



ISTITUTO SUPERIORE DI SANITÀ

**Il Congress on rare diseases.
Genetic disorders related to dysfunction
of cellular organelles**

Istituto Superiore di Sanità
Rome, November 20-22, 2000

ABSTRACT BOOK

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II Congress on rare diseases. Genetic disorders related to dysfunction of cellular organelles. Istituto Superiore di Sanità. Rome, November 20-22, 2000. Abstract book.

2000, viii, 45 p. ISTISAN Congressi 69

The aim of the Congress is to present public health problems related to the increasing developments of genetic research, with particular regard to rare diseases. In this context, the molecular and biochemical basis of lysosomal and peroxisomal diseases, the use of animal models for a better understanding of the physiopathology of these diseases and results of new therapeutical approaches are discussed. The final session deals with the National Project on Rare Diseases of the Istituto Superiore di Sanità.

Key words: Animal models, Genetic testing, Lysosomal diseases, Peroxisomal diseases, Public health, Rare diseases, Therapy

Istituto Superiore di Sanità

II Congresso sulle Malattie rare. Disordini genetici correlati a disfunzioni degli organelli cellulari. Istituto Superiore di Sanità. Roma, 20-22 novembre 2000. Riassunti.

2000, viii, 45 p. ISTISAN Congressi 69 (in inglese)

Obiettivo del Congresso è quello di approfondire i problemi di sanità pubblica correlati ai crescenti sviluppi nel settore della ricerca genetica, con particolare riguardo alle malattie rare. In tale ambito vengono discusse le basi molecolari e biochimiche delle malattie lisosomiali e perossisomiali, i risultati di approcci terapeutici innovativi ed il ruolo di modelli animali per una migliore comprensione della patogenesi di queste malattie. La sessione finale è dedicata al Progetto Nazionale sulle malattie rare dell'Istituto Superiore di Sanità.

Parole chiave: Malattie lisosomiali, Malattie perossisomiali, Malattie rare, Modelli animali, Sanità pubblica, Terapia, Test genetici

PROGRAMMA

Monday, November 20

9.00 Welcome
G. Benagiano
The Director of Istituto Superiore Sanita'

SESSION I
GENETICS AND PUBLIC HEALTH
Chairs: G. Berlinguer, D. Greco

9.15 Introduction
D. Taruscio

9.30 The Human Genome Project in retrospect and prospect
A. Piazza

10.15 Genetics and public health policy
A. Holtzman

11.00 Break

11.30 Equity, quality, participation: a European view
H. Scicluna

12.00 Equity and health care
B. Starfield

12.30 Ethics, quality, resources and health policies
G. Berlinguer

SESSION II
MOLECULAR AND BIOCHEMICAL BASIS OF LYSOSOMAL AND PEROXISOMAL
DISEASES
Chairs: K. Suzuki, S. Di Donato

14.30 Mechanism of brain dysfunction in lysosomal diseases
K. Suzuki

15.00 Peroxisomal diseases
P. Aubourg

15.30 Break

15.50 Krabbe disease: genetic analysis and prospects for therapy
D. Wenger

16.20 Niemann-Pick type C
M. Vanier

16.50 The pathogenesis of X-Adrenoleukodystrophy (ALD)
M. McGuinness

17.20 Saposins and lysosomal diseases
A.M. Vaccaro

Tuesday , November 21

SESSION III

NEW THERAPEUTICAL APPROACHES FOR LYSOSOMAL AND PEROXISOMAL DISEASES

Chairs: M. Vanier, S. Garattini

9.30 Recent advances in Fabry disease
R.J. Desnick

10.00 Enzyme therapy for Pompe disease: from science to industrial enterprise
A. Reuser

10.30 New approaches for the treatment of Gaucher's disease
A. Zimran

11.00 Break

11.20 Ten years of enzyme replacement therapy in neuropathic forms of Gaucher's disease
B. Bembi

11.50 Recent advances in pharmacological therapy for X-ALD
I.Singh

12.20 Gene therapy for X-ALD
N. Cartier

12.50 Bone marrow transplantation for peroxisomal and lysosomal diseases
W. Krivit

13.30 Break

ITALIAN EXPERIENCE ON LYSOSOMAL AND PEROXISOMAL DISEASES

Chairs: A.M. Vaccaro, S. Salvati

15.00 C. Dionisi-Vici, C. Rizzo, G. Parenti, S. Martino, R. Salvioli, E. Bertini,
M. Cappa, Di Biase, A. Salviati, G. Uziel

Wednesday, November 22

SESSION IV

ANIMAL MODELS

Chairs: S. D'Azzo, G. Andria

9.30 Genetic manipulations of mouse models of lysosomal diseases

K. Suzuki

10.00 Sialidosis and galactosialidosis: lessons from the animal models and implications for
therapy

S. D'Azzo

10.30 Mouse models for human leukodystrophies

K.A. Nave

11.00 Break

SESSION V

THE ITALIAN PROJECT ON RARE DISEASES

Chairs: G. D'Agnolo, D. Taruscio

11.30 The Italian Project on Rare Diseases of Istituto Superiore di Sanità

D. Taruscio

12.00 Approaches to public health monitoring and prevention

L. Botto

12.30 Discussion

13.00 Break

14.00 Round Table of the Project on Rare Diseases of Istituto Superiore di Sanità

B. Bembi, F. Bianchi, L. Botto, P. Casali, S. De Virgiliis, R. Gatti, P.

Mastroiacovo, R. Mingarelli, G. Sabetta, A. Schieppati, M.A. Stazi, D. Taruscio.

Representatives of the Associations of Patients and Families

CHAIRS AND INVITED SPEAKERS

- G. Andria - Dipartimento di Pediatria Università "Federico II", Napoli, Italy
P. Aubourg - INSERM U342 Hopital Saint Vincent de Paul, Paris, France
B. Bembi - Dipartimento di Genetica, "Istituto per l'Infanzia Burlo Garofolo", Trieste - Italy
G. Berlinguer - Presidente Comitato Nazionale per la Bioetica, Roma, Italy
E. Bertini - Ospedale Pediatrico "Bambino Gesù", Roma, Italy
F. Bianchi - Istituto di Fisiologia, CNR, Grezzano, Pisa, Italy
L. Botto - Center for Disease Control and Prevention, Atlanta - USA
M. Cappa - Ospedale Pediatrico "Bambino Gesù", Roma, Italy
N. Cartier - INSERM U342 Hopital Saint Vincent de Paul, Paris, France
P. Casali - Istituto Nazionale Tumori, Milano, Italy
G. D'Agnoletto - Laboratorio di Biologia Cellulare, Istituto Superiore di Sanità, Roma, Italy
E. Daina - Centro di Ricerche Cliniche per le Malattie Rare, Ranica, Bergamo, Italy
D'Azzo - St. Jude Children's Research Hospital, Department of Genetics, Memphis, USA
R. J. Desnick - Mount Sinai School of Medicine, Human Genetics Department, New York, USA
S. De Virgiliis - Istituto di Clinica e Biologia dell'Età Evolutiva, Università degli Studi, Cagliari, Italy
A. Di Base - Laboratorio Metabolismo e Biochimica Patologica, Istituto Superiore di Sanità, Roma, Italy
S. Di Donato - Istituto Nazionale Neurologico "Carlo Besta", Milano, Italy
C. Dionisi Vici - Ospedale Pediatrico "Bambino Gesù", Roma, Italy
S. Garattini - Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy
R. Gatti - Istituto "Giannina Gaslini", Genova, Italy
D. Greco - Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanità, Roma, Italy
N.A. Holtzman - Genetics and Public Policy Studies, The Johns Hopkins Medical Institutions, Baltimore, USA
W. Krivit - Department of Pediatrics, University Minnesota, USA
S. Martino - Dipartimento di Scienze Biomediche e Biotecnologie Molecolari, Università degli Studi, Perugia, Italy
P. Mastroiaco - Università Cattolica del Sacro Cuore, Roma, Italy
R. Mingarelli - Ospedale "Casa Sollievo della Sofferenza" San Giovanni Rotondo, Foggia, Italy
M. McGuinness - Department Neurogenetics, Kennedy Krieger Institute, Baltimore, USA
K.A. Nave - Department Neurogenetics-Max-Planck, Goettingen, Germany
G. Parenti - Dipartimento di Pediatria Università "Federico II", Napoli, Italy
A. Piazza - Dipartimento di Genetica, Università degli Studi, Torino, Italy
A. Reuser - Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands
C. Rizzo - Ospedale Pediatrico "Bambino Gesù", Roma, Italy
G. Sabetta - Ospedale Pediatrico "Bambino Gesù", Roma, Italy
S. Salvati - Laboratorio di Metabolismo e Biochimica Patologica, Istituto Superiore di Sanità, Roma, Italy
A. Salviati - Istituto Neurologico, Università degli Studi, Verona, Italy

R. Salvioli - Laboratorio di Metabolismo e Biochimica Patologica, Istituto Superiore di Sanità, Roma, Italy
H. Scicluna - Consiglio d'Europa, Strasburgo, Francia
I. Singh - Department Pediatrics, Medical University of South Carolina, Charleston, USA
B. Starfield - The Johns Hopkins Medical Institutions, Baltimore, USA
M.A. Stazi - Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanità, Roma, Italy
K. Suzuki - Neuroscience Center, University of North Carolina, Chapel Hill USA
D. Taruscio - Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, Roma, Italy
G. Uziel - Istituto Neurologico "Carlo Besta", Milano, Italy
A.M. Vaccaro - Laboratorio di Metabolismo e Biochimica Patologica, Istituto Superiore di Sanità, Roma, Italy
M. Vanier - Laboratoire Fondation Gillet-Merieux, Centre Hospitalier Lyon, France
D. Wenger - Thomas Jefferson University, Department of Neurology, Philadelphia, USA
A. Zimran - Gaucher Clinic, Department of Medicine, Shaare-Zedek Medical Centre, Jerusalem, Israel

Scientific and Organizing Committee

A.M. Vaccaro
Laboratory of Metabolism and Pathological Biochemistry
Istituto Superiore di Sanità, Roma
Phone: ++30 06 49902416
Fax: ++39 06 49387149
E-mail: avaccaro@iss.it

S. Salvati
Laboratory of Metabolism and Pathological Biochemistry
Istituto Superiore di Sanità, Roma
Phone: ++30 06 49902574
Fax: ++39 06 49387149
E-mail: salvatis@iss.it

D. Taruscio
Laboratory of Ultrastructure
Istituto Superiore di Sanità, Roma
Phone: ++39 06 49902805
Fax: ++39 06 49387140
E-mail: taruscio@iss.it

Congress Secretariat

N. MANCINO, A. MECCHIA, M.C. PETRASSI, V. RAIA N., L. TRANQUILLI
Laboratory of Metabolism and Pathological Biochemistry and Laboratory of Ultrastructure
Istituto Superiore di Sanità, Roma
Phone: ++39 06 49902598, 2805
Fax: ++39 06 49387149, 7140
E-mail: taruscio@iss.it
E-mail: mbpsegr@iss.it

INTRODUCTION

Domenica Taruscio

Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, Roma

The welfare policies are undergoing important changes, due to both the constraints of resource limitation and the need for new procedures to assess resource allocation. As a consequence, the identification of needs and priorities is of paramount importance to orient welfare actions, of which public health is a major component.

Now, a possible question would arise about the weight given to diseases who are themselves defined as “rare”. In fact, the principles of equity in public health and, in Italy, the National Public Health Plan (1998-2000) stress the importance of a broad-range intervention toward subjects and families affected by rare diseases.

Because of low prevalence, the individual diseases might be considered a relatively little social burden; however, the whole complex of the numerous rare diseases affect a considerable fraction of the population; moreover, rare diseases are generally severe, leading to early death or chronic impairment. Finally, for many such conditions the very fact that they are “rare” lead to a limited interest by the scientific community and the pharmaceutical industry.

Therefore, the Italian National Public Health Institute (ISS) is coordinating and developing the National Project on Rare Diseases. The “*II Congress on rare diseases. Genetic disorders related to dysfunction of cellular organelles*” (ISS, 20-22 November 2000) is taking place one year after the start of the Project. The topics of this international meeting are:

- genetics and public health
- molecular and biochemical basis of lysosomal and peroxisomal diseases
- animal models
- new therapeutical approaches for lysosomal and peroxisomal diseases
- the Italian project on rare diseases

The first session deals with the medical and social consequences of the recent progress of genetics, first of all the results of the Human Genome Project. A major increase is expected of genetic tests available for diagnosis, identification of disease gene carriers or identification of subjects with higher predisposition to certain diseases, etc. In Italy, the national Guidelines on Genetic Testing (available also on the ISS website: <http://www.iss.it>) illustrate the general principles and criteria for the use of genetic tests in public health; examples of major items include quality assurance, equity of access to services and ensuring an adequate information and participation of the public.

