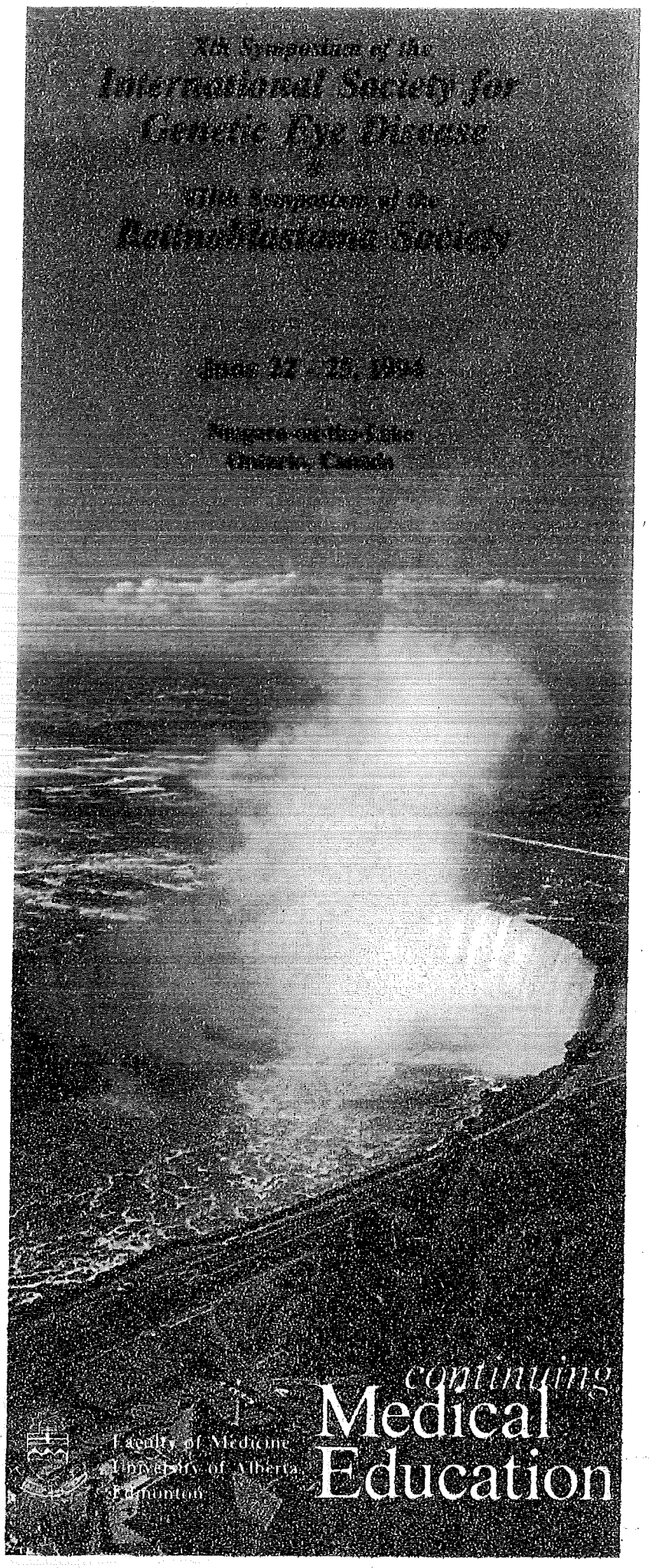
XIth Symposium of the
International Society for
Genetic Eye Disease
&
VIIth Symposium of the
Retinoblastoma Society
June 22 - 25, 1994
Niagara-on-the-Lake
Ontario, Canada
continuing
Medical
Education
Faculty of Medicine
University of Alberta
Edmonton

TIME	FUNCTION	LOCATION
Friday June 24		
0700	CONTINENTAL BREAKFAST	Outside Room 19
0750	Introduction	Rooms 17 & 18
0755	Franceschetti Lecture **Dr Irene Maumenee**	Rooms 17 & 18
0855	Presentation of Franceschetti Medal	Rooms 17 & 18
0900	*Scientific Session* Joint II	Rooms 17 & 18
1000	REFRESHMENT BREAK & POSTER DISPLAYS	Room 19
1100	*Scientific Session* Joint III	Room 17 & 18
1215	LUNCH (on your own)	
1345	*Scientific Sessions:* a) Genetics II	Room 18
	b) RB II	Room 17
1530	REFRESHMENT BREAK	Outside Room 19
1545	*Scientific Sessions:* a) Genetics III	Room 18
	b) RB III	Room 17
1650	Genetics Adjourn	
1755	RB III Adjourn	
1900	BANQUET Reception & Cocktails	Ballroom
1930	BANQUET Dinner & Dance	Ballroom

TIME	FUNCTION	LOCATION
Saturday, June 25		
0700	Retinoblastoma Bus leaves for Toronto Ocular Tumor Meeting	Hotel Lobby
0830	CONTINENTAL BREAKFAST	Outside Room 19
0915	*Scientific Session* Genetics IV	Room 18
1000	REFRESHMENT BREAK	Outside Room 19
1200	Adjourn	
1330	Business Meeting of ISGED Planning Committee	Room 2 (Boardroom)
1600	Genetics Bus leaves for Toronto Sheraton Centre	Hotel Lobby

LIST OF REGISTRANTS
International Society of Genetic Eye Disease & Retinoblastoma Society Symposia
June 22-25, 1994

ABRAMSON Dr David
New York USA

AL MESFER Dr Saleh
Riyadh SAUDI ARABIA

ALCORN Dr Debby
Palo Alto

ANTONELI Dr Celia B
Gianotti
Sao Paulo BRAZIL

BASCOM Dr Roger
Toronto CANADA

BATEMAN Dr Bronwyn
Los Angeles USA

BECH-HANSEN Dr Torben
Calgary CANADA

BHATT Dr Sucheta
Los Angeles USA

BLACH Dr Laurie
New York USA

BOYCOTT Ms Kym
Calgary CANADA

BRODIE Dr Scott E
New York USA

BUDNING Dr Andrew S
Toronto CANADA

CHARLES Dr Steve
Wilmslow ENGLAND

CHEONG Dr Pauline
Singapore

CHRISTENSEN Dr Laurie
Portland USA

CLARKE Dr Michael
New Castle Upon Tyme
ENGLAND

DASILVA Dr Lisa
Toronto CANADA

DE BECKER Dr Inge
Halifax CANADA

DEL MONTE Dr Monte
Ann Arbor USA

DESJARDINS Dr Laurence
Paris FRANCE

DI PISA Dr Francesco
Siena ITALY

DOZ Dr Francois
Paris FRANCE

DUNKEL Dr Ira
New York USA

DUNN Dr James M
Toronto CANADA

ELDER Dr James
Parkville AUSTRALIA

ERWENNE Dr Clelia M
Sao Paulo BRAZIL

ESPIRITU Dr Romeo
Manila PHILLIPINES

ESPIRITU Mrs Bella G
Manila PHILLIPINES

FIORE Dr Cesare
Perugia ITALY

FONTANESI Dr James
Memphis USA

...cont'd..

