neurons and in KO mice; 3) the molecular and functional role of the *in silico* identified target of the MeCP2/HIPK2 co-expression, *Mdm2*. Thus far, we have found, by kinase assays, that HIPK2 phosphorylates MeCP2, at least in one specific serine residue. Results from co-transfection experiments of cells from different lines with wild-type or mutant forms of MeCP2 and HIPK2 indicate that ectopic expression of MeCP2 leads to apoptosis and this effect is increased by the presence of HIPK2 and strongly inhibited by depletion of MeCP2. Evaluation of *Mdm2* transcription has evidenced a repressive activity of MeCP2 on p53-mediated induction. This has been observed analyzing both *Mdm2* promoter activity through luciferase assay as well as *Mdm2* mRNA by quantitative RT-PCR.

P NATURAL HISTORY OF CMT1A INCLUDING QOL WITHIN 2 YEAR

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The Italian CMT Study Group performed a multicentre multidimensional longitudinal study (with a follow-up of 2 years), using validated measurements of neurological impairment, disability and QoL. The aim of the study was to evaluate the natural history of clinical features, disability and quality of life (QoL) in patients with CMT1A.

On clinical examination, CMT1A patients showed a significant reduction in muscle strength and sensory function during the 2-year follow-up period.

However, there was no worsening of QoL or disability, nor was depression observed.

The discrepancy between the evolution of clinical features and the evolution of QoL and disability may be due to the development of compensatory strategies that help patients cope with the slow progression of the disease. Our observations provide information which may be useful when designing clinical trials in CMT.

P VARIABLES INFLUENCING QUALITY OF LIFE AND DISABILITY IN CMT PATIENTS: ITALIAN MULTICENTER STUDY

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To assess the variables that influence QoL and disability in patients with CMT.

Background: Any studies assessed which variables determine deterioration of Quality of Life (QoL) and disability in Charcot Marie Tooth (CMT).

We performed a large prospective multicenter (six centers) study through the use of validated clinical, disability and QoL measurements. Multivariate analysis was performed using QoL as dependent variables and duration of symptoms, age, gender and CMT type, depression and disability measurements as independent variables.

We enrolled 211 patients (60% females, mean age 42.5 yrs). QoL was highly significantly deteriorated with respect to the Italian normative sample. The physical aspect of QoL was mainly related to disability but it does not increase with the age, probably because of an adaptation between expectation and reality. The mental QoL is influenced by depression (hence we have to consider this aspect approaching CMT patients). Moreover, we observed that women complained of more severe symptoms than men. Finally, some CMT subtypes are related to more severe bodily pain symptoms than others.

Multiperspective assessment of CMT showed new aspects of this disease; mainly concerning 1) differences between men and women; 2) the crucial role of pain and depression.

MOLECULAR APPROACHES FOR THE DIAGNOSIS OF GENETIC LYMPHEDEMA

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Hereditary lymphedemas, are characterized by a chronic swelling of the extremities due to impaired lymphatic drainage which causes disability and predisposition to infection and chronic ulceration. Congenital hereditary lymphedema (Milroy disease) was found to be caused by mutations in the kinase domains of vascular endothelial growth factor receptor 3. This receptor is specifically expressed in lymphatic endothelium and is necessary for a correct differentiation of these cells. Other types of lymphedema (lymphedema-distichiasis) have been found to be due to truncating mutations in the forkhead-related transcription factor FOXC2. The genetic causes of other types of hereditary lymphedema, remain essentially unknown. Recently, few families have been described with a hereditary condition in which Hypotrichosis, Lymphedema and Telangiectasia are associated (HLT syndrome). Interestingly the characteristics of the disease mirror those described in mice with spontaneous mutations of Sox 18 transcription factor. Genetic analysis of three families with HLT syndrome showed that Sox 18 was mutated and accounted for both recessive and dominant forms of the disease. Sox 18 belongs to the SRY related HMG domain family of developmental transcription factors. The natural mutations in mice give rise to dominant negative forms of the transcription factor, these mutations are comparable to those found in HLT syndrome in men as well as the phenotype.

Using an *in vitro* system of endothelial cell differentiation from mouse embryonic stem cells we found that Sox-18 is upregulated at early stage of cell differentiation and precedes Prox-1 expression. Prox-1 is a homeodomain transcription factor considered the “master” gene in lymphatic differentiation. This gene acts to switch the fate of vascular endothelial cells to a lymphatic endothelial phenotype and so initiate lymphatic development. Infection of ES cells with lentiviral vectors expressing Sox 18 wt showed that upregulation of Sox 18 induced expression of different lymphatic markers including Prox-1. Overexpression of the dominant negative SOX18 prevented upregulation of Prox-1 and of the other lymphatic markers. All together these results strongly suggest that Sox 18 is upstream of Prox-1 during lymphatic differentiation of endothelial cells. This would explain the early and dramatic effects observed in mice and in men when the gene is mutated. Work is underway to characterize the mechanism of action of Sox 18 in lymphatic development and to relate mutations to HLT type of lymphedema in men.

DEVELOPMENT OF NEW DIAGNOSTIC APPROACHES FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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Transmissible Spongiform Encephalopathies (TSEs) or prion disorders are fatal neurodegenerative conditions affecting humans and animals. Human TSEs are diffused worldwide with an incidence of about 1-2 cases per million people per year. Most of them (about 90%) occur as sporadic Creutzfeldt-Jakob disease (sCJD) with an unknown aetiology.

TSEs are characterized by the presence in the central nervous system and, at exceedingly lower levels, in other peripheral organs, of a host-derived amyloid protein termed PrP^{TSE} that is the only available marker of TSEs and its apparent absence in accessible tissues hampers the identification of infected individuals during the preclinical phase. The presence of infectivity traces in some body fluids (e.g., blood and cerebrospinal fluid) during this phase can only be demonstrated through expensive, poorly sensitive and time-consuming animal bioassays that have no practical utility in the current clinical practice or in surveillance activity. A definite diagnosis of TSE is only obtained post-mortem by detection of PrP^{TSE} in the brain. The absence of an ante-mortem confirmatory test represents an obstacle for the design of targeted strategies for prevention, control, and treatment of these diseases.

The objective of this project is to improve the diagnosis and the prognosis of human TSEs through the development of new biochemical tests and the optimization of already available diagnostic tools. During the first part of this work we have developed a specific protocol for the collection of body fluids from TSE patients and started the collection. We are also collecting control samples from individuals affected by other neurodegenerative conditions and from non-neurological patients. These samples are central for the search, through mass spectrometry and electrophoresis techniques, of diagnostic proteomic patterns. Mass spectrometry results are not yet available whereas two-dimensional gel electrophoresis analyses of cerebrospinal fluid showed that sCJD cases are characterized by the presence of the γ , ϵ and ζ isoforms of 14-3-3 proteins, and that this pattern is different from that encountered in inflammatory and vascular disorders of the CNS.

To increase the sensitivity of PrP^{TSE} based tests we are applying the novel Protein Misfolding Cyclic Amplification (PMCA) technology to human biological fluids: preliminary results indicate that it is possible to detect PrP^{TSE} in blood of experimental TSE diseases.

Regarding the improvement in the classification of human sCJD subtypes, which is important for an early differential diagnosis with other dementias, we have found that the presence and relative amount of truncated forms of PrP^{TSE} in the brain correlate with the disease subtype.

P EFFICIENT MOLECULAR DIAGNOSTICS AND GUIDELINES FOR RARE GENODERMATOSES AFFECTING EPITHELIAL ADHESION

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The Epithelial Adhesion Diseases (EAD) are a clinically and genetically heterogeneous group of inherited blistering disorders due to defective epithelial-mesenchymal adhesion. EAD comprise the inherited epidermolysis bullosa (EB) forms, due to mutations in 10 different genes, and the Kindler syndrome (KS) whose causative gene, *KIND1*, has been identified in 2003. The spectrum of mucocutaneous manifestations is highly variable, spanning from minor blistering tendency limited to extremities to severe phenotypes in which the extreme skin and mucosal fragility results in early lethality. EB variants can be diagnosed by ultrastructural analysis and by immunofluorescence antigen mapping of a skin biopsy. In addition, molecular analysis of the causative gene allows disclosing the specific mutation/s in the affected family, bearing significant clinical implications in terms of prenatal diagnosis and genetic counselling as well as prognosis prediction in specific cases. Molecular analysis of EB families performed at IDI and University of Brescia has identified several recurrent mutations in the Italian population, allowing for optimal screening strategy for future patients, and provided clues on genotype-phenotype correlation. We are currently setting up more effective screening protocols for laminin 5 genes (*LAMA3*, *LAMB3* and *LAMC2*) and *ITGB4* gene based on heteroduplex scanning of PCR products by DHPLC technology. For a lethal variant of EB, EB with pyloric atresia (EB-PA), we are also developing a novel early prenatal test based on chorionic villi immunodetection of the proteins defective in the disease, $\alpha 6\beta 4$ integrin and plectin. This procedure represents the only possible prenatal test in pregnancies at risk for recurrence of EB-PA when mutations are unknown. On the other hand, assessment of skin biopsies by electron microscopy and immunomapping analyses does not provide definitive clues to the diagnosis of KS, rendering molecular analysis of particular relevance in this rare and often misdiagnosed disease. We have identified the causative mutations in 13 KS cases, 4 of which in the last three months, and characterised 3 recurrent mutations confined to the Italian ancestry of specific geographical areas. Aware of the complexity of the procedures and the costs associated with EAD diagnostics, an expert task force coordinated by the Istituto Superiore di Sanità has been established in July 2007 to develop and validate diagnostic guidelines for the entire group of EAD. The guideline development procedure has been agreed upon and guideline release is expected by July 2008.

P DEVELOPMENT OF AN EPIDEMIOLOGICAL AND MOLECULAR INTEGRATED APPROACH FOR THE PREVENTION OF CONGENITAL HYPOTHYROIDISM: A MODEL FOR OTHER RARE DISEASES

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Congenital Hypothyroidism (CH) is a rare disease with a prevalence of 3.5 cases/10,000 citizens in Italy. Despite this low prevalence, CH represents the most frequent endocrinopathy in infancy and the most common cause of preventable mental retardation. A high risk of extra-thyroid congenital malformations has been also demonstrated to be associated with CH. The availability of effective screening procedures, the efficient network composed of the 26 Screening Centres for CH active in Italy and the surveillance of the disease performed by the Italian National Register of Infants with CH, have allowed an efficient "secondary prevention" for CH. This represents one of the most important results in the field of preventive medicine in our country and can be a model of intervention for other rare diseases.

It has been recently demonstrated a multifactorial origin of CH in which genetic and environmental risk factors contribute to the aetiology of the disease. However, as the occurrence of mutations in candidate genes have been observed only in a small proportion of the patients and the role of specific environmental risk factors has not yet completely elucidated, the aetiology of CH is still largely unknown. Moreover, although early diagnosis and treatment of CH has led to the disappearance of severe mental retardation, it has been clearly recognised that persistent selective neuropsychological impairments may still occur in these children.

On the basis of the above mentioned findings, the aims of our project are: 1) to use the almost 30-year experience of screening for CH in Italy to evaluate the possible impact of "extended newborn screening program" (at least for 30 rare metabolic diseases) at the national level; 2) to investigate possible spatial variations in CH risk (spatial clusters) in our

country and the possible relationship with local environmental exposures (iodine deficiency, endocrine disrupting chemicals), by using data collected in the Italian National Registry of Infants with CH; 3) to verify the possible occurrence of a geographical overlapping of CH spatial clusters with spatial clusters of specific congenital malformations (to identify possible common environmental risk factors), by using data collected by the Italian National Register of Rare Diseases; 4) to investigate how genetic variability may influence individual responses to environmental factors (particularly iodine deficiency) in CH infants with *in situ* gland, and to identify new genes involved in thyroid dysgenesis both in humans and experimental *in vivo* models; 5) to identify possible risk factors (familial, maternal, neonatal, environmental) associated with a poor neuropsychological outcome.

The findings deriving from this project will contribute to nationwide prevention strategies for CH.

AUTOIMMUNE PEMPHIGUS: QUALITY OF LIFE, ALTERNATIVE THERAPEUTIC APPROACHES AND DYNAMICS OF AUTOREACTIVE B CELLS

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Autoimmune Pemphigus (AP) is a rare but highly disabling and, if untreated, almost always fatal immunobullous disease of the skin and mucous membranes. AP is characterized immunopathologically by the presence of circulating and tissue-bound autoantibodies against keratinocyte cell surface antigens, specifically desmoglein 1 and 3 (Dsg).

At present the involvement of anti-Dsg antibodies in causing blister formation is well-established, while the relative role of B and T cells in the pathogenesis of the disease remains to be defined. Systemic glucocorticoids are the mainstay of therapy for pemphigus and are often used in association with immunosuppressive drugs, to decrease the need for steroids and the related side-effects.

Among alternative therapies, rituximab, a chimeric anti-CD20 monoclonal antibody directed against pre-B lymphocytes and mature B lymphocytes, has been used in a small number of patients affected with severe pemphigus after the failure of conventional therapeutic approaches. We have recently treated with rituximab 12 recalcitrant pemphigus patients (375 mg/m² once weekly for 4 weeks). The treatment was well tolerated, and all the patients showed a good clinical response during an 18-month follow-up period, along with a consensual decline of the serum anti-Dsg titres. No infectious complications were observed. Our results confirm and extend previous reports showing that rituximab is able to induce a prolonged clinical remission in pemphigus patients after a single course of treatment. We are at present designing an open clinical trial for treatment of refractory pemphigus with rituximab to get better insight on clinical and immunologic dynamics in treated subjects. In particular, we intend to apply a previously developed method for the efficient immortalization of human B cells to the study of the repertoire of autoreactive memory B cells in pemphigus patients at various disease stages and following rituximab treatment. Preliminary results obtained in two untreated patients indicate that this technique allows to determine frequency and specificity of autoreactive memory B cells and to isolate anti-Dsg3 human monoclonal antibodies.

In parallel, we have started to evaluate the impact of disease burden on the patients' quality of life and psychological status. Fifty-eight patients were assessed using 36-item Short Form of the Medical Outcome Study (SF-36), and Institute for Personality and Ability Testing questionnaires for anxiety and depression. A compromised health status on both the physical and psychological scales was observed. Patients with anxiety had severe or more recent onset disease. Anti-Dsg3 antibody levels correlated with clinical severity and with lower SF-36 scores.

GENOTYPE-PHENOTYPE CORRELATIONS IN THE CMT NEUROPATHIES: DEFINITION OF A CLINICAL AND GENETIC DIAGNOSTIC FLOW-CHART

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Charcot-Marie-Tooth (CMT) diseases are the most common inherited disorders of the peripheral nervous system, with a general incidence of 1:2500. CMTs are motor and sensory neuropathies characterized by progressive muscular atrophy and weakness affecting distal extremities. On the basis of electrophysiology and histopathology, CMT has been divided into primary demyelinating, CMT1, primary axonal, CMT2 and intermediate CMT-I, with features of both CMT1 and CMT2.

CMTs are a very heterogeneous group of disorders not only from the clinical point of view but also from the genetic one. To date, at least 40 loci have been identified and 22 genes have been isolated for this pathology, responsible for autosomal recessive, autosomal dominant, or X-linked forms. It is mostly impossible to predict the type of locus/gene associated with a specific CMT on the basis of the phenotype with few exceptions. Otherwise, some additional elements derived from morphological evaluation of the sural nerve may facilitate the identification of the specific CMT. Due to the rare occurrence of each specific clinical form, a proper genotype-phenotype correlation has not been yet established.

This indeterminacy produces in clinical practice an expansive and time-consuming wide molecular screening for patients affected by CMT, mostly following as a unique criteria the frequency of incidence of the various forms of CMT.

In order to facilitate a definite diagnosis, we are collecting clinical, histopathological, and molecular data from CMT patients. To provide a broad range of molecular screening of the most of the genes responsible for CMT, we designed a flow-chart for genetic analysis of 12 genes involved in different forms of CMTs (PMP22, MPZ, GJB1, EGR2, GDAP1, MTMR2, MTMR13, MFN2, NF-L, LMNA, HSP22 and HSP27) (Southern Blot and MLPA are performed to identify duplication/deletion of PMP22 gene which is responsible for the majority of demyelinating CMTs, while DHPLC and/or direct sequencing were performed to identify point mutations in all genes analysed).

To date, we screened 40 patients affected by demyelinating (20), axonal (10) or intermediate (10) forms of CMTs. We found mutations in 24 of the enrolled patients, in particular in the 65% of familiar cases and in 25% of sporadic ones.

A better clinical and genetic characterization of different forms of CMT will allow to establish genotype-phenotype correlations to address molecular diagnosis facilitating the diagnostic protocols and to run hypothesis for pathogenetic mechanisms at present still poorly understood. Moreover, the availability of a well-characterized cohort of patients will allow to identify subclasses of patients, which might constitute potential target for pharmacological treatment.

INNOVATIVE BURKITT'S LYMPHOMA THERAPY

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Burkitt Lymphoma (BL), one of the most aggressive human cancers, is a very rare malignancy in the western world (sporadic form), while is frequent among children from areas like Central Africa (endemic form). BL is a monoclonal proliferation of B lymphocytes characterized by small non cleaved cells producing a diffuse tissue involvement. Most BL are characterized by t(8;14, 2 or 22) chromosomal translocations juxtaposing the c-myc oncogene to the Ig loci. Consequently c-myc becomes upregulated by the now proximal E μ enhancer, at the 5' of the Ig locus, promoting cell hyperproliferation.

Systemic chemotherapy, the present treatment of choice for BL at all stages, has an overall survival rate correlated to the stage of the disease at diagnosis and concomitant pathologies (e.g. HIV-seropositivity). In our previous studies we devised new strategies for BL therapy based on anti-gene PNA.

PNAs are nucleic acids analogues where the natural backbone is substituted with uncharged pseudo peptidic 2-aminoethyl-glicine units. They are resistant to proteases and nucleases and in viable cells can, as anti-gene reagent, inhibit specific gene expression. Indeed we have shown that PNAs targeted to the second c-myc exon prevent its transcription in BL cells. We also showed that the expression of the c-myc oncogene translocated in proximity of the E μ enhancer of the Ig can be blocked by a PNA (PNAE μ) specifically targeting E μ core sequence. PNAE μ inhibits the translocated c-myc leaving the normal proto-oncogene unaffected.

We just completed a series of studies required to start testing PNAE μ in phase I/II in patients:

- first we showed that BL cells reproducibly grow in SCID mice;
- in this animal model system toxicity was never observed following PNAE μ administration;
- however persistence of the therapeutically active portion of PNAE μ was demonstrated in all analyzed tissues and particularly within BL cells derived tumours;
- we subsequently demonstrated that chronic administration of PNAE μ to mice, already inoculated with BL cells, caused: increased latency of tumour appearance, relevant decrease of final tumour size and necrosis;
- we also showed that PNAE μ neither induces mutations nor has clastogenic effects as detectable by standard test;
- lastly when immunocompetent mice were inoculated with PNAE μ in Freund's Adjuvant antibodies were undetectable, although both IgG and IgM antibodies were present in serum of positive control mice inoculated with PNA-KLH conjugate.

GENOMIC DIAGNOSIS AND CLASSIFICATION OF RARE DISORDERS WITH MENTAL RETARDATION USING HIGH THROUGHPUT TECHNOLOGIES

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Mental Retardation (MR) has a prevalence of ~2-3% and can result from different causes, including non-genetic and genetic mechanisms. Chromosomal abnormalities account for up to 5-10% of the cases, when the analysis is performed with standard techniques, but no etiology is recognized in ~50% of affected individuals. The original aim of this study was the analysis of 100 consecutive patients presenting with severe MR using two different technologies, an oligonucleotide microarray, with an average resolution of 75 Kb (Human Genome 44K; Agilent Technologies; Waldbronn, Germany), and an Affymetrix 500K (GeneChip Mapping Arrays; Affymetrix Inc., Santa Clara, CA) platform, to detect eventual pathogenetic cryptic genomic changes. Patients were consecutively recruited by expert clinical geneticists and were included in this study based on the presence of moderate to severe MR (DSM-IV criteria; American Psychiatric Association) with at least one of the additional clinical features: one major malformation, dysmorphisms, multiple minor anomalies. During this study the cohort of patients was expanded to include 204 subjects. Standard karyotype was normal in 191 subjects, while 13 had an apparently balanced rearrangement. Array-CGH analysis disclosed 35 copy number variations (16%), with a range size of 0.5 to ~10 Mb. 18 were deletions and 13 duplications.

Moreover, in 2 cases one deletion and one duplication were coexisting in the same patient. Among the 13 patients with structural rearrangements, 3 showed a chromosomal imbalance (23%). All aberrations were confirmed by FISH and/or quantitative-PCR and microsatellite analysis. Whenever possible, analyses were carried out also in the parents, which showed that 5 (15%) copy number variations were inherited. In 4 cases, an X chromosome pathogenic rearrangement was segregated from healthy mothers to affected sons, the derived X chromosomes having a skewed inactivation in the heterozygous females. The fifth inherited imbalance consisted in a duplication of the imprinted 15q11-q13 region, which had segregated from a clinically normal mother to her two affected male sibs. In conclusion, this study has proved that the oligonucleotide array-CGH is a powerful tool for studying MR. These cases are now being re-evaluated by the Affymetrix 500K platform, which includes two arrays containing a total of 500,000 SNPs, with an average spacing of 6 Kb. This second analysis will allow to compare the sensitivity of the two platforms.