The following sessions of the Congress deal with lysosomal and peroxisomal diseases; emphasis will be given to the understanding of the pathogenesis at molecular and biochemical level, possible animal models and new therapeutical approaches.

The last session will illustrate the progress of the National Project on Rare Diseases. A Round Table will provide ample space for an open discussion involving scientists, public health operators and representatives of the Associations of patients and families.

As coordinator of the National Project, I am fully confident that this yearly appointment at the ISS will represent a fruitful occasion to exchange ideas and experiences on different groups of rare conditions. A general interdisciplinary approach will be followed, involving from molecular biology through clinics and epidemiology to ethics.

THE HUMAN GENOME PROJECT IN RETROSPECT AND PROSPECT

Alberto Piazza

Dipartimento di Genetica, Università degli Studi Torino

ABSTRACT NON PERVENUTO

GENETICS AND PUBLIC HEALTH POLICIES

Neil A. Holtzman

*Genetics and Public Policy Studies, The Johns Hopkins Medical Institutions,
Baltimore, USA*

What are the benefits and what are the dangers of having government (public health) agencies involved in genetics? Involvement includes research support, surveillance and data collection, provision of genetic services, regulation of new genetic technologies, and use of DNA analysis for forensic purposes. (Genetics in agriculture will not be discussed.)

Research. Several countries have spent large sums in supporting the mapping and sequencing of the human genome. This has paid off handsomely in increasing our understanding of the role of single genes in many rare diseases. Its yield regarding the common diseases is more problematic, raising the question of whether heavy investment should be made in the genetics of common diseases or whether other approaches to these disorders will have higher yield.

Surveillance and data collection. Governments can require reporting of various conditions on a population-wide basis in order to get complete information on the incidence and prevalence of specific disorders and clues to their etiology. Birth defects monitoring systems have not been extremely helpful in identifying environmental exposures that increase the risk of congenital malformations, but the identification of genotypes responsible for Mendelian forms of birth defects may lead to the identification of related genotypes for which infants in putative clusters can be screened. Surveillance also raises issues of privacy and misuse of information. Some surveillance, e.g., to estimate allele frequencies, can be done anonymously.

Provision of genetic services. In most developed countries all newborns are screened for genetic and some other disorders as part of universal, national health programs. As a result, inequities are avoided. Prenatal screening and testing programs are also provided under state auspices and raise the danger of eugenics. Informed consent and non-directive counseling are important safeguards.

Regulation of new technologies. Before new technologies are made available under either public or private auspices, their safety and effectiveness must be established. The quality of laboratories performing genetic tests must also be assured. Government regulation in the pre-market and post-market phases will be needed. Criteria for assessing safety and effectiveness must be established.

Use of DNA for forensic purposes. Performed in quality-controlled laboratories, DNA analysis can establish paternity and, in criminal cases, guilt or innocence. Collection of specimens on the entire population (e.g., all newborns) for eventual use for identification purposes raises possibilities of abuse.

EQUITY, QUALITY, PARTICIPATION: A EUROPEAN VIEW

Henry Scicluna

Consiglio d'Europa, Strasburgo, Francia

Socially marginalized people eventually develop bad health. On the other hand, people in bad health become socially marginalized because of their low health status.

The Council of Europe, whose vocation is the protection of human rights and the respect of the dignity of the individual has in its work on health placed its emphasis on equity in access to health services, good quality services and patient participation in decisions affecting the health of the population.

These three principles are implicit in several basic Council of Europe texts: the European Social Charter, the European Convention on Human Rights and the Convention on Human Rights and Biomedicine.

With the increasing number of vulnerable groups (older persons, chronically ill persons, single parent families) the dangers of discrimination are considerable.

Equity in access to good quality health services has to be ensured if discrimination is to be avoided. Similarly, all members of society should have their say in decision-making affecting their health.

These rules are valid in all aspects touching health. They are equally valid in issues relating to genetics. In this area the Council of Europe has condemned any form of discrimination against a person on grounds of his / her genetic heritage and requires that applicants for a job should not in principle be submitted to genetic testing.

INCOME EQUITY AND PRIMARY CARE--NEW DIRECTIONS FOR HEALTH POLICY?

Barbara Starfield

The Johns Hopkins Medical Institutions, Baltimore, USA

New paradigms for thinking about the determinants of health are rapidly emerging. Whereas individual social (as well as biological) factors have long been thought to influence health, it is only within the past decade that the characteristics of the social environment themselves have been added to models of disease causation and health promotion..

The two most prominent «new» determinants concern income inequity and the health services environment. The adverse effects of income inequality are now well documented by a variety of studies at various levels of aggregation: nations, states, and counties. Not so well known are the recent findings on the impact of health services organization itself. Whereas it is well known that health services themselves have a relatively minor effect on the health of populations, it is only recently that a distinction has been made between «health services» and the components of those health services. (1) When the distinction between primary care and specialty care is made studies show that health systems with stronger primary care infrastructures are more likely to achieve better levels of health. (1) Moreover, studies in the United States are showing that the availability of primary care services can, in part, reduce the adverse effects of income inequality on health. (2)

The conventional assumptions of disease causality are based largely on the notion of a single, or at least a prime, factor. These assumptions, which stem from over a century of the model of the germ theory, are rapidly being replaced by the notion of a web of causality in which multiple factors at both the ecological level as well as the individual level operate to increase or decrease vulnerabilities and resiliencies.

Future research, both epidemiological as well as health services, requires a much expanded view of disease origins and causes, whether the primary focus is on genetic, biological, or environmental characteristics. Furthermore, the ways in which characteristics are operationalized and measured need to be standardized, in order to facilitate comparisons of the results of different studies. This is particularly the case for new categories of variables, such as those reflecting health system and other contextual (ecological) characteristics.

The importance of the issues of income inequity and health services is heightened in this era of increasing polarization between the privileged and the non-privileged, including the effects of the privatization and increasing specialization of health services. A concerted and coordinated program of research is needed to help devise strategies for altering social policies.

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2. SHI L., STARFIELD B., KENNEDY B., KAWACHI I. Income inequality, primary care, and health indicators. *J Fam Practice* 1999; 48:275-84.
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ETHICS, QUALITY, RESOURCES AND HEALTH POLICIES

Giovanni Berlinguer

Presidente Comitato Nazionale per la Bioetica, Roma

1. The main ethical debate related to health is, as far as principles are concerned, around the question: does it exist a right to health? Since the foundation of the WHO, the answer has been generally positive, but two affirmations have gained ground in recent years: a) that only a right to health care should be recognized; b) that even the right to a minimum health cannot be based on recognized ethical principles. We should therefore discuss why and how health should be considered a right, and who should implement this right.

2. The main ethical debate, as far as reality is concerned, is around the question: why the inequities in health are increasing both between and inside the countries? This situation is connected with the quality of life and of health care, but implies also an analysis of the moral tendencies prevailing in the world and of the activities of the international organizations.

3. The main political debate, on the relations between globalization and health, is around the question: how globalization is affecting the health of the people? To this descriptive question another should be added: can *global health* become one of the main aims of the globalization?

4. The main debate on resources for health is around the question: how to allocate the scarce economic resources existing? This is important, in order to define priorities (non "rationing"), but we should add two other questions: a) why are they so scarce?, and: b) which are the resources for health?

5. Finally, the paper considers the international debate on these issues and its implications for health policy.

MECHANISM OF BRAIN DYSFUNCTION IN LYSOSOMAL DISORDERS

Kunihiko Suzuki

Neuroscience Center, Departments of Neurology and Psychiatry, University of North Carolina School of Medicine, Chapel Hill, USA

Progressive clinical and pathological impairment of the brain is one of the most prominent manifestations of many genetic lysosomal disorders. Pathologists classified these diseases as “storage disease” because many cells are distended with abnormal “storage materials”, which displace nuclei, mitochondria and other cellular organelles to the periphery. The pathogenetic mechanism of brain dysfunction in lysosomal disorders poses a major puzzle. The earlier notion based on the pathological appearance that neurons cannot function normally when they are filled with the storage materials was obviously simplistic and merely gave a false sense of having answered the question without really answering it. In recent decades, attempts have been made to understand the molecular mechanisms underlying the brain dysfunction. It is fair to say, however, that they all remain speculations/hypotheses rather than definitive explanations of how brain dysfunction results from the basic genetic defects in lysosomal hydrolases.

In the mid-seventies, Purpura observed that the prominent axonal spheroids often found in these disorders at the proximal portion of axons just beyond the axon hillock (“meganeurite”) developed aberrant synapses receiving inputs from other neurons. Such abnormal synapses would create electrical short circuits and thus would disrupt the normal propagation of electric impulses from the neuronal perikarya down through the axons. This concept has been followed up by Walkley, who suggested that accumulation of GM2-ganglioside that occurs not only in GM2-gangliosidosis but also to a much smaller extent in many other lysosomal disorders is responsible for the meganeurite formation. Although attractive, the hypothesis of Purpura/Walkley was reduced from observed phenomena and is yet to be tested by active experimental manipulation. Perhaps one of the oldest and best developed hypothesis is the “psychosine hypothesis” first proposed in 1972 in an attempt at explaining the unusual characteristics of globoid cell leukodystrophy (Krabbe disease). While the primary natural substrate of the deficient enzyme is galactosylceramide, which is uniquely concentrated in the myelin sheath, the enzyme also hydrolyzes a metabolic byproduct, galactosylsphingosine (psychosine). Psychosine is highly cytotoxic and, if not quickly hydrolyzed, causes rapid cell death. Inhibition of protein kinase C was suggested earlier as the possible mechanism of its cytotoxicity. Galactosylceramide and psychosine are synthesized nearly exclusively within the myelinating cells, oligodendrocytes in the CNS and the Schwann cells in the PNS. No evidence exists for enzymatic degradation of galactosylceramide to psychosine. In Krabbe disease, psychosine accumulates abnormally because of the enzymatic deficiency and exerts its cytotoxic effects on the myelinating cells. This hypothesis has survived the test of time for over 25 years. It is likely that an analogous mechanism operates in Gaucher disease, in which the corresponding “psychosine” is glucosylsphingosine. Different degree of glucosylsphingosine accumulation in the brain appears to correlate well with the degree of brain dysfunction in different types of Gaucher disease. Apoptotic cell death as a general mechanism underlying brain dysfunction is attracting attention. Our recent data indicate that psychosine is as

potent an apoptosis inducer as C6 ceramide. In the twitcher mutant, which is a mouse model of human Krabbe disease, oligodendrocytes undergo rapid apoptotic death. We recently observed increased apoptotic cell death in cultured fibroblasts of total sphingolipid activator (saposins) protein deficiency. Images of apoptotic neuronal death have also been described in other lysosomal disorders. In recent years our laboratory has been utilizing mouse models to understand the pathogenesis of some of the lysosomal diseases. Results obtained by cross-breeding of pairs of mutants to generate not only the double knockout mice but also mice with all possible combinations of genotypes indicate importance of genetic background in brain dysfunction in these disorders.