FRANCESCHETTI Dr Albert
Geneva SWITZERLAND

FUCILLO Dr Lucy
Toronto CANADA

GALLIE Dr Brenda L
Toronto CANADA

GEORGE Mr Nicolas
Cambridge UK

GIBLIN Dr Michael
Sydney AUSTRALIA

GREEN Dr Jane
St John's CANADA

GREENWALD Dr Mark
Chicago USA

GROTTE HILL Dr Rhian
Constantia SOUTH AFRICA

HADJISTILIANOU Dr
Theodora
Siena ITALY

HAYAKAWA Dr Mutsuko
Bunteyo-ku JAPAN

HEON Dr Elise
Iowa City USA

HUNGERFORD Mr John
London UK

IKEDA Dr Kozo
Nagoya JAPAN

IMHOF Dr Saskia
Amsterdam HOLLAND

KANEKO Dr Akihiro
Tokyo JAPAN

KASTE Dr Sue C
Memphis USA

KINGSTON Dr Judith
London ENGLAND

LEVIN Dr Alex
Toronto CANADA

LING Dr Yvonne
Singapore SINGAPORE

LISCH Dr Walter
Hanau GERMANY

LORENZ Dr Birgit
Regensburg GERMANY

LUEDER Dr Gregg
St Louis USA

MA Dr Colin
Portland USA

MACDONALD Dr Ian
Edmonton CANADA

MACKEY Dr David
East Melbourne
AUSTRALIA

MAGLI Dr Adriano
Naples ITALY

MAJIMA Prof Akio
Nagoya JAPAN

MANZITTI Dr Julio
Cap. Federal ARGENTINA

MASTRANGELO Dr Domenico
Siena ITALY

MAUMENEE Dr Irene
Baltimore USA

MCINNES Dr Rod
Toronto CANADA

MCKENZIE Dr John
East Melbourne
AUSTRALIA

METS Dr Marilyn
Chicago USA

MILLER Dr Marilyn
Chicago USA

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Los Angeles USA

NEESON Mr Michael
N Olmsted USA

NIKSARLI Dr Kevork
New York USA

ONADIM Dr Qerrin
London UK

PATON Dr Katherine
Vancouver CANADA

PEARCE Dr Bill
Edmonton CANADA

PHILLIPS Dr Robert
Toronto CANADA

PILLERS Dr De-Ann
Portland USA

PRATT Dr Charles
Memphis USA

RAYMOND Dr Vincent
Ste-Foy CANADA

ROARTY Dr John
Detroit USA

SALAMA Dr Hesham
Baltimore USA

SASABE Dr Tetsuo
Habikino JAPAN

SCRIVER Dr Charles
Montreal CANADA

SENFT Dr Susan
San Francisco USA

SERRA Dr Alicia
Barcelona SPAIN

SERVIDIDIO Ms Camille
New York USA

SHIELDS Dr Jerry
Philadelphia USA

SNEAD Mr Martin
Cambridge UK

SQUITIERI Dr Nicola
Siena ITALY

STIRN KRANJC Mrs Branka
Ljubljana SLOVENIA

STRAUSS Dr Lewis
Chicago

STURGIS-BUCKHOUT Dr Lee
Sanibel USA

SUSMAN Dr David
New York USA

...cont'd...

SUTHERLAND Ms Joanne
Toronto CANADA

TAN Dr Karel
Amsterdam THE NETHERLANDS

TIJMES Dr Nel
Amsterdam THE NETHERLANDS

TRABOULSI Dr Elias
Baltimore USA

TREMBLAY Dr Francois
Halifax CANADA

VAN DORP Dr Dieuwke
Amsterdam NETHERLANDS

WALTER Dr Michael
Edmonton CANADA

WEBSTER Dr Andrew
Cambridge ENGLAND

WHALEN Ms Mary
New York USA

WIGGS Dr Janey
Boston USA

ZHANG Dr Qingjiong
Ichihara JAPAN

ZHU Dr Danping
Baltimore USA

Join us for the

Buffet Dinner and Dance

Friday, June 24
in the "Ballroom", Main Floor

Reception & Cocktails–1900 hours
Dinner (wine with dinner)–1930 hours
Dance–2115 hours