Preliminary data suggest that the integration of results is allowing to reach a tiling coverage of the whole genome, including the possibility to detect anomalies involving even a single gene.

NOVEL EXPERIMENTAL APPROACHES FOR INVESTIGATION ON NEW THERAPIES AGAINST RARE HUMAN BONE TUMOURS

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Human bone malignancies including osteosarcoma (OS) and Ewing's Sarcoma (ES) are rare tumours in both adults and children, characterized by a high biologic aggressiveness. The strategy of treatment is based on a combined modality of surgery and chemotherapy. Chemotherapy (a combination of 4 drugs), is delivered before and after surgical removal of the tumour (neoadjuvant chemotherapy). However, the most recent improvements in the cure rate of patients with localized disease have been achieved by dose-intensification, in turn paying the price of acute severe toxicity and secondary malignancies. Moreover, 35% of patients show tumour resistance to systemic therapies and treatments for high-risk patients are still completely inadequate and no new drugs have reported to be really active and useful in sarcomas. Thus, innovative treatment modalities are indeed very welcome and needed. In this project we proposed some *in vitro* and *in vivo* models, to evaluate potential new therapeutic strategies aimed at inhibiting the growth and metastatic behaviour of human bone tumours. The first issue of the project was to investigate the significance of ezrin in bone malignancies.

Moreover, we investigated expression and function of V-ATPases in osteosarcoma and Ewing's sarcoma cell lines. We also performed experiments aimed at analyzing molecular composition of microvesicles released from bone-marrow malignancy cells.

Based on previous studies we have also further investigated the role of the Insulin-like growth factor 1 (IGF), of the antigen CD99 and s-src tyrosine kinase family in the pathogenesis and progression of Ewing's sarcoma.

Preliminary results have shown that ezrin is involved in many activities of bone marrow malignancies, including multidrug resistance and microvesicle release. Moreover, also V-ATPases have shown to participate to the malignant behaviour of rare bone tumours and some V-ATPases inhibitors have proven to interfere with the metastatic potential of these tumours. MRI-guided MRS approaches have shown that bone marrow malignancies are clearly acidic and that inhibition of V-ATPases activity, through proton pump inhibitors (PPI) increase the pH of these tumours. Lastly, treatment of an extensive panel of osteosarcoma and Ewing's sarcoma cell lines bone-tumours derived cells with small molecule inhibitors against IGF-IR or c-src, recently identified as specific tyrosin kinase inhibitors, have shown to be effective in inhibiting the tumour cell growth.

Altogether these preliminary results suggest that newly-identified molecular pathways associated to rare bone malignancies may represent valuable targets for future anti-tumour approaches.

GENOTYPE/PHENOTYPE ANALYSIS OF NEURODEGENERATIVE AND AGING-PRONE SYNDROMES CAUSED BY MUTATIONS IN THE DNA DAMAGE RESPONSE/REPAIR PATHWAY

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Human hereditary syndromes with genetic defects in the repair of DNA single-strand breaks (SSBs) and double-strand breaks (DSBs) are associated with early-onset progressive neurodegeneration, premature ageing and other major extra-neurological features. These include Ataxia-Telangiectasia (A-T), Ataxia-Telangiectasia-Like Disease (ATLD) and Nijmegen Breakage Syndrome (NBS), caused by mutations in ATM, Mre11 and NBS1 genes, respectively and conferring defects in DSBs repair; Werner Syndrome (WS) mutated in WRN gene involved in DNA replication, recombination repair, transcription and incorrect handling of stalled forks with accumulation of DSBs; ataxia oculomotor apraxia type 1 (AOA1) and type 2 (AOA2) caused by the genes APTX and SETX involved in SSBs repair. Despite considerable progress in understanding the function of these disease-causing gene products, it is unclear how their involvement in common repair pathways result in different clinical phenotypes. This project aims to elucidate the role of these genes in determining specific phenotypes. Our strategy will start from the identification of patients with well defined clinical features and their molecular characterization. The second step will be the establishment of Lymphoblastoid Cells (LCLs) from selected patients and their relatives, to have a source of biological material on which to study the responses to SSBs- and DSBs-inducing agents. LCLs will be used to analyze the cell-cycle phase in which SSBs and DSBs originate and to find out the enzymatic activities necessary for DSBs avoidance after cell cycle or replication arrest, and to determine how the cellular phenotype of the mutant cells depend on their genomic defect. The project aims also to identify by mass spectrometry proteins that co-interact with Aprataxin and Senataxin and to link the interacting partners to known cellular pathways. Finally we will establish an *in vitro* model system for neurodegeneration using human neural stem-cells made defective for SETX, APTX and WRN expression by stable shRNA interference. The result will provide a deeper understanding of the basic genotype/phenotype mechanisms.

AUTOSOMAL RECESSIVE SPASTIC PARAPLEGIA WITH THINNING OF *CORPUS CALLOSUM* AND PERIVENTRICULAR WHITE MATTER CHANGES: CLINICAL, MOLECULAR AND NEUROIMAGING STUDIES

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The leukodystrophies represent a heterogeneous group of rare inherited disorders that involve primarily and preferentially the white matter of the brain, and remain undetermined in about 50% of cases. At times, white matter disorders complicate neurological syndromes affecting the cerebellum or the corticospinal motor system, including the heterogeneous group of familial spastic paraplegias (HSP). In particular, neuroimaging evidences of periventricular white matter changes and rostral Thinning of *Corpus Callosum* (TCC) are observed in the single most common form of Autosomal Recessive Spastic Paraplegia (ARHSP), which also presents severe mental deterioration. The majority of the families with this clinical form appear to be linked to SPG11 on chromosome 15q13-q15. We recently narrowed the SPG11 chromosomal interval and identified the disease gene (SPG11). Moreover, we showed that a subset of families were unlinked to the SPG11 locus proposing genetic heterogeneity in this clinical entity.

The identification of the disease gene permits the genetic determination in a relatively common form of HSP+leukodystrophy but it leaves open a number of questions. As an example, the spectrum of associated mutations is still limited and further allelic variants are expected. Also, no known function can at present be established for the gene product (spatacsin). Moreover, not all families manifesting clinical SPG11 phenotype are linked to 15q13-q15 and not all linked families have TCC or mental retardation, all of which call for better clinical/molecular correlations.

The aim of this project is to study clinically and genetically families with ARHSP-TCC linked to the SPG11 locus, to gain insight into the function, in health and disease condition, of a new gene, and its overall relationship to the clinical phenotype related to both corticomotor and white matter degenerations.

The project will thus be based on the pursuit of the following objectives: 1) identification and sampling of further patients; 2) molecular analyses to determine spectrum and frequency of SPG11 mutations in affected families, but also in other forms of autosomal recessive hereditary spastic paraplegia and leukodystrophies as well as in sporadic cases; 3) linkage analysis in families non-SPG11 in order to identify novel disease

loci/genes; 4) functional neuroimaging to evaluate the consequences of mutations in SPG11 at the level of brain and spine in relationship to different mutations and severity of the disease; 5) better understanding of the gene function; 6) complete genotype/phenotype correlations to estimate the penetrance of different mutations, a property that has important implications for presymptomatic testing.

P CYTOREDUCTIVE SURGERY AND HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY IN THE TREATMENT OF DIFFUSE MALIGNANT PERITONEAL MESOTHELIOMA

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Diffuse Malignant Peritoneal Mesothelioma (DMPM) is a very rare disease with a poor prognosis. In the former times the condition was considered terminal and amenable only to palliative interventions. The median survival was approximately 1 year after systemic chemotherapy. The emergence of a combined modality of Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy (CRS+HIPEC) seems to have changed positively the outcome of DMPM patients. In the present study we propose to report the experience of NCI of Milan in the treatment of DMPM with CRS+HIPEC focusing results in terms of survival, morbidity and clinical prognostic factors.

From a data base of 82 cases of peritoneal mesothelioma we selected 63 patients (27M/36F) with DMPM histology submitted to CRS+HIPEC with a curative intent. Cases with well differentiated multicystic and papillary histologies, second malignancy (ies) were excluded from the analysis. CRS was performed using peritonectomy procedures. HIPEC through the closed abdomen technique was conducted with cisplatin (CDDP 25mg/m²/L of perfusate)+mitomycin C (MMC 3.3 mg/m²/L of perfusate) or CDDP (43mg/L of perfusate)+doxorubicin (Dx 15.25 mg/L of perfusate), at 42.5°C. We tested the prognostic significance of the followings: age, sex, previous surgical score, carcinomatosis extension, completeness of cytoreduction (CC) and HIPEC drug schedule. The survival was calculated from the date of operation until the date of death or of the last contact. The median follow-up was 30.4 months (range: 1-118). The adverse events were graded according to NCI CTCAE v3 criteria. The survival curve distribution was calculated by the Kaplan-Meier method. The Log-rank test was used to assess the significance of survival distributions.

Five-year OS and PFS were 47% and 21%, respectively. Median OS and PFS were 40 months and 23 months, respectively. Only the completeness of cytoreduction was proven to be of prognostic significance. The postoperative surgical morbidity G3-5 and systemic toxicity G3-5 rates were 32% and 33%, respectively.

Unfortunately the rarity of the disease represents the major drawback for the conduction of a prospective randomized study, in an acceptable timeframe, to confirm these promising results. Notwithstanding the peritoneal surface malignancy program of NCI of Milan is also exerting efforts in the molecular biology field, in order to obtain a better understanding of the underlining tumour kinetics, identify new prognostic markers and try to validate new targeted therapies.

P CHARACTERIZATION OF A NOVEL SPLICING MUTATION CAUSING GLYCOGEN STORAGE DISEASE TYPE II

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Pompe disease (Glycogen Storage Disease type II, MIM# 232300) is an autosomal recessive disorder caused by the deficit of Acid Alpha-Glucosidase (GAA; E.C.3.2.1.20), which results in impaired glycogen degradation and its accumulation within the lysosomes. The GAA gene (MIM# 606800) is located in human chromosome 17q25.2-25.3 and up to date more than 150 mutations have been described [<http://www2.eur.nl/fgg/ch1/pompe>].

We report here a novel GAA mutation, c.1626C>G, which leads to an unexpected effect on the mRNA splicing process.

The c.1626C>G is located in exon 11 and apparently corresponds to a silent mutation (p. P542P). Instead, the *in silico* analysis predicted the creation of a putative donor splice site 11 nucleotides upstream of the exon 11 donor site, which might result in the exclusion of 11 nucleotides. Since patient's fibroblasts were not available, we used the functional splicing assay to study the effect of this mutation on the mRNA splicing process.

We prepared a wild-type and a mutant minigene, cloning the genomic sequence from exon 10 to exon 12 of GAA gene and the corresponding sequence bearing the c.1626C>G substitution, in the pcDNA3 expression vector.

The constructs were expressed in different cells lines (HeLa, Hep3B, COS-1 and CHO). Surprisingly, RT-PCR analysis revealed that the mutant mRNA lacks of 57 nucleotides. Direct sequence of the PCR product demonstrated that the presence of c.1626C>G causes the activation of a cryptic acceptor splice site in exon 12, which leads to the exclusion of 46 nucleotides from this exon and to the formation of a stop codon 393 nt downstream (p.P542PfsX393). This result, which is different from the *in silico* prediction, confirms the importance of the functional splicing assay in the characterization of splicing mutations.

MOLECULAR GENETICS OF INFANTILE POMPE DISEASE IN ITALY

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Glycogen Storage Disease type II (GSDII) is an autosomal recessive disorder in which deficiency of Acid Alpha Glucosidase (GAA) results in impaired glycogen degradation that accumulates within lysosomes.

The GAA gene (MIM 606800) localizes to human chromosome 17q25.2-25.3; the enzyme is synthesized as an inactive precursor of 110 kDa and then processed, in the lysosomal compartment, to the fully active forms of 76 and 70 kDa. More than 150 mutations have been described up to date [<http://www2.eur.nl/fgg/ch1/pompe>]. Recently we carried on the mutational analysis in Italian patients affected by the late onset of the disease.

In this study we analyzed 37 unrelated patients affected by infantile Pompe disease coming from different parts of Italy. Genomic DNA was analyzed by PCR followed by automatic sequencing. RNA was extracted from patient fibroblasts, amplified by RT-PCR and sequenced.

Functional characterization of novel missense mutation was performed by transient expression of wild-type and mutant proteins in a human GAA deficient cell line.

Among the 32 different alleles identified, nine were novel. Five single base substitutions were studied by functional expression assay: c.572A>G (p.Y191C); c.1124G>T (p.R375L); c.1202A>G (p.Q401R); c.1564C>G (p.P522A) and c.1796C>A (p.S599Y); none of the mutants tested expressed residual activity. This is in agreement with Western blot analysis which indicates that p.S599Y is retained as the inactive 110kDa while no immunoreactive protein can be detected for p.Y191C, p.R375L and p.P522A mutant constructs. The c.742delC deletion cause a shift in the reading frame introducing a premature stop codon that lead to a truncated protein p.L248PfsX20. Three mutant alleles were found in the intronic region (c.-32-3C>A, c.1075+13C>T, c.1636+5G>C) and might affect RNA processing.

This study integrated to the one regarding the late onset form offers a complete picture of the molecular genetics of Pompe disease in Italy and may contribute in the understanding of the natural history of the disease. The functional study of mutant alleles contribute to a better understanding of genotype-phenotype correlation and provide valuable insights into the molecular basis of the disease.

P THE ITALIAN EXTERNAL QUALITY ASSESSMENT IN GENETIC TESTS: DEVELOPMENT OF A WEB-BASED SYSTEM

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Several government agencies and advisory bodies elaborated various international policies aimed to assure the safe and effective use of genetic tests. An objective that can be achieved through recommendations and the monitoring of the quality of laboratories performing genetic tests, including the implementation of molecular diagnostic techniques relevant to all laboratories. In 2001 at the Istituto Superiore di Sanità started the Italian External Quality Control (EQC).

This scheme covers Cystic Fibrosis, Beta-Thalassemia, Fragile-X, Adenomatous Polyposis Coli APC gene and prenatal, postnatal and oncological cytogenetics. A total of 80 public laboratories distributed on the National territory, participated at the initiative on a voluntary basis. Five trials have currently been performed.

The results showed an improvement in the use and interpretation of molecular genetic tests. Moreover the percentage of complete reports in cytogenetics increased over the period. However analytical and interpretative errors can be still observed.

In the same time, as indicated in the international surveys for quality assessment, a significant reduction in laboratory error will be possible only after several years of testing experience and participation in quality assessment schemes.

Relying on the experience acquired until now, we are developing a brand new approach to EQA using a web-based system that will be illustrated.

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RARE DISEASES: INFANT BOTULISM

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Infant Botulism (IB) first recognized in 1976 is an orphan ("rare") disease that affects infants between one and 52 weeks of age. The laboratory confirmation of suspected cases needs a rapid and specific method to detect BoNT producing *clostridia* in clinical samples. With the exception of the USA, the incidence rate of IB is low, but underestimated. In USA, IB is the most common form of human botulism and about 100 cases are confirmed per year. The "Infant Botulism Treatment and Prevention Program" (IBTPP) in California, provides diagnostic and consultative medical services; and distributes the new Orphan Drug BabyBIG.

The NRCB at the ISS, performs an active surveillance of the disease with laboratory confirmation of suspected cases. Twenty-seven cases of IB occurred in Italy between 1984 and 2006 and the diagnosis is generally done by clinicians familiar with the clinical manifestations and maintaining a very high index of clinical suspicion. After the United States and Argentina, Italy has the third highest number of IB cases.

This project, in collaboration also with the California IBTPP is primarily focused on improving IB knowledge and having it included in the Italian National Register of Rare Diseases.

The project is structured in three WPs:

WP1 - The Pavia Poison Control Centre and National Toxicology Information Centre is involved in developing this WP. The main objective is to formulate an educational medical program, to fill the identified information gaps and to improve physician awareness of IB.

WP2 - The objective of the WP is to develop rapid molecular biological methods for the detection of BoNTs producing *clostridia* in clinical samples for the rapid diagnosis of the disease.

WP3 - This WP deals with the dissemination of collected data on clinical and microbiological aspects of cases. All the informations will be easily available on the website that can be consulted by all stakeholders.

The preliminary results obtained from this project are:

WP1 - A very simple questionnaire sent to European Poison Centers (PCs) with the aim to collect preliminary information concerning epidemiological data, diagnosis and management of botulism disease showed that the IB remains a rare disease. During 2004 to 2006 no cases of IB have been observed in Europe, with the exception of Italy, where 7 cases were diagnosed. Laboratories for analysis exist, but only some are open 24 hours. At present no PCs report having BabyBIG available.

WP2 - A multiplex PCR method to detect simultaneously type A,B,E and F BoNTs-genes in fecal samples has been developed and a international European evaluation is in progress.

WP3 - A meeting is about to take place with experts of IBTPP in California to discuss items to be incorporated onto the website.

DOES NMMHCIIA (MYH9) PLAY A ROLE AS A TRANSCRIPTIONAL REGULATOR?

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Prep1 is a member of the TALE superfamily of homeodomain proteins. These transcription factors heterodimerize with the PBC family members in a DNA-independent manner and this affects their nuclear localization and transcriptional activity. *Prep1* is essential for embryonic development: in particular hypomorphic *Prep1^{hi}* embryos, that express 2-3% of *Prep1* mRNA and up to 10% of the protein, show a leaky embryonic-lethal phenotype and defects in angiogenesis, hematopoiesis and eyes development.

To isolate new *Prep1* interactors we used a tandem affinity purification protocol that allowed the isolation of proteins that copurified with a *Prep1*-TAP chimeric protein from both the cytoplasm and the nucleus.

Among the isolated interactors were Non-Muscle Myosin Heavy Chain IIA and β -actin. The former is protein involved in four autosomal dominant disorders (May-Hegglin anomaly; Sebastian syndrome; Fechtner syndrome and Epstein syndrome), going under the collective name of "MYH9-related disease" and is known to be a cytoplasmic protein. On the other hand β -actin is also known to be mainly a cytoplasmic proteins, but recent findings have emphasized its involvement in the transcriptional regulation of rRNA genes together with unconventional myosin I. Thus, the interaction of NMMHCIIA with *Prep1* suggested a possible nuclear localization, which we observed in preliminary immunofluorescence experiments in NT2-D1 cells by confocal microscopy.

The transcription of genes of the HoxB cluster (among which the HoxB2 gene is a known target of *Prep1*) can be induced by Retinoic Acid (RA) treatment of NT2-D1 cells, whereas treatment with trichostatin A (TSA) reverts the induction. Thus, we performed chromatin immunoprecipitation experiments on material from untreated, RA- and RA+TSA-treated NT2-D1 cells, asking if NMMHCIIA was bound to the HoxB2 enhancer. The results showed that in uninduced cells we could detect only the presence of the initiating form of RNA Polymerase II (RNAPII). In RA-treated cells we could observe the presence of the elongating form of RNAPII, myosin VI (known to be associated with this form of the polymerase) and β -actin. In RA+TSA-treated cells we again observed the presence of the initiating form of RNAPII, which was now associated with NMMHCIIA and β -actin.

Our results strongly indicate a nuclear localization of NMMHCIIA and a possible role for in transcriptional regulation. They also suggest a possible interplay with other unconventional myosins in this cellular compartment.

P RELIABILITY AND EFFICACY OF THE CURRENT DIAGNOSTIC APPROACH IN NARCOLEPSY AND SEARCH FOR NEW GENETIC MARKERS

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Narcolepsy is a chronic central nervous system disease characterized by Excessive Daytime Sleepiness (EDS), typically associated to cataplexy and other phenomena due to the abnormal occurrence of REM sleep elements during wakefulness and sleep/wake transition. Probably, its pathogenesis is due to a dysfunction in hypothalamic neurons which produce hypocretin (Hcrt or orexin), a neurotransmitter involved in the complex interaction of neuron networks responsible for the regulation of the sleep/wake cycle. Some HLA system antigens seem to play a role of predisposing factors because they are present in at least 95% of patients. The descriptive-clinical epidemiology of narcolepsy has still many unclear aspects because of the relatively low prevalence of this condition and because of the difficulty in defining reliable and reproducible epidemiological study tools.

Currently, the diagnosis of narcolepsy is still based on the contemporary use of clinical criteria and expensive (sometimes invasive) laboratory data (PSG, MSLT, HLA typing, Hcrt CSF levels). On the basis of the rationale reported above, with this project we plan to follow a complete clinical and laboratory diagnostic protocol in two groups of patients with EDS (one with Narcolepsy/Cataplexy and another with sleep apnea syndrome); the main scope will be to determine the subset of diagnostic interventions able to discriminate these two groups from each other.

This would minimize the load of diagnostic procedures needed for the correct diagnosis of individuals with EDS due to Narcolepsy/Cataplexy. At the same time, we plan to evaluate with an advanced molecular genetics methodology (gene microarrays) the eventual involvement of genes in the pathogenesis of Narcolepsy/Cataplexy and its CSF characteristic feature, i.e. decreased Hcrt levels. In fact, despite the repeated confirmation of this biochemical trait, its genetic basis remains unknown.