PEROXISOMAL DISEASES: UP DATE

Patrick Aubourg

Hôpital Saint-Vincent de Paul., Paris, France

A deficiency in one or more peroxisomal enzymes has been linked to at least 20 genetic disorders that affect mainly the central nervous system (CNS) and are often lethal. These disorders can be assigned to 3 groups:

1) In the first group, a dramatic loss of peroxisome functions result from impaired peroxisome biogenesis. Patients with peroxisome biogenesis disorders (PBDs) synthesize peroxisomal proteins normally but display defect in the import of peroxisomal enzymes into peroxisomes. Patients with PBD have basically the same kind of peroxisomal protein import defect but show marked phenotypic heterogeneity that includes Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), infantile Refsum's disease (IRD) and variants and rhizomelic chondrodysplasia punctata (RCDP). Cell-fusion complementation studies have established the existence of 11 distinct complementation groups (Cgs), the ZS, NALD and IRD phenotypes being represented in most CGs. Gene-identification strategies (homology probing from yeast and functional complementation) has allowed the determination of the molecular basis in all known PBD CGs other than CG8. Most patients with ZS, NALD and IRD belong to CGs 1 and 4 (Pex1 and Pex 6 genes). A founder effect is found in CG1 patients with the presence of a missense mutation (G843D) that is present in half of the patients and cause only partial reduction in Pex1 function. Similarly, a L292ter mutation is found in the Pex7 gene from patients with RCDP (CG11). Whereas most pex gene mutations lead directly to impaired peroxisomal enzyme import with enlarged peroxisomes that are decreased in number, pex 3 and pex 16 gene mutations (corresponding to CG12 and 9) lead to impaired insertion of peroxisomal membrane proteins and abnormal or no division of peroxisomes. Patients with pex3 and pex16 gene mutations have a severe ZS phenotype and 4-10 peroxisomes per cells. Molecular studies have also provided some explanation for patients with milder PBD phenotype such as IRD. Patients with IRD have missense mutation of pex genes that are "leaky" and thermo-sensible *in vitro*. The expression of the biochemical and clinical phenotype is consequently variable between tissues. Targeted disruption of the pex2 and pex5 genes in mice has provided a good model to study the abnormal neuronal migration disorder found in patients with ZS.

2) This group include patients with isolated peroxisomal enzyme deficiencies. Important progress has also been accomplished in the molecular characterization of these disorders during the past few years. Deficiency of D-bifunctional protein (D-BFP) is the most frequent defect of peroxisomal β -oxidation. Patients with D-BFP deficiency show a clinical phenotype that is strikingly similar to patients with NALD and accumulate VLCFA, pristanic acid and bile acid intermediates. Some patients with D-BFP does not accumulate bile acid intermediate and have mutation in the enoyl-CoA hydratase domain of D-BFP. Mice with acyl-CoA oxidase or L-bifunctional protein deficiency does not develop CNS abnormalities. Mice with sterol carrier protein (SCP-2) develop hepatic carcinoma, increased production of reactive oxygen species and CNS demyelination. A defect in peroxisomal SCP-2 has not yet been found in human. SCP is however involved in the metabolism of 2-methyl-branched fatty acids and bile acid intermediates, a step below the

D-BFP. One recent breakthrough was the discovery that distinct mutations in the mevalonate kinase gene cause two distinct phenotype: 1) mevalonic aciduria; 2) and periodic fever syndrome with hyper IgD (HIDS). Lastly, mutations in the gene encoding peroxisomal alpha-methylacyl-CoA racemase (that converts the (2R) pristanoyl, DHCA and THCA-CoA stereoisomers into respective (2S) stereoisomers) were shown to cause late-onset sensory motor neuropathy. Patients have only elevation of pristanic acid and C27-bile intermediates with mild elevation of phytanic acid and normal VLCFA in plasma.

3) The last group of peroxisomal disease includes only adrenoleukodystrophy (ALD). The ALD gene encodes an half-peroxisomal ABC transporter that forms homodimers and heterodimers with related partners (PMP70 and ALD related protein). The ALD protein is not necessary to the import of VLCF-CoA synthetase (VLCAS) whose activity is deficient in ALD fibroblasts. The ALD protein could import the VLCFA into peroxisomes (where they are then converted into CoA derivatives) or a factor necessary to the activity of the VLACS enzyme. The ALD mice do not develop cerebral demyelination but show mild involvement of peripheral nerves and spinal cord after 2 years of age that mimicks adrenomyeloneuropathy. Based on the observation that the ALDR gene could have redundant function(s) with the ALD gene, different therapeutic strategies aims at upregulating the ALDR gene expression in ALD patients. The 4-PBA (4-phenyl-butyrate) is effective in ALD mice (decrease of VLCFA in brain) but fails to show any biochemical effect in ALD patients. Although it has been demonstrated that lovastatin can reduce the concentration of plasma VLCFA in ALD patients, recent studies indicate that lovastatin does not reduce the concentrations of VLCFA in the brain from ALD mice. Recent studies have confirmed the long-term beneficial effect of bone marrow transplantation (BMT) in patients with cerebral ALD when the procedure is performed at an early stage of the disease.

KRABBE DISEASE: GENETIC ANALYSIS AND PROSPECTS FOR THERAPY

David A. Wenger

Department of Neurology, Jefferson Medical College, Philadelphia, USA

Krabbe disease or globoid cell leukodystrophy (GLD) is an autosomal recessively inherited disease resulting from mutations in the gene for galactocerebrosidase (GALC). GALC is responsible for the lysosomal catabolism of certain galactolipids including galactosylceramide and galactosylsphingosine (psychosine) that are maximally synthesized during active myelination. The accumulation of psychosine appears to be toxic to myelin-forming cells resulting in their apoptotic death. This leads to demyelination and to all of the symptoms recognized in human patients and the animal models. GALC is synthesized as an 80 kD glycosylated precursor that is proteolytically cut into 30 and 50-52 kD subunits that are extremely hydrophobic. The precursor has little, if any, activity. Molecular analysis has identified nearly 70 mutations in human patients and the animal models. While the active site is not known, it is clear that both subunits are required for activity. There are some more common mutations, including the 30 kb deletion, that make up just over 50% of the total mutant alleles. The presence of one copy of the G809A (G270D) mutation always results in late-onset GLD. Although there are a number of individuals with the identical GALC genotype, including G809A in one allele and the 30 kb del in the other allele, their clinical features differ significantly, indicating that other genes and environmental factors play a significant role in determining the phenotype of the late-onset patients. In humans, treatment has been limited to hematopoietic stem cell transplantation. Similar treatment in twitcher (twi) mice has prolonged their lives to more than 100 days but they still die with similar pathology to untreated mice. Attempts to decrease substrate accumulation by inhibiting synthesis of certain galactolipids has shown some promise but serious hurdles to the use of these methods probably will prevent their use in human patients. The human GALC cDNA has been placed in several viral vectors and these have been used for in vivo and as well as cell-mediated gene therapy trials in twi mice. Injection of transduced and untransduced murine neural stem cells into neonatal twi mice resulted in a widespread distribution of donor cells and some evidence for remyelination, but no consistent prolongation of the lives of treated mice. Overall the results have been less than successful, and this could be for several reasons. Studies in vitro have demonstrated correction of the twi oligodendrocyte phenotype by giving GALC activity via viral transduction or uptake from over-expressing cells. Using the animal models with GALC deficiency new approaches to therapy must be taken before this genetic disease will be effectively treated.

NIEMANN-PICK C DISEASE: A FUNCTIONAL APPROACH TO GENOTYPE /PHENOTYPES CORRELATIONS

M.T.Vanier (a), G.Millat (a), I.Ribeiro (b), C.Tomasetto (c), C.Marçais (a), A.H.Fensom (d), K.Harzer (e), D.A.Wenger (f), C.Sa Miranda (b), K.Ohno (g).

(a)*Lyon-Sud Medical School, and Fondation Gillet-Merieux, Lyon-Sud Hospital, Oullins, France*

(b)*Genetic Neurobiology, IBMC, Porto, Portugal*

(c) *IGBMC, Illkirch, France*

(d)*Medical and Molecular Genetics, Guy's Hospital, London, UK*

(e)*Neurochemical Laboratory, Institut für Hirnforschung, University of Tübingen, Tübingen, Germany*

(f)*Department of Neurology, Jefferson Medical College, Philadelphia, PA, USA,*

(g)*Neurobiology, School of Life Sciences, Tottori University Medical School, Yonago, Japan*

Lysosomal sequestration of endocytosed LDL-derived cholesterol and accompanying anomalies in intracellular sterol trafficking are the hallmark phenotypic features of the Niemann-Pick C (NP-C) lesion. Cell hybridization and linkage studies have established that mutations in either the *NPC1* (95% of the cases) or the *NPC2* gene can underly the disease and all available data comparing NP-C1 and NP-C2 phenotypes suggest that the two gene products function in tandem or sequentially in a common metabolic pathway. The NPC1 protein has been shown to reside in late endosomes, and to interact transiently with lysosomes and the trans-Golgi network. In NP-C1 cells, general retroendocytic trafficking and mobilization of multiple lysosomal cargo appeared defective at a late endosomal trafficking step. Certain glycolipids also accumulate in NP-C tissues, such as minor gangliosides GM3 and GM2, as well as glucosylceramide and lactosylceramide. Current opinion favours a key role of the NPC1 protein in modulating vesicular trafficking of both glycolipids and cholesterol.

Recent studies on mutations and NPC1 protein in NP-C1 patients have shed some light on various functional domains of the protein. Any truncation, even at the C-terminal end, appeared very deleterious, unvariably leading to the most severe phenotype. The putative sterol-sensing domain (SSD) of the NPC1 protein (similar to those in HMG-CoA reductase and SCAP) was also found to play an essential role. All missense mutations located in the sterol-sensing domain led to an absence of NPC1 protein (as detected in cultured fibroblasts by Western blot) and corresponded to patients with a severe infantile neurological onset and pronounced alterations of cellular cholesterol trafficking. A conserved NPC1-specific cystein-rich domain (luminal loop between TM-8 and TM-9) with resemblance to a RING finger motif was initially described as a mutational "hot-spot". Indeed, more than 1/3 of the NPC1 missense mutations were on this loop. They resulted in a wide variation of clinical phenotypes, ranging from the most severe infantile neurological form to adults with isolated splenomegaly, and in either severe ("classic") or mild ("variant") alterations of cellular cholesterol transport, without correlation with the clinical picture except for the most severe form. Characterization of mutant alleles leading to a "variant" biochemical phenotype disclosed that, in vast majority, they were located on this domain, and more

specifically, clustered within an inner part of the loop. Taken together, these observations suggest that while most of NPC1 protein govern transport of both cholesterol and glycolipids, some subdomains may have a more targeted function. We also observed that NPC2-deficient fibroblasts contain large amount of apparently normally processed NPC1 protein. However, whether NPC1 is functional in these cells is not known, and work is in progress to specify its subcellular location. One hypothesis that could explain the identical phenotypes in genetic complementation groups 1 and 2 and the rarity of the NPC2 mutational event would be that NPC2 is a small protein that triggers functionality of NPC1.

THE PATHOGENESIS OF X-LINKED ADRENOLEUKODYSTROPHY

M.C. McGuinness(a) and K.D. Smith(b)

Kennedy Krieger Institute and Department of

(a) Neurology and

(b) Pediatrics, Johns Hopkins, University School of Medicine, Baltimore

X-linked adrenoleukodystrophy (X-ALD; McKusick 300100) is associated with elevated levels of very long chain fatty acids (VLCFA) resulting from a deficiency in peroxisomal VLCFA β -oxidation. X-ALD maps to Xq28 and the gene encodes a peroxisomal membrane transporter protein of unknown function. The X-ALD phenotype is remarkably variable with no correlation among phenotype, genotype and VLCFA levels. The two major X-ALD phenotypes are the severe childhood cerebral form (CCER) and a milder form, adrenomyeloneuropathy, (AMN). CCER and AMN differ in (1) the age of disease onset, (2) the duration from age of onset to death and (3) the presence of a cerebral white matter inflammatory demyelinating reaction in CCER which is mild or absent in AMN.

The pathogenetic mechanism of the cerebral inflammatory demyelinating reaction in CCER and the role of the protein product of the X-ALD gene at Xq28 in destructive demyelination is, as yet, unknown. Whatever the initiating event in the cerebral inflammatory demyelinating reaction in CCER, it has been hypothesized that myelin breakdown resulting from excess VLCFA initiates a cytokine cascade which may lead to breakdown of the blood brain barrier followed by a cellular based immune reaction in the brain. Recent studies, in our laboratory of (1) the X-ALD mouse model and (2) the effects of various pharmacological agents in X-ALD *in vitro* and *in vivo*, lead to a reevaluation of the biochemical and metabolic bases for pathology and therapy in X-ALD.

SAPOSINS AND LYSOSOMAL DISEASES

Anna Maria Vaccaro, Rosa Salvioli, Fiorella Ciaffoni and Massimo Tatti

Department of Metabolism and Pathological Biochemistry, Istituto Superiore Sanita', Italy

Saposins (Sap) A, B, C and D are generated in lysosomes from a common precursor, called prosaposin, whose proteolytic cleavage begins in the late endosomes. It has been shown that saposins are required for the degradation of sphingolipids. Deficit of saposins causes lysosomal diseases characterized by the accumulation of the sphingolipids whose hydrolysis is promoted by a specific saposin. Mutations affecting Sap B cause a variant form of metachromatic leukodystrophy with storage of sulfatides, while mutations of Sap C cause glucosylceramide accumulation in variant forms of Gaucher disease. A patient lacking the four saposins in consequence of a mutation in the prosaposin initiation codon showed a combined sphingolipid storage disorder. So far an isolated deficiency of Sap A or D has not been reported, thus their actual physiological function has not been established.