Sandy Vine and the Midnights will entertain you with dinner music and a dance to follow at 2115 hours.

~~~CASH BAR~~~

* * * **Dinner & Dance Complimentary** * * *
for registrants only

Extra tickets, please contact the Registration Desk by Thurs at 1400 hrs. Tickets can be purchased for $35/ea-No Refunds. Tickets will be collected at the door.

DO NOT MISS this evening of entertainment with an INTERNATIONAL FLAIR..
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SHAW FESTIVAL THEATRE

Thursday, June 23, 1994

2000 - 2200 hours

Join us for an entertaining evening and play entitled:

Sherlock Holmes

by William Gillette

A young woman held hostage! A blackmail scheme! The evil Professor Moriarty in his undeground lair! Sherlock Holmes walks straight into a trap! This is the celebrated hit play based on the Sherlock Holmes stories, written in collaboration with Conan Doyle himself.

The Shaw Festival Theatre is only a 10 minute walk from the Queen's Landing Hotel. If you wish to walk as a group, we will meet in the Hotel Lobby at 1920 hours.

The *TICKETS* for the play will be distributed at the Registration Desk. As you know, one ticket was included in your registration fee. There will be NO refunds.

Thursday June 23

0745 **Welcome - Dr Bill Pearce, Edmonton, Canada**

0750 **Greetings - Dr Rod McInnes, Toronto, Canada**
RP Eye Research Foundation (Canada)

0755 **Introduction - Dr Rod McInnes, Toronto, Canada**
0800 **GUEST SPEAKER: Dr Charles Scriver, Montreal, Canada**

"The Human Genome Project: Implications for Medicine"

Joint Session

JOINT I ROOMS 17 & 18 CHAIRPERSON: IRENE MAUMENEE

0900 **Dr Jane Green, St John's, Canada**

Molecular and Clinical Screening for von Hippel Lindau Disease

0915 **Dr Andrew Webster, Cambridge, England**

Natural History. Treatment and Genetics of Retinal Angiomas in von Hippel-Lindau Disease

0930 **Dr Alicia Serra, Barcelona, Spain**

A Point Mutation in the RDS/Peripherine Gene in a Family Affected of Central Areolar Choroidal Dystrophy

0945 **Dr Roger Bascom, Toronto, Canada**

Putative Disease-Causing ROM1 Mutations in Retinitis Pigmentosa

1000 **Dr Annette Moll, Amsterdam, Netherlands**

Parental Age in Sporadic Hereditary Retinoblastoma

1015 **Dr Susan Senft, San Francisco, USA**

Influence of Paternal Age on the Incidence of Retinoblastoma

1030 **Dr Robert Phillips, Toronto, Canada**

The Retinoblastoma Tumor Suppressor Gene

1045-1200 **REFRESHMENT BREAK AND POSTER DISPLAYS**

THURSDAY JUNE 23

Concurrent Session

GENETICS I ROOM 18 CHAIRPERSON: JANE GREEN

1215 **Dr Elise Heon, Iowa City, USA**

Identification of a Novel Rhodopsin Mutation Responsible for Retinitis Pigmentosa: Implication for Genetic Counselling

1230 **Dr Inge De Becker, Halifax, Canada**

Congenital Nystagmus as the Presenting Sign of Aniridia?

1245 **Dr Elias Traboulsi, Baltimore, USA**

Studies of the Pax 6 Gene in Patients with the Anophthalmia Microphthalmia/Coloboma Spectrum of Ocular Malformations

1300 **Dr Bill Pearce, Edmonton, Canada**

Autosomal Dominant Keratitis: A Possible Aniridia Variant

1315 **Dr Michael Walter, Edmonton, Canada**

Analysis of the Genetic Defect in Autosomal Dominant Keratitis

1330 **LUNCH (ON YOUR OWN)**

1500 **Trip to Niagara Falls**

2000 **Shaw Festival Play - "Sherlock Holmes"**

Thursday June 23

Concurrent Session

RBI — ROOM 17 — CHAIRPERSON: THEODORA HADJISTILIANOU

1200 **Dr James Fontanesi, Memphis, USA**

Electron Beam Irradiation for Treatment of Retinoblastoma: The St Jude Children's Research Hospital Experience

1215 **Dr David Abramson, New York, USA**

Local Failures in Irradiated Unilateral Retinoblastoma

1230 **Dr Scott Brodie, New York, USA**

ERG Abnormalities in Eyes with Retinoblastoma

1245 **Dr John Hungerford, London, England**

Conservative Treatment of Sporadic Unilateral Retinoblastoma

1300 **Dr Francesco Di Pisa, Siena, Italy**

A Cognitive Approach to Database Semantics of Clinical Data of Retinoblastoma Cases

1315 **Dr Charles Pratt, Memphis, USA**

Proposal for a New Staging System for Retinoblastoma

1330 **Dr Jerry Shields, Philadelphia, USA**

Unusual Intraocular Infections Simulating Retinoblastoma

1345 **LUNCH (ON YOUR OWN)**

1500 **Trip to Niagara Falls**

2000 **Shaw Festival Play - "Sherlock Holmes"**

Friday June 24

0750	**Introduction - Dr Bill Pearce**
0755	**FRANCESCHETTI LECTURER: Dr Irene Maumenee, Baltimore, USA**
	"The Marfan Syndrome"
0855	**Presentation of Franceschetti Medal** **Dr Albert Franceschetti, Geneva, Switzerland**

Joint Session

JOINT II **ROOMS 17 & 18** **CHAIRPERSON: BILL PEARCE**

0900	**Dr Birgit Lorenz, Regensburg, Germany**
	The Clinical Spectrum of Point Mutations in the Norrie Disease Gene
0915	**Dr Danping Zhu, Baltimore, USA**
	Mutations in the ND gene in families with Norrie Disease
0930	**Dr Quingjiong Zhang, Ichihara, Japan**
	Detection of RB1 Germline Mutations in RB Patients by SSCP
0945	**Dr James Dunn, Toronto, Canada**
	Retinoblastoma Gene Mutation Detection
1000-1100	**REFRESHMENT BREAK AND POSTER DISPLAYS**

Friday June 24

Joint Session

JOINT III ROOMS 17 & 18 CHAIRPERSON: BRENDA GALLIE

1100 **Dr Vincent Raymond, Ste-Foy, Canada**

Linkage Disequilibrium Studies Confine the Juvenile Open Angle Glaucoma Locus within a 5 cM Interval on Chromosome 1q21-31

1115 **Dr Janey Wiggs, Boston, USA**

Further Evidence for a Locus for Autosomal Dominant Juvenile Glaucoma on Chromosome 1q and Evidence for Genetic Heterogeneity

1130 **Dr James Fontanesi, Memphis, USA**

Asynchronous Bilateral Retinoblastoma: The St Jude Children's Research Hospital Experience

1145 **Dr David Abramson, New York, USA**

The Topography and Age of Retinoblastoma Tumors

1200 **Dr Domenico Mastrangelo, Siena, Italy**

Frequent Loss of Both Copies of a VNTR from Within Intron 16 of the RBI Gene in Retinoblastoma

1215-1345 LUNCH (ON YOUR OWN)

Friday June 24

Concurrent Session

GENETICS II ROOM 18 CHAIRPERSON: TORBEN BECH-HANSEN

1345 **Dr Walter Lisch, Hanau, Germany**

Differential Diagnosis of Primary Microcysts of the Corneal Epithelium

1400 **Dr Alex Levin, Toronto, Canada**

X-Linked Megalocornea - A Four Generation Pedigree

1415 **Dr Steve Charles, Wilmslow, England**

Microfibrillar Abnormalities in Ectopia Lentis

1430 **Dr Marilyn Mets, Chicago, USA**

Usher's Syndrome in Congenitally Deaf Children

1445 **Dr Hesham Salama, Baltimore, USA**

Relation of Visual Loss to Neurologic Dysfunction and New Ocular Findings in 23 Patients with Batten's Disease

1500 **Dr Bronwyn Bateman, Los Angeles, USA**

Cataract Formation in Spielmeyer-Vogt

1515-1545 **REFRESHMENT BREAK**

Friday June 24

Concurrent Session

RBII ROOM 17 CHAIRPERSON: LINN MURPHREE

1345 **Dr Judith Kingston, London, England**

Is There a Role for Chemotherapy in the Management of Intraocular Retinoblastoma

1400 **Dr Francois Doz, Paris, France**