SALIVARY GLAND TUMOURS: COMPARATIVE GENOMIC HYBRIDIZATION IN PARAFFINE-EMBEDDED SAMPLES

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Salivary Gland Tumours (SGTs) are rare tumours of the neck and head with an overall incidence in the Western world of approximately 2.5-3/100.000/year. SGTs involve the major glands (parotid, submandibular and sublingual) and the minor glands (oral mucosa, palate, uvula, floor of mouth, posterior tongue, retromolar area and peritonsilla area, larynx and paranasal sinuses).

SGTs are remarkable for their histopathological and biologic diversity; they include benign and malignant tumours of epithelial, mesenchymal and lymphoid origin.

Although exposure to ionizing radiation has been implicated as a cause of SGTs, the aetiology of most of these tumours cannot be determined; moreover it's difficult to determine the prognosis and select the optimal therapeutic modality. The study of molecular pathogenesis of SGTs is a challenging task because of the rarity and histopathological diversity of these malignancies.

We are collecting STG samples with different histotypes in order to identify DNA copy number variations and to correlate anomalies with clinico-pathological data; moreover recurrent alterations will give information about regions containing potential candidate tumour suppressor genes and oncogenes involved in the tumourgenesis of SGTs.

Comparative Genomic Hybridization (CGH) is a powerful tool for detecting chromosomal aberrations in archival paraffin embedded tumour samples.

CGH analysis performed in eight adenoid cystic SGT samples (with different histotypes) revealed a prevalence of gains over deletions; in particular gains detected in our study, have been already reported in literature as regions potentially involved in the pathogenesis of this subtype of tumours.

The correlation of CGH results with clinical-pathological data and a comparison with literature data will be discussed.

This work has been funded in the frame of the Projects "Identification of genetic markers for diagnosis and prognosis of rare tumours" "Programma di collaborazione Istituto Superiore di Sanità-National Institute of Health, Office for Rare Diseases " Fasc. 526/B and "Salivary gland tumours: different approaches to identify genetic and prognostic markers" Fasc 7GR1.

A NOVEL PHARMACOLOGICAL APPROACH AND IDENTIFICATION OF PERIPHERAL CELLULAR BIOMARKERS IN NIEMANN-PICK C DISEASE PATIENTS

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Aim of our project is to understand the contribute of lipid rafts in the occurrence of neurological disturbs in Niemann-Pick Disease type C (NPDc) and in particular their possible involvement in the incorrect development of neuronal networks. In fact, the loss of a correct dynamic of cholesterol-sphingolipids-enriched microdomains in the neuronal and glial plasma membrane, caused by an imbalance in the lipid trafficking due to NPDc gene mutation, could have a key role in neuronal dysfunction and the consequent clinical pathologies. Lipid rafts constituents can be manipulated with drugs either interacting with the metabolism of their components (i.e. inhibitors of the synthesis of glycosphingolipids or cholesterol), or extracting/adding single components by using liposomes or molecules that bind cholesterol (i.e. cyclodextrines).

Although the genetic defects causing NPDc are well known, very few information is available on the causes of neurological symptoms that eventually lead to a fatal end the patient affected by NPDc. Changes in the functioning of neuronal plasma membranes are good candidates in the research of NPDc pathophysiogenetic mechanisms.

Recently, it has been shown that neuronal mitochondria membranes are altered and unable to effectively participate in ATP synthesis, a defect that could be antagonized by normalizing cholesterol with methyl- β -cyclodextrin. Until now, no study addressed the possible changes in the functioning of neuronal plasma membrane receptors as a putative determinant of NPDc neurological disturbs. Changes in the plasma membrane cholesterol content and in the glycosphingolipids/cholesterol ratio is particularly important in affecting lipid rafts and neurotransmitter receptor activity. In particular, lipid rafts are important regulators of glutamate receptor functioning.

Electrophysiological recordings and calcium imaging analysis have been performed on rodent hippocampal slices and cell cultures to study the effects of modification of lipid rafts composition both on synaptic transmission and on calcium influx in a control group of animals. We evaluated the effects of beta-cyclodextrine induced cholesterol depletion on synaptic transmission and on the expression of Paired Pulse Facilitation (PPF) in CA1 hippocampal region. We found that cyclodextrine strongly reduced synaptic transmission whereas it did not affect PPF.

The role of glutamatergic receptors in cholesterol depletion mediated effects was evaluated by applying specific pharmacological agents. Our data indicate that, in cholesterol depleted neurons, reduction of synaptic transmission is sustained by AMPA and kainate receptors altered activities. Moreover, the involvement of these receptors has been confirmed by fluorimetric measurements of intracellular calcium concentration. These results support the hypothesis that modulation of receptors activity by manipulation of membrane lipids component may represent a possible therapeutical strategy in neurological aspects of NPDc.

STIMULATION OF ERYTHROPOIESIS AND FETAL HEMOGLOBIN REACTIVATION INDUCED BY STEM CELL FACTOR IN HUMAN β -THALASSEMIA

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Although the relationship between phenotype and genotype in human β -thalassemia is complex, the imbalance between α and non α globin chains correlates with the severity of disease and is responsible for the ineffective erythropoiesis and haemolysis. In β -thalassemia intermedia, for example, one or two β -globin genes are defective, but concurrent genetic factors (HPFH, α and $\delta\beta$ -thal determinants) can reduce the globin chain imbalance thus ameliorating or exacerbating the clinical conditions; on the contrary, in homozygous β -thalassemia major a very high α /non α ratio is associated with severe ineffective erythropoiesis and dependence on red blood cell transfusions for survival.

These observations supported the conviction that the severity of disease can be reduced by the γ -globin reactivation and considerable progress has been achieved, in the last two decades, in the pharmacological induction of fetal haemoglobin (HbF) synthesis in animal models and in patients with β chain hemoglobinopathies; however, until now, previous clinical trials of HbF reactivation in β -thalassemia produced inconsistent results. Previously, we demonstrated that Stem Cell Factor (SCF) induces cell proliferation and HbF synthesis in normal adult erythroid cultures and pharmacological doses of dexamethasone (Dex) potentiate its effect.

Here, we investigated the *in vitro* response of β -thalassemic haematopoietic progenitor cells to SCF-based treatments in terms of erythropoietic stimulation, inhibition of apoptosis and HbF reactivation. In unilineage erythroid cultures of 20 patients with either intermedia or major β -thalassemia, addition of SCF, alone or in combination with Dex, remarkably stimulated cell proliferation (3-4 logs over control cultures), while decreasing the percentage of apoptotic and dyserythropoietic cells (<5%).

More important, in both thalassemic groups the addition of SCF+Dex induced a strong increase of γ -globin chains reaching values of HbF content three fold higher than those observed in erythroid controls (81% vs 27%, mean values, in the β -thalassemia major).

These *in vitro* studies clearly indicate that scf, in combination with dex, represents an effective alternative in the expansion of erythroid compartment and γ -globin gene reactivation in β -thalassemia and may be considered as a therapeutic agent in preclinical models for this disease.

INHIBITION OF PDGFR PHOSPHORYLATION: A PATHOGENETIC TREATMENT OF SYSTEMIC SCLEROSIS

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Systemic Sclerosis (scleroderma, SSc) is a rare disease, characterized by extensive fibrosis of the skin and of visceral organs due to exaggerated production of collagen. Disease modifying therapy for scleroderma fibrosis is an unmet medical need. Excessive oxidative stress has been implicated, since ROS generation is strictly linked to a pathway linking the Ha-Ras, and growth-factor activated extracellular signal-regulated kinases 1/2 (Ha-Ras, ERK 1/2), with the Ha-Ras- ERK 1/2-ROS circuitry amplified in scleroderma fibroblasts. Stimulatory autoantibodies against PDGF receptor possibly provide a link between autoimmunity and fibrosis in SSc: they induce ROS production via Ha-Ras and ERK1/2 signalling and are ultimately responsible for SSc fibroblast activation via the intracellular kinases system, with consequent collagen overproduction. Inhibition of this pathway is therefore a candidate strategy for molecular intervention in SSc patients. Imatinib mesylate, the standard therapy for chronic myeloid leukaemia, is a specific inhibitor of ABL kinases, which normally phosphorylate the PDGFr. Recent reports suggests its possible therapeutic application in different diseases, such as PAH, in which an activation of PDGFr is involved, and in the Bleomycin (BLM)-induced lung fibrosis model.

The objective of the present study is to treat scleroderma skin fibrosis using a therapeutic strategy based on pathogenetic mechanisms. The study is an explanatory, open, controlled, randomized trial, in which 30 SSc patients with refractory disease (worsening of skin involvement or visceral damage despite adequate immunosuppressive and vasoactive therapy at standard doses for at least 3 months) will be randomly be assigned to receive or not Imatinib 100 or 200 mg die orally for 6 months (+6 months of follow-up) in addition to the conventional treatment.

Primary outcomes include the evaluation of the safety of Imatinib administered in low dose in SSc patients and the efficacy of the drug to revert *in vitro* the functional alterations of the SSc fibroblasts (i. Ros generation; ii. collagen production, iii. PDGFr phosphorylation). Skin biopsies will be performed prior to treatment, at the end of treatment and then after 3 and 6 months of follow-up. Secondary outcomes include the evaluation of the efficacy by assessment of: i. skin thickness evaluated by the modified Rodnan skin score, which reflects skin fibrosis and directly correlates with systemic involvement and progression of the disease; ii. scleroderma disease activity; iii. HAQ; arrest of visceral progression of the disease according to ACR 1995 guidelines for clinical trials in SSc.

P. SURVEILLANCE OF RARE CANCERS IN EUROPE

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The burden of rare cancers in Europe is unknown, and no generally accepted definition exists. There are large variations in survival, with poorer outcome among older patients and in eastern European countries.

Aims:

- to provide an operational definition of “rare cancers”, and a list of cancers that meet this definition;
- to estimate the burden of rare cancers in Europe;
- to improve the quality of data on rare cancers;
- to develop strategies and mechanisms for the diffusion of information among all the key players involved in Europe-wide surveillance on and treatment of rare cancers.

Actions:

- incidence, survival, prevalence and mortality for all rare cancers will be estimated;
- data quality will be analysed for a subset of cancers, by confirming the diagnostic data and, if possible, analysing additional data on stage and treatment;
- a web-site on rare cancers will be designed to disseminate the results of the project, and in particular, to inform clinicians, patients and health planners.

ADIPOSE TISSUE-DERIVED STEM CELLS FOR THE TREATMENT OF MUSCULAR DYSTROPHY

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Duchenne Muscular Dystrophy (DMD) is a progressive X-linked muscle wasting disease, leading to early disability and death. DMD is caused by a mutation in the dystrophin gene that precludes the production of a stable protein, causing disruption of muscle contractile structures.

Therapeutic approaches to DMD aim at rescuing muscle damage by delivery of cells able to differentiate into skeletal muscle. Skeletal muscle-derived progenitor cells represent one choice because of their intrinsic myogenic potential. Unfortunately, these cells are recovered in low number from DMD muscle biopsies and are poorly expandable *in vitro*. Stem cells of different origin may also restore dystrophin expression in several mouse models of muscular dystrophy. However, the frequency of stem cells incorporation into skeletal muscle is often too low to result in an important amelioration of the dystrophic phenotype. Thus it is important to identify alternative stem cell sources and to establish which population better contributes to skeletal muscle regeneration.

Adipose Tissue (AT) provides a uniquely abundant and accessible source of multipotent cells. We have recently shown that in addition to mesenchymal stem cells, adipose tissue contains a subpopulation of cells, referred to as adipose tissue-derived autonomously myogenic cells (AT-AMCs), which are able to spontaneously differentiate into contractile skeletal myotubes.

By seeding AT-derived cells at low density, we have been able to isolate clones of AT-AMCs expressing markers characteristic of muscle progenitor cells such as Pax3, Pax7, c-Met and Flk-1. *In vitro* amplified clones display a homogeneous morphology and the ability to readily convert to multinucleated contractile myotubes upon switching to low serum conditions. Transplanted AT-AMCs are incorporated with high efficiency in skeletal muscle fibers of regenerating tissue in a mouse model of hindlimb ischemia. Moreover, donor-derived Pax7⁺ mononucleated cells able to differentiate into multinucleated myotubes can be re-isolated from previously injected, regenerated muscle. Such results indicate that in addition to participate in the formation of new muscle fibers, transplanted AT-AMCs are retained as myogenic progenitors and may contribute to the replenishment of the muscle progenitor pool.

PHARMACOLOGICAL AND GENETIC REGULATION OF TSC2-/- CELL PHENOTYPE. A NOVEL INSIGHT FOR TSC AND LAM

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The TSC2 gene functions as a tumour suppressor and tuberin, the TSC2-encoded protein, regulates cell growth and cell cycle progression, and its loss may lead to abnormal cell proliferation. Cultured cells from human TSC2 angiomyolipomas may be an optimal means of studying and developing appropriate pharmacological strategies aimed at blocking the life-threatening growth of TSC2-/- smooth muscle cells in TSC and LAM. We have recently reported the novel isolation of a pure culture of LOH smooth muscle-like cells derived from the angiomyolipoma of a TSC2 patient.

EGF supplementation to the culture medium is necessary to promote proliferation and maintenance of TSC2-/- cells, and its proliferative action cannot be replaced by the addition of IGF-1.

Exposure to antibodies to EGF and IGF-1 receptors led to the death of TSC2-/- cell; IGF-I might act as a survival factor as it is secreted in autocrine fashion and regulates the expression of survivin in TSC2-/- cells. Treatment with rapamycin failed to affect TSC2-/- cell phenotype, particularly when added after cell plating, while anti-EGFR normalized the biochemical phenotype within 48 hours. The reintroduction of the TSC2 gene eliminated the EGF-dependency for cell growth, promoted tuberin expression, and normalized the function of intracellular pathways constitutively activated in TSC2-/- cells; thus the extent of phosphorylation of Akt, PTEN, ERK, p70S6K and its ribosomal protein S6 substrate, and mTOR was down regulated to normal and the typical expression of the HMB45 antigen was abolished.

Proliferation and diffusion of TSC2-/- cells *in vivo* were studied in nude mice and cells were applied by intraperitoneal injection or endonasal application. The lymphatic system was invaded in a couple of days and cells diffused throughout the body with accumulation in uterus and lungs. After endonasal application, TSC2-/- cells survived and proliferated in lung parenchyma creating a condition that somewhat resembled LAM. VEGF-D blood levels were dramatically increased and lungs were massively invaded by the lymphatics. We are now evaluating whether treatment with the above mentioned drugs may reverse the lung damage and kill the invading TSC2-/- cells.

In conclusion the *in vitro* work suggest that anti-EGFR and anti-IGF-IR treatment may kill human TSC2-/- cells and partially reverse their phenotype, while rapamycin has a cytostatic effect only when added at plating time. The reintroduction of the TSC2 gene into TSC2-/- cells promotes tuberin expression, and normalizes proliferation and phenotype of these cells.

MESENCHYMAL STEM CELLS FOR THE TREATMENT OF TIBIAL CONGENITAL PSEUDARTHROSIS ASSOCIATED WITH TYPE 1 NEUROFIBROMATOSIS

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Type 1 Neurofibromatosis (NF1), the most common single gene disorder found in humans, usually occurs in infant or young child with multiple *café-au-lait* spots. In childhood NF1 shows several bone growth disorders like spinal deformities, scoliosis and congenital tibial dysplasia. Five percent of NF1 patients shows fractures in long bones followed by pseudarthrosis. Tibial congenital pseudarthrosis is one of the most challenging conditions in pediatric orthopedics. The treatment of the tibial non-union is characterized by repeated surgical procedures which often failed with the inevitable outcome of severe disability. New strategies based on the use of autologous Mesenchymal Stem Cells (MSC) have been recently suggested, because the MSC pool contains precursors of osteogenic differentiation, that in turn may enhance bone repair and regeneration. In the current study, we compared the osteogenic potential of MSCs derived from tibial lesion with those obtained from the iliac crest. Our goal was to establish the biological basis for the use of the autologous MSC transplantation as an effective tool for the treatment of the tibial non-union in patients with NF1. Bone marrow samples were collected from the Iliac Crest (IC) and from the pseudarthrosis site (P) of 6 patients. Both IC-MSC and P-MSC were cultured in differentiating medium containing α -MEM added with 10% Fetal Bovine Serum (FBS) or with 10% Autologous serum (AUT), 10^{-8} M Dexamethasone (DEX), and 50 μ g/mL ascorbic acid. The differentiation of MSC into osteoblasts varied among patients. IC-MSC cultures showed an increased cell number and viability in comparison to P-MSC. Pseudarthrosis-derived cells did not show the typical morphology of osteoblasts, at an early culture time. Moreover, the number of Alkaline Phosphatase (ALP)-positive colonies was higher in IC-MSCs than in P-MSCs. The amount of ALP transcripts reflected the ALP activity viewed in CFU cultures. The addition of FBS or AUT in the culture medium did not change the osteogenic potential of IC-MSC.

Our preliminary results show that autologous MSCs derived from the iliac crest are able to differentiate into osteoblasts better than MSC derived from the site of pseudarthrosis. These findings suggest that the autologous MSC transplantation may be a promising strategy for the therapy of congenital pseudarthrosis and could lead an improving tissue healing and consolidation where traditional techniques fail.

The study was performed with the collaboration and contribution of Istituto Superiore di Sanità (Italy-USA programme on Rare Diseases) and "Io ci sono" Association.

P NEURAL TUBE DEFECTS AND FOLIC ACID: AN INTEGRATED, EVIDENCE-BASED APPROACH TO PRIMARY PREVENTION IN THE ITALIAN CONTEXT

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Our interdisciplinary project aims at: providing scientific evidence to support strategies for primary prevention of Neural Tube Defects (NTD) in the Italian scenarios through appropriate Folic Acid (FA) supplementation; estimating the association of FA supplementation and twin pregnancy; evaluating the impact of the adopted strategies on NTD prevalence in Italy; performing risk-to-benefit assessment of different approaches to increase FA intake in Italy. As for periconceptional FA and multiple pregnancy, a pilot study was performed on 40 mothers of twins (Association “Il Mondo dei Gemelli”) to estimate i) the target population size of the main study, and ii) how long after delivery women could recall information on FA supplementation. Women received four questions on FA supplementation and involved a friend, mother of singletons, to answer the same questions. Preliminary results indicate that all women recall whether and when they took FA, with no difference between twin and singleton mothers. Some women, with children >5 y.o., do not remember the brand name of FA supplementation. The minimum sample size should identify low relative risks like, supposedly, for the association between multiple pregnancy and FA and/or MTHFR677T polymorphism, together with their interaction. Accordingly, the minimum sample size for a 80% power is 1,350 women (450 mothers of twins from the Italian Twin Registry - ITR and 900 mothers of singletons, with children <5 y.o.); this will allow to estimate a minimum detectable relative risk of 1.4. Each mother, using a standardized procedure implemented by the ITR, received a mail questionnaire and a saliva kit for DNA collection. As for risk-to-benefit analysis, in Europe the Tolerable Upper Intake Level of FA has been defined at 1,0 mg/day (2000), based on limited data showing how a high FA consumption could mask the development of vitamin B12 deficiency, a possible problem in the elderly. Recent data on FA and cancer have somewhat complicated the picture. Observational studies indicate that FA might be effective at decreasing some cancer (colon and breast), especially in people with a lifestyle profile characterized by low folate intakes. However, recent experimental and epidemiological studies hint that high levels of FA may feed cancer growth. Thus, in Italy the risk-to-benefit evaluation of policies aimed at higher FA intake by the general population (e.g., fortification of flours) has to take into account that i) NTD incidence is relatively low, while ii) the consumption of wheat-based foods is high and iii) the aging population steadily increases.

P NEW GENETIC SYNDROMES WITH AORTIC TORTUOSITY AND DISSECTION

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Four new syndromes have been recently characterised and linked to TGFBR1 and TGFBR2 genes. The four syndromes share disease genes and major cardiovascular traits, in particular aortic aneurysm and dissection. Inherited aortic aneurysms and dissections may occur as isolated traits or combined with other arterial aneurysms (Thoracic Aortic Aneurysm Dissection, TAAD, MIM#608967) or in the context of complex syndromes such as classical Marfan Syndrome (MFS1, MIM#154700), MFS2 (MIM#154705), and Loeys-Dietz syndrome (LDS, #609192). The four phenotypes are caused by mutations of the FBN1 (*134797) (MFS1) and TGFBR2 (*190802) genes (MFS2, LDS and TAAD), respectively.

Diagnostic objectives: to implement the molecular diagnosis of MFS2, LDS, TAAD, and ATS. Transcriptomic objectives: to set up expression signatures in mutated versus wild type individuals, in peripheral blood samples. Clinical objectives: definition of the phenotype traits specifically and uniquely associated with TGFBR1 and TGFBR2 gene defects; generation of a complete multidisciplinary diagnostic work up; providing the ISS with all necessary information for including these new syndromes in the list of rare diseases. Genetic epidemiology objectives: generating prevalence data of different disease gene defects in our series and launching a call, with the ISS support, for a national registry for future larger research projects. Treatment objective: analysis of the effects of beta-blockers to the progression of aortic dilation, and define an optimal surgery timing for these patients whose risk of aortic dissection seems to be higher than that established in FBN1+MFS, Methods: Clinical evaluation, genomic studies.