In order to shed light on the molecular mechanisms underlying the role of saposins in sphingolipid degradation, the interactions of saposins with enzymes and lipids have been a matter of extensive research. It has been reported that saposins bind to lysosomal hydrolases and/or to sphingolipids. Conversely we have demonstrated that at least two of them, Sap C and Sap D, preferentially interact with anionic phospholipids and that the trigger for the interaction with membranes is a pH-dependent conformational change that exposes their hydrophobic regions. The binding of the two saposins to the lipid surface occurs only at pH values lower than 5.5 and is affected by the content of anionic phospholipids, such as phosphatidylserine (PS) or phosphatidylinositol (PI), suggesting that anionic phospholipids have the potential to modulate the insertion of Sap C and D in the hydrophobic environment of lysosomal membranes. Upon binding Sap C, but not Sap D, promotes the association of glucosylceramidase with the lipid surface, provided that the PS or PI concentration exceeds 5-10%. Thus, the role of Sap C is that of mediating the interaction of glucosylceramidase with membranes, allowing the contact between the enzyme and its substrate, glucosylceramide.

While Sap C can bind also to phosphatidylcholine vesicles, Sap D requires the presence of anionic phospholipids to tightly interact with membranes. Upon lipid binding, Sap D causes a clearance of vesicles turbidity due to transformation of large to smaller vesicles. The solubilizing effect is highly dependent on lipid composition as well as on membrane surface curvature and is inhibited by an increase of pH. The great affinity of Sap D for anionic phospholipid-containing membranes and its effect on the lipid organization strongly suggests that lysosomal membranes are the primary target not only of Sap C, but also of Sap D.

Our findings offer a new approach for understanding the physiological role of saposins in lysosomes and stress the importance of pH and anionic phospholipids in the regulation of their function/s.

ENZYME REPLACEMENT THERAPY FOR FABRY DISEASE

R. J. Desnick

Department of Human Genetics, Mount Sinai School of Medicine of New York University, New York, USA

Fabry disease is an X-linked inborn error of glycosphingolipid metabolism caused by the deficient activity of the lysosomal enzyme, α -galactosidase A (α -Gal A).¹ In classically affected males, the enzyme deficiency results in the pathogenic accumulation of globotriaosylceramide (GL-3) primarily in the vascular endothelium, leading to vascular disease of the heart, brain, and kidney. Treatment is currently palliative and affected patients usually die in their forties to fifties.

Early studies of enzyme replacement therapy indicated the feasibility of this therapeutic approach; however, the major obstacles were the inability to produce sufficient quantities of enzyme for clinical trials and the absence of an animal model test system for preclinical studies. These obstacles were overcome following the cloning of the human cDNA,² its overexpression and selective secretion in Chinese hamster ovary (CHO) cells,³ and the generation of an α -Gal A deficient mouse.⁴ With these advances, preclinical studies of four recombinant human α -Gal A glycoforms administered to α -Gal A deficient mice were performed. Intravenously injected enzyme glycoforms were rapidly cleared from the circulation ($T_{1/2} \sim 3-5$ min), the more highly sialylated glycoform circulating the longest. The tissue distributions of the enzyme glycoforms were similar and dose dependent. At all doses, all four glycoforms were distributed primarily to liver (~30% of dose) with low levels of each detected in heart, lung, spleen and kidney. The clearance of tissue and plasma GL-3 also were dose-dependent; with increasing dose, greater clearance was observed in heart, spleen and kidney. These studies provided "proof of concept" for clinical trials of enzyme replacement in patients with Fabry disease.

Based on the preclinical studies, a Phase 1-2 FDA-approved clinical trial was conducted at the Clinical Research Center of the Mount Sinai School of Medicine.⁵ Sponsored by the Genzyme Corporation, this was an open label, dose-escalation study involving 15 classically affected males who received five doses of recombinant human α -Gal A (produced by the Genzyme Corporation) in one of the following dose regimens: 0.3, 1.0 or 3.0 mg/kg every 14 days or 1.0 or 3.0 mg/kg every 48 hr. The enzyme was well-tolerated, and rapid and marked reductions in plasma and tissue GL-3 were observed biochemically, histologically, and ultrastructurally. Mean GL-3 content decreased 84% in liver ($n = 13$) and was markedly reduced in kidney in 4 of 5 patients who had pre- and post-treatment biopsies. GL-3 deposits also were reduced in the vascular endothelium of the kidney, heart, skin and liver by light and electron microscopic evaluation. In addition, trends toward improvements of pain, quality of life, and autonomic function were observed. These results provided dose-response data and explored possible primary efficacy endpoints for a Phase 3 pivotal trial.

Subsequently, a multinational, double-blind, randomized, placebo-controlled Phase 3 trial was conducted (sponsored by the Genzyme Corporation).⁶ This pivotal trial involved

58 patients and eight centers in four countries. Each patient received an infusion of α -Gal A or placebo every two weeks for 20 weeks. The predetermined primary efficacy endpoint was the clearance of stored GL-3 in the kidney vasculature. Twenty of 29 (69%) patients treated with α -Gal A achieved the primary endpoint, while the other nine treated patients all had improved renal vasculature. In contrast, no patient receiving the placebo achieved the primary endpoint. These results were highly significant ($p \leq 0.0001$). The GL-3 content in the vasculature of the heart and skin also were evaluated. The amount of GL-3 decreased significantly in both tissues in response to the α -Gal A infusions ($p \leq 0.001$). The enzyme infusions were well tolerated, and adverse event profiles of both groups were comparable with the exception of transient mild to moderate infusion-related reactions in the α -Gal A treated group which were managed conservatively. Twenty-four of 29 treated patients developed IgG antibodies to α -Gal A; however, seroconversion did not appear to influence the efficacy of treatment. All patients originally randomized to the placebo group received enzyme after completion of the phase 3 trial. After six months of treatment with α -Gal A, all of these patients had cleared GL-3 from their kidneys. In conclusion, α -Gal A cleared GL-3 from the vascular endothelium of the kidney, heart, and skin, the key sites of pathology in this disease. These results demonstrated the efficacy and safety of α -Gal A replacement in Fabry disease.

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ENZYME THERAPY FOR POMPE DISEASE: FROM SCIENCE TO INDUSTRIAL ENTERPRISE

Arnold Reuser (a), Ans Van der Ploeg (b), Hannerieke Van den Hout (b), Marian Kroos (a)
(a) *Department of Clinical Genetics, Erasmus University Rotterdam*
(b) *Department of Paediatrics, Sophia Children's Hospital, Rotterdam*

Enzyme replacement therapy for lysosomal storage disorders is based on concepts formulated by Christian DeDuve, Henry Hers and colleagues (1955-1963) about lysosomal function and involvement of lysosomes in disease processes. It has taken three decades to bring enzyme therapy for Gaucher disease in practice and another decade is about to pass before similar therapies for other lysosomal diseases will enter into the market. Clinical trials are currently ongoing for Fabry-, Pompe- and Niemann-Pick B disease, and for MPS1. New insights in cellular mechanisms for enzyme transport and persistence of few scientists have played an essential role in the revival of enzyme therapy after 1985. The advance of molecular biology and biotechnology has played a crucial role by enabling large-scale production of therapeutic proteins. Industrial companies, stimulated by the orphan drug legislation, have brought in the third essential element in the form of production, registration, and marketing know-how.

Pompe's disease or Glycogen storage disease type II is a muscular disorder caused by inherited deficiency of acid α -glucosidase resulting in lysosomal glycogen storage. The infantile form presents with a characteristic hypertrophic cardio-myopathy and generalised muscle weakness. Milestones like rolling over, sitting and standing are not achieved. Patients die within the first year of life. Late onset forms are more slowly progressive and present as a proximal myopathy with involvement of respiratory muscles.

Seven patients were enrolled in a single centre pilot study conducted in the Sophia Children's Hospital Rotterdam to test the efficacy of enzyme therapy. Four of these patients have the infantile and three the juvenile form of Pompe disease. The recombinant human α -glucosidase being tested in the trial is produced in milk of genetically modified rabbits. The infantile patients are currently treated for over a year. All four patients had a severe deficiency of α -glucosidase and a characteristic cardiomyopathy. Ages ranged from 2.5 to 8 months at start. The enzyme dose was 15 to 20 mg/kg/week and was later increased to 40mg/kg. α -Glucosidase activities normalized in skeletal muscle in all patients, and tissue morphology improved. By 36 weeks of treatment cardiac size had decreased significantly to less than 30% of baseline. Motor function improved and respiratory insufficiency could be prevented when treatment started at a young age. In conclusion recombinant α -glucosidase from transgenic rabbit milk has therapeutic effects in patients with Pompe disease.

The various aspects and complications of the development program will be discussed and the results of the trial will be highlighted.

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**NEW APPROACHES FOR THE TREATMENT OF GAUCHER DISEASE.
SUBSTRATE BALANCE WITH N-BUTYL-DEOXYNOJIRIMYCIN (OGT
918) : A NOVEL ORAL THERAPY FOR GAUCHER DISEASE.**

T.Cox (a), R.Lachmann (a), C.Hollak (b), J.Aerts (b), S.van Weely (b), M.Hrebicek (c), F.Platt (d), T.Butters (d), R.Dwek (d), C.Moyses (e), I.Gow (e), D.Elstein (f), A.Zimran (f).
(a)University of Cambridge, Department of Medicine, Addenbrooke's Hospital, Cambridge
(b) University of Amsterdam, Academic Medical Centre, Amsterdam
(c)Institute of Inherited Metabolic Disorders, Prague
(d)Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford
(e)Oxford GlycoSciences, Oxon
(f) Shaare Zedek Medical Center, Jerusalem

Introduction: Current therapy for Gaucher disease involves intravenous infusions of the enzyme glucocerebrosidase to degrade glucocerebroside stored in lysosomes. Attenuating the biosynthesis of glucocerebroside should reduce lysosomal storage and this approach has been beneficial in several animal models of glycosphingolipid storage disease. N-butyl-deoxynojirimycin (OGT 918) is an inhibitor of glucosyltransferase, the first committed step in the biosynthesis of glycolipids. Herein we report the results of the European multi-center clinical trial of OGT 918 in adult patients with type I Gaucher disease (Lancet 2000,355:1481-85) as well as the data from the extension period up to 18 months.

Methods Twenty-eight adult patients (7 splenectomized) with type I Gaucher disease who were unable or unwilling to receive enzyme therapy were recruited. Liver and spleen volume were measured by CT or MRI at baseline and at months 6 and 12; hematological and biochemical parameters were monitored at monthly intervals. OGT 918 was given orally at a dose of 100 mg three times daily, increasing to 200mg three times daily in three patients.

Findings. Baseline liver volumes ranged from 1.1 to 2.7 times normal and spleen volumes from 5.1 to 24.8 times normal. At the end of the 12 month protocol, mean liver and spleen volumes were significantly reduced from baseline (by 12% and 19% respectively, $p < 0.001$). Haematological parameters showed a small improvement. Chitotriosidase fell by 16.4% over the 12 month period ($p < 0.001$). Of the 28 patients enrolled, six withdrew: two early in the study because of gastrointestinal complaints, two for personal reasons, and two because of severe pre-existing disease. The most frequent adverse effect in this study was diarrhoea; most cases were mild and either resolved spontaneously or responded to anti-motility agents. Twenty-two patients completed 12 months of therapy and 18 of these entered into an optional extension phase. An update of trial progress will be provided.

Conclusion: These results demonstrate that substrate reduction using OGT 918 is showing proof of principal and is capable of affecting key clinical features of type I Gaucher disease. Because of the relatively slight effect on hematological parameters OGT 918 may be clinically useful for patients, who do not suffer from significant cytopenia. These include many patients with mild to moderate disease severity, those who have undergone splenectomy in the past, and most patients who have been on enzyme therapy and have achieved a plateau in these parameters.

TEN YEARS OF ENZYME REPLACEMENT THERAPY IN NEURONOPATHIC FORMS OF GAUCHER'S DISEASE

Bruno Bembi, Giovanni Ciana, Cristina Martini.