VP 16 & Carboplatin in Extra-ocular Retinoblastoma (ERB): A Study of the Societe Francaise D'Oncologie Pediatrique

1415 **Celia Gianotti Antoneli, Sao Paulo, Brazil**

Response of Chemotherapy (ct) in Extraocular Retinoblastoma

1430 **Dr Judith Kingston, London, England**

Adjuvant Chemotherapy Following Enucleation for Retinoblastoma in Children with Adverse Histological Features

1445 **Dr Theodora Hadjistilianou, Siena, Italy**

Regression Patterns Following Chemotherapy as a Primary Treatment of Intraocular Retinoblastoma

1500 **Dr Linn Murphree, Los Angeles, USA**

Chemoreduction of Intraocular Retinoblastoma

1515 **Dr Brenda Gallie, Toronto, Canada**

Cyclosporin-Modulated Chemotherapy use with Focal Therapy: A New Approach to Retinoblastoma

1530-1545 **REFRESHMENT BREAK**

Concurrent Session

GENETICS III ROOM 18 CHAIRPERSON: IAN MACDONALD

1545 **Dr Alex Levin, Toronto, Canada**

Corneal Topography in Down Syndrome (PB #33)

1550 **Dr Kozo Ikeda, Nagoya, Japan**

A Sibling with Mesenchymal Dysgenesis of the Ocular Anterior Segment (PB #36)

1555 **Dr Sucheta Bhatt, Los Angeles, USA**

Videokeratography in Family Pedigree Analysis of Familial Keratoconus (PB #38)

1600 **Dr Steve Charles, Wilmslow, England**

Congenital Alacrima Assessment of an Autosomal Dominant Pedigree (PB #37)

1605 **Dr Cesare Fiore, Perugia, Italy**

Corneal Degeneration in Severe Turner Syndrome with a Peculiar Karyotype: A Sign of Syndrome or a New Mutation? (PB #41)

1610 **Dr Alex Levin, Toronto, Canada**

Prenatal Diagnosis of Retinal Detachment in the Walker-Warburg Syndrome (PB #34)

1615 **Dr Alex Levin, Toronto, Canada**

Ocular Abnormalities in the Prenatal Ultrasonographic Delineation of Genetic Disorders (PB #35)

1620 **Dr Sucheta Bhatt, Los Angeles, USA**

Familiarity in Age Related Macular Degeneration (PB #39)

1625 **Dr Cesare Fiore, Perugia, Italy**

Evaluation of Activity of Functionality of Cochlear Outer Hair Cells in Patients with Retinitis Pigmentosa and in their Relatives (PB #42)

1630 **Dr Inge De Becker, Halifax, Canada**

Severe Infantile Cardiomyopathy cone-rod degeneration: Alstrome syndrome (PB #43)

1635 **Mr Martin Snead (presented by Nicolas George) Cambridge, UK**

Stickler Syndrome: Correlation Between Vitreo Retinal Phenotype and Linkage to COL 2A1 (PB #44)

1640 **Ms Kym Boycott, Calgary, Canada**

Development of a YAC Contig in the Minimal Region of X-Linked CSNB in Xp11 (PB #46)

1645 **Dr Joanne Sutherland, Toronto, Canada**

RFLP Analysis of Choroideremia is Complicated by Questionable Paternity (PB #45)

1900 **Pre-Banquet Reception and Cocktails**

1930 **Banquet Dinner Buffet and Dance**

Concurrent Session

RBIII ROOM 17 CHAIRPERSON: DAVID ABRAMSON

1545 **Dr Mark Greenwald, Chicago, USA**

Clinicopathologic Correlation of Retinoblastoma Successfully Treated with Systemic Chemotherapy (PB #1)

1550 **Dr Akihiro Kaneko, Tokyo, Japan**

Treatment of Vitreous Seeds of Retinoblastoma Recurrent after Radiotherapy using Vitreous Injection of Melphalan Combined with Ocular Hyperthermia (PB #2)

1555 **Dr Linn Murphree, Los Angeles, USA**

Thermal Chemotherapy for Retinoblastoma: Protocol, Instrumentation and Outcomes (PB #3)

1600 **Dr Francois Doz, Paris, France**

N-myc amplification, loss of heterozygosity (LOH) in 1p and DNA Indec in Tumor Specimen of 45 Patients with Retinoblastoma (PB #4)

1605 **Dr David Mackey, East Melbourne, Australia**

DNA Testing in Retinoblastoma Families is Cost Effective (PB #5)

1610 **Dr Domenico Mastrangelo, Siena, Italy**

The Polymerase Chain Reaction and its Usefulness in the Genetic Characterization of Retinoblastoma Patients: A Tool for Clinical Laboratory (PB #6)

1620 **Dr Laurie Blach, New York, USA**

Trilateral Retinoblastoma Incidence and Outcome: A Decade of Experience (PB #8)

1625 **Dr John Roarty, Detroit, USA**

Multidisciplinary Management of Trilateral Retinoblastoma (PB #9)

1630 **Dr Sue Kaste, Memphis, USA**

Orbital Volume of Longterm Survivors of Retinoblastoma treated with Orbital Irradiation (PB #10)

1635 **Dr Sue Kaste, Memphis, USA**

Orbital Volume of Longterm Survivors of Retinoblastoma treated with Orbital Irradiation (PB #11)

1640 **Dr James Fontanesi, Memphis, USA**

Second Malignant Neoplasms in Patients with Retinoblastoma: St Jude Children's Research Hospital Experience (PB #13)

1645 **Dr Saskia Imhof, Amsterdam, Holland**

Non-Ocular Tumors in Patients with Megavoltage External Beam Irradiation for Retinoblastoma (PB #14)

1650 **Dr Jerry Shields, Philadelphia, USA**

Unusual Intraocular Infections Simulating Retinoblastoma (PB #15)

1655 **Dr James Elder, Parkville, Australia**

A Rhabdomyosarcoma and Retinoblastoma in a Nine Year Old Child with Ring Chromosome 13 - A Case of the Second Tumor First (PB #16)

1700 **Dr Tetsuo Sasabe, Habikino, Japan**

RGDS Peptide Interferes with the Attachment and Spreading of Retinoblastoma Cells in Vitro (PB #17)

1705 **Dr Charles Pratt, Memphis, USA**

Outcome for Five Patients with Constitutional 13q Chromosomal Abnormalities and Retinoblastoma (PB #18)

1710 **Dr Karel Tan, Amsterdam, Netherlands**

Mutation Frequency and Number of Retinoblasts (PB #19)

1715 **Dr Gregg Lueder, St Louis, USA**

Retinoma Associated with Vitreous Seeding (PB #20)

1720 **Dr Andrew Budning, Toronto, Canada**

Work up of New Retinoblastoma Patients (PB #21)

1725 **Dr Julio Manzitti, Cap Federal, Argentina**

Treatment of Retinoblastoma at Hospital Garrahan in Argentina (PB #22)

1730 **Dr Julio Manzitti, Cap Federal, Argentina**

Local Therapy of Bilateral Retinoblastoma at the Hospital Garrahan Argentina (PB #23)

1735 **Dr Kevork Niksarli, New York, USA**

Trends in Retinoblastoma Management (PB #25)

1740 **Dr John Roarty, Detroit, USA**

Assessment of Retinoblastoma Viability with Doppler Flow Ultrasonography (PB #26)

1745 **Dr David Susman, New York, USA**

Ambulatory Anesthesia for Retinoblastoma the New York Experience 1980-1993 (PB #27)

1750 **Dr Sue Kaste, Memphis, USA**

Persistent Hyperplastic Primary Vitreous: Imaging Findings with Pathologic Correlates (PB #12)

1900 **Pre-Banquet Reception & Cocktails**

1930 **Banquet Dinner Buffet & Dance**

Saturday June 25

GENETICS IV ROOM 18 CHAIRPERSON: ELIAS TRABOULSI

0915 **Dr Dieuwke Van Dorp, Amsterdam, Netherlands**

Pseudoxanthoma Elasticum

0930 **Prof Akio Majima, Nagoya, Japan**

A Pedigree of Presumed X-Linked Familial Exudative Vitreoretinopathy

0945 **Mr Nicolas George, Cambridge, UK**

Clinical and Genetic Features of X-Linked Retinoschisis

1000 **REFRESHMENT BREAK**

1030 **Dr De-Ann Pillers, Portland, USA**

Deletion Mapping of Effects of the Duchenne Muscular Dystrophy Gene on the Electroretinogram

1045 **Dr Inge De Becker, Halifax, Canada**

Correlation of Electroretinogram Findings with Molecular Analysis in the Duchenne Muscular Dystrophy Phenotype

1100 **Dr Francois Tremblay, Halifax, Canada**