Expected results: standardised diagnostic work up for these new syndromes, optimised molecular diagnostic tests, validation of the transcriptomic studies for the measurement of the expression signature of the mutated and wild type genes as well as of the FBN 1 gene, definition of the spectrum of the clinical phenotypes associated with defects of TGFBR1 and TGFBR2 gene; definition of overlapping traits in LDS, TAAD, MFS1, MFS2; generation of a first cohort of patients for future registry programs.

P CLINICAL AND DIAGNOSTIC COURSE FOR PATIENTS AFFECTED BY PRADER-WILLI SYNDROME

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The Prader-Willi syndrome is a complex pathology of genetic origin that can affect 1 newborn in 15000. Among the numerous phenotypical aspects of this syndrome (muscular hypotonia, low stature, narrow forehead, fragile hair and of light complexion, prominent lower lip, hypogonadism, obesity, diabetes mellitus, delay of psychomotor development, intellectual deficit, squint, myopia, sleeping disorder with nighttime apnea, ligamentous hyperlaxity, clinodactily, valgus knee with instability, osteoporosis) there are severe deformities of the vertebral column. Rates of deformity of the spine are described on the frontal plane, that is 80% scoliosis and a 40% rate of prevailing deformity of the spine on the sagittal plane, that is of hyperkyphosis.

Considering that this syndrome presents at birth with muscular hypotonia, with subsequent improvement of the muscular tone in the first year of life and onset of childhood obesity, some clinical situations arise that can divert or delay the treatment of the deformities of the spine with all the pejorative consequences on the quality of life and also on the duration of life of those affected by it. The inadequate, delayed, or lack of treatment of a deformity of the spine can jeopardize the respiratory and cardiocirculatory "compliance" already penalized seriously by the other not negligible phenotypic aspects of the syndrome.

We aim at designing a project that can define a clinical diagnostic course for all the cases that will have access to our Institute for vertebral column deformity combined with Prader-Willi syndrome, with diagnosis already defined or with diagnostic suspicion. The objective is to define from a diagnostic point of view every case, and to follow the spine deformity, according to a univocal protocol, that takes into account the genetic disease and its particular evolutionary aspects. This course would be a reference for any specialist or association that it deals with this pathology in a city, regional or national context, by transferring the data that will be collected in the national register of rare diseases of the National Institute of Health, to be able to have from the Istituto Superiore di Sanità validation of the course itself that becomes accessible not only to all the orthopedic doctors, but also pediatricians, and family doctors that have a diagnostic suspicion make a diagnosis of combined deformity of the spine and Prader-Willi Syndrome.

CLASSIFICATION OF PARATHYROID TUMOURS FOR OPTIMAL DETECTION OF HRPT2 MUTATIONS

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Germline and somatic mutations of HRPT2 are a cause of the Hyperparathyroidism-Jaw Tumour (HPT-JT) syndrome and Parathyroid Carcinoma (PC), consistent with a tumour suppressor role for the gene product, parafibromin. The pathological diagnosis of PC is difficult and the loss of expression of parafibromin by immunostaining has been suggested as a diagnostic tool. The purpose of our study was to identify and characterize HRPT2 mutations from patients affected by PC and evaluate parafibromin immunostaining in parathyroid tissues. Our cohort was 21 subjects with PC (including 1 with HPT-JT and 1 with familial recurrence). Genomic DNA from non-neoplastic tissues or peripheral blood was extracted and HRPT2 gene screened by dHPLC followed by direct sequencing of the entire coding sequence. Tumour DNA was similarly analyzed and Loss of Heterozygosity (LOH) across the HRPT2 locus assessed. Overall, we identified 8 mutations [4 somatic (c.231C>G, c.13C>T, c.42delG, and c.94insTA) and 4 Germline (c.415C>T, c.518_521delTCTC, c.679_680delAG and c.685_688delAGAG)] in 11 out of 21 (52%) subjects with PC. The c.679_680delAG deletion was found at the Germline level in three apparently unrelated subjects, in keeping with recurrence of the same mutation in other series. We found LOH in half of the ten subjects negative at mutation screening, but in only 2 subjects as a double hit. Parafibromin immunostaining was performed on tissues from 16 patients. In 7, 5 and 4 specimens there was diffuse loss, focal loss and diffuse positive staining, respectively. The high rate of positive parafibromin immunostaining and the relatively low rate of HRPT2 mutations prompted us to re-examine the pathological specimens. Seven tumours formerly classified as definite carcinomas were reclassified as equivocal carcinomas. According to our reclassification, HRPT2 gene mutations were identified in 10 out of 14 (71%) definite carcinomas and only in 2 out of 7 (29%) equivocal carcinomas; moreover diffuse/focal loss of parafibromin staining was observed in 9 definite and 3 equivocal carcinomas, while diffuse positive staining was observed in 1 definite and 3 equivocal carcinomas. In conclusion, we describe 5 novel HRPT2 mutations and confirm the association of mutations with parathyroid cancer. A high number of HRPT2 gene mutations should be identified if a correct pathological diagnosis is performed. Finally parafibromin immunostaining is an adjunctive tool to the diagnosis of definite carcinoma.

STUDY OF THE GENETIC SUSCEPTIBILITY AND ENVIRONMENTAL FACTOR INVOLVEMENT IN THE ETIOPATHOGENESIS OF AUTISM

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Autistic Spectrum Disorders (ASD) are a group of neurodevelopmental syndromes with onset in childhood, probably associated with brain abnormalities, not fully defined yet.

Various studies suggest a genetic influence in ASD with the involvement of different genes. Some Major Histocompatibility Complex (MHC) alleles like Human Leukocyte Antigen (HLA) B44-SC30-DR4 haplotype resulted more frequent among autistic children and their mothers. Moreover, HLA DQ2/8 and Tumour Necrosis Factor (TNF) alpha A-308G, which are located in the MHC region too, are related to celiac disease that is frequently observed in ASD subjects suggesting a possible involvement of these genes in both diseases. Also the SLC6A4 gene, coding for the human serotonin transporter (5-HTT) is regarded as a good candidate gene for autism susceptibility, as serotonin seems to play a key role in a range of behaviours and psychological processes, and an increase in whole blood serotonin has been observed in subjects with ASD.

A recent study performed in our laboratory on a group of Sardinian families with autistic subjects, evidenced a linkage between the HLA and 5-HTT polymorphism in the promoter region (5-HTT LPR) and ASD.

A group of 30 ASD patients and their relatives will be enrolled, at different centres of Don Gnocchi Foundation and at the Institute of Child Neuropsychiatry, University of Sassari. Informed consent, previously approved by the Ethical Committee of the Don Gnocchi Foundation, will be signed from all participants/legal guardians prior to inclusion in the study. Molecular genotyping analysis will be performed to study the linkage between the HLA and 5-HTTLPR genetic regions and ASD. Then the genomic regions in and around the HLA loci will be scanned to find out a genetic polymorphism specifically related to ASD susceptibility (for this reason TNF A-308G polymorphism, will be evaluated too). In the same time patients will be subjected to Neuropsychiatric and neuropsychological tests, EEG and MRI. The present study will bring to a definition of the

genetic background and clinical assessment underlying the development and the severity of ASD, by the creation of a clinical, neuropsychiatric, neuroimaging and biological database of ASD patient.

This multiparametric approach should allow to better understand the mechanisms responsible for the presence and progression of ASD and to identify markers predictive of its development. Moreover, it could be possible to distinguish the different subtypes within the spectrum of ASD, which is the most important bias of all the studies on these diseases.

A FAMILY-BASED LINKAGE ANALYSIS OF HLA AND 5-HTTLPR GENE POLYMORPHISMS IN SARDINIAN CHILDREN WITH AUTISM SPECTRUM DISORDER

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Autism Spectrum Disorders (ASD) are heterogeneous disorders with a wide range of additional phenotypic characteristics and IQ ranging from mental retardation to above average intelligence. ASD seem to be on the increase however, no information on their prevalence is available at European level. Support for genetic influences in autism come from different sources. Family-based studies suggest that a multigenic pattern may be responsible for susceptibility, but most results are conflicting and have yet to be replicated. The purpose of this investigation was to evaluate the distribution and inheritance of two genetic markers as possible candidates for autism susceptibility, namely the Human Leukocyte Antigen (HLA) and the human serotonin transporter coding (5-HTTLPR) polymorphisms, among Sardinians, a population characterized by a peculiarly homogenous genetic distribution.

A family based linkage analysis in ASD was conducted in a group of 37 families of Sardinian ethnicity. A total of 135 Sardinians, that is, forty autistic children 71 parents, and 24 siblings all of whom born in Sardinia and of Sardinian descent, were enrolled. The diagnosis of autism was made according to DSM-IV criteria. Informed consent was obtained from all participants/legal guardians prior to inclusion in the study.

Class I and class II HLA typing was performed by the standard sequence specific primer PCR, while variants of the 5-HTTLPR were investigated by molecular genotyping of the short (434bp) and long (528bp) alleles.

Linkage analysis was performed using LINKAGE software (shareware software developed by J. Ott at the Rockefeller University of New York). The degree of association of the various loci with autism was analysed by means of the Transmission Disequilibrium Test (TDT). Our results indicated linkage between the HLA and 5-HTTLPR genetic regions and ASD susceptibility. The statistical evaluation of possible linkage between autism and HLA, 5-HTTLPR or none of the genetic loci considered, revealed that in all

cases, there was linkage with one or the other or sometimes with both genetic loci. However, not even one allele in these polymorphisms proved to be related to ASD in a specific way, suggesting these loci as markers of other genes mapped in their close proximity that may be more directly involved.

Thus, in light of these considerations, our results are not revelations but premises for further linkage analyses, to scan the genomic regions in and around the HLA and 5-HTTLPR loci.

P IS THERE A ROLE OF OXIDATIVE DNA DAMAGE IN COCKAYNE SYNDROME?

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Cockayne Syndrome (CS) is multi-system autosomal recessive disorders defective in functions involved in transcription as well as in DNA repair. This syndrome shows progressive physical and mental impairment, precocious ageing and lack of cancer. Since oxidative damage has been involved in neurodegeneration, we examined the oxidative stress response in primary keratinocytes and fibroblasts established from the same skin biopsy of CS patients. CS-A cells were hypersensitive to oxidizing agents and accumulated 8-oxoguanine upon exposure to oxidants. In addition, CS-A fibroblasts showed an increased sensitivity to inhibitors of poly (ADP-ribose) polymerase, a nuclear enzyme that signals the presence of DNA damage and is implicated in the repair of DNA Single-Strand Breaks (SSB). Accordingly CS-A cells accumulated DNA-SSB induced by hydrogen peroxide.

Altogether these findings suggest that lesions and/or mechanisms other than 8-oxoguanine accumulation are involved in neurodegeneration and provide the first evidence of the involvement of the CSA protein in the signaling of SSB.

PROPOSAL FOR AN INTEGRATED APPROACH TO RARE DISEASES: A STUDY BETWEEN BASIC LABORATORY MODELS AND CLINICAL EPIDEMIOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Our project is addressed to Amyotrophic Lateral Sclerosis (ALS) epidemiology, including the evaluation of incidence in different times and areas, to pathogenesis at cellular and molecular level, and to integrated studies. Therefore, there is need for collecting data on large populations, using different methods (Work-Packages 1-3) as well as for integrating epidemiological data and experimental studies (WP4).

WP1. The National Registry of ALS (NRALS) was started, on the basis of the experience of the National Registry Rare Diseases and the existing regional Registries (PARALS-Piemonte; SLALOM-Lombardia; SLAP-Puglie). The NRALS is elaborating a common database at national level in order to collect clinical and epidemiological data. The NRALS has the following objectives:

- prevalence and/or incidence, natural history;
- pathogenetic studies, from the physiopathological mechanisms to genotype-phenotype correlation;
- causes and risk factors;
- clinical studies.

WP2: Aetiological evaluation of genetic and environmental factors started with identification of affected twins: ALS patients from 3 regional Registries and 4 ALS diagnosis reference Centres were obtained. Records lacking identification variables necessary for linkage to the Italian Twin Registry (ITR), and any duplicated records

resulting after merging the lists were removed. Overall, number of patients summed up to 3307 (71% males). Year of diagnosis ranged from 1979 to 2004. Record linkage with ITR yielded 33 possible twins. Ascertainment of their status and gathering of relevant information are ongoing activities.

WP3: Mortality for Motor Neuron disease by gender and residence area was analysed using data from Italian Mortality Data Base. Age-adjusted mortality rates per 100,000 inhabitants were calculated from 1970 to 2002 (1991 reference census) by gender, region of residence and age group. Mortality rate increased from 0.87 in 1970 to 1.63 in 2002 (men), and from 0.56 to 1.46 (women). In 1970 were recorded 322 deaths, *versus* 1,020 in 2002; South Italy showing the lowest mortality levels.

WP4: We studied bio-energetic (mitochondrial) abnormalities in animal models of ALS, through an imaging technique based on physico-chemical properties of NAD(P)H molecule to react if stimulated through UV light, and the possibility to quantify the NAD(P)H redox in *ex-vivo* brain cortex slices of wild-type 120 days-old C57BL/6J and G93A-SOD1 mice. Consistent differences in the first part (electron discharge) of responses were found, whereas the second part (energy charges) appeared less affected. Further experiments with *ex-vivo* mice, with proteomic experiments on mitochondria complexes, and on cybrid cells are in progress.

P COMPOUND HETEROZYGOSITY FOR MUTATIONS IN *LMNA* IN A PATIENT WITH A MYOPATHIC AND LIPODYSTROPHIC MANDIBULOACRAL DYSPLASIA TYPE A PHENOTYPE

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Mandibuloacral dysplasia type A is a rare progeroid syndrome characterized by dysmorphic craniofacial and skeletal features, lipodystrophy and metabolic complications. Most Italian patients carry the same homozygous missense mutation (p.R527H) in the C-terminal domain of *LMNA* gene, encoding lamin A/C, a component of the nuclear envelope. We report a 27-yr-old Italian girl showing a MADA-like phenotype (MADA-het). Features include hypoplastic mandible, acro-osteolysis, pointed nose, partial loss of subcutaneous fat and progeric appearance. Due to the absence of clavicular dysplasia, this phenotype can be considered an atypical laminopathy.

We identified a patient compound heterozygote for the p.R527H and p.V440M alleles. Patient's cells showed nuclear shape abnormalities, accumulation of pre-lamin A and irregular lamina thickness. Lamins A and C were normal. Electron microscopy revealed heterochromatin defects, typical of laminopathies. The clinical and cellular features of this patient show overlapping laminopathy phenotypes, probably due to the combination of p.R527H and p.V440M alleles. The p.R527H mutation cannot cause clinical effects in the heterozygous state. The p.V440M mutation has been described in the compound heterozygous state (p.V440M-p.R482Q), causing a severe lipodystrophy, while it was found alone in apparently healthy people, suggesting it might modulate the phenotype severity in subjects with another *LMNA* mutation. Our patient showed also a limb-girdle-like myopathy, never detected in MAD phenotypes.

The MADA-het mild progeroid phenotype could be explained by light alterations in the distribution of heterochromatin proteins observed in her fibroblasts. The MADA-lipodystrophy is attributable to an adipocyte differentiation defect caused by anomalous interaction of prelamin A with the adipocyte transcription factor SREBP1.

In conclusion, we present a case with a MAD-A phenotype without clavicular hypoplasia or metabolic unbalances and resembling limb-girdle myopathy. Nuclear envelope disorganization confirms the crucial role of lamin A/C C-terminal domain in nuclear lamina assembly.

Our data support the phenotypic heterogeneity of laminopathies and suggest that *LMNA* gene analysis could be proposed in patients with myopathies of unknown origin.

P BIOCHEMICAL AND CELLULAR REAL-TIME BIOMARKERS OF DIAGNOSTIC AND PROGNOSTIC VALUE IN THE MANAGEMENT OF KAWASAKI'S AND HENOCCH-SCHONLEIN PURPURA DISEASES

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The etiology of Kawasaki's Disease (KD) and Henoch-Schonlein Purpura (HSP), two rare systemic diseases occurring in children, is still unknown. These diseases are characterized by vessel inflammation (vasculitis) with alterations of different sized blood vessels and if not treated, different serious complications have been described so far. While KD involves skin, mouth, lymph nodes, and may affect coronary arteries causing serious cardiac complications; HSP affects capillaries in the skin and may cause damage at kidney level. The main clinical manifestations of HSP are vascular purpura, predominantly on the lower limbs, articular gastrointestinal and renal symptoms. With both diseases it is of crucial importance to make an early diagnosis, as their prognosis may be different and the complications avoided. Being both KD and HSP acute inflammatory illnesses, a role for oxidative stress has been suggested, although not investigated in deep.

Actually, for both diseases diagnostic tools are only clinical. The search for biochemical and cellular biomarkers in peripheral blood from KD and HSP patients as possible real-time biomarkers of diagnostic or prognostic value to be translated in the clinical practice could thus be of great importance. After informed consent, we tested blood samples from these patients taking into account: i) the possibility to find plasmatic or cellular redox markers in the peripheral blood and ii) the possible presence of immunological alterations. Preliminary data seem to indicate that this approach could provide in a near future some useful clue to improve our knowledge of the pathogenetic mechanisms and, supposingly, of the peripheral blood bio-indicators of progression of these diseases.

P ANTI-TUMOUR ACTIVITY OF THE NEW DUAL PI3K/MTOR INHIBITOR, NVP-BEZ235, IN SARCOMAS

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Osteosarcoma and Ewing's sarcoma are the most common primary malignant bone tumours in children and adolescents whereas rhabdomyosarcomas are the most common soft tissue sarcoma of childhood. Combination chemotherapy associated with local control with surgery or radiation therapy has become a standard practise in the treatment of patients with sarcomas. However, treatments for high-risk sarcoma are still completely inadequate since one third of patients with non metastatic disease and the great majority of patients with metastases at diagnosis do not survive. Although recent clinical studies indicated that the cure rate of patients with local disease can be improved with dose intensification of conventional therapeutics; this raise with severe toxicity and secondary tumours.

For these reasons, identification of new selective drugs are urgently needed for these tumours. Protein Kinase B or Akt (PKB/Akt) is a serine/threonine protein kinase involved as crucial regulator of divergent cellular processes including apoptosis, proliferation, differentiation and metabolism. Disruption of normal PKB/Akt signaling has been documented as a frequent occurrence in several human cancers and the enzyme appears to play an important role in their progression.

In this study, we analyzed the preclinical therapeutic potential of a novel inhibitor of PI3K/mTOR, NVP-BEZ235, in Ewing's sarcoma, osteosarcoma and rhabdomyosarcomas. The compound induced a G₁ cell cycle block in all cell tested, whereas apoptosis rate remained unchanged. Growth in soft-agar, as well as migration and adhesion to ECM components were significantly reduced in cells treated with NVP-BEZ235.

Combined *in vitro* treatments identified in the interaction of NVP-BEZ235 with vincristine the best drug-drug combination and this was confirmed against Ewing's sarcoma xenografts in nude mice. No toxic effects were observed with respect to metabolism. These results encourage the potential inclusion of this drug in the treatment of patients with musculoskeletal tumours.

THE BLADDER EXTROPHY-EPISPADIAS COMPLEX AND EXOGENOUS RISK FACTORS: THE BLADE PROJECT

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The Bladder Extrophy-Epispadias Complex (BEEC) include congenital malformations of genitourinary tract such as epispadia, bladder extrophy and cloacal extrophy. BEEC is considered a multifactorial disorder. Although the specific risk factors are still unknown, limited experimental studies indicate that endocrine disrupting chemicals may induce BEEC-like abnormalities. Among endocrine disrupters, persistent pollutants such as PCB deserve special attention because of widespread dietary exposure due to bioaccumulation. The many PCB congeners are divided in Dioxin-Like (DL) and Non Dioxin-Like (NDL). DL-PCB are agonists of the Aryl-hydrocarbon Receptor, thus activating target genes (CYP enzymes, etc.) and inducing antiestrogenic effects. NDL-PCB may alter steroid synthesis, cell-cell communication and redox cycling; the relationship of their different mechanisms and targets with chemical structure is still unclear.

BLADE aims at clarify the possible involvement of PCB in BEEC pathogenesis through an integrated approach by:

- setting up established *in vitro* cell cultures from the epithelial and mesenchymal components of the main urogenital structures (bladder, penis and urethra), obtained from human fetuses. *In vitro* fetal organ cultures will be also established to study responses at organ level (WP1, Florence University);
- *in vitro* studies on PCB using the models developed by WP1 to detect modulation of selected cellular and molecular parameters. PCB mixtures will be investigated concentrations relevant to the Italian population exposure. Previous attempts to define structure-activity relationships for NDL-PCB will be considered (WP2, ISS);
- investigating PCB risk assessments in correlation with genomic data on the basis of Toxicity Equivalency Factors (WHO, 2006); in particular cell pathways relevant to genitourinary development and dysmorphogenesis will be evaluated (WP3, University of Washington).

As first results:

- WP1 has established smooth muscle cell primary cultures from fetal corpora cavernosa 9 and 10 wk fetuses. Human foetal tissues are obtained from spontaneous and therapeutic abortion following patient approval consensus (the study protocols were approved by the Local ethical committee);
- WP2 selected PCB congener patterns and experimental levels for WP2 activities.