Istituto di Ricovero e Cura a Carattere Scientifico "Burlo Garofolo", Trieste, Italy

Gaucher's disease is one of the most common lysosomal storage disorder characterised by the deficiency of the enzyme acid β glucosidase. It recognises an autosomic recessive inheritance. The coding gene has been cloned and mapped to chromosome 1q21. More than 100 mutations have been identified but three are the more frequent: N370S, L444P and recombinant alleles. Three phenotypes have been described: GD1, non-neuronopathic form, GD2, acute neuronopathic form, and GD3, sub-acute neuronopathic form. The genotype/phenotype analysis has demonstrated the association of the N370S allele with the non-neuronopathic form of the disease, whereas the L444P mutation in homozygosis or in heterozygosis with mutations different from N370S correlates with neuronopathic forms.

Enzyme replacement therapy (ERT) has demonstrated to be effective in the treatment of GD: it corrects haematological parameters, reduces liver and spleen accumulation and improves the patients' quality of life. In contrast with the visceral response, still open remains the question of the ERT effectiveness on skeletal involvement.

The availability of ERT has also led to more systematic studies of the different phenotypic and genetic aspects of the neuronopathic forms, GD2 and GD3, as well as of its clinical effectiveness. Preliminary results have demonstrated the ineffectiveness of ERT in GD2, while in GD3 they have demonstrated to reverse, stabilise or slow the progression of neurological symptoms. An analysis of available clinical and laboratory data, ten years after introduction of ERT, will be done.

MECHANISM OF PHARMACOLOGICAL THERAPY FOR X-LINKED ADRENOLEUKODISTROPHY

Inderjit Singh

Department of Pediatrics, Medical University of South Carolina, Charleston,

X-Adrenoleukodistrophy (X-ALD), a common peroxisomal disorder is caused by a deficit in the degradation of very long chain (VLC) fatty acids (>C22:0). The metabolic defect subsequently leads to neuroinflammatory disease associated with mononuclear cell infiltration, induction of cytokines (e.g. TNF and IL-1) and iNOS resulting in demyelination of cerebral white matter. The molecular defect in X-ALD is in a 84 kDa peroxisomal membrane protein half-transport, adrenoleukodystrophy protein (ALDP). The precise function of ALDP is not known. Studies from our laboratory have shown that Lovastatin, an inhibitor of 3-HMG.CoA reductase, upregulates the β -oxidation of VLC fatty acids in cultured skin fibroblasts from both control and X-ALD patients. Consistent with tissue culture studies, 40mg daily dose of Lovastatin also partially corrected VLC fatty acid levels in plasma of X-ALD patients. The percentage decline from pretreatment values varied and did not correlate with the type of ALD gene mutation (point mutation vs deletion). Moreover, red cell membrane fatty acid composition showed approximately 50 percent correction after six months of therapy, indication prolonged and sustained benefit. A direct cause and effect relationship was noted by reversion of plasma C26:0 levels to pretreatment values in patients who withdrew from the study. There was no significant adverse effects in any of these patients.

To understand the molecular mechanism of correction of the metabolic defect, the regulation of expression peroxisomal half transporters (e.g. PMP70, PMPR70, ALDP, ALDRP) was examined. Lovastatin corrected the metabolic defect in VLC fatty acids accumulation in X-ALD by inducing the expression of ALDRP, product of ALD related gene, and thus complements the function of ALDP.

We have previously shown that lovastatin blocks the induction of proinflammatory process in cultured primary astrocytes, microglia and macrophages, the cells that play a role in the pathophysiology of X-ALD (*J.Clin.Invest.*100:2671-2679,1997) and in brains of animal with experimental allergic encephalitis (EAE), an animal model of Multiple Sclerosis. These results indicate the potential usefulness of lovastatin therapy for patients with X-ALD.

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GENE THERAPY FOR X-ALD

Nicole Cartier

Hopital Saint-Vincent de Paul, Paris, France

X-linked adrenoleukodystrophy (ALD) is a common (1/20 000) neurodegenerative disorder characterized by progressive demyelination of the central nervous system, adrenal insufficiency and accumulation of very long chain fatty acids (VLCFA) in tissues. The ALD gene encodes an ABC transporter involved in the transport of VLCFA in peroxisomes. Patients with cerebral ALD (70%) are entirely normal until they develop cerebral demyelination leading to death within 3-5 years. ALD is improved by bone marrow transplantation when the procedure is performed at an early stage of the disease. Toward a gene therapy approach for ALD, two strategies are currently developed:

- Ex vivo gene transfer into hematopoietic stem cells:

We have reported the correction of hematopoietic stem cells (HSC) from ALD patients using a Moloney-derived retroviral vector (Hum. Gene Therap. 9:1025-1036, 1998). However, murine retroviral vector transduction of HSC requires strong cytokine stimulation and is poorly efficient (25-30%), since classical retroviral vectors are only able to infect dividing cells and normal cells have no advantage upon ALD cells *in vivo*. In contrast, HIV-based lentiviral vectors can transduce more efficiently quiescent HSC, due to specific nuclear import properties. We have constructed an HIV-ALD vector (pTRIP-EF1-ALD) containing a central DNA flap that enhances DNA nuclear import (Cell 101:173-185, 2000) and thereby increases markedly transduction of HSC.

CD34+ cells mobilized from peripheral blood of ALD patients were transduced overnight in low-cytokine serum-free medium. 56-75% CD34+ cells expressed ALDP, depending on MOI. ALDP expression was demonstrated in colonies derived from progenitor cells, especially in myeloid-derived cells, the target cells for ALD gene therapy. Long-term culture confirmed transduction of stem cells. Our data demonstrate that our pTRIP-EF1-ALD vector allows very efficient transduction of CD34+ cells from ALD patients using a short clinically applicable protocol (low cytokine stimulation, short transduction).

-Direct targeting of ALD gene in the brain:

Although the lesions of ALD become widespread after years of evolution, the demyelinating process starts always in specific brain region suggesting that specific sub-populations of glial cells are vulnerable to the ALD gene mutation. The targeting of ALD gene in such specific brain regions would possibly prevent the further extension of cerebral demyelination. Using an AAV2-ALD vector, we have evaluated the efficacy of stereotaxic injection into the brain from newborn (injection into the subventricular zone) and adult mice (corpus callosum, thalamus, spinal cord and brain stem). Our results demonstrate that neurons were efficaciously transduced, whereas glial cells were not or poorly, confirming previous studies. Importantly, expression of the non secreted ALD protein was observed at large distance (cerebral cortex, thalamus, hippocampus, brain stem, cerebellum) from the various injection sites both in newborn and adult mice. The expression of the ALD protein remained unchanged up to 12 month. The efficacy of neurons transduction was at least similar to that reported recently with AAV5 vectors. Ongoing studies are aimed to determine whether the extensive transduction of neurons that we observed could result from diffusion of AAV vector within extracellular spaces or from retro-axonal transport.

BONE MARROW TRANSPLANTATION AS EFFECTIVE TREATMENT OF CENTRAL NERVOUS SYSTEM IN PATIENTS WITH LYSOSOMAL AND PEROXISOMAL ENZYME DEFICIENCIES

William Krivit, Charles Peters, Elsa Shaprio, Lawrence Lockman, Daniel Loes, John Day, Richard Ziegler, Kathryn Dusenbery, John Wagner, and Norma K.C. Ramsay.
University of Minnesota, Minneapolis, MN.

One hundred ninety (190) patients with lysosomal and peroxisomal diseases have received bone marrow transplantation in order to correct their disease at the University of Minnesota. This represents half of the total number treated world-wide.

Central nervous system function has been analyzed by serial surveillance of neurological, neuropsychological, neuroradiologic and neurophysiological characteristics for each patient for their respective diseases.

Globoid cell leukodystrophy (galactocerebrosidase deficiency) late-onset and early-onset patients have had major clinical positive responses following engraftment from bone marrow transplantation (NEJM 1998). These have included repair of hemiplegia, return of sight, major improvements in cognitive behavior, normalization of cerebrospinal fluid abnormalities and lessening of MRI findings.

Adrenoleukodystrophy (ALDP deficiency) patients with acute childhood type of severity have had documentation of their positive clinical responses (Lancet 2000).

Metachromatic leukodystrophy (ASA deficiency) patients with juvenile and adult forms of disease have had similar long-term salutary effects. (J. Inherit Metab Disease 1995)

Hurler disease (a-L-iduronidase deficiency) patients have had maintenance and improvement in intelligence and normal MRI subsequent to long term engraftment (Blood 1996, 1998).

Documentation of similar observations in patients with diverse diseases will be presented. Experience in treatment of experimental animals will be presented.

The thesis will be established that the cells provided by the transplant process can have access to the central nervous system. These cells provide correction of morbid pathology and physiology. There is now sufficient evidence for dismissal of the concept that "blood-brain-barrier" prevents such correction.

URINARY ORGANIC ACIDS IN PEROXISOMAL DISORDERS

C. Rizzo (a), P. Bertucci (a), G. Federici (a), R.J.A. Wanders (b), G. Sabetta (a), C. Dionisi Vici (a).

(a) *Departments of Metabolism & Clin Biochem Bambino Gesù Hospital, Rome, Italy*

(b) *University Hospital Amsterdam, The Netherlands*

Peroxisomal disorders (PD) are classified in two major categories: a) disorders with abnormal peroxisome assembly with multiple defective peroxisomal function (Zellweger syndrome, NALD, infantile Refsum's disease, RCP); b) disorders with a single peroxisomal protein deficiency (i.e. X-ALD).

We evaluated the urinary organic acids in three patients with different PD: Zellweger syndrome (ZS), NALD, and an isolated β -oxidation defect.

All patients showed increased excretion of a) epoxydicarboxylic acids (C10; C12; C13; C14); b) odd-chain C7–C15 dicarboxylic acids (mainly pimelic and azelaic); c) 2-hydroxy-sebacic acid; d) saturated and unsaturated C6-C10 dicarboxylic acids.

Epoxydicarboxylic aciduria, which could derive from the abnormal oxidation of ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid), was prominent in ZS compared to NALD and the isolated β -oxidation defect. Raised odd-chain dicarboxylic acids and 2-hydroxy-sebacic acid reflect increased production and oxidation of 2-hydroxy-VLCFA, which usually accumulate in some PD. Differing from mitochondrial beta-oxidation defects, the sebacic/adipic and the suberic/adipic ratios were found to be >1 in ZS and NALD. Increased 4-hydroxy-phenyllactic and 4-hydroxy-phenylacetic acids were detectable only in ZS patient as a sign of liver dysfunction.

We conclude that careful evaluation of urinary organic acids could be a useful tool for the differential diagnosis of PD and dicarboxylic acidurias of mitochondrial origin.

GAUCHER DISEASE. THE EXPERIENCE OF THE DEPARTMENT OF PEDIATRICS OF THE FEDERICO II UNIVERSITY, NAPLES

G. Parenti (a), L. Titomanlio (a), A. Perretti (b), M. Filocamo (c), P. Balbi (d), M.G. Marzano (a), M. Sibilio (a), G. Andria (a)

(a)Department of Pediatrics, Federico II University, Naples, Italy

(b)Department of Neurological Sciences, Federico II University, Naples, Italy

(c)Laboratory of pre- and postnatal diagnosis, G. Gaslini Institute, Genoa, Italy

(d)U. Maugieri Foundation IRCCS, Benevento, Italy

During the past 20 years 35 patients with Gaucher disease (GD) from 24 families, all originating from the Italian region Campania, have been diagnosed at the Department of Pediatrics, Federico II University, Naples. The diagnosis of GD was confirmed by enzymatic assay in 32 patients; molecular analysis was performed in 30.

Thirty-one patients presented with phenotypes compatible with GD type 1. Two showed early presentation associated with signs of neurological involvement (squint, laryngospasm) suggesting GD type 3a. Two patients with early and severe visceral involvement were classified as type 3b.

Molecular analysis showed a relatively high frequency of the L444P mutation in Campania (38.9% of independent alleles), as compared to the frequency of the mutation in the whole country; 3 patients were homozygous for the L444P mutation; in 12 patients from 9 families the L444P mutation was associated with other alleles. The frequency of the N370S mutation was 27.5%. Other rare mutations were found in 20% of the alleles, while 13.6% of the alleles could not be characterized.

Three patients with GD type 3 were L444P homozygotes (two patients with severe visceral involvement and one with neurological signs); in another patient with neurological involvement the L444P allele was associated with the G202R mutation, previously described in patients of Italian origin. Two of these patients died of choking. The other two patients, presently 7 and 9.5 years old have been on enzyme replacement therapy for 6 and 7 years, respectively, and do not show overt neurological involvement

The degree of lung involvement was studied by standard chest X-ray and high resolution CT scan and was found to be more severe in patients homoallelic for the L444P mutation.