Duchenne Muscular Dystrophy (Xp21) and Congenital Stationary Night Blindness (Xp11.3) Similarities and Differences of Two Distinct Retinal Conditions

1115 **Dr Torben Bech-Hansen, Calgary, Canada**

Further Mapping of X-Linked Congenital Stationary Night Blindness in Region Xp11.22

1200 **Adjourn**

1330 **Business Meeting of ISGED Planning Committee**
Room 2 (Boardroom)

Thursday, June 23 *0755 hours*

Guest Speaker: Dr Charles SCRIVER, Montreal, Canada

The Human Genome Project and Genetic Epidemiology

MOLECULAR AND CLINICAL SCREENING FOR VON HIPPEL LINDAU DISEASE.

Green JS[1], Crossey PA[2], Bowmer MI[1], Maher ER[2], [1]St. John's, Canada. [2]Cambridge, U.K.

Von Hippel-Lindau disease (VHL) is a potentially lethal autosomal dominant disorder predisposing to benign and malignant tumours including retinal angioma, cerebellar or spinal cord hemangioblastoma, renal cell carcinoma and pheochromocytoma. The age of onset, and combination and order of occurrence of tumours is variable even within families. Ideal clinical management includes genetic screening (direct mutation detection or linkage analysis) to identify those with the gene, and clinical screening for this group to permit early diagnosis and treatment of individual tumours. Seventy-five affected and at risk members of a large VHL family have been screened clinically from age 3 until at least age 50. A missense mutation (C→T) has now been identified in codon 238 of the VHL gene in affected members of this family. DNA was obtained from 65 family members, including 32 at 50% risk, and tested for the mutation. Only 4 patients at risk (age 13, 14) have the VHL mutation; 28 others (age 11-53) are negative for the mutation and do not require clinical screening. The excess of mutation negative results indicates that in the age group so far tested the majority of gene carriers have been identified by clinical screening. Because of the early age of onset of VHL and availability of treatment, genetic testing in childhood is justified and will reduce personal and medical care costs when clinical screening is concentrated on those with the VHL gene.

NATURAL HISTORY, TREATMENT AND GENETICS OF RETINAL ANGIOMAS IN VON HIPPEL-LINDAU DISEASE.

Maher E, Moore AT, Webster AR ; Cambridge, England.

In von Hippel-Lindau disease little is known about the natural history of untreated retinal angiomas nor of the rate of development of new angiomas. We report our experience in observation and treatment of optic disc and peripheral retinal angiomas detected in a screening program for VHL disease which has been running for 5 years.

61 individuals (23 affected and 38 at-risk relatives) from 18 kindred with VHL disease underwent a full ophthalmological assessment including fluorescein angioscopy and angiography. 5 optic disc and 14 peripheral angiomas were detected in 16 individuals. One optic disc angioma and 12 peripheral angiomas were treated with cryotherapy or laser photocoagulation. 4 optic disc angiomas and 3 small peripheral angiomas that were detected on angioscopy were observed untreated for between 2 and 4.5 years and have not shown signs of progression. Overall, all 19 tumors were present at the first examination and no new tumors have developed during follow up of up to 5 years.

The VHL gene has recently been cloned and molecular genetic analysis using linked DNA markers and gene deletion detection has been performed on 14 at-risk individuals. 11 of these were found to be of low risk and their screening protocol adapted appropriately. Correlation of the results of mutation analysis with clinical phenotype is in progress; initial results do not show a difference in the rate of angiomatosis in individuals with genetic deletions and those without.

A POINT MUTATION IN THE RDS/PERIPHERINE GENE IN A FAMILY AFFECTED OF CENTRAL AREOLAR CHOROIDAL DYSTROPHY

Serra A, Reig C, Carballo M, Calzada M D, Vidal M, Gean E, Arumi J, Antich J.

Different mutations in the RDS-peripherine gene have been related with different phenotypes of retinal dystrophies, including Retinitis Pigmentosa, Retinitis Punctata Albescens, Butterfly dystrophy, Vitelliform macular dystrophy (Best's disease) and other macular dystrophies.

We describe a family with a macular dystrophy that clinically corresponds to a Central Areolar Choroidal Dystrophy, inherited in an autosomal dominant pattern. A point mutation in codon 172 (Arg-172-Trp) in RDS/peripherine gene was found in all the affected members of the family.

Clinically, electrophysiological and genetical characteristics will be discussed.

PUTATIVE DISEASE-CAUSING *ROM1* MUTATIONS IN RETINITIS PIGMENTOSA.
Bascom RA, Liu L; Toronto, Canada. Heckenlively JR;Torrance, USA. Sheffield VC, Stone EM; Iowa City, USA, and McInnes RR; Toronto, Canada.

Rom-1 and peripherin/rds are related transmembrane proteins of the photoreceptor disk rim. Given their sequence similarity, their non-covalent association at the disk rim where they may act as adhesion molecules (Bahtia & Travis, ARVO #2675, 1994), and the occurrence of *RDS* mutations in patients with several inherited retinopathies, we searched for disease-causing mutations in *ROM1* in 298 adRP patients. We previously reported six polymorphisms and three rare sequence variants at the *ROM1* locus. We have recently found several putative disease-causing *ROM1* mutations in both typical and in atypical adRP patients. The macula of the atypical RP proband is affected with an atrophic lesion. All of the affected families are small, but where it has been possible to study cosegregation, the mutant allele has cosegregated with the disease. None of the mutant alleles have been detected in any other of the adRP families or 50 controls. Because no simple, functional assay currently exists to determine whether a mutation in *ROM1* or *RDS* is disease-causing or not, we are evaluating the use of the ER/Golgi quality control system of several different cultured cell lines to assess the effects of amino acid substitutions on the transport of the rom-1 protein to the cell membrane. To date, we find that the wild-type rom-1 and peripherin proteins become "stuck" in the ER of COS-1 and 293s cells, even if the two proteins are expressed together. Expression of rom-1 in *Drosophila* Schneider cells, however, demonstrates transfer of rom-1 to the plasma membrane in a significant fraction of this heterogeneous cell population. Evaluation of the transfer of rom-1 mutant proteins is underway using this system as well as several other cell types. We conclude that i) *ROM1* mutations may be a third identified cause of adRP, in addition to rhodopsin and *RDS* mutations, and ii) *Drosophila* Schneider cells may be a suitable cell type for the demonstration that some mutant rom-1 and peripherin proteins fold abnormally and are not exported to the the plasma membrane.

PARENTAL AGE IN SPORADIC HEREDITARY RETINOBLASTOMA