This was performed also with the support of novel human exposure data: i) DL-PCB levels range from 1ng/g lipid (serum or adipose tissue) to 5 ng/g lipid with similar patterns, with the 118, 156 126 and 169 PCB congeners being the most abundant; ii) notwithstanding the limited data on NDL-PCB in humans, the concentration of congeners identifies as most persistent (153, 180, etc.) range from 140 to 300 ng/g lipid.

P CALLOSAL AGENESIS: A BRAIN MALFORMATION WITH POLIGENIC ORIGIN. IDENTIFICATION OF CANDIDATE GENES AND LOCI THROUGH A MULTIDISCIPLINARY APPROACH OF CLINICAL, CYTOGENETIC AND MOLECULAR STUDIES OF A LARGE SET OF PATIENT WITH *CORPUS CALLOSUM* ANOMALIES

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The *corpus callosum* is the largest fibre in brain and connects neurons in the cerebral hemispheres. Its main cognitive function is to coordinate and transfer information between left and right brain. Agenesis of *Corpus Callosum* (ACC) can manifest as partial or complete agenesis or hypoplasia across the entire structure; moreover ACC may occur as an isolated malformation or as a component of a more complex malformation syndrome (over 50 syndromes with ACC are described).

Mutations in genes encoding guidance molecules/receptors, transcription factors, extracellular matrix, signaling/cytoplasmic molecules, growth factors have been shown to cause malformation in *corpus callosum* development in mouse. Therefore one group of candidate genes for ACC in humans includes human homologues of proteins regulating *corpus callosum* formation in mice. In addition, several cytogenetic abnormalities were found in human patients with ACC or callosal dysgenesis. Consequently, the regions involved in these rearrangements are candidate loci for *corpus callosum* anomalies in man. Clinical, neuroradiological and cytogenetic analysis of a selected cohort of 63 ACC patients allowed us to identify 7 cytogenetically detectable chromosomal abnormalities involving chromosomes 8p23, 4p15, 10q11, 10p and 21 (trisomy). Moreover, by fluorescent *in situ* hybridization analysis using subtelomeric probes we identified three subtelomeric rearrangements involving chromosome 13 and chromosome 1 (1p36 and 1q44). In all cases presenting callosal agenesis associated with chromosome and subtelomeric rearrangements, mental retardation, major congenital malformations and facial dysmorphisms were present.

In this proposal we decided to focus on in three directions:

- expanding the previously characterized patients' cohort, extending the analysis on new ACC patients referred to our Institute by a complete clinical, neuropsychiatric, and dysmorphologic evaluation (WP1: clinical);
- performing cytogenetic analysis (high-resolution karyotype and FISH analysis using subtelomeric probes in all subjects added to the previous study, and CGH-arrays analysis in selected patients) in order to detect patients with chromosomal rearrangements. (WP2: cytogenetics);

- performing molecular analysis of ACC candidate genes, selected on the basis of chromosomal location or functional homology with mouse genes [genes: AKT3 (1q44), Netrin-1 (17p13-12), Emx2 (10q26), Hesx1 (3p21), ARX (Xp22-21), Npn1 (10p12), MCPH (8p23.1)] (WP3: molecular).

The workpackages 1, 2 and 3 are now in progress. When concluded, the research should provide relevant information about chromosomal regions and candidate genes involved in callosal agenesis. These will thus represent invaluable genetic markers and diagnostic tools for ACC in all those cases in which the genetic cause is still unknown.

P PRELIMINARY DATA OF A TISSUE MICROARRAY (TMA) - BASED MULTICENTER STUDY OF THYMIC EPITHELIAL CELL TUMOURS WITH CLINICAL IMPLICATIONS

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Thymic Epithelial Tumours (TET) are rare neoplasias as they constitute <2% of all tumours. Their association with a variety of autoimmune disorders and their clinical behaviour, often unpredictable, render them relevant to immunologists and to oncologists. Moreover, TET oncogenic pathways are largely unknown, either for Thymoma, either for Thymic carcinoma.

Recently, the Tissue Microarrays (TMA) technology has proved to be suitable for simultaneous *in situ* analysis of hundreds of primary tumours at the protein level as well as for DNA investigations. TMAs allow identification and mapping of novel markers of neoplastic transformation and progression/response to therapy.

In TET analysis, Immunohistochemistry (IHC) play a relevant role, as mRNA-based microarray data have to consider contamination from the Thymoma lymphoid populations. Therefore the variety of molecular methods applicable could not overcome the primary role of the morphological / immunohistochemical approach. The initial purpose of the study is a multiparametric evaluation of phenotypical characters and of metabolic pathways involved in TET growth. Particular interest resides on the family of Tyrosine kinase Receptors: the EGFR family and their phosphorylated forms, and -c-kit (CD117).

The overall aim is to contribute to the setting of biologically targeted therapies in Thymoma and in Thymic carcinoma. Based on a long-lasting collaboration among Pathologists and Clinicians in different Institutions, the recruitment of archival paraffin-embedded tumour tissue was started. 206 cases, occurring between 1996 and 2007, have been collected until now. Moreover, the study is open to further contribution from other Centres. In some Institutions, a contribution to the study deriving from the existing frozen tissue and serological bank was planned.

The cases derived mainly from Centre and South Italy. Among the 206 cases collected, 57 derived from the Regina Elena Cancer Institute, Rome, 107 from the Catholic University of Rome, 17 from the University "Sapienza", II Faculty, S. Andrea Hospital in Rome, and 25 from the University of Perugia, Terni Medical Centre. Additional cases provided by Dr. G. Palmieri with known outcomes contributed data to biomarker's choice for TMA. Cases derived from the Catholic University of Rome, at variance with cases contributed from other Institutions, were mostly affected by Myasthenia Gravis (MG). As first step, a retrospective pathological review was planned to update the diagnoses to the WHO classification consensus terminology for Thymic tumours (2004) and to select adequate and representative pathological material to TMA establishment.

P DIVERSE MECHANISMS UNDERLIE THE INVARIANT OCCURRENCE OF THE T42A, E139D, I282V AND T468M SHP2 AMINO ACID SUBSTITUTIONS CAUSING NOONAN AND LEOPARD SYNDROMES

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Missense mutations in *PTPN11* cause Noonan Syndrome (NS) and the clinically related Leopard Syndrome (LS), two developmental disorders with a pleiomorphic phenotype.

PTPN11 encodes SHP2, an SH2 domain-containing protein tyrosine phosphatase that relays signals from activated cell-surface receptors to RAS. NS-causing mutations promote SHP2's gain of function, whereas LS-causing lesions engender loss of SHP2 catalytic activity.

While the identity of substitution does not seem to be critical in some cases, indicating a crucial role in SHP2's function for the residue being replaced, an invariant amino acid change is frequently observed, suggesting a specific role for the introduced residue. Here, we characterized functionally and structurally the invariant NS- or LS-causing T42A, E139D, I282V and T468M mutations. By analyzing *in vitro* biochemical behaviour and ligand-binding properties of all possible substitutions arising from a single base change affecting such codons, we show that different mechanisms drive the specificity in the amino acid substitution. While the NS-causing T42A, E139D and I282V changes are the only activating mutations among all possible substitutions affecting such codons, deamination of methylated cytosine represents the driving factor leading to the high prevalence of the T468M change in LS.

* *Questi autori hanno contribuito in egual misura al progetto*

CLINICAL, GENETIC AND MORPHOLOGICAL INVESTIGATION OF HRPT2-RELATED FAMILIAL HYPERPARATHYROIDISM

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Hyperparathyroidism-Jaw tumOr syndrome (HPT-JT, OMIM #145001) is an autosomal dominant syndrome with incomplete penetrance, characterized by the familial occurrence of parathyroid tumours, ossifying fibromas of mandible or maxilla, and, less frequently, uterine tumours and renal lesions. Germline mutations of the oncosuppressor gene *HRPT2* are responsible for more than half of cases of HPT-JT but also for a subset of Familial Isolated Hyperparathyroidism (FIHP, OMIM 145000), another rare disorder.

The protein encoded by *HRPT2* is named parafibromin, whose expression is absent or impaired in parathyroid tumours from HPT-JT subjects. Clinical, genetic, and morphological study of HPT-JT and FIHP kindreds in order to investigate the presence of genotype-phenotype correlates. The study population includes 6 affected subjects and 3 carriers from large HPT-JT kindred, 1 unrelated patient with HPT-JT, and 2 unrelated FIHP kindreds, including 4 and 2 affected members, respectively.

Clinical, radiological, and laboratory investigation for the occurrence of parathyroid tumours and other disorders; analysis of *HRPT2* germline and somatic mutations; methylation analysis of *HRPT2* promoter in parathyroid tumours; immunohistochemical analysis of parafibromin expression in parathyroid and jaw tumours.

Both HPT-JT and FIHP patients showed hyperparathyroidism and associated diseases, such as uterine polyposis, kidney cancer, and other neoplasms, but parathyroid carcinomas and atypical parathyroid adenomas occurred only in HPT-JT kindreds. Germline *HRPT2* mutations were identified in HPT-JT kindred (c.433_442delinsAGA) and in the two FIHP kindreds [c.(136_144)del5, Leu63Pro].

A somatic mutation (Glu29X) was demonstrated in the parathyroid carcinoma of the index case patient of the second HPT-JT kindred. Loss of nuclear parafibromin expression was demonstrated in all the parathyroid tumours examined at immunohistochemistry, while intense immunostaining was present in available normal parathyroids obtained at surgery from both the above patients and in ossifying fibromas of the jaw. No methylation in CpG site of the *HRPT2* were demonstrated in parathyroid tumours.

These findings, critically related with the literature, indicate that FIHP and HPT-JT associated with *HRPT2* mutations do not have a distinct genetic signature, but represent two variants of the same genetic disease. Thus, other co-factors could be responsible for

different clinical expression of the diseases in different subjects and kindreds. The mutations we found are typically inactivating, in agreement with a tumour-suppressor role for *HRPT2*, and occur all along the sequence of the gene, without evidence of *hot spots*.

P USEFULNESS OF MLPA IN THE MOLECULAR DIAGNOSIS OF LISSENCEPHALIES AND NEURONAL MIGRATION DISORDERS: HIGH DIAGNOSTIC YIELD IN P>A LISSENCEPHALY

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Lissencephaly (LIS) is a neuronal migration disorder characterized by absent (agyria) or reduced gyration (pachygyria) of the cerebral surface and a thickened, simplified four layered cortex. Patients with LIS have intractable epilepsy, spasticity, mental retardation and reduced longevity. Lissencephaly is rare with a prevalence of 11.7 per million births. Classical LIS or type 1 LIS is caused by mutations of either the LIS1 gene (PAFAH1B1) on 17p13.3 or by the DCX gene (doublecortin, or XLIS) on Xp22.3. LIS1 mutations result in more severe LIS in the posterior brain regions (p>a gradient), whereas DCX mutations cause a more severe LIS in the anterior brain regions (a>p). LIS1 gene is involved in both Isolated Lissencephaly Sequence (ILS) and Miller-Dieker Syndrome (MDS). ILS is caused by intragenic mutations or by internal deletions of the LIS1 gene whereas MDS is caused by deletions of contiguous genes in 17p13.3, including LIS1.

We studied 45 patients with p>a LIS not including MDS, in whom FISH for the 17p13.3 region gave negative results. We initially performed DNA sequencing of LIS1 and, subsequently, Multi Length Probe Amplification (MLPA) in those who were mutation negative.

The latter method identified small genomic deletions/duplications of LIS1 in about 82% (19/25) of patients who had previously been tested unsuccessfully with both FISH and DNA sequencing. Overall, small genomic deletions/duplications, represented 49% (19/39) of genomic alterations and brought to 87% (39/45) the number of patients in our series in whom any involvement of LIS1 could be demonstrated. In order to characterize the breakpoint regions, we performed Long Range PCR in five patients with deletions. We demonstrated that, in four out of five patients, deletions were caused by Alu elements mediated recombination, suggesting that LIS1 is particularly prone to undergo recombination between Alu elements.

We suggest MLPA to be used as first line molecular diagnosis for p>a LIS.

FROM PROTEOMIC TO STRUCTURAL BIOLOGY: A COMPREHENSIVE APPROACH TO AMYLOID DISEASES

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Amyloidosis is a prototype of diseases caused by “abnormal protein conformation” and a molecular approach to diagnosis and therapeutic strategies is based on proteomics and protein biochemistry.

At the National Center for Systemic Amyloidosis of the Fondazione IRCCS Policlinico San Matteo, we have developed a research strategy aimed at elucidating the molecular basis of the disease in order to design a rational therapeutic intervention. Our approach involves the molecular characterization of biological material and its correlation with the corresponding clinical phenotype. The clinical data-base is associated with a tissue bank in which the diagnostic specimens are stored for diagnostic and research purposes.

We have defined a proteomic approach for the diagnosis of systemic amyloidoses, which is complementary to the genetic and biochemical approach, which we are now opening for validation to the Italian national network for systemic amyloidosis (www.amiloidosi.it). Beside the immediate diagnostic implications of proteomics, that corroborates the genetic and histopathological approach, we have several examples of how the proteomic data can redirect experimental research in the field. Just recently, the analysis of natural amyloid fibrils obtained from patients affected by dialysis-related amyloidosis has provided evidence of the absence in the fibrils of a form of beta-2 microglobulin cleaved at position 58, whereas this species is apparently highly represented in serum of patients undergoing haemodialysis.

In the case of amyloidosis caused by apolipoprotein A-I, we have determined the pathogenic domain of the protein and consequently prepared a recombinant protein suitable for experimental studies.

Full description of the structure of the natural amyloidogenic proteins and identification of local co-factors that contribute to protein fibrillogenesis and local tissue toxicity represents now the core of our research activity.

Significant progresses have been achieved in the case of amyloidosis caused by beta-2 microglobulin in which we have demonstrated that the tissue localization is driven by the interaction with collagen. In the case of amyloidogenic monoclonal light chains new data have been obtained on the putative toxic role of pre-fibrillar, oligomeric light chains, on myocardial cells.

P CYTOKINE-BASED IMMUNOTHERAPY IN PRECLINICAL MODELS OF CUTANEOUS AND OCULAR MELANOMA

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Uveal melanoma is a rare and distinct disease, since metastatic patients develop only liver metastases, differently from the metastatic pattern of cutaneous melanoma. An Italian Reference Center for ocular melanoma will provide materials suitable for the study of the tumour microenvironment and of the antigenic profile of uveal melanoma. Our project will study new vaccine treatments in preclinical syngeneic models of both cutaneous and ocular melanoma. So far, clinical response rates to vaccine or chemo-immunotherapy in metastatic melanoma are variable, but the impact on survival rates is negligible. A possible reason for such failure is related to melanoma-induced immunosuppressive mechanisms, such as the activity of CD25+CD4+ regulatory T cells (Tregs). One research Unit will focus on the role of leaderless cytokines such as HMGB1, thioredoxin, MIF and galectin-1, and of the redox status (as free thiols) in the generation of an immunosuppressive and tumour-promoting melanoma microenvironment. The definition of the suppressive factors in melanoma may provide suitable targets to potentiate immunotherapy.

In this context other Units involved in the project will attempt to combine drugs (cyclophosphamide, melphalan) or antibodies blocking regulatory T cells (Treg) cells (anti-CD25, anti-OX40) in conjunction with tumour-targeted cytokines to achieve synergistic effects in melanoma immunotherapy. Strategies of selective delivery of cytokines at the tumour site may allow to reach high local concentrations and to reduce systemic adverse side effects.

This goal can be achieved by engineering cytokines, with tumour-specific ligands such as recombinant antibodies to tumour-associated antigens or the RGD peptide, which binds to the α -V/ β -3 integrins. GM-CSF will be chimerized with an RGD or a scFV tumour-binding domain, with the aim to reduce toxicity and the possible induction of immature Myeloid Suppressor Cells by GM-CSF. A chimeric protein (L19-mTNF- α) formed by TNF- α , linked to a recombinant antibody fragment recognizing a fibronectin isoform of the tumour extracellular matrix, allows TNF targeting at the tumour site. The study of scFvs directed to different molecular components of the tumour matrix is in progress. We will also attempt to use melanoma-targeted TNF (L19-mTNF) and GM-CSF-RGD as adjuvants for a syngeneic melanoma cellular vaccine by incubating these molecules with melanoma cells, to be used as a vaccine.

The study of the combination of L19-mTNF or GM-CSF-RGD with drugs or antibodies acting on Treg cells is an attractive possibility. Preliminary data obtained in the first months of this project will be illustrated.

P GENETIC ABNORMALITIES OF COMPLEMENT REGULATORY MOLECULES IN HEMOLYTIC UREMIC SYNDROME N. 526D/9

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Haemolytic Uremic Syndrome (HUS) is a thrombotic microangiopathy with manifestations of haemolytic anaemia, thrombocytopenia and renal impairment. Genetic studies have shown that mutations in complement regulatory proteins predispose to atypical forms of the disease that are not caused by Shiga-toxin producing *E.Coli* infection (non-Stx-HUS). We undertook genetic analysis of Complement Factor H (CFH), Membrane Cofactor Protein (MCP), and Factor I (CFI) in more than 200 patients with non-Stx-HUS. We screened 215 patients for CFH and we found 53 mutations (24.6%). The majority (75%) of mutations affect the last two Short Consensus Repeats (SCR) of the protein. For MCP we analysed 256 subjects and 23 mutations have been identified (10%). Seventeen of the 18 independent mutational events (94%) cluster in the 4 SCRs at the amino-terminal region of MCP, thus confirming previously reported data on the importance of this region for complement regulation. Nine CFI mutations have been found in a cohort of 233 patients (4%). Seven out of nine mutations (78%) are located in the last five exons (IX-XIII) of the gene causing alterations in the serine-protase region of the protein. Identification of CFH, MCP and CFI mutations could potentially translate into an improvement in the management and therapy of patients and will hopefully provide the way to design tailored treatments. However, despite these advances in our understanding of the molecular bases of non-Stx-HUS, no genetic defects have been found yet in about 60% of patients. Recent studies have shown gain-of-function mutations of Complement Factor B in patients with non-Stx-HUS, suggesting its role in the pathogenesis of the disease. In addition to mutations in complement regulators, anti-CFH autoantibodies have been reported in 4-8% of non-Stx-HUS patients. Screening of CFB and search for anti-CFH autoantibodies is undergoing in our patients. To better understand genetic causes of the disease we have also undertaken molecular analysis in additional candidate genes, involved in complement system regulation, encoding for: CD59, Clusterin (CLU), Osteopontin (OPN), CRIg a new Complement Receptor of the Immunoglobulin Superfamily (VSIG4). However no CD59, CLU, OPN nor VSIG4 mutations have been found till now in any of 48 patients. Additional candidate genes have been chosen, including C1-inh that encodes C1-inhibitor, a protein that regulates the classical and the alternative pathways of complement and CFP, encoding a plasma protein, properdin, that increases the stability of the alternative pathway convertases 10-fold on target surfaces. Screening of these two genes is ongoing.

CSB PATIENTS WITH UNUSUAL CLINICAL FEATURES

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The *CSB* protein plays a key role in transcription-coupled repair, the Nucleotide Excision Repair (NER) subpathway that rapidly removes damage from the transcribed strand of active genes. In addition, *CSB* is involved in the removal of some types of oxidative damage that are repaired via base excision repair and in different aspects of transcription by RNA polymerase I, II and possibly III. The many roles of *CSB* provide an explanation for the variety of pathological phenotypes associated with *CSB* defects, which comprise a significant number of patients affected by Cockayne Syndrome (CS) and rare cases affected by Cerebro-Oculo-Facio-Skeletal Syndrome (COFS), an autosomal recessive disorder sharing a lot of clinical features with CS, including severe mental and physical retardation and precocious ageing. Mutations in *CSB* have also been found in rare NER-defective patients affected by disorders sharing with CS only cutaneous photosensitivity, namely a severe form of xeroderma pigmentosum and the UV-sensitive syndrome. A clue to clarify the intriguing genotype–phenotype relationships in patients mutated in *CSB* has not been found yet.

We have recently assigned to the *CSB* group four new cases, two with the extremely severe clinical symptoms typical of COFS and two that are the first reported *CSB* cases affected by the mild form of CS. In all four patients we found the alterations of the cellular response to UV typical for CS. Sequencing of the *CSB* gene demonstrated that one COFS patient was homozygous for a mutation already described in CS cases while the other was carrier of a new combination of mutated *CSB* alleles. As well as amplifying the spectrum of mutations in the *CSB* gene associated with COFS, these findings demonstrate that COFS may be associated with the same inactivating mutations in the *CSB* gene responsible for the CS phenotype.

Both mild CS patients appeared to be compound heterozygous for differentially mutated *CSB* alleles not described previously, which are predicted to result in severely truncated products or in a protein of 1,243 aa in which the last 1,025 aa are out of frame. Immunoblot analysis suggested that the amount and/or length of the mutated *CSB* protein correlate with the severity of CS symptoms.