Mild neurological involvement was found in three affected members of a sibship. These patients were homoallelic for a novel mutation (R353G) and had seizures or subclinical neurological involvement detectable by evoked potential (EP).

Multimodal evoked potentials (VEP, BAEP, SEP, MEP) were performed in 15 patients. In 13 patients we found abnormalities of at least one EP. Four patients showed abnormal patterns in 2 or 3 EP. The results of electrophysiological studies suggest that this approach is highly sensitive in detecting subclinical neurological involvement in GD, and that EP abnormalities are more frequent in GD patients than in the general population.

GENE THERAPY OF TAY-SACHS DISEASE

S.Martino (a,b), C.Bordignon (b) and A.Orlacchio (a)

(a)*Dipartimento di Scienze Biochimiche e Biotecnologia Molecolari, Università di Perugia*

(b)*San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET)*

Tay-Sachs (TS) disease is a genetic lysosomal storage disorder due to the deficiency of the β -N-acetylhexosaminidase A activity (Hex A, EC 3.2.1.52). Hex A is an α - and β -subunit heterodimer, responsible, in the presence of the GM2 activator protein, for the hydrolysis of GM2 ganglioside to GM3. Inherited defects in the α -subunit gene leads to the absence of Hex A with a massive accumulation of the GM2 ganglioside and related lipids mainly in the neuronal lysosomes, resulting in severe cellular dysfunction and rapid progressive neurodegeneration.

Up today, there is no available treatment for TS disease. Therefore a gene transfer approach could in the future provide a solution for this disease and give valuable information which can be applied to the treatment of other lysosomal diseases.

We evaluated the efficacy of two different gene transfer strategies to produce a functional Hex A in TS cells. The first strategy is based on the introduction of the missing gene into the deficient cells. With this direct correction strategy the enzyme is produced by the deficient cell itself. To this aim, we have produced a retroviral vector carrying the human α -subunit gene (L α HexTN) of Hex A and transduced the human fibroblasts from TS patients.

The second strategy consists in a cross-correction of the enzyme defect and it is based on the rationale that the secreted lysosomal enzyme can be recaptured by neighbouring cells through binding with the plasma membrane mannose-6-phosphate receptor. For this purpose we have overexpressed Hex A in murine NIH3T3 fibroblasts transduced with the retroviral vector L α HexTN as source of enzyme that will be delivered to the TS cells.

Our results demonstrate that although with both strategies we are able to deliver the missing enzyme to the Hex A deficient cells in adequate levels, the metabolism of GM2 ganglioside was restored only after direct transduction of cells from TS patients but not after cross-correction of TS cells. Preliminary results suggest that Hex A is correctly uptaken by the TS cells from the murine transduced fibroblasts but the intracellular delivery of Hex A to lysosomes is impaired.

These data demonstrate that the restoration of the metabolic defect in TS cells can be achieved by the direct gene transfer strategy.

DIAGNOSTIC ASPECTS OF GAUCHER DISEASE AND THE RELEVANCE OF THE ITALIAN GAUCHER REGISTRY.

R. Salvioli

Laboratorio di Metabolismo e Biochimica Patologica, Istituto Superiore di Sanità, Roma

Gaucher disease is an autosomal recessive disorder characterized by the deficient activity of the lysosomal enzyme, glucosylceramidase. As consequence, undegraded glucosylceramide accumulates in several tissues.

Three clinical phenotypes of Gaucher disease (type 1, 2 or 3) are distinguished according to the clinical symptoms (hepatosplenomegaly, skeletal complications, anemia, neurological involvement, etc.). The symptoms are not sufficient to diagnose Gaucher disease; the definitive diagnosis requires the demonstration of glucosylceramidase deficiency, which is apparent in all tissues and cells. The enzyme can be assayed with two different types of substrates: artificial (chromogenic or fluorogenic substances which contain in their molecule the bond on which the enzyme acts) and natural (the glucosylceramide). The use of each type of substrate has advantages, disadvantages and requires precautions. Artificial substrates, although poorly specific, allow to perform rapidly the enzymatic assays. Conversely, unequivocal analysis of atypical cases or research on the aspects of the molecular mechanisms responsible for the disease impose the use of the natural substrate. An other advantage of the natural substrate is the possibility of eliminating the interferences of other β -glucosidases.

Gaucher disease is a single gene disorder. More than 100 mutations have been identified in the glucosylceramidase gene, located on chromosome 1q21; few of them occur frequently, the others are rare or unique. Most of the known mutations in Gaucher disease are missense mutations that result in catalytically inefficient or unstable glucocerebrosidases (i.e. N370S, L444P). These two mutations represent about 65% of mutant alleles in the non-Jewish population. Molecular analysis is now an integral component of the evaluation of patients with Gaucher disease. Some prognostic correlation between genotype and phenotype provides a convincing rationale for DNA testing of high-risk populations.

Gaucher disease is now accessible to therapy by infusions of purified glucosylceramidase modified in the oligosaccharide chains for macrophage targeting. Only after an accurate diagnosis Gaucher patients can begin the enzyme replacement therapy (ERT), that has proven to be very effective in treating the disease. The infusions of the enzyme can differ in doses and schedules of administration according to the age of the patient and the severity of the disease.

In response to the rising interest in the management of patients an Italian Gaucher Registry has been established. A cooperative group of physicians, biochemists and epidemiologists has collaborated to collect and evaluate data.

The main aims of the Italian Registry are:

- 1) Evaluation of the number of patients and their distribution in the Italian regions.
- 2) Comparison of the effectiveness of the different therapeutic protocols.
- 3) Evaluation of possible side effects of therapeutic treatments.
- 4) Evaluation of follow-ups.
- 5) Evaluation of the cost/benefit ratio.

PROGRESS IN THE COMPREHENSION OF HEREDITARY SPASTIC PARAPLEGIA AND DIFFERENTIAL DIAGNOSIS WITH ADRENOMYELONEUROPATHY

Enrico Bertini, Filippo Santorelli, Marco Cappa
Laboratory of Molecular Medicine, Ospedale Bambino Gesù, Rome.

Hereditary spastic paraplegias (HSP) are a heterogeneous group of neurodegenerative disorders of the motor system characterized by slowly progressive weakness and spasticity of the lower extremities (Behan WM and Maia M, 1974). The disease is characterized pathologically by axonal degeneration in the long ascending and descending tracts of the spinal cord, especially in their terminal portions. HSPs are conventionally classified as "pure", when spastic paraplegia exists in isolation, and as "complicated" (Harding AE, 1981). The complicated HSPs are defined by the association of spastic paraplegia with peripheral neuropathy, ataxia, extrapyramidal disturbances, dementia, ichthyosis, optic atrophy, retinopathy, and deafness. These same features are often seen in patients with mitochondrial disorders associated with defective oxidative phosphorylation, and mitochondrial DNA alterations (DiMauro S et al, 1998) as well as in mild and late-onset variants of leukodystrophies such as metachromatic leukodystrophy, Krabbe's disease and adrenomyeloneuropathy. Adrenomyeloneuropathy is frequently associated with peripheral neuropathy mimicking the clinical picture of complicated HSP.

Little is known about the pathogenesis of most cases with HSP. Autosomal dominant, autosomal recessive, and recessive X-linked inheritance have been described for both pure and complicated forms of HSP. Genetic heterogeneity for autosomal dominant HSP (ADHSP) has been shown, for which at least seven loci have been mapped-on chromosomes 2p (SPG4; Hazan J et al, 1994), 2q (SPG11; Fontaine B et al, 2000), 8q (SPG8; Hedera P et al, 1999), 10q (SPG9; Seri et al, 1999), 12q (SPG10; Reid E et al, 1999), 14q (SPG3; Hazan J et al, 1993), 15q (SPG6; Fink JK et al, 1995), and 19q (SPG12; Reid E et al, 2000). In addition, three loci for autosomal recessive type (ARHSP), 8q (SPG5A Hentati et al. 1994), 16q (De Michele G et al, 1998) and 15q (Martinez-Murillo F et al, 1999). The much rarer X-linked forms have been associated with mutations in the L1CAM (Ruiz JC et al, 1995) and PLP (Sauger-Veber P et al, 1994) genes on Xq28 and Xq22, respectively.

Casari et al. (1998) reported mutations in a new gene (SPG7) in families linked to chromosome 16q24.3. The gene product, named paraplegin, is a nuclear encoded mitochondrial metalloprotease, with both proteolytic and chaperon-like activities at the inner mitochondrial membrane. Muscle biopsies from two affected patients harbouring paraplegin mutations showed mitochondrial abnormalities typical of myopathies associated with mtDNA mutations. It is still unknown how OXPHOS impairment affects specific axonal populations such as the corticospinal tracts and the dorsal columns. These are the longest axons in the human body and may be more sensitive to an impairment of the mitochondrial proteome.

Spastin, a protein of the AAA family, members of which are ATPases associated with diverse cellular activities, is encoded by SPG4 on chromosome 2p, the only ADHSP gene identified, thus far. Mutations in spastin have been linked to ADHSP in one report (Hazan J et al, 1999). Among 33 ADHSP kindreds, Fink et al (1996) found linkage to 2p in 15

(45%), to 14q in 2 (6%), to 15q in 1 (3%). Fifteen families (45%) were not linked to any of these three loci. The relative frequency of SPG4 alterations in Italian families is unknown. No data are available on the frequency of ARHSP. A recent paper suggested that a locus on 15q may be the commonest (Murillo et al 1999).

Metabolic screening by plasma very long chain fatty acids should be performed in all patients with HSP, particularly the in the complicated forms.

ADRENOLEUKODYSTROPHY: THERAPEUTIC APPROACHES IN FIFTEEN YEARS EXPERIENCE

M.Cappa (a), C.Bizzarri (a), E.Bertini (b), S.Salvati (c), A.Di Biase (c), C.Avellino (c), S.Cianfarani (d)

(a)UO di Auxologia

(b) Neurologia, Ospedale Bambino Gesù, IRCCS, Roma

(c) Istituto Superiore di Sanità, Roma

(d)Clinica Pediatrica Università Tor Vergata, Roma

X-linked adrenoleukodystrophy (X-ALD) is a genetic disorder characterized by progressive demyelination of central nervous system (CNS) and adrenal insufficiency. Very long chain fatty acid (VLCFA) are abnormally present in tissue and in body fluids of these patients and their plasma levels are considered marker for the diagnosis of this disorder. Many therapeutic approaches were attempted to arrest the progression of the disease. Herein we present our experience of treatment with different therapies in our cohort of patients. Bone marrow transplantation in very selective case seems to be the best method, but in our experience only one out of three treated patients survives. High dosage of i.v. immunoglobulin fail to cure six of our patients who died shortly after treatment. Lorenzo's oil and VLCFA-restricted diet seem to be the best tool to normalize serum VLCFA levels while very poor effect are observed on clinical evolution. Recently, to reduce VLCFA levels, an approach with HMGCoA reductase inhibitors has been attempted. Our observations on simvastatin treatment are in progress. We treated one patient with IGF-1, since this substance plays a role in the development of oligodendrocytes and myelin metabolism. After three months of treatment we observed an improvement of clinical course with a gain of 4kg in the body weight and an improvement of Visual Evocate Potential. We propone IGF-I therapy as one of the possible therapeutic approach.

TH-1 CYTOKINE PRODUCTION BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN X-LINKED ADRENOLEUKODYSTROPHY

Antonella Di Biase (a), Nicolò Merendino (b), Cristina Avellino (a), Marco Cappa (c), Serafina Salvati (a).

(a) *Istituto Superiore di Sanità, Rome*

(b) *Tuscia University, Viterbo*

(c) *Bambino Gesù Children Hospital, Palidoro (Rome)*

Cerebral adrenoleukodystrophy (ALD) and adrenomyeloneuropathy (AMN) are the two most frequent clinical phenotypes of the same genetic defect leading to the accumulation of very long chain fatty acids (VLCFA). Previous studies have suggested that inflammatory cytokines may play a role in the cerebral demyelination and in phenotype expression of the disease. To investigate if peculiar immunological responses are correlated to different clinical forms of the disease, we analyzed cytokine production by stimulated peripheral blood mononuclear cells (PBMC) from 17 patients (4 asymptomatic subjects, 8 AMN and 5 ALD). Our results show that lipopolysaccharides (LPS) stimulated PBMC from both symptomatic and asymptomatic patients have an increased production of IL-12 and TNF α compared to controls, while after phitoemoagglutinin (PHA) stimulation we observed a decreased production of IL-6 and IL-10. These data indicate that, following an immunological stimulus, PBMC from patients have an increased production of cytokines typical of a Th1 cell response which is able to promote the inflammatory process. This characteristic profile of cytokine production could be related to the biochemical defect and could have a role in central nervous system (CNS) pathogenesis.