Moll AC, Imhof SM, Bezemer PD, Tan KEWP; Amsterdam, The Netherlands

purpose

Investigation of the impact of the paternal and maternal age on the incidence of sporadic hereditary retinoblastoma (RB). In the literature it has been suggested that RB new germ-line mutations usually orginate from the father.

material and methods

From 1862 to 1993 940 RB patients were registered in The Netherlands. The ages of the parents at the time of birth of the RB patients were found. The ages of parents of sporadic hereditary RB patients were compared to the ages of non-hereditary RB patients.

results

The mean age of the fathers at birth of their sporadic hereditary RB child was 32.9 years, this was 32.2 years in the non-hereditary group ($p<0.1$ one-sided). The mothers were 30.2 years in the sporadic hereditary group and 29.3 years in the non-hereditary group ($p<0.05$ one-sided).

conclusion

The mean age of the mothers at the birth of their children with sporadic hereditary retinoblastoma is significantly higher than the mean age of the mothers at the birth of their children with non-hereditary retinoblastoma. This is, surprisingly, not the case for the fathers. These findings will be discussed.

INFLUENCE OF PATERNAL AGE ON THE INCIDENCE OF RETINOBLASTOMA

Senft, SH, Moll, AC, Dabas, KH, Bezemer, PZ, Tan, KEWP
San Francisco, CA; Riyadh, Saudi Arabia; The Netherlands

Advanced parental age has been suggested as a factor increasing the incidence of sporadic hereditary retinoblastoma (der Kinderen, et al). Recently, the incidence of retinoblastoma in Saudi Arabia was reported to be considerably elevated compared to worldwide statistics--1:11,000 vs. 1:18,000 (Al-Idrissi, E. et al). This study was undertaken to determine whether an increased *paternal* age not correlated to maternal age could explain the noted higher prevalence of hereditary retinoblastoma in Saudi Arabia.

41 parents of children with sporadic hereditary retinoblastoma, and 206 age and sex-matched controls obtained from pediatric ophthalmology strabismus patients were interviewed to determine the ages of the parents at the time of birth of the affected child. Conversions from the Hegorian calendar year to Gregorian ages were made accordingly.

The mean age of the fathers from the hereditary retinoblastoma group was 36 years, (range of 18-74) whereas the mean age of the control group fathers was 32.3 (range 18-65) , significant to a p-value of 0.031 one side. The mean age of mothers from the hereditary group was 26.8 (range of 14-42) and that of the mothers control group was 25.0 years (range of 13-43) , with a corresponding p-value of 0.066 one side.

Our conclusion was that the significantly higher age of the fathers is likely to be the main factor responsible for the increased incidence of retinoblastoma in Saudi Arabia.

THE RETINOBLASTOMA TUMOR SUPPRESSOR GENE
Dr Robert Phillips

IDENTIFICATION OF A NOVEL RHODOPSIN MUTATION RESPONSIBLE FOR RETINITIS PIGMETOSA: IMPLICATION FOR GENETIC COUNSELLING

Heon E, Vandenburgh K, Seiler R, Levin A, Sheffield V, Stone EM; Iowa, USA.

Retinitis pigmetosa is a heterogeneous group of disorders characterized by aprogressive degeneration of the photoreceptors that results in night blindness, visual field loss and electroretinographic changes. In the 50% of cases where the inheritance pattern can be demonstrated, 20% are classified as autosomal dominant (adRP), 20% as autosomal recessive (arRP) and a further 7-20% are due to an X-linked form of inheritance. The remaining 50% (simplex cases) are usually felt to be automsomal recessive in nature. The recogition that multiple point mutations within the coding sequence of the rhodopsin gene have been associated with adRP, has improved our understanding and management of this disorder. We describe a family in which the proband was affected with what was thought to be arRP, in which mutational analysis of the coding region of the rhodopsin gene by GC-clamped denaturing-gradient gel electrophoresis (DGGE) revealed a point mutation at codon 51 of exon 1 resulting in a glycine to alanine amino acid substitution. This rhodopsin mutation was also present in her two sons, also screened by DGGE. The clinical relevance of this autosomal dominant mutation will be discussed.

HEREDITY OF SQUINT: A STUDY IN ESOTROPIC MONO AND DIZYGOTE TWINS

Magli A, Pansini M, Sebastio L, D'Esposito M, de Berardinis T; Naples, Italy.

In the past 10 years we have examined 20 pairs of esotropic Mz twins, with ages ranged from 2 - 9 years. The data have been compared with the same number of pairs of Dz twins of the same sex. In all patients we have performed refraction, ophthalmoscopy and examination of motor and sensory anomalies by means of tests in common use. Hereditability of every trait was calculated using Holzinger's formula, and the collected data were examined by means of the X^2 test of contingency. From our research it can be deduced that hypermetropia, esotropia, in particular microesotropia, and marked amblyopia are traits with inheritability higher than 0.50, according to Holzinger's formula, and therefore of genetic importance. From the high percentage of concordance found in Mz twins in comparison with the low percentage in Dz twins, 3 times higher, it can be deduced that all the anomalies related to squint are strictly connected; they can be partly recognized in heredity and the characteristics are not those of a monomer inheritance but follow the laws of a multifactorial genetic system.

CONGENITAL NYSTAGMUS AS THE PRESENTING SIGN OF ANIRIDIA?

De Becker I, Tremblay F, Walter M, Halifax and Edmonton, Canada.

Aniridia is a disorder causing abnormal development not only of the iris but also of the lens, cornea and retina. When the iris is absent the diagnosis of aniridia is obvious, but when the iris is well-formed it is much less so. We present two patients, a mother and her infant son, who have no obvious iris abnormalities. The mother carried a diagnosis of "congenital nystagmus" , until at age 27 her corneal changes were recognized as being typical for aniridia. Her 8 week old son was refered for work-up of nystagmus. He had striking blue irides with well-formed and well-centered pupils. We will present the ocular findings, the ERGs, and results of the analysis of the PAX6 gene on these two patients.

STUDIES OF THE PAX-6 GENE IN PATIENTS WITH THE ANOPHTHALMIA/ MICROPHTHALMIA/COLOBOMA SPECTRUM OF OCULAR MALFORMATIONS.

Traboulsi EI, Rutherford GW, Zhu D, Smith EA, Maumenee IH; Baltimore, USA