P CHARACTERIZATION OF THE MOLECULAR AND CELLULAR MECHANISMS UNDERLYING THE LIVER PATHOGENESIS IN HEMOPHAGOCYTIC SYNDROMES (HS)

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Hemophagocytic Syndrome (HS) is a severe and often fatal syndrome resulting from potent and uncontrolled activation and proliferation of T lymphocytes, leading to excessive macrophage activation and multiple deleterious effects. This project is devoted to a better characterization of the mechanisms underlying the liver pathology of the HS. Our preliminary results indicate at least two new mechanisms potentially involved in HS progression.

We have analyzed a series of patients who presented with symptoms of HLH and/or abnormal NK cytolytic activity. We have identified 6 patients with heterozygous mutations of PRF1: FHL2, OMIM 603553 and two patients with composite heterozygous mutations of AP3B1: OMIM 603401. Mutations of the AP3B1 led to absence of the β 3A subunit with subsequent loss of stability of the heterotetrameric complex to which β 3A is directly bound, while the others subunits are variably affected. This condition which is known as Hermansky-Pudlak Syndrome type 2 (HPS2) is characterized by oculocutaneous albinism, platelet defects due to absence of platelet bodies (leading to defective secondary aggregation response), and immunodeficiency. AP-3 absence differentially affects vesicular trafficking in certain cell types, including melanocytes, platelets, Cytotoxic T Lymphocytes (CTL) and NK cells. These two siblings affected by HPS2 who presented a dramatic reduction of cytolytic activity of freshly isolated and of IL-2 activated NK cells against multiple targets. Perforin content was significantly reduced in NK cells, thereby accounting for the impairment of NK cytolytic activity. We are in the process to analyze the effects of AP-3 deficiency in other cell types expressing the AP-3 complex including CD8 and dendritic cells.

We are also characterizing the chemotactic ability of two proteins highly expressed in the liver, namely chemerin and activin A. These two proteins share the ability to induce the migration of both dendritic cells and NK cells and to potentially act as co-localization factors of these two cell types in pathological tissues. Both proteins acted as potent chemotactic factors but apparently failed to induce a general activation program in the two cell types. Indeed, neither chemerin and activin A induced the production of inflammatory cytokines (e.g. TNF, IL-6, CXCL8 and IL-12) by dendritic cells or by NK cells (IFN γ) and NK cell degranulation. We propose that chemerin and activin A, in addition to certain chemokines known to be produced by activated Kupffer cells, such as CCL20, may induce the colocalization of innate immunity effector cells in pathological conditions, such as HS.

P GAIN-OF-FUNCTION RAF1 MUTATIONS CAUSE NOONAN AND LEOPARD SYNDROMES WITH HYPERTROPHIC CARDIOMYOPATHY

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Noonan and Leopard Syndromes (NS and LS) are developmental disorders with overlapping features including cardiac abnormalities, short stature and facial dysmorphisms. Increased RAS signalling due to *PTPN11* and *KRAS* mutations cause approximately 50% of NS, while *PTPN11* mutations cause 90% of LS. Here, we report that 18 of 231 NS and two of six LS patients without known mutation have missense mutations in *RAF1*, which encodes a serine/threonine protein kinase that activates MEK1 and MEK2. Most mutations altered a motif flanking Ser259, a residue critical for RAF1's autoinhibition through 14-3-3 binding. *RAF1* mutations in two hotspots were strongly associated with Hypertrophic Cardiomyopathy (HCM; 95% vs 18% of all NS). Ectopically expressed RAF1 mutants from HCM clusters had increased kinase activity and enhanced ERK activation, while non-HCM-associated-cluster mutants were kinase impaired. Our findings further implicate increased RAS signalling in pathological cardiomyocyte hypertrophy.

* Questi autori hanno contribuito in egual misura al progetto

P DIVERSITY, PARENTAL GERMLINE ORIGIN AND PHENOTYPIC SPECTRUM OF *DE NOVO* *HRAS* MISSENSE CHANGE IN COSTELLO SYNDROME

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Activating mutations in *HRAS* have recently been identified as the molecular cause underlying Costello Syndrome (CS). To investigate further the phenotypic spectrum associated with germline *HRAS* mutations and characterize their molecular diversity, subjects with a diagnosis of CS (N=9), Noonan syndrome (N=36), cardiofaciocutaneous syndrome (N=4) or with a phenotype suggestive of these conditions (N=12) were screened for the entire coding sequence of the gene. A *de novo* heterozygous *HRAS* change was detected in all the subjects diagnosed with CS, while no lesion was observed with any of the other phenotypes.

While eight cases shared the recurrent c.34G>A change, a novel c.436G>A transition was observed in one individual. The latter affected residue p.Ala146, which contributes to GTP/GDP binding, defining a novel class of activating *HRAS* lesions that perturb development. Clinical characterization indicated that p.Gly12Ser was associated with a clinically homogeneous phenotype.

By analyzing the genomic region flanking the *HRAS* mutations, we traced the parental origin of lesions in nine informative families and demonstrated that *de novo* mutations were inherited from the father in all cases. We noted an advanced age at conception in unaffected fathers transmitting the mutation.

* *Questi autori hanno contribuito in egual misura al progetto*

P ELABORATION OF NOVEL VACCINES FOR THE TREATMENT OF SELECTED SOFT-TISSUE SARCOMA HISTOTYPES

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We have recently found that NG2 predicts metastasis formation and constitutes a novel independent prognostic factor in certain soft-tissue sarcomas. In parallel, the group of Michele Maio has recently coordinated a successful Phase I/II immunotherapeutic trial involving vaccination of advanced melanoma patients with anti-idiotypic antibodies mimicking NG2. We have further observed that NG2 promotes the tumour-host microenvironment interaction *in vivo* by acting as an ancillary receptor for collagen type VI (Col VI). Accordingly, a first objective is to clarify the modes by which the above “receptor-ligand” pair regulates metastasis formation in soft-tissue sarcomas. In this first phase of the work we are studying the motility behaviour of sarcoma cells in response to purified Col VI and Col VI-containing matrices/tissues using 2D/3D motility assays routinely used in the laboratory, whereas tumour progression and metastasis formation *in vivo* are examined in wild type, immunodeficient and transgenic mice harbouring deletion of the Col VI gene. In both *in vitro* and *in vivo* paradigms we are testing human and murine sarcoma cells with various relative surface levels of endogenous NG2, cells in which the endogenous NG2 is knocked down by RNAi, cells harbouring stable misexpression of NG2, and cells that we have stably transduced with NG2 variants encompassing loss-of-function mutations. The second objective is to generate suitable vaccines to substitute for anti-idiotypic antibodies, to substitute for anti-idiotypic antibodies previously utilized on melanoma patients. For this purpose, we will pursue bioinformatic “reversed immunology” and a proteomic strategies in order to pinpoint the immunodominant sequences of NG2 and generate the corresponding synthetic peptides. Effective immunogenic peptides will subsequently be screened *in vivo* in NOD/SCID mice in which the deficient haematopoietic system has been reconstituted via human CD34⁺ stem cell transplantation. These “humanized” mice will receive subcutaneous sarcoma cell implantations alone, or combined with co-infusion of *in vitro* selected NG2-specific cytotoxic T lymphocytes, and will be adopted as tumour models to evaluate vaccination efficacy of the selected immuno-active peptides. The cumulative outcome of the proposed research is expected to provide cellular, molecular and clinically relevant information about crucial metastasis-promoting molecular interplays and generate the necessary tools for immediate therapeutic intervention on soft-tissue sarcomas patients.

THE *TP53* NOINS-PRO HAPLOTYPE IS NOT ASSOCIATED WITH BREAST AND OVARIAN CANCER IN HEREDITARY CASES NEGATIVE FOR MUTATIONS IN *BRCA1* AND *BRCA2*

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Germline disease-causing mutations in *BRCA1* and *BRCA2* genes confer high risk of Breast and Ovarian Cancer (BOC), but account approximately for only 15% of familial cases. Theoretical models and experimental observations have indicated that a variable number of low penetrance alleles would explain BOC in the majority of familial and sporadic cases. Moreover, genetic factors may act as risk modifiers in carriers of *BRCA* mutations. Recently, it has been shown that the *TP53* haplotype NoIns-Pro (a combination of the polymorphisms c.97-147ins16bp, and c.215C>G, p.Arg72Pro termed hereafter Ins16bp and Arg72Pro respectively) had significantly increased frequency in cases with *BRCA* mutations and cancer onset before 35 years, with most of the effect among *BRCA2* carriers. Aim of this study was to investigate the possible role of the NoIns-Pro haplotype of *TP53* as a low-penetrance allele in hereditary BOC cases negative for mutations in *BRCA* genes. The Ins16bp and Arg72Pro polymorphisms were genotyped in a total of 423 unrelated cases and 380 controls. The cases were ascertained through the Unit of Medical Genetics of Istituto Nazionale Tumori (INT) in Milan, and fulfilled the criteria of eligibility to *BRCA1* and *BRCA2* mutation testing. All cases included in this study were those who tested negative for disease-causing mutations and variants of uncertain significance after screening in coding exons and exon-intron boundaries of both *BRCA1* and *BRCA2*. The controls were blood donors collected through the Service of Immunohematology and Transfusion Medicine of INT. Comparison of genotypes and allele frequencies in cases and controls was performed by resorting to a logistic regression model for the two Ins16bp and Arg72Pro polymorphisms and the haplotypes. The Ins16bp and Arg72Pro polymorphisms were studied separately, and as haplotypes. As regards the haplotypes, the frequencies of the four alleles were examined separately; we also considered the three most common

haplotype combinations, grouping all the remaining in a forth class; finally, we specifically investigated the effect of the NoIns-Pro haplotype by examining the distribution of all combinations containing at least one copy of this haplotype. None of the performed analyses resulted statistically significant. These observations indicated that neither the Ins16bp or Arg72Pro polymorphisms considered separately, nor the NoIns-Pro haplotype, or any other haplotype, were associated with breast and ovarian cancer risk in *BRCA*-mutation negative hereditary cases.

P **ROLE OF THE DYSTROPHIN-ASSOCIATED GLYCOPROTEIN COMPLEX IN LIMB-GIRDLE AND CONGENITAL MUSCULAR DYSTROPHIES: FROM MOLECULAR PATHOPHYSIOLOGY TO POTENTIAL THERAPY (7DR1)**

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Different forms of muscular dystrophy are caused by mutations in genes coding components of the dystrophin associated-glycoprotein complex, the multimeric transmembrane protein complex first isolated from skeletal muscle that links the cytoskeleton to the extracellular matrix. Muscular dystrophies are multi-system disorders affecting in addition to skeletal, smooth and cardiac muscles, the endocrine system and the central nervous system. Cognitive impairment and mental retardation are often characteristic features associated with muscle atrophy in some muscular dystrophies. The aim of our project, involving four groups, is to improve our understanding of the underlying mechanisms that produce a dystrophic phenotype by focusing our studies on i) the role of dystroglycan in cell adhesion and cell signalling, ii) the potential of hyperglycosylation strategies as a means for therapeutic intervention in several forms of muscular dystrophy, including dystrophinopathies and dystroglycanopathies; iii) the emerging functions of dystrobrevin as motor adaptor and signalling scaffold protein. Although many animal models of Congenital Muscular Dystrophy (CMD) are at present available, our knowledge over the molecular mechanisms, underlying the pathophysiology of CMD are still poor.

To characterize the muscle tissue gene expression profile in cases of CMD due to merosin deficiency or associated with α -DG hypoglycosylation by using oligonucleotide microarrays technology we have started to perform histology, histochemistry and immunocytochemistry analysis on muscle biopsies of patients with a known diagnosis of CMD or LGMD and to set up primary cultures from diagnostic muscle biopsies. In patients affected by CMD or LGMD, the interaction between the two (α and β) subunits of dystroglycan is often altered or completely disrupted leading to severe sarcolemmal instability. To understand the molecular details underlying the DG complex function we used a mutagenic approach to evaluate the effect of a series of individual point mutations on the affinity of α -DG(485-630) towards β -DG(654-750) and to analyse the post-translational fate of mutated full-length DG-constructs in transfected human cells.

We are also analysing biopsies of patients affected by rare neuromuscular disorders to assess possible alterations in the α/β interface. With the aim to elucidate the molecular organization of signalling proteins associated with components of the DGC and to analyse

whether and how it is altered in CMD and LGMD, we started the characterization of the interaction between dystrobrevin and its binding partners, pancortin and iBRAf, two proteins thought to play a major role in neurogenesis and brain development and dysbindin, which is involved in protein trafficking and organelle biogenesis.

ESTABLISHMENT OF A EUROPEAN NETWORK OF RARE BLEEDING DISORDERS

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Rare Bleeding Disorders (RBDs), representing 3-5% of all the inherited coagulation deficiencies, are autosomal recessive diseases leading to lifelong bleeding, relatively neglected by health care providers and drug manufacturers. Based on a decade-long research, an International Registry on 400 patients from 19 countries, was compiled (www.rbdd.org). Major cohorts were from Iran (155) and Italy (154); remaining patients were spread worldwide. According to coagulant activity, 41% of patients were severe (with activity <1% and severe bleedings), 20% moderate (with activity >5% and <10% and non spontaneous bleedings, only after trauma or post-surgery episodes) and 39% mild (activity >10% and very few bleeding episodes after trauma or post surgery); 77% of patients was fully characterized for 165 different mutations (70% novel) increasing knowledge on RBDs genetics by 15%.

Despite the existence of this and other RBDs databases, data are not yet sufficient to indicate which course of action is needed to improve diagnosis and treatment. This lacuna could be made up by the collection and organization of clinical, laboratory and treatment data and their statistical analysis using a unique and homogenous model. As the most readily available data come from Europe, we chose to create a network among 10 European Centres in order to develop a novel communication tool for managing, editing and viewing collected information. Due to the great interest raised by this issue, this project was submitted and funded in the frame of both PHEA and ISS. Each Centre will insert and manage patients' data through a protected access area on the www.rbdd.eu web-site,

following the same data collection scheme. Queries and reports are deemed to be fundamental for the research and interrogation of the database. The final goal will be the creation of a unique on-line tool available to all Italian and European Centres, dealing with RBDs.

Statistical results derived by all the clinical, therapeutic and genetic information will be available to clinicians and patients, as well as National and Supranational organizations and regulatory agencies (FDA, EMEA). Moreover, data obtained on distribution and treatment of patients could stimulate the interest of pharmaceutical industries in developing new products.

A GENOME WIDE NON-SYNONYMOUS SNP SCAN OF AMYOTROPHIC LATERAL SCLEROSIS

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The objective of our project is to identify susceptibility genes for sporadic Amyotrophic Lateral Sclerosis (ALS). This was to be achieved through a genome wide scan in a set of Italian ALS patients and controls using a set of approximately 15,000 non-synonymous functional single-nucleotide polymorphisms (nsSNPs) on the Infinium BeadChip HNS-12 (Illumina). Recently, the results from three independent genome wide association (GWA) studies have been published and have led to a better understanding of the genetic architecture of ALS. The three studies of 276 cases and 271 neurologically normal controls, 1,152 cases and 1,292 controls and 1,337 cases and 1,356 controls have similar findings, in that no substantial genetic associations were found. This suggests that there are no common variants with a large genetic effect acting individually in sporadic ALS, but rather a complex interaction between environmental factors and many susceptibility genes of small effect.

The GWA approach has been validated by several studies, including a study of age-related macular degeneration, studies of type 2 diabetes and a large series of studies from the Wellcome Trust Case Control Consortium. In the WTCCC study of 14,000 cases and 3,000 shared controls, undertaken in the British population, 24 independent loci associated with seven common multifactorial diseases were identified and important methodological issues in the case-control design resolved.

As a consequence of these advances in the field, we have refocused our project. So far we have collected 1,385 cases and 1,400 neurologically normal controls and we are recruiting more cases, in the first large-scale Italian sporadic ALS screen. The GWAs currently published or being undertaken in ALS has generated a large number of signals, many of which will be statistical false positives and some of which will be genuine associations. We are collaborating in this study with Dr Ammar Al-Chalabi in UK who has completed the GWA analysis of a large sporadic ALS cohort comprising more than 1,000 cases and 1,000 controls, and who therefore has experience of many of the methodological issues involved. We will perform multiple candidate gene associations using regulatory rSNPs and coding nsSNPs combined with common tagSNPs and rare SNPs, all placed in the candidate genes so far found. For this purpose we will use an Illumina GoldenGate custom chip for genotyping.

This approach will allow us to simultaneously validate previous findings and establish risk genes of relevance to the Italian population.

P EFFECTIVE COMMUNICATION AND COUNSELLING: IMPROVING LISTENING IN RARE DISEASES

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An effective interchange of information among health centres, patients, their families and medical workers could help to focus on the un-met needs in rare disease management, and in return fulfil them.

In order to achieve this goal the Italian National Centre for Rare Diseases has promoted a multi-centric, nation-wide project:

- to identify the planning criteria for the communication process in rare diseases, and to improve the scientific and technical expertise;
- to implement more diffused and comprehensive communicative procedures based on integration and participation models;
- to favour a cooperative network exchange among people involved in rare diseases.

The project will be organized in four phases:

- 1. Organization and development of a qualitative study to identify needs and crucial areas in rare disease management using a Focus Group approach. The Focus Group participants will be health operators, members of advocacy groups, patients and their relatives. A questionnaire will be formulated on the results derived from the various Focus Groups. This questionnaire will be addressed to health operators to track their knowledge, behaviours and attitudes on rare disease management;
- 2. An effective formative training programme will be developed to fulfil the requirements derived from the analysis of collected data from the questionnaire;
- 3. The expertise acquired from the formative programmes will be monitored by completed *Evaluation Forms* given to the participant operators;
- 4. A final meeting will be organized to present and discuss the obtained results and to propose further implementations.

Currently Focus Groups have been organising according to the following functional patterns:

- Group 1: parents of underage patients with mental and motor deficits;
- Group 2: relatives of adult patients with severe mental disorders;
- Group 3: adult patients with motor deficits;
- Group 4: representatives and members of patients associations;
- Group 5: medical doctors dealing with rare diseases;
- Group 6: other health operators dealing with rare diseases.

Each Focus Group session is 90 minutes in duration and will be video recorded. About 10 participants will be recruited for each identified Focus Group after having completed an *Assent Form*.

A moderator and an assistant will oversee each Focus Group to guide the discussion and to gather the most relevant participant opinions on rare disease management. The recorded contents will be computerised and analysed to produce the necessary questionnaire to be sent to health operators, nation wide.

This project aims at identifying the growing needs of people involved in rare diseases. This concept might promote an active participation of patients, relatives, parents, and Associations to plan relevant public health programs. Therefore, it could improve quality of life of patients and their relatives through an effective self-empowerment.

P MOLECULAR CHARACTERISATION OF ITALIAN PATIENTS AFFECTED BY METACHROMATIC LEUKODYSTROPHY

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Metachromatic Leukodystrophy (MLD) is a demyelinating lysosomal storage disorder due to the defective degradation of the glycolipid Cerebroside Sulphate (CS). MLD is inherited as autosomal recessive trait and is caused by allelic mutations of the Arylsulfatase A (ARSA) locus except for few cases that are due to mutations of Saposin B (Sap-B), a small protein involved in CS solubilisation.

The series of 60 MLD patients included 55 patients with ARSA defect and 5 others with Sap-B deficiency. All of them, selected on the basis of the biochemical diagnosis, were molecularly characterised. ARSA activity was determined on leukocytes and/or cell lines (fibroblasts) using a colorimetric assay. Urinary sulfatide excretion was evaluated by a qualitative method based on thin layer chromatography. Molecular analysis of the ARSA gene was carried out by PCR and/or RT-PCR and sequence analysis. The identified alterations were confirmed by PCR-RFLP methods or by the sequencing of independent PCR products or clones. *In vitro* expression of ARSA mutations and variants was performed using the pSVL4BAT3A and pSVL4BAT expression vectors containing the complete ARSA cDNA with or without the first ARSA polyadenylation signal, respectively. The desired mutation or variant was inserted into the vector by site-specific mutagenesis and transfected into COS7 cells. The effect of putative splice site mutations was determined by the *in vitro* expression of the mutation in a properly constructed recombinant minigene cloned in an expression vector. The coding portion of the PSAP cDNA, encoding Sap-B, was amplified by RT-PCR, cloned and sequenced.

Overall we found a total of 52 mutant alleles including 24 new. Among these 8 alleles carried more than one alteration. The novel mutations were confirmed on at least two independent PCR products and on the parents' DNA when available. The newly identified alterations were tested in at least 100 normal alleles and, whenever possible, their deleterious effect was confirmed by *in vitro* expression studies.

The comprehensive examination including biochemical and molecular characterisation of the patients enabled us to confirm the diagnosis of MLD and to offer a reliable genetic counselling to the families at risk. The *in vitro* expression studies of new mutant alleles

allowed us to demonstrate their pathogenic role and, in case of complex mutant alleles carrying more than one alteration, to investigate for their hypothetically cumulative effect in modulating the phenotype of the patients.

P X-LINKED OR AUTOSOMAL RARE MENTAL RETARDATION SYNDROMES: PHENOTYPIC ANALYSIS IN TRANSGENIC MOUSE MODELS

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Some rare defects caused by mutations in genes encoding transcriptional regulators that orchestrate the brain differentiation and functioning are involved in Mental Retardation (MR). A gene mutation, namely the X-linked Methyl-CpG-binding protein 2 (MeCP2), is responsible for Rett Syndrome, a severe neurodevelopmental disorder, and the second genetic cause of mental retardation affecting 1 on 20,000 girls. Clinical characteristics appear between 6 and 18 months of age.