Five patients were submitted to reevaluation of cytokine production after 4 months of simvastatin treatment. Recently statins treatment has been suggested for X-linked ALD in the light of the fact that these compounds are able to inhibit the induction of proinflammatory cytokines in astrocytes and microglia. Our preliminary results show that monocyte production of IL-12, such as that of TNF α , is decreased in 3 of 5 treated patients. In spite of the small size of analyzed samples, these observations highlight the possibility, by HMG-CoA reductase inhibitors, to modify the cytokine production at the periphery. It is interesting to point out that the two patients who did not show modifications of cytokine production, belong to the same kindred.

THERAPEUTICAL EXPERIENCE AND PERSPECTIVES IN RARE DISORDER. THE EXAMPLE OF ADRENOLEUKODYSTROPHY

Marianna Bugiani, Graziella Uziel

Child Neurology Department, Istituto Nazionale Neurologico C. Besta, Milan, Italy

Most genetic diseases of the central nervous system have been considered without treatment for a long time. Increasing knowledge in pathophysiology and unceasing advances in molecular biology have recently led up to new perspectives that will make a turning point and require a great effort from clinicians involved in the field of metabolic disorders.

Experimenting the treatment of X-linked adrenoleukodystrophy for more than ten years, we can now draw some concluding remarks, but also point out difficulties, pitfalls and warnings desumed from this experience.

Adrenoleukodystrophy is a genetic disease with many different clinical phenotypes. In the infantile onset form, central nervous system involvement is invariably present and the clinical course is often dramatically progressive. When we first approached the disease, the molecular defect was still to be determined and the diagnosis rested on the detection of increased levels of very long chain fatty acids (VLFA) in plasma and tissues. At that time, the rationale for treatment aimed to limit intake and reduce endogenous synthesis of potentially toxic VLFA by means of a low lipid content diet along with trierucic and trioleic acids supplementation.

Twelve years afterwards, in spite of accurate clinical follow-up, the experience of many different Centers had to be joined and complex statistical evaluation was needed to reach still partial conclusions about the non-efficacy of this treatment.

What happened for adrenoleukodystrophy reflects a common experience in approaching the treatment of rare genetic diseases. The reasons for such difficulties include:

- The rarity of these diseases does not allow the fast enrolment of enough patients, and follow-up has to be protracted for a long time.
- The gravity and relentless progression of these diseases are up against the design of controlled clinical trials, especially if double-blind.
- The extreme clinical heterogeneity, typical for adrenoleukodystrophy but also common to other genetic disorders, makes it difficult to quantify the exact worsening of these diseases. These considerations, specifically fitting for adrenoleukodystrophy but compassing other rare disorders, must be object for further discussion.

GENETIC MANIPULATION OF MOUSE MODELS OF LYSOSOMAL DISEASE: AN AMINO ACID SUBSTITUTION, C106F, IN THE SAPOSIN A DOMAIN OF THE SPHINGOLIPID ACTIVATOR PROTEIN GENE RESULTS IN A LATE-ONSET, CHRONIC FORM OF GLOBOID CELL LEUKODYSTROPHY.

Junko Matsuda (a), Marie T. Vanier (b), Yuko Saito (a), Jun Tohyama (a), Kinuko Suzuki (a), and Kunihiko Suzuki (a)

(a)Neuroscience Center, Department of Pathology and Laboratory Medicine, and Departments of Neurology and Psychiatry, University of North Carolina, Chapel Hill

(b)Lyon-Sud School of Medicine and Fondation Gillet-Mérieux, Lyon-Sud Hospital, France

Sphingolipid activator protein (SAP, prosaposin) gene generates four homologous proteins (saps, saposins) that activate in vivo degradation of sphingolipids with short carbohydrate chains. Each is relatively specific with respect to the sphingolipid it activates but some overlapping specificities are also indicated. Human patients with point mutations in the saposin B and C show phenotypes of metachromatic leukodystrophy and Gaucher disease, indicating that their primary in vivo substrates are sulfatide and glucosylceramide, respectively. In addition, two mutations are known in humans that result in complete inactivation of all four saposins and prosaposin. Total saposin deficiency is a devastating disease with involvement of multiple organs and multiple sphingolipids. We earlier generated a mouse model of total saposin deficiency with the gene targeting technology.

The model closely mimics the human disease. Experimental evidence exists to indicate that saposin A may be an activator for galactosylceramidase and saposin D for ceramidase. Since no human diseases or animal models due to specific defects of saposin A or D are known, we decided to introduce mutations in these domains in order to obtain definitive answers regarding the in vivo functions of saposin A and D. To date, we have successfully generated mice with an amino acid substitution in the saposin A domain.

The targeting vector was constructed using appropriate mis-matched primers to introduce a mutation in exon 4 that changed the 4th cysteine in saposin A to phenylalanine and simultaneously to introduce a new restriction enzyme recognition site for convenient genotyping. All saposins have six strictly conserved cysteines. In humans a mutation in the 4th cysteine to phenylalanine in saposin C causes specific saposin C deficiency and a mutation of the 5th cysteine to serine in saposin B causes specific saposin B deficiency.

The critical feature of the targeting vector was the Cre/loxP system. The neomycin resistance (neo) gene inserted within an intron was flanked by the loxP sequence. This design allowed initial selection of targeted ES cells with neomycin and subsequent removal of neo by transient transfection with a Cre expression plasmid. Targeted ES cells after removal of neo were injected into blastocysts and the standard procedures followed to generate homozygous mice with the mutation. Clinically, homozygous mice appeared completely normal until about 45 days, at which time independent observers could identify them by their slight sluggishness but only with careful comparison with normal littermates.

However, slowly progressive hind leg weakness became apparent by 2.5 months. As the weakness and atrophy of hind legs progressed, affected mice stopped gaining weight. Twitching, prominently seen in twitcher and other myelin mutants, was not obvious. Both

males and females were fertile, and mothers were able to raise their offspring normally at least twice. At 50 days, there were occasional typical globoid cells in the brain and spinal cord, and evidence of myelin degeneration was evident in the PNS. Similar to twitcher mice, there is a prominent accumulation of galactosylceramide in the kidney and of the seminolipid precursor (1-alkyl,2-acyl,galactosylglycerol) in the testis. In the brain, galactosylceramide and monogalactosyldiglyceride may be slightly increased, but this must be confirmed by more quantitative studies. Brain psychosine level at 60 days was approximately three times normal in contrast to the 15-fold increase in twitcher brain. These findings not only confirm the earlier in vitro evidence by O'Brien, Wenger, Harzer and others that saposin A may be a galactosylceramidase activator but also establish that it is in fact essential for normal catabolism of galactosylceramide. However, the metabolic block due to saposin A deficiency appears less than complete because it causes a disease much milder than the complete inactivation of galactosylceramidase in the twitcher mouse. These findings may anticipate genetic saposin A deficiency among human patients with undiagnosed late-onset chronic leukodystrophy with normal galactosylceramidase activity.

SIALIDOSIS AND GALACTOSIALIDOSIS: LESSONS FROM THE ANIMAL MODELS AND IMPLICATIONS FOR THERAPY.

Alessandra d'Azzo.

Department of Genetics, St. Jude Children's Research Hospital, Memphis

Galactosialidosis (GS), GM1-gangliosidosis (GM1) and sialidosis are three neurodegenerative disorders of metabolism affecting the lysosomal system. They are caused either by single or combined deficiency of three lysosomal enzymes, β -galactosidase (β -gal), neuraminidase and protective protein/cathepsin A (PPCA). We have generated mouse models for these diseases that are faithful phenocopies of the corresponding human conditions. GS and sialidosis mice develop extensive lysosomal storage in some cells of most of the systemic organs as well as the central nervous system (CNS). As observed in children with these diseases, the mouse models have several overlapping phenotypic abnormalities. GM1 mice display a progressive and generalized CNS and PNS pathology with little involvement of the systemic organs. These animal models represent the ideal experimental paradigm for the study of gene therapy approaches to these and related lysosomal deficiencies. We have tested the therapeutic potential of an 'autologous' BM transplantation in GS mice, using a high titer MSCV-retroviral vector expressing human PPCA. Myeloablated $-/-$ recipients were transplanted with genetically modified BM and tested between 1-10 months post transplantation for GFP expression in peripheral blood cells, and increased cathepsin A activity in tissues. PPCA-positive, BM-derived cells were detected in all tissues, with the highest expression in liver, spleen, BM, thymus, and salivary glands. In liver and kidney, a clear punctated staining was seen in non-hematopoietic cells, indicating efficient internalization of the corrective protein. Expression in the brain occurred throughout the parenchyma and was mainly localized on perivascular areas. Although the results of these studies are encouraging, clearance of neuronal storage was only partial. For this reason, we have also used AAV-based vectors containing the PPCA or β -gal cDNAs for stereotaxic injections into affected brain regions of deficient mice. In particular, AAV-PPCA was stereotaxically injected into the cerebellum of 10-day-old GS mice. Treated recipients were analyzed for expression of the corrective protein at different time points post injection. Immunocytochemistry revealed the presence of human PPCA in scattered Purkinje cells and cerebellar nuclear cells. In many instances expression was seen at significant distance from the injection site. We are currently assessing the extent of correction of lysosomal storage after rAAV-mediated gene transfer into the brain. The complete correction of systemic organ pathology and clear improvement of CNS disease support the use of gene therapy approaches for the cure of the GS phenotype.

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PLP GENETICS AND DEMYELINATION IN A TRANSGENIC MOUSE MODEL OF PELIZAEUS-MERZBACHER DISEASE

K.A. Nave, I.R. Griffiths, M. Klugmann, and H. Werner

Department of Neurogenetics- Max-Planck Institute- Goettingen

Mutations in genes encoding structural myelin membrane proteins have been associated with dys- and demyelinating diseases. In humans, structural alterations of the CNS-specific proteolipid protein (PLP) underlie Pelizaeus-Merzbacher disease (PMD). Using PMD mouse models, we have followed the disease mechanism from the genomic to the cellular and systems level. Spontaneous and engineered mutants of the PLP gene cover a wide spectrum of phenotypic expression which parallels the different clinical forms of PMD. Surprisingly, in the absence of PLP, compact myelin is elaborated, merely lacking the normal physical stability. This suggests that misfolded protein is toxic in the natural PLP mutants which are associated with glial cell death (Klugmann, M. et al., (1997). *Neuron* 18, 59). Whereas PLP-deficiency does not interfere with the acquisition of motor functions, it does affect long-term maintenance of myelinated axons. Axonal swellings which emerge throughout the CNS cause numerous axons to degenerate (Griffiths, I. et al. (1998). *Science* 280, 1610) and indicate that oligodendrocytes are required to maintain axonal integrity. Moreover, PLP may be directly involved in the communication between glial cell and axon. The latter is supported by a genetic interaction and the severe phenotype of mice lacking both PLP and MAG, a cell adhesion molecule facing the myelinated axon (Klugmann, M. et al., submitted).

THE ITALIAN PROJECT ON RARE DISEASES OF THE ISTITUTO SUPERIORE DI SANITÀ

Domenica Taruscio and the Task Force on Rare Diseases of the Istituto Superiore di Sanità
Istituto Superiore di Sanità

The Italian National Institute of Public Health (Istituto Superiore di Sanità - ISS) is coordinating and developing the National Project on rare diseases (RD). This project, besides a number of ISS scientists, involves several national (Ministry of Health, Universities, specific Centers, etc.), international Institutions (European Agency for the Evaluation of Medicinal Products, Centers for Disease Control and Prevention) and the Associations of Patients and Families. The general objectives include the potentiation and implementation of existing activities on RD as well as the promotion of public health interventions following an adequate evaluation of priorities. Such objectives fit within the Italian National Health Plan (1998-2000), which identifies as important target the intervention toward RD. This include:

- improvement of efficacy and efficiency of diagnosis;
- identification of reference centers for diagnosis and treatment of specific RD;
- promotion of scientific research.