Purpose. PAX-6 is a homeobox and paired-box-containing gene that belongs to the vertebrate Pax gene family. It is specifically expressed in the developing eye and brain. PAX-6 is the AN2 (aniridia) gene in man and maps to 11p13. Mutations in the murine Pax-6 gene result in microphthalmia and colobomatous malformations, while mutations in the human homologue lead to aniridia and/or to anterior segment malformations. The focus of this study is to screen patients with the anophthalmia/ microphthalmia/coloboma spectrum of malformations for mutations in the PAX-6 protein coding region. **Methods.** DNA was extracted from blood leukocytes and PCR was used to amplify some exons (including those containing the homeobox and paired-box) of the PAX-6 gene using exon-specific primers (Glaser et al., Nat. Genet. 1992; 2:232). The amplified products were then analyzed using Single Strand Conformation Polymorphism (Orita et al., Proc. Nat. Acad. Sci. 1989; 86:2766) to detect alterations in the electrophoretic mobility of DNA fragments resulting from mutations. **Results.** We studied samples from 25 patients with anophthalmia, microphthalmia and coloboma, as well as combinations of the above. PAX-6 comprises 14 exons which code for a 422 amino acid protein product. To date ~~one~~ patient with microphthalmia was found to have a rearrangement in the paired-box region. **Conclusions.** PAX-6 is a candidate gene for the anophthalmia/microphthalmia/ coloboma spectrum of ocular malformations and for other ocular malformations, especially those with anterior segment dysgenesis.

AUTOSOMAL DOMINANT KERATITIS: A POSSIBLE ANIRIDIA VARIANT

Pearce B, Mielke B, Hassard D, Climenhaga H, Climenhaga D, Hodges E; Edmonton, Canada.

A 3 generation family is reported in which 12 members, 5 male and 7 female, are affected with an hereditary keratitis. The pattern of transmission is consistent with autosomal dominant inheritance. The disorder is characterized by the presence of a circumferential band of opacification and vascularization of Bowman's membrane adjacent to the limbus. Progression towards the central cornea occurred in some instances. Penetrating keratoplasty was performed in certain cases when the visual axis was involved and the acuity deteriorated. Histopathology confirmed the inflammatory nature and the anterior stromal localization of the keratitis.

Other ocular features included minor iris abnormalities in several cases. The main additional finding present in each affected member was macular hypoplasia. The association of macular hypoplasia, minor iris abnormalties, and anterior stromal keratitis suggests that autosomal dominant keratitis may be a variant of aniridia. This possibility is currently being explored at a molecular level.

ANALYSIS OF THE GENETIC DEFECT IN AUTOSOMAL DOMINANT KERATITIS.

Walter, M.A., Mirzayans, F., Pearce, W.G., Bamforth, F., and MacDonald, I.M. Edmonton, AB., CANADA

Aniridia and autosomal dominant keratitis (ADK) are two eye diseases with similar modes of inheritance and overlapping clinical manifestations. Aniridia is a bilateral congenital anomaly that is defined by structural defects of the iris, frequently severe enough to cause an almost complete absence of iris. This may be accompanied by other anterior segment manifestations, including cataract, and keratitis. Posterior segment involvement in aniridia is characterized by foveal hypoplasia resulting in a highly variable impairment of visual acuity, often with nystagmus. Aniridia is usually inherited as an autosomal dominant disease that occurs in 1 in 50,000 to 100,000 people. In contrast, ADK is a much rarer disorder with only two known families. The major clinical feature of ADK is anterior stromal corneal opacification and vascularization in the peripheral cornea. Progression into the central cornea may compromise visual acuity. Other anterior segment features include minimal radial defects of the iris stroma. Posterior segment involvement is characterized by foveal hypoplasia with minimal affect on visual acuity. Aniridia and ADK are characterized by similar corneal changes, iris defects, and foveal hypoplasia, although ADK patients generally have less serious manifestations. It is our contention that these similar clinical findings, in conjunction with the similar pattern of inheritance, is compelling evidence that these conditions are variants of the same genetic disorder. Aniridia has been shown to result from mutations in PAX-6, a gene thought to regulate fetal eye development. We are determining whether ADK is also caused by mutations in the PAX-6 gene. We are testing for linkage between PAX-6 and ADK within an ADK family with 33 members over three generations, including 11 affected individuals. We are using a CA-repeat polymorphism located within the PAX-6 gene and two additional CA-repeat polymorphisms that flank the PAX-6 gene (D11S914 and D11S907). The results of this linkage analysis determining if ADK and aniridia are allelic will be presented at this meeting.

ELECTRON BEAM IRRADIATION FOR TREATMENT OF RETINOBLASTOMA: THE ST. JUDE CHILDREN'S RESEARCH HOSPITAL EXPERIENCE

Fontanesi J, Pratt CB, Meyer D, Parham D, and Jenkins JJ; Memphis,TN, USA

Between 8/89 and 8/93, 6 patients who presented with bilateral retinoblastoma (5 synchronous, 1 asynchronous) received electron beam irradiation (10-12 MeV electrons) for treatment of both eyes (n=2) or in the eye with the greatest chance of vision preservation. Two patients had history of familial retinoblastoma. Each child received once daily irradiation utilizing 160 - 180 cGy (prescribed to ensure complete dosimetric coverage of the globe based on CAT measurements) to a total dose of 36 Gy. Reese-Ellsworth stage at presentation included Group I (n=4); Group II (n=2) and Group III (n=2). Follow-up has ranged from 2-48 months (median = 16 months). One eye has experienced local failure which developed at 16 months post irradiation and required enucleation; the remaining 7 eyes have been in continuous local control since completion of irradiation without the use of supplemental cryotherapy. Acute reactions include loss of eye lashes and skin changes in all patients. There have been no significant late complications reported to date. No patient has been diagnosed with either metastatic retinoblastoma or second malignant neoplasm. Based on this initial experience, we continue to investigate the use of electron beam irradiation in the treatment of selected retinoblastoma patients and will discuss the differences in irradiation techniques between electron and photon therapy and will present recommendations for the future.

LOCAL FAILURES IN IRRADIATED UNILATERAL RETINOBLASTOMA

Abramson DH, Niksarli K, DeLillo AR, Gamell LS, Kruger EF, McCormick B, Servodidio CA: New York, United States