Recently however, subtle deficits have been shown to appear in younger infants. So far, mouse models have been behaviourally and neurochemically characterized mainly at the adult stage. We will focus the early phases of development (spontaneous motor and emotional behaviour) of the *Mecp2*-308 mutant mice. Such analysis can be especially informative in models of human neurodevelopmental disorders with neurological and cognitive symptoms already during infancy.

To evaluate possible neuronal effects of MeCP2 gene alterations, morphological analysis on growth and remodeling of dendrites and spines as well as evaluation of neurotrophin (NGF, BDNF) levels will be carried out. We will perform a preclinical evaluation of two different therapeutic strategies: i) perinatal supplementation with choline on onset and time-course of those behavioral endpoints sensitive to *Mecp2* mutation; ii) rearing in socially and physically enriched environment early in development. Both these strategies are reported to stimulate growth factors and synaptic plasticity. Results are expected to identify early markers of the disease and provide useful relevant indications for the development of non-pharmacological interventions within clinical settings.

Regarding a rare autosomal form of MR, we will analyze Kruppel-Like Factor 7 (KLF7) alterations (polymorphism and point mutations), a gene mapping on chromosome 2q31-33, a region associated with MR and autism. KLF7 is a strong candidate as a brain developmental gene associated with altered neuronal plasticity and intellectual disorders. A systematic search of KLF7 genomic alteration in a cohort of 110 Sardinian patients of both genders with various degree of mental retardation (with or without autism) will be performed. Such analysis will allow to test KLF7 involvement in hereditary neurodevelopmental disorders associated with cognitive disabilities, and will offer an important and valuable tool to identify new targets for innovative drugs and therapeutic strategies.

IS THE “CHROMOSOMAL PHENOTYPE” A CLUE FOR THE DIAGNOSIS OF *DE NOVO* CRYPTIC CHROMOSOMAL REARRANGEMENTS IN PEOPLE WITH MENTAL RETARDATION?

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Mental Retardation (MR) occurs in 2-3% of the general population. Chromosome abnormalities represent an important cause of MR, detectable in 4-28%, depending on techniques used. Recently, array-CGH studies demonstrated Cryptic Chromosomal Rearrangements (CCRs) in about 15-25% of people with MR, who resulted normal to routine cytogenetic analysis. Frequently, the candidates to array-CGH analysis are patients with “chromosomal phenotype”. The first two questions of the project “Usefulness of 244K array-CGH in the ascertainment of Cryptic Chromosomal Rearrangements in Mental Retardation and Autism (ASD)” are the percentage of CCRs among MR and ASD samples, and the influence of “chromosomal phenotype” in their ascertainment. We designed two groups, made up of 50 patients each, one (group 1) with a “chromosomal phenotype” and the other (group 2) without dysmorphic features, all affected by MR, diagnosed according to the DSM-IV-TR criteria.

The patients were included in the appropriate group according to the score reached following the administration of the clinical checklist published by De Vries in 2001.

Each subject reached a score between 0 and 10, depending on the presence or absence of some clinical features such as MR in family history, small birth weight, abnormal postnatal growth, facial dysmorphisms and congenital abnormalities. We established a total score of 3 as cut-off, separating group 1 (scoring 3 or above) from group 2 (scoring 2 or less). Until now, we analyzed a total of 63 subjects (31 males and 32 females), 43 from group 1 and 20 from group 2. A *de novo* CCR, validated by MLPA, has been diagnosed in 14 (8 from group 1 and 6 from group 2) out of 63 MR patients. The preliminary resulting percentages are: a *de novo* CCR is present in 22.2% (14/63) of MR patients, while in 18.6% (8/43) of group 1, and in 30% (6/20) of group 2.

These preliminary results confirm that CCRs have an overall prevalence of 22.2% in MR, and show that the “chromosomal phenotype” may not be the best clue for the diagnosis of CCRs in MR. However, it should be noted that the sampling in the first group is almost complete, while further 30 subjects should be enrolled in the second group, and this could modify the results. Furthermore, 8 patients with ASD, MR and “chromosomal phenotype” have been analyzed. Three of them showed a *de novo* CCR.

P OXIDATIVELY DAMAGED DNA REPAIR DEFECT IN COCKAYNE SYNDROME AND ITS COMPLEMENTATION BY HETEROLOGOUS REPAIR PROTEINS

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Repair of the oxidized purine 8-oxo-7, 8-dihydroguanine (8-oxoG) is inefficient in cells belonging to the B complementation group of Cockayne Syndrome (CS-B), a developmental and neurological disorder characterized by defective transcription coupled-repair. We have observed that cells belonging to the A complementation group (CS-A) are also defective in repair of 8-oxoG.

To conceive new therapeutic strategies for patient treatment, we are investigating whether the oxidatively damaged DNA repair defect in CS might be complemented by heterologous repair proteins, such as the *Escherichia coli* Formamidopyrimidine-DNA Glycosylase (FPG) and endonuclease III (NTH).

We have recently developed a vector that allows functional expression of the *Escherichia coli* FPG protein in human cells. This protein has a broad substrate range, being able to repair AP sites, 8-oxopurines and FaPy purines. We are investigating whether expression of FPG in CS cells may improve repair of oxidation damage and reverse partially or totally their mutant phenotypes. FPG may be a suitable candidate for relieving oxidatively damaged DNA repair defects in human cells. Similar studies will be carried out with the NTH protein from *E. coli*. This enzyme has substrate specificity different from FPG, being mainly devoted to repair of oxidized pyrimidines.

In summary, the complementation studies will shed light on the important lesions for the CS phenotype. Furthermore they will hopefully offer new tools for future therapies aimed at counteracting the neurological consequences of oxidatively damaged DNA accumulation in CS patients.

P GENETIC AND CLINICAL ASPECTS OF RARE LYMPHOMAS

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The application relates to orphan human lymphomas especially those belonging to the sphere of dermatological disease and those frequently arising in patients with type II mixed cryoglobulinemia associated with chronic HCV infection. The aim of this proposal are: 1) to use genome-wide approach enable to delineate the genes and pathways which might be responsible or might contribute to the onset, development and progression of these lymphomas creating a complete molecular dataset of information that would surely improve our pathogenetic knowledge of these rare diseases and patients' clinical management; 2) to assess the quality of life (QoL), and some psychological aspects of patients with these rare lymphomas.

Preliminary results: during the first period of this research we have analyzed a series of rare lymphomas arising in patient with HCV infection and we have identified two distinct genetic events in these tumours. Gain of human chromosome 3q is a hallmark of B-cell clonal expansion rather than malignancy, as it occurs in low-grade SMZL as well as in the non-malignant lymphoproliferative phase of type II mixed cryoglobulinemia. Conversely, deletion of 850 kb at 2q22.3 is a genomic rearrangement which is associated with more aggressive B-cell lymphomas. Interphase FISH probes for the 2q deleted region has been validated in this study and are available for clinical screening.

Further we have started a collection of cases regarding cutaneous B-cell lymphomas of the leg type for analysis of mutational screening in the CDKN2A gene by meaning of Denaturing High Performance Liquid Chromatography.

Finally we are analyzing data from QoL, psychological distress, and alexithymia (i.e., difficulty in recognizing and expressing emotions) in patients with cutaneous lymphoma. So far we have collected data on 95 patients, including 24 patients with CBCL, 59 with CTCL, and 12 with Sézary syndrome. The most frequent problems appearing from the utilized instruments, such as, QLQ-C30 analysis were fatigue, pain, and insomnia. The differences among CL types were particularly high in the global health status and emotional functioning scales.

P INVESTIGATION OF GENETIC AND EPIGENETIC MECHANISMS UNDERLYING BECKWITH-WIEDEMANN SYNDROME (BWS) ON A LARGE COHORT OF ITALIAN PATIENTS

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Beckwith-Wiedemann (BWS) (MIM130650) is an overgrowth disorder with an incidence of 1:15.000 determining organ hyperplasia and increased risk of embryonal tumours. According to clinical signs complete, incomplete BWS or Isolated Hemihypertrophy (IH) are diagnosed. The genetic basis involves genetic/epigenetic alterations within two 11p15 domains of imprinted growth regulatory genes. Genetic defects include 11p15 chromosomal rearrangements (2-3%), segmental chromosome 11 paternal UPD (20%) and mutations in the CDKN1C gene. Epigenetic alterations affect either IC1 (~2-7%) associated with H19 and IGF2 or IC2 associated with KCNQ10T1, which rules the expression of KCNQ and CDKN1C (~50%). The high incidence of sporadic cases suggests that many epigenetic defects arise in early development without sequence alteration. However ICs microdeletions resulting in disruption of imprinted methylation and altered expression were found in a few patients. >250 DNA samples from patients fulfilling the BWS spectrum (138 UO1, >120 UO2) constitute the resource of the project. Molecular data have been achieved on a notable fraction of cases allowing to address genotype-phenotype correlations.

This aim will be pursued with UO4, which is in charge of follow-up of the recruited patients; peculiar features of rare alterations such as microdeletions of H19-DMR and CDKN1C are a major focus. The prevalence of genetic mechanisms of tumour predisposition and the genomic profile of the associated tumours is being examined using molecular-cytogenetic tools. No alteration has been so far detected in approximately 50% familial and 30% sporadic BWS and most IH cases, suggesting: a) the existence of additional molecular abnormalities, b) not easily detectable tissue mosaicism or c) misdiagnosis. Continuing a recent cooperative work UO1 and UO2 plan to search for microdeletions/point mutations in the ICs and additional regulatory elements, which could be targets of genetic/epigenetic defects. The role of imprinted regions other than 11p is under study (UO2). Terminally mutated and wt CDKN1C alleles are investigated by molecular and bioinformatic analyses (UO1) to get insights on the structure of the protein and the role of its COOH-terminal.

The applicability of BWS prenatal diagnosis, currently undertaken by karyotyping and microsatellite analysis is addressed by UO3 which specialists in Obstetrics, ultrasonography, foetal medicine, clinical genetics, and pathology have created a protocol to evaluate the management of foetal abnormalities and to improve the quality of genetic counselling. So far one of 6 CVS/amniotic cells from fetuses presenting with small omphalocele was found to carry KvDMR1 hypomethylation.

THROMBIN GENERATION IN SEVERE HAEMOPHILIACS WITH DIFFERENT CLINICAL PHENOTYPE

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Some severe haemophiliacs (FVIII/FIX<1%) exhibit a mild bleeding tendency, but the basis for this heterogeneous clinical expression is poorly understood. This study investigated the relationship between the values of endogenous thrombin potential (ETP) and clinical phenotype in severe haemophiliacs. The impact of FVIII/FIX gene mutations and thrombophilic polymorphisms into the modulation of the phenotype was also evaluated.

Severe hemophiliacs older than 18 years without inhibitor history and treated on demand were eligible. Mild bleeders (MB) and severe bleeders (SB), representing the extremes of the clinical spectrum, were defined according to the following criteria: spontaneous bleeding episodes per year ≤ 2 (MB) or ≥ 25 (SB) and concentrate consumption <500 (MB) or >2000 (SB) IU/Kg/year. Patients who did not fit these criteria were considered as intermediate bleeders (IB). Plasma samples were obtained after a minimum wash-out period of 5 days. FVIII was measured by chromogenic assay. ETP was measured in platelet-rich plasma after addition of tissue factor.

22MB, 22SB and 28IB were enrolled. MB showed an older median age at first bleed compared to SB (3 yrs, range: 1-10 vs 1 yr, range: 0-4 in SB; $p<0.005$) and p for trend among the 3 groups was also significant ($p<0.05$). The prevalence of severe FVIII/FIX gene defects (null mutations) was extremely low in MB (6% vs 59% in SB; $p<0.005$). Median ETP values were higher in MB compared with both IB and SB (850, 478 and 414 nM, respectively; $p<0.05$); p for trend among the 3 groups was also significant ($p<0.05$). No differences were found with respect to the prevalence of PTG20120A and FV Leiden polymorphisms among the 3 groups.

This study shows that the measurement of thrombin generation in platelet-rich plasma may allow to identify patients with mild bleeding diathesis among severe hemophiliacs, in contrast with the features of conventional functional assays.

ROLE AND PREVALENCE OF GATA4, NKX2.5, FOG2 AND NOVEL CANDIDATE GENES' MUTATIONS IN SPECIFIC SUBSETS OF CONGENITAL HEART DEFECTS

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Congenital Heart Defects (CHDs) are individually rare, but altogether affect about 6-8/1000 newborns. Their aetiologies are largely unknown. In addition to environmental factors, a number of single gene mutations have been shown to cause CHD. In particular, researchers have highlighted the role of some cardiogenic transcription factors, such as *NKX2.5*, *GATA4* and *FOG2*. However, first screenings indicate that the role of each of these genes is marginal, and analysis of small or heterogeneous patients' cohorts is often negative. In this study, we have collected and analyzed a wide cohort of patients affected by Conotruncal (CT) CHDs, for mutation in known and candidate genes.

The patients' cohort included 143 individuals affected by tetralogy of Fallot (ToF), 30 with atresic pulmonary valve associated with ventricular septal defects, 20 with truncus arteriosus and 10 with other CT defects. In addition to *NKX2.5*, *GATA4*, and *FOG2*, other molecules expressed in the developing and adult heart, such as *ISL1* and *GJA5*, have been also screened. The *ISL1* gene, deleted in a unique patient with velocardiofacial syndrome, encodes for a LIM/homeodomain transcription factors.

The null mice show severe CT defects, suggesting a role for *ISL1* in CHD pathogenesis. The *GJA5* gene encodes for a gap junction protein and its expression is regulated by *NKX2.5*. Mice lacking one or two alleles of *GJA5* show CT malformations, such as ToF. Molecular screening has been performed by PCR, DHPLC analysis and direct sequencing. While no mutations have been identified in *GATA4*, *NKX2.5* mutations have been detected in 2% of ToF patients.

A novel *FOG2* mutation was detected in a ToF patients, giving a total prevalence of 2% of *FOG2* gene mutations in this CHD. *ISL1* gene screening identified a novel sense variant, while a *GJA5* missense mutation has been identified in 2 unrelated patients with ToF. Screening of more than 400 control chromosome was negative for both *ISL1* and *GJA5* gene mutations and segregation analysis for these changes is ongoing. These results confirm the minor role of *GATA4*, *NKX2.5* and *FOG2* genes in ToF. Moreover, our results indicate the possible role of *GJA5* mutations in the pathogenesis of CT defects. The negative

results in the other cohorts might be related to the small patients' samples. The observed prevalence of cardiogenic genes' mutations confirms the genetic heterogeneity and the multifactorial origin of CHDs, suggesting that only a few genes have a *major* pathogenetic effect on distinct phenotypes.

TESTING *IN VITRO* AND *IN VIVO* TREATMENTS FOR INCLUSION BODY MYOSITIS

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Sporadic inclusion body myositis sIBM is the most common progressive muscle disease in elderly people; aetiology and pathogenesis are unknown and there is no definitive treatment. sIBM shares biochemical features with Alzheimer's Disease (AD) and other central nervous system degenerative diseases, and is now thought to be a disease of muscle aging. Hereditary Inclusion-Body Myopathies (hIBMs) are hereditary muscle diseases with pathology resembling that of sIBM, but usually lacking inflammation.

There is increasing evidence that oxidative stress, is involved in neurodegenerative disorders as well as in IBMs. We evaluated Nitric Oxide (NO) basal content in sIBM and GNE-mutated hIBM myoblasts and found 20-30% increase in NO content in hIBM myoblasts compared to control cells, and 30-80% increase with greater variability in sIBM cells. NO production was not significantly changed in dermatomyositis or polymyositis myoblasts. We also evaluated the enzyme Heme Oxygenase-1 (HO-1), that is increased in the brain of AD patients and induced by a variety of disparate stimuli, including oxidative stress. Preliminary results indicate that HO-1-positive myoblasts were more numerous in sIBM than in control cells, but were variable in hIBM, polymyositis and dermatomyositis myoblasts.

Our *in vitro* preliminary results indicate that increased oxidative stress and, possibly, inadequate detoxification systems, are likely to play a role in IBM muscle fibers.

We proposed a pilot, open-label study, comparing simvastatin with Immunoglobulin intravenous injection (I.V.Ig) treatment in sIBM patients. The study has been approved by the Ethical Committees of Catholic University, Rome, and Besta Institute, Milan. Until now 13 patients have been recruited at the Catholic University, Rome, after informed consent, independently of disease duration and age, and randomized (11 with oral simvastatin therapy and 2 with I.V.Ig). Eight patients have reached the full dosage of 40 mg/d. No secondary adverse effects were observed, including no sign of rhabdomyolysis and no significant CK increase after simvastatin treatment. Two patients dropped out for reasons unrelated to the treatment. Six patients were contemporarily under corticosteroid treatment. None of the patients treated with simvastatin showed any worsening, in three patients an objective improvement of strength was observed and two others reported a subjective improvement. In the two patients with I.V.Ig treatment no clinical variations were observed. Our preliminary results indicate that simvastatin treatment in IBM is safe, well tolerated and has no significant collateral effects both as monotherapy or in association with corticosteroids.

ALTERATION OF STRIATAL SYNAPTIC ACTIVITY IN A MOUSE MODEL OF DYT1 DYSTONIA

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Early-onset generalized torsion dystonia is an autosomal dominant disease caused by a deletion in the DYT1 gene encoding the protein torsinA. Recently, a mouse model expressing a transgene encoding either the human Wild Type TorsinA (hWT) or Mutant TorsinA (hMT) proteins has been generated. These mice do not have overt dystonia, but exhibit a motor learning deficit.

Long-lasting changes in synaptic plasticity at corticostriatal synapses have been proposed to underlie motor learning. Since an impaired motor learning has been shown in asymptomatic carriers of DYT1 dystonia, we performed electrophysiological recordings from striatal Medium Spiny Neurons (MSNs) from hWT, hMT, and control mice. High-frequency stimulation induced striatal Long-Term Depression (LTD) in MSNs of hWT mice, but failed to cause LTD in hMT mice. Pre-treatment with dopamine agonists partially restored corticostriatal LTD. Additionally, to detect possible changes in transmitter release, we recorded spontaneous Excitatory (sEPSCs) and Inhibitory (sIPSCs) postsynaptic currents from MSNs. Glutamate-mediated activity was normal, since neither frequency, nor amplitude of sEPSCs was significantly modified in MSNs of hMT mice with respect to controls. In contrast, we found an altered GABA-mediated synaptic activity. Frequency of sIPSCs from hMT was increased with respect to their controls, whereas amplitude of sIPSCs was unchanged between controls and hMT.

To explore possible changes in transmitter release probability of MSNs, we recorded miniature GABAergic (mIPSCs) and Glutamatergic (mEPSCs) currents in the presence of TTX. Frequency and amplitude of mEPSCs were unaltered, whilst the mean frequency of mIPSCs from hMT was increased with respect to control mice. Amplitude of mIPSCs was unchanged. In order to study the presynaptic inhibitory control exerted by dopamine on the GABAergic transmission we tested the effect of D2 dopamine receptor agonist quinpirole. First, we tested the quinpirole effect on sIPSC-frequency of MSNs. In control animals, quinpirole reduces the frequency but not the amplitude of the recorded sIPSCs. This inhibitory effect is lost in hMT mice. No change was detected on the sIPSC amplitude. Then, we tested the quinpirole effect on the evoked Inhibitory Postsynaptic Current (eIPSC). In control and mutant mice quinpirole reduces the eIPSC but in hMT this reduction is smaller than the control.

In conclusion, loss of corticostriatal LTD is paralleled by an increase in GABA release, and both phenomena appear to be dependent on an altered dopaminergic transmission. Together, these results provide a possible explanation for the abnormalities in motor learning observed in DYT1 dystonia.

P ANALYSIS OF DYSTROGLYCAN IN HUMAN PATIENTS AFFECTED BY NEUROMUSCULAR DISORDERS

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Dystroglycan (DG) is a membrane receptor formed by two subunits, α -DG, a peripheral membrane protein, and β -DG, a transmembrane protein, held together by noncovalent interactions. In patients affected by muscular dystrophy, this interaction is often altered or completely disrupted leading to severe sarcolemmal instability. Our research focuses on the interaction between the two subunits and the possible role that their noncovalent association has for cells and tissues. Via a mutagenic approach we are characterizing the interface between α -DG and β -DG, carrying out an alanine scanning within two recombinant polypeptides, α -DG(485-630) and β -DG(654-750), containing the amino acidic sequences contributing to the α/β interaction. We evaluated the effect of a series of individual point mutations on the affinity of α -DG(485-630) towards β -DG(654-750) by solid-phase binding experiments using biotinylated β -DG(654-750) as soluble ligand and α -DG(485-630) coated onto wells of microtiter plates. The same point-mutations were introduced in full-length DG-constructs in order to analyse their post-translational fate in transfected human cells. The DG gene was associated to a reporter gene encoding for the Green Fluorescent Protein (GFP) enabling a direct analysis of the wild-type and mutated DGs in Ebna-cells by fluorescence microscopy. A previous data set highlighted the importance of a triad of Phe residues within the ectodomain of α -DG. Our new experiments indicate that two amino-acids, namely G563 and P565, belonging to α -DG(485-630), are involved in the α -DG/ β -DG interaction, as their substitution with alanine increases 3-fold the apparent dissociation constant, whereas the alanine substitution of M561 and Q559 decreases the affinity to a minor extent. The substitution of other α -DG(485-630) amino acids, such as L564, Y562 and S556, do not significantly alter the dissociation constant. None of the individual point mutations affect either the DG expression level in transfected human Ebna-cells and membrane targeting, as confirmed by fluorescence and Western blot analysis. Interestingly, the expression of DG gene carrying the four mutations G563A, P565A, F692A and F718A is highly perturbed resulting in the inhibition of the post-translational processing of the DG precursor confirming the importance of these aminoacids for the stability of the DG complex. In order to highlight possible correlations between neuromuscular disorders and alterations in the α/β interface we are also analysing biopsies of patients affected by rare neuromuscular disorders. We are also developing new polyclonal and monoclonal antibodies directed against the two DG subunits, that could represent innovative tools for the analysis of DG in pathological tissues.