Accordingly, the main tasks of the National Project on RD are:

- to build up the National Register of RD (<http://www.iss.it/sanita/index.htm>)
- to optimize and diffuse protocols for diagnosis and treatment;
- to build up the national inventory of orphan drugs;
- to promote the professional education of health operators on RD;
- to organize the web site of the ISS on RD.

An update of the work in progress will be presented.

Moreover, the ISS is coordinating the European project "Network of Public Health Institutions on Rare Diseases" (NEPHIRD), which will be funded within the frame of the European Commission Programme on RD. NEPHIRD involves the participation of institutions and centres from fourteen Countries (either EU or associated countries) as well as the international Association on birth defects "EUROCAT" and the Associations of Patients and Families.

The main objective of NEPHIRD is to build a model for epidemiological data collection on selected RD, ending up with recommendations and guidelines for data base standards, sources, information content and presentation, which will be diffused at EU level.

APPROACHES TO PUBLIC HEALTH MONITORING AND PREVENTION

Lorenzo Botto

Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA

Applying the framework of public health monitoring and prevention to rare diseases is both challenging and powerful. It is powerful because such public health approach can provide important data for patients, families, and health care providers, and also, and importantly, for policy and decision makers. Such data include not only the population-based prevalence of such conditions, and its ethnic and geographic distribution, but also the mortality, morbidity, and disability related to the conditions. In addition, gaps in prevention practices, where such practices are possible, can also be documented and improved.

For example, in the case of spina bifida, we might think that we know all we need to know for prevention and intervention. However, even for such a well-known condition, it is difficult to find accurate and timely population-based data on its prevalence (including terminated pregnancies), and its associated mortality, disability, and costs. In addition, we often lack data on the use, knowledge and attitudes relative to multivitamin and folic acid supplements, both among women and health care providers.

In addition to generating data on rare diseases, public health monitoring can provide a vital contribution to intervention and prevention. Such contribution is based on three aspects of public health monitoring, which are particularly relevant to rare diseases. First, public health monitoring highlights not only the collection and analysis of health data but on its dissemination, i.e., on its application. Second, public health monitoring is linked to public health practices such as intervention and prevention programs; third, and most importantly, public health monitoring is, by definition, ongoing, systematic, and timely and thus can provide a ongoing evaluation of the impact of rare diseases and the effectiveness of intervention and prevention efforts.

Where to start in public health monitoring and prevention of rare diseases? A powerful approach is to start by developing and fostering local model systems in well-defined areas where integrated, multisource, and population-based monitoring programs can be promoted and piloted. These model systems should make use to the extent possible of existing information systems, such as disease registries, support organizations, administrative data sources. In the process, it is particularly important to involve from the beginning the main constituents, including the public, the medical professionals, and the public health community. The steps in the development of such public health monitoring systems are many, but should include, at the beginning, the assessment of data gaps, and, throughout the process and at the conclusion, a careful evaluation of the system. Such experience will then help guide the development of more extensive and perhaps nation-wide programs.

Rare diseases are not new, but have only recently reached public health visibility in many countries. Applying the lessons of public health monitoring to rare diseases in a systematic and flexible way will contribute substantially to preventing disease and improving the health of those affected.

TEN YEARS OF ENZYME REPLACEMENT THERAPY IN NEURONOPATHIC FORMS OF GAUCHER'S DISEASE

Bruno Bembi, Giovanni Ciana, Cristina Martini

Istituto di Ricovero e Cura a Carattere Scientifico "Burlo Garofolo", Trieste

Gaucher's disease is one of the most common lysosomal storage disorder characterised by the deficiency of the enzyme acid β glucosidase. It recognises an autosomic recessive inheritance. The coding gene has been cloned and mapped to chromosome 1q21. More than 100 mutations have been identified but three are the more frequent: N370S, L444P and recombinant alleles. Three phenotypes have been described: GD1, non-neuronopathic form, GD2, acute neuronopathic form, and GD3, sub-acute neuronopathic form. The genotype/phenotype analysis has demonstrated the association of the N370S allele with the non-neuronopathic form of the disease, whereas the L444P mutation in homozygosis or in heterozygosis with mutations different from N370S correlates with neuronopathic forms.

Enzyme replacement therapy (ERT) has demonstrated to be effective in the treatment of GD: it corrects haematological parameters, reduces liver and spleen accumulation and improves the patients' quality of life. In contrast with the visceral response, still open remains the question of the ERT effectiveness on skeletal involvement.

The availability of ERT has also led to more systematic studies of the different phenotypic and genetic aspects of the neuronopathic forms, GD2 and GD3, as well as of its clinical effectiveness. Preliminary results have demonstrated the ineffectiveness of ERT in GD2, while in GD3 they have demonstrated to reverse, stabilise or slow the progression of neurological symptoms. An analysis of available clinical and laboratory data, ten years after introduction of ERT, will be done.

ORPHAN DRUGS REGISTRY

Arrigo Schieppati , Erica Daina

Clinical Research Center for Rare Diseases Aldo e Cele Daccò, Mario Negri Institute, Bergamo

Millions of people in Europe suffer from one form of rare disease. Little is known about most rare diseases and this lack of knowledge is a barrier to their diagnosis, treatment and prevention. Once diagnosed, patients with rare diseases have difficulty obtaining information about treatments, research advances and location of research centers investigating on their condition. Pharmaceutical industry does not consider profitable to invest money in research and product development when the potential market is limited. The process of developing a product to treat persons with rare diseases - actually to treat any disease - is complex, lengthy, and expensive. Therefore the revenues from its sales should compensate all these efforts. When the potential market for a drug is small, as in the case of rare diseases, the development of a new product is not considered cost-effective, unless economic incentives are not warranted. The Orphan Drug Act in the U.S.A. has proved a valid tool to promote development of orphan products.

On 27 April 2000, the European Commission adopted a regulation laying down implementing rules and setting out definitions essential for the application of the regulation on Orphan Medicinal Products. This means that companies may submit applications for designation as Orphan Medicinal Products to the EMEA for consideration by the newly created Committee of Orphan Medicinal Products. Orphan medicinal products eligible for incentives should be easily and unequivocally identified. The EU has funded research on orphan medicinal products in the past under the Fourth RTD Framework Programme's Biomed II programme and funding continues under the Fifth RTD Framework Programme's Quality of Life and Management of Living Resources programme.

This latest Regulation lays down a Community procedure for the designation of such drugs as orphan medicinal products; to provide incentives for their research and development; and to bring them to market. The legislation defines the criteria for a drug to be labelled 'orphan' and sets up a committee for orphan medicinal products. The Regulation also sets down legislation relating to the procedure for designation and removal of an orphan medicinal product from the register, protocol assistance, Community marketing authorisation, market exclusivity and other incentives.

Whereas the Community is beginning implement the Orphan Medicinal Product regulation, orphan drugs to treat certain conditions are already available through the American legislation. However it may be difficult for patients, and health authorities, to obtain adequate and updated information on such products, and how they can be provided to patients. The Information Center for Rare Diseases of the Mario Negri Institute in the frame of the project of the "Istituto Superiore di Sanità" on rare diseases is willing to fill the gap and will established a database of relevant data on:

- * Approved Orphan Products
- * Designated Orphan Products
- * Antidotes and galenics for rare diseases
- * Ongoing Clinical Studies on Rare Diseases

PREVENZIONE E TERAPIA DI ALCUNE MALATTIE RARE IN SARDEGNA

Franco Lilliu, Georgios Loudianos, Stefano De Virgiliis
*II° CLINICA PEDIATRICA, Dipartimento di Scienze Biomediche e Biotecnologie,
Università degli Studi di Cagliari.*

L'Unità operativa di Cagliari è compresa nell'ambito del Dipartimento di Scienze Biomediche e Biotecnologie dell'Università degli Studi di Cagliari.

Questo Dipartimento comprende la Clinica Pediatrica, il Centro Regionale per le Microcitemie ed il Centro Regionale per le Malattie Ereditarie del Metabolismo, inseriti nell'Azienda U.S.L. n. 8 della Regione Autonoma della Sardegna

Questa unità operativa da numerosi anni è ampiamente impegnata nel settore delle malattie rare sia per la prevenzione, diagnosi e cura sia per promuovere l'informazione e la formazione a livello della classe sanitaria e della popolazione sulle malattie genetiche in generale e in particolare sulla talassemia e sulle malattie ereditarie del metabolismo.

I livelli di intervento nella prevenzione delle malattie genetiche sono essenzialmente:

- screening degli eterozigoti;
- diagnosi prenatale;
- screening e diagnosi neonatale;
- diagnosi e terapia presintomatica.

Tutti questi obiettivi, almeno per molte malattie rare, sono stati raggiunti da questa unità operativa negli anni precedenti.

Esempi dei risultati ottenuti sono gli screenings degli eterozigoti per le β microcitemie (punto a), la diagnosi prenatale sia per le β microcitemie che per altre malattie genetiche, lo screening e diagnosi neonatale per la Fenilchetonuria (b, c), la diagnosi e terapia presintomatica per alcune aminoacidopatie come la Tirosinemia di tipo I e per la malattia di Wilson (d).

La malattia di Wilson è un disordine ereditario, autosomico recessivo, del trasporto del rame cui consegue un eccessivo accumulo di questo metallo nel fegato, nel sistema nervoso centrale e in altri organi come rene, cornea (anello di Keiser-Fleischer), scheletro, etc..

Il gene della malattia di Wilson, localizzato nel cromosoma 13, è stato recentemente clonato da due diversi gruppi di ricerca.

La storia naturale della malattia prevede l'evoluzione verso la cirrosi e il grave danno neurologico.

La terapia con chelanti del Rame, iniziata prima dell'insorgenza di lesioni irreversibili, è capace di normalizzare il bilancio del Rame e determinare la scomparsa dei sintomi. E' evidente quindi la grande importanza della diagnosi precoce di questa malattia che viene, così, a rappresentare un modello unico nella patologia umana.

L'incidenza di questa malattia è di 1/50.000-100.000 nati vivi, ma in Sardegna, considerato il numero di nuove diagnosi negli ultimi anni l'incidenza è molto più elevata. In questo anno abbiamo condotto un'indagine retrospettiva sull'incidenza della malattia in Sardegna: abbiamo calcolato l'incidenza della malattia di Wilson sulla base dei casi diagnosticati a partire dal 1960. L'incidenza della malattia nel decennio 60-70 è stata di 1:10.446, mentre nel periodo dal 70 al 92 è stata di 1:6.985 nati vivi.

Alla luce di questi dati appare evidente la necessità e l'utilità di una diagnosi precoce e di uno screening di massa per arrivare alla individuazione e alla terapia presintomatica della malattia. Meno chiara è la distribuzione e la frequenza della malattia nel restante territorio nazionale.

Da un preliminare studio collaborativo appare evidente come la malattia sia distribuita su tutto il territorio nazionale, ma non vi sono dati precisi sulla sua reale frequenza, e soprattutto se vi siano delle regioni in cui una elevata frequenza della malattia giustifichi l'utilità di uno screening di massa per una diagnosi e terapia precoce come in Sardegna.

Abbiamo preparato un protocollo diagnostico clinico genetico per la malattia di Wilson (allegato).

PRENATAL DIAGNOSIS OF METABOLIC DISEASE

Rosanna Gatti, Mirella Filocamo

Lab. Diagnosi PrePostnatale Malattie Metaboliche - Istituto G. Gaslini - Genova

Prenatal diagnosis of a metabolic defect is generally only carried out for pregnancies known to be at high risk for a severe disorder for which there is no affective treatment. Most requests for prenatal monitoring of a pregnancy will be from parents who have previously had a child with a metabolic disease and for whom termination of the pregnancy is acceptable. Approximately 150 metabolic disease can be diagnosed prenatally in foetal samples (particularly in chorionic villus samples obtained in the first trimester).

Therefore the genetic counselling service of a laboratory of prenatal metabolic disorder diagnosis collects a series of indirect information about the frequency of a remarkable group of rare diseases. Since 1975 seven hundred and seventeen prenatal diagnoses of metabolic diseases have been performed at G. Gaslini Institute. Among these, 626 were carried out for lysosomal diseases and 91 for other metabolic diseases. The frequency of the different metabolic diseases monitored during the pregnancy will be reported and discussed.

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*Direttore dell'Istituto Superiore di Sanità
e Responsabile scientifico: Giuseppe Benagiano*

Direttore responsabile: Vilma Alberani

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