A retrospective analysis of 65 unilateral retinoblastoma patients treated initially with external beam radiation between 1958 and 1988 (allowing a minimal follow-up of 5 years) was conducted to determine which patients (tumors) failed radiation therapy and reasons for failure. Twenty-five eyes (38.5%) developed local recurrence after treatment; 96% of the local failures occurred within 2 years of initial diagnosis than those who were successfully treated (1.8 years vs 0.9 years, $p<0.001$). Larger tumors recurred more often (10.7DD vs 5.9DD $p<0.001$). Sixty-nine percent of eyes in Group III-V developed recurrencewhile only 10% of those in Group I-II had recurrence. All but one eye that developed recurrence went on to enucleation. Family history of retinoblastoma, gender, laterality and superior/inferior, nasal/temporal location did not correlate with the development of recurrence. Although no patient has died of retinoblastoma yet, one patient has developed a second tumor. We advise this form of treatment only in very select cases.

ERG ABNORMALITIES IN EYES WITH RETINOBLASTOMA

Brodie SE, Notis CM, Boxrud C, Abramson DH; New York, United States

Purpose: To determine the likelihood of widespread retinal dysfunction in eyes which have survived retinoblastoma, as reflected in abnormalities of the electroretinogram.

Methods: Patients cured of retinoblastoma were drawn from the registry of the Ophthamic Oncology Center at The New York Hospital. ERG recordings were obtained from 15 eyes of 13 patients, using ganzfeld stimulation and a contact lens electrode. ERG's were classified as abnormal if the photopic B-wave amplitude was less than 75uV, if the photopic B-wave implicit time was greater than 33 msec, or if the scotopic B-wave amplitude was less than 250uV.

Results: Overall, electroretinograms were abnormal in 7 of 15 eyes. All ERG abnormalities occurred among the 13 eyes which had been treated with external beam irradiatin. One clinically normal eye in a patient with the germinal mutation for retinoblastoma had a normal ERG. ERG abnormalities were independent of tumor location, and showed no selectivity for rod or cone function. The mean age at initial irradiation of eyes with ERG abnormalities was 4 months, compared with nearly 9 months for irradiated eyes with ERG abnormalities (p=0.044).

Conclusion: These findings suggest a substantial risk of widespread retinal dysfunction following external beam irradiation treatment for retinoblastoma. The risk may be greatest in the youngest eyes.

Supported by Research to Prevent Blindness.

CONSERVATIVE TREATMENT OF SPORADIC UNILATERAL RETINOBLASTOMA

Allamby D.L., Hungerford J.L., Kingston J.E., Plowman P.N., London U.K.

The purpose of this study is to evaluate the role of conservative therapy in the management of retinoblastoma presenting in one eye.

The study comprises a retrospective, consecutive series of children without a family history of retinoblastoma and presenting with uniocular and apparently unifocal involvement. All the children have been followed for at least 12 months.

A total of 32 children fulfilled the inclusion criteria. They received a variety of treatments including plaque therapy, lens-sparing external beam radiotherapy, and whole-eye radiotherapy. The results are presented in terms of eye retention, vision, side effects, cosmesis, and second eye involvement.

A CONGNITIVE APPROACH TO DATABASE SEMANTICS OF CLINICAL DATA OF RETINOBLASTOMA CASES

DI PISA F., HADJISTILIANOU T., MASTRANGELO D., SQUITIERI N. and FREZZOTTI R.; SIENA, ITALY

Clinical data can be useful only if they are validated reliable entities filed in a database system, easily accessible by the medical community. Before any speculative support tool, like statistical or deductive procedures, can be applied, data must satisfy binding constraints to preserve their integrity in (variables) space and in time.

The validation process to produce reliable clinical data is often expensive and needs cooperative resounces. Computer assisted procedures for data entry can produce high quality information, stored permanently and available on the network. A uniform taxonomy, an integrated on-line dictionary of clinical terms, a coherent temporal layout and a persistent spatial integrity on values the variables can assume, all together, can contribute to form a very consistent information basis for data manipulation. The system developed at the Institute of Ophthalmology of the Siena tries to comply the requirements of a valitated and relible database system for the clinics and the epidemiology of retinoblastoma. Cases are analized in detail and examples are provied to substantiate the system characteristics.

PROPOSAL FOR A NEW STAGING SYSTEM FOR RETINOBLASTOMA. Pratt CB, Fontanesi J, Meyer D, Parham D, Elfervig J, Kaste S. Memphis, TN, U.S.A.

The treatment of retinoblastoma is dependent upon many factors, including laterality, lesion size, lesion number, lesion location, lesion extension and metastasis. The systems presently utilized for early treatment decisions often are of limited value because the factors which affect the ability to control ocular disease differ from those factors that affect patients with locally advanced or metastatic disease, where survival is the problem to be addressed. Based on these discrepancies between the most commonly used systems (Reese - Ellsworth, International, St. Jude), we propose a new staging system which includes patient information in regards to ocular control and also addresses the significant findings noted only on histologic examination, including evidence of extraocular disease. This staging system considers tumor(s) confined to the retina (single, multiple, size, location), tumor(s) confined to the globe (optic nerve head, choroid [with or without replacement, anterior chamber), extrachoroidal extension (regional disease with extension to the emissary vessels of the sclera, beyond cut end of optic nerve, through sclera into orbital contents, or a combination of these factors), and distant metastases (to brain, soft tissue bone, bone marrow). Application of this staging system to information from 93 globes from 64 children will be presented, with comparison of the value of this staging schema to that of Reese and Ellsworth.

UNUSUAL INTRAOCULAR INFECTIONS SIMULATING RETINOBLASTOMA

Shields JA, Shields CL, De Potter P, Barrett J, Eagle RC Jr, Philadelphia USA

Among conditions that can simulate retinoblastoma, infectious endophthalmitis is quite rare. A prior review of 500 consecutive children with leukocoria, who were referred with the diagnosis of possible retinoblastoma, revealed that persistent hyperplastic primary vitreous, Coat's disease and presumed ocular toxocariasis were the most frequent simulating conditions. Only 1% of pseudoretinoblastomas were due infectious endophthalmitis. The authors have subsequently been referred several children with unusual forms of infectious endophthalmitis that were difficult to differentiate from retinoblastoma. Examples will be illustrated of streptococcal endophthalmitis, meningococcal endophthalmitis, Candida endophthalmitis, cytomegalovirus endophthalmitis, idiopathic retinovitreal abscess and idiopathic choroidal abscess that simulated retinoblastoma. All of