GENOTYPE/PHENOTYPE CORRELATION IN CDLS: ITALIAN EXPERIENCE

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Cornelia de Lange Syndrome (CdLS) is a rare multisystem disorder characterised by facial dysmorphisms, upper limb abnormalities, growth and cognitive retardation. About half of patients carry mutations in *NIPBL* gene, mutations in *SMC1L1*, coding another component of cohesin complex, account for about 5% of patients with an X-linked heredity. The gene *SMC3*, also part of the cohesin complex, has been identified in one patient on 93 who did not show mutations of the previous two genes.

The main goals of the project are:

- to define the prevalence of different main genetic defects (*NIPBL* and *SMC1L1* mutations) in a large population of patients;
- to improve the knowledge on the genotype/phenotype correlations in CdLS for every type of genetic defect;
- to identify new genes related to the syndrome by functional/positional approaches.

To achieve these results all CdLS patients, confirmed clinically by experienced professionals, have been characterized at clinical, functional and behavioural level.

The first Italian CdLS cohort involving 52 patients (including 4 related members) was screened for *NIPBL* and *SMC1L1* mutations after a clinical evaluation using a quantitative score that integrates auxological, malformation and neurodevelopmental parameters. The patients were classified as having a “severe”, “moderate” or “mild” phenotype.

NIPBL screening showed 23 mutations so classified: truncating (11), splice-site (7), missense (3), in-frame deletion (1) and regulatory (1). Eighteen of them are novel. The truncating mutations were most frequently found in patients with a high clinical score, whereas most of the splice-site and all missense mutations clustered in the low-medium score groups.

The *NIPBL*-negative group included patients covering the entire clinical spectrum. The prevalence of a severe phenotype in the mutated group and a mild phenotype in the non-mutated group was statistically significant.

In terms of isolated clinical signs, the statistically significant differences between the mutation-positive and mutation-negative individuals were pre- and post-natal growth deficits, limb reduction, and delayed speech development.

We applied a refinement of *NIPBL* mutation analysis on negative patients by using *NIPBL* MLPA kit and FISH analysis without a positive result.

The proposed score seems to be a valuable mean of prioritising the patients to undergo an *NIPBL* mutation test.

SMC1L1 screening showed 2 mutations: an in frame deletion and a missense; the last one is present in the 4 members of the family group.

The project will be completed with the enlargement of the cohort, the refinement of genotype/phenotype correlation and the identification of new genes.

P IMMUNOBIOLOGIC AND CLINICAL ACTIVITY OF DNA HYPOMETHYLATING AGENTS IN HUMAN SARCOMAS

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Sarcomas are a heterogeneous group of mesenchymal tumours that are frequently locally aggressive and/or metastasize, accounting for approximately 1% of human malignancies. Current standard therapy includes surgical resection plus adjuvant radio- and/or chemotherapy. Adjuvant therapy reduces local disease recurrence, although no convincing effects on overall survival have been demonstrated yet; thus, newer treatments are urgently needed for human sarcomas. Utilizing human cutaneous melanoma as a “model disease” we have recently characterized the immunologic effects of DNA Hypomethylating Agents (DHA) on tumour cells. In particular, 5-aza-2'-deoxycytidine (5-AZA-CdR) was shown to induce/up-regulate the expression of Cancer Testis Antigens (CTA) in melanoma cells, and to functionally revert their intratumour clonal heterogeneity. 5-AZA-CdR also up-regulated a set of “immune molecules” (e.g., HLA class I and II, costimulatory molecules, components of the antigen processing machinery), which positively modulate the recognition of tumour cells by immune effectors. These data provide the scientific background to design a phase I/II trial with 5-AZA-CdR in metastatic melanoma patients that will identify the optimal time-/dose schedule of drug administration and define its immunotherapeutic potential alone or in prospective combination with CTA-based vaccines. Initial literature data show that CTA are expressed in different subtypes of human primary sarcomas, with a heterogeneous pattern of inter- and intratumour expression, and that treatment of sarcoma cells with DNA hypomethylating agents induces/upregulates their expression of distinct CTA. Our research proposal aims to evaluate the immunotherapeutic potential of DHA in human sarcomas both *in vitro* and *in vivo*, and to eventually design novel chemio-immunotherapeutic strategies capable to overcome their clinicopathological and biological heterogeneity. Initial dose-response *in vitro* experiments have unveiled an exquisite responsiveness of sarcoma cells of different histotype to the immunomodulating activities of 5-AZA-CdR: even very low doses (0.1-0.2 mM) of the drug generated high levels of CTA expression in treated cells. This sensitivity to the immunomodulating properties of 5-AZA-CdR seems not to be restricted to CTA; in fact, HLA class I antigens and ICAM-1 expression were also up-regulated by treatment. These data, though very preliminary, strongly suggest that DHA may represent useful therapeutic agents to comprehensively increase immunogenicity and immune recognition of sarcoma cells.

P GENETIC ANALYSIS OF ARRHYTHMOGENIC INHERITED DISEASES

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Inherited arrhythmogenic diseases, as Brugada Syndrome (BS), Long QT Syndrome (LQTS) and Short QT Syndrome (SQTS), are rare conditions associated with cardiomyocyte electrical instability and Sudden Cardiac Death (SCD). From the genetic point of view, BS, LQTS and SQTS are partially overlapping syndromes: 25% of the cases of BS are associated with defects in the cardiac sodium channel gene *SCN5A*. Loss of function mutations in potassium channels genes *KCNQ1* and *KCNH2* cover about 80% of LQTS patients, while a gain of function in *SCN5A* covers 5-10%. Other genes have been associated with LQTS, but account for a minority of patients. The SQTS associated genes so far identified are *KCNH2*, *KCNQ1* and *KCNJ2*. Moreover, the co-segregation of modifier variants may modulate clinical presentation and prognosis of these disorders. According to current guidelines, the main therapy for BS, LQTS and SQTS is prevention of SCD by cardioverter defibrillator implant, but often the choice is debatable. Notably, genetic data are not considered for risk stratification.

To date we have collected 50 BS patients, 39% displaying spontaneous type I ECG pattern and 26% with history of syncope, documented ventricular tachycardia or aborted cardiac arrest. Electrophysiologic study by intracardiac catheter stimulation induced sustained ventricular arrhythmias in 70% of patients. 90% patients underwent *SCN5A* testing, leading to the identification of genetic alterations in 18% of them. Five new mutations were discovered, whose effect on channel function is currently being studied *in vitro*.

This project will be expanded by screening a wide panel of ion channels genes in all patients with BS, LQTS and SQTS. This will have a first diagnostic impact, confirming the clinical diagnosis in affected patients and identifying asymptomatic at-risk relatives. Secondly, genetic data will be correlated with the results of clinical evaluation to determine genotype-phenotype correlations. The follow-up will individuate *a posteriori* higher risk patients and the parameters that may be predictive of major arrhythmic events. Moreover, the role of genetic variants in channels function will be tested by *in vitro* functional studies to evaluate the contribution of putative modifier alleles.

The close interaction between genetists and clinicians will allow the drawing of up-to-date guidelines.

P EVALUATION AND REHABILITATION OF SWALLOWING DYSFUNCTION IN PATIENTS WITH RARE NEUROLOGICAL DISORDERS AND MOVEMENT DISORDERS

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In recent years there has been increasing awareness of the feeding difficulties experienced by patients with neurological disorders children with neurodevelopmental disability and adults with movement disorders. The majority of the studies have been in children with cerebral palsy and in patients with Parkinsonism in particular Progressive Supranuclear Palsy (PSP). Many have been found to have major problems with eating and swallowing.

Only a few studies have suggested that nutritional problems are also frequent in patients with inherited neuromuscular disorders. Patients with Duchenne muscular dystrophies, spinal muscular atrophy, congenital muscular dystrophies and other congenital myopathies often have feeding difficulties, gastrointestinal dysfunction and excessive or reduced weight gain but this has not been systematically explored. Patients with PSP can develop a severe impairment of speech and swallowing. These problems are linked to neuronal damage within the brainstem and their connection to higher brain centres, especially the basal ganglia.

Choking episodes may be frequent in the above mentioned populations but videofluoroscopy or similar investigations are often not routinely performed and these patients are at risk of food aspiration and *ab ingestis* pneumonia.

The work will be carried on into 2 workpackages, one focusing on the assessment and rehabilitation of feeding difficulties in the different forms of neuromuscular disease and the other relating these aspects to patients with PSP and to the various phenotypes observed in the different forms of neuromuscular diseases and top other aspects of clinical management. The aims of this study are:

- to conduct a survey using a questionnaire on feeding difficulties, gastrointestinal involvement and weight gain in a large cohort of neuromuscular and PSP patients;
- to assess swallowing problems by use of clinical and instrumental tools;
- to correlate these findings with other variables such as age, level of motor impairment, use of ventilatory support and other aspects of clinical management such as scoliosis, heart involvement etc.;
- to evaluate quality of life (qol) and validate in Italian two English language specific questionnaires (swall qol and swall-care questionnaire);
- to suggest therapeutic options and management guidelines according to the results of the research.

ACTIVATING MUTATIONS IN *SOS1* CAUSE A DISTINCTIVE FORM OF NOONAN SYNDROME

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Noonan Syndrome (NS) is a genetically heterogeneous disorder characterized by short stature, facial dysmorphisms, congenital heart defects and skeletal anomalies. Increased RAS-Mitogen-Activated Protein Kinase (MAPK) signalling due to *PTPN11* and *KRAS* mutations cause 50% of NS.

Here, we report that 26 of 224 NS patients without *PTPN11* or *KRAS* change have a germline heterozygous mutation in *SOS1*, which encodes a RAS-specific Guanine nucleotide Exchange Factor (GEF). The majority of mutations were missense, and clustered at residues implicated in the maintenance of *SOS1* in its autoinhibited form. Expression of two NS-causing mutants induced enhanced RAS and ERK activation. The phenotype associated with *SOS1* defects was distinctive, although within NS spectrum, with a high prevalence of ectodermal abnormalities but generally normal cognitive development and growth.

No mutation was observed in 10, 4 and 2 subjects respectively with cardiofaciocutaneous syndrome, Leopard syndrome and neurofibromatosis/NS, without defects in known genes.

Our findings implicate for the first time gain-of-function mutations in a RAS GEF in inherited disease and define a new mechanism by which upregulation of the RAS pathway can profoundly change human development.

* *Questi autori hanno contribuito in egual misura al progetto*

TACKLING RARE DISEASES YET LACKING DIAGNOSIS AND/OR PROGNOSIS: A PILOT PROJECT INTEGRATING DATA COLLECTION AND EXPERIMENTAL STUDIES

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Our project aims to implement a system for appropriate collection, storage and evaluation of epidemiological/clinical data and biological specimens on cases of “undiagnosed” rare diseases, as a basis to target new interdisciplinary research. Our integrated study on Hepatoblastoma (HB) will represent a pilot experience on an important, inadequately known, rare condition. HB is the most important primary liver cancer in children accounting for up to 1% of all paediatric malignancies and affecting mainly infants and young children (6mo-3yr old). Predisposing genetic factors for HB have been identified and scientific evidence clearly points to HB as a multi-factorial condition, including overgrowth syndromes (i.e. Beckwith-Wiedemann Syndrome/BWS) and inheritance of a mutated APC gene in the dominant disorder Familial Adenomatous Polyposis (FAP).

Environmental factors contribute to HB pathogenesis, although their actual role has to be clarified yet. Intrauterine Growth Retardation (IUGR) is the most important non-genetic factor so far identified for HB. Exposure to phthalates (diethyl-2-exhyl-phthalate/DEHP) is also suggested as a potential risk factor: phthalates are widespread chemical additives of plastics, particularly present in PVC-based devices in maternal care units. Noticeably, DEHP and other phthalates are able to interact with nuclear receptors/NRs (PPAR- γ , PXR and sex steroid receptors), hence with a number of signalling pathways. Few models exist for HB: a HB-like neoplasm can be induced in aged mice by exposure to some chemicals.

The objectives of the project are: i) to elucidate signalling pathway(s) and molecular mechanism(s) of HB induction and progression with special attention to the role of Wnt/ β -catenin and IGF pathways; and ii) to characterize possible early markers for HB diagnosis and/or prognosis.

Histological and immuno-histochemical evaluation as well molecular analysis at DNA, RNA and protein levels will be performed on frozen and paraffin-embedded human tissues from HB patients, starting a new, *ad hoc* collection of HB specimens. Furthermore, to investigate the role of phthalate exposure, animal and cell line studies are in progress to

characterize the effects of DEHP, in comparison with Benzofuran (BF), a recognized HB inducer in aged mice.

Human cell lines (primary hepatocytes, hepatoblastoma HuH6, hepatocarcinoma HepG2 and Hep3B, hepatoma HLE) and tissues from mice treated with DEHP and BF are also used to elucidate the mechanism(s) of action involved in HB. In particular, we are currently performing: β -catenin and APC gene DNA mutational analysis (by sequencing), modulation of gene expression analysis (by qPCR and cDNA microarray) of genes of the Wnt/ β -catenin and IGF signalling pathways as well as of NRs and their cofactors, genomic post-transcriptional regulation analysis through microRNA and protein expression patterns. Finally, in order to assess *in vitro* cytotoxicity of DEHP and BF on HuH6 cell line, dose-response curves (0.1nM-500 μ M range) have been performed and the established EC10 used to perform the above mentioned experimental investigation.

So far, the expression of microRNAs and their putative targets in normal liver and HB cell lines revealed that two of these microRNAs show an altered expression level and an active role in the Wnt/ β -catenin and IGF signalling pathways. Further studies are in progress regarding the expression of other genes and proteins (by the Panorama Antibody Microarray, Sigma-Aldrich) involved in the pathology, the role of the expression of the two microRNAs in the different classes of HBs and if there is a correlation between clinico-pathological features and microRNA levels.

P FUNCTIONAL CHARACTERIZATION OF DIFFERENT CONNEXIN 26 MUTANTS IDENTIFIED IN KID SYNDROME PATIENTS: IMPAIRMENT OF MEMBRANE TRAFFICKING AND CONNEXON ASSEMBLY

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The KID (MIM 148210) syndrome is a congenital disorder that affects the epidermis and other ectodermal tissues such as the inner ear, and has been associated to mutations in the Cx 26 (GJB2) gene. Connexins (Cx), share a common pattern of structural motifs or domains, four trans-membrane (M1 to M4), two extra cellular (E1, E2), and three cytoplasmic (NT, CL, CT), and they are components of the gap junctions allowing the exchange of different molecules.

In this work, we analysed two heterozygous mutations (G11E, D50N) found in two unrelated KID patients. Both patients presented xerodermic eyelids, diffuse ichthyosis with large hyperkeratotic; erythematous and verrucous plaques, and Palmo-Plantar Keratoderma (PPK). In addition, both patients presented bilateral, profound sensorineural hearing loss. The G11E mutation is located in the cytoplasmic NT domain of Cx26, that presumably control the voltage gating and the calcium fluxes, while the D50N mutants is localized in the trans-membrane domain M1. Normal Human Epidermal Keratinocytes (NEHK), transfected with wild type Cx26 showed a classical punctate staining at the plasma membrane, indicating that the wt Cx26 localizes correctly. On the other hand, both Cx26 mutants are miss-localized, they did not form aggregates at the plasma membrane, while showed cytoplasmic localization with some perinuclear distribution. In addition, overexpression of G11E mutant is lethal in different cell lines. Preliminary experiments indicate a different behaviour of this mutant in the management of the calcium fluxes.

In conclusion both mutants cause, by the impairment of protein trafficking to the plasma membrane, failure in the assembly of connexons and gap junction. Moreover, the G11E mutation is lethal for cell by deregulation of calcium management.

P PHENOTYPE CORRECTION OF ADAMTS13 DEFICIENCY AND PROTECTION FROM THE DEVELOPMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA THROUGH INTRAVASCULAR AND SKELETAL MUSCLE ADAMTS13 GENE DELIVERY IN MICE

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Thrombotic Thrombocytopenic Purpura (TTP) is a life-threatening syndrome characterized by microangiopathic haemolytic anemia, thrombocytopenia, neurological abnormalities and variable renal dysfunctions. TTP is caused by congenital or acquired deficiency of ADAMTS13, a plasma metalloprotease. ADAMTS13 cleaves the endothelium-derived ultra-large multimers of von Willebrand Factor into smaller and less adhesive forms preventing platelet deposition and thrombus formation. Plasma exchange is the therapy of choice for these patients but needs the availability of adequate structures and may expose patients to the risk of infections and of serious complications.

Moreover in patients with TTP recurrences of the disease are common.

The aim of our study is to establish a gene therapy approach to achieve measurable levels of plasma ADAMTS13 in ADAMTS13 KO mice. In order to evaluate the more suitable gene delivery system we produced a recombinant adeno-associated (AAV) and an adenovirus vector (Ad) both encoding for human ADAMTS13 cDNA. AAV recombinant vector was obtained by co-transfection in HEK293 cells of a plasmid carrying ADAMTS13 cDNA with the packaging/helper plasmid (pDG), expressing AAV2 and adenovirus helper functions. The adenoviral construct was prepared using Cre/Lox sitespecific recombination system to achieve homologous recombination between a plasmid carrying the right hand portion of the adenoviral genome and a shuttle plasmid, containing the left end of the viral genome together with ADAMTS13 cDNA.

As first, we tested their ability in infecting and producing the recombinant protein by *in vitro* experiments. Both vectors efficiently infected human fibrosarcoma HT1080 cells and induced ADAMTS13 mRNA as evaluated by RT-PCR. Adenovirus also induced the expression and secretion of a functionally active ADAMTS13 protein as detected by western blot analysis and collagen-binding-assay. We are now analysing the expression and activity of ADAMTS13 produced by adeno-associated virus-infected cells.

We will then test Ad-ADAMTS13 and AAV-ADAMTS13 *in vivo*. We will inject both construct into the tail vein of ADAMTS13 KO mice and we will then evaluate the expression of recombinant ADAMTS13 into the liver at different time points together with levels and activity in the plasma of transfected mice. Since ADAMTS13 KO mice did not develop TTP, we are now attempting to obtain a TTP mouse model to evaluate whether gene transfer of ADAMTS13 is able to induce phenotype correction and protection from the development of TTP.

P TELOMERASE ACTIVITY IS LARGELY EXPRESSED AND SIGNIFICANTLY AFFECTS CLINICAL OUTCOME IN DIFFUSE MALIGNANT PERITONEAL MESOTHELIOMA

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In the context of a large project aimed at validating new prognostic indicators and therapeutic targets for diffuse malignant peritoneal mesothelioma (DMPM), we took advantage of a relatively large mono-institutional series of DMPM patients with long follow-up and investigated the prevalence of telomerase activity (TA) in this disease and whether it contributes to clinical progression. Telomerase is an RNA-dependent DNA polymerase that stabilizes telomeres and allows cells to avoid the senescence checkpoint, and may therefore contribute to tumourigenesis and neoplastic progression. In addition to its role in maintaining chromosome ends, telomerase activation has recently been implicated in providing growth-promoting properties to tumour cells.

It is known that TA is expressed in over 90% of malignant pleural mesothelioma, and the enzyme's activity has been proposed as a diagnostic marker to distinguish malignant from benign mesothelial lesions. Conversely, no information is available thus far concerning the presence of TA in DMPM.

In the present study we evaluated the expression of TA in 44 DMPM specimens obtained from 38 patients. TA was determined using the telomeric repeat amplification protocol and its expression was found in 68.2% (30 out of 44) of tested lesions. Although investigated in only 6 patients, the expression of TA appeared to be consistent during disease progression. The prognostic significance of TA was analysed in a subset of 29 patients, who underwent a uniform treatment regimen consisting of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. TA proved to be associated with clinical outcome. Specifically, 48 months after treatment, patients with TA-positive tumours had a significantly lower probability of being disease-free (12% vs 69%; hazard ratio, 3.32; 95% CL, 1.09-10.12; $P=0.03$) and exhibited a trend in favour of a lower probability of being alive than patients with TA-negative tumours (42% vs 85%; hazard ratio, 3.69; 95% CL, 0.79-17.13; $P=0.09$). Overall, our results indicate that TA is expressed in a large percentage of DMPM and significantly affect patient's prognosis.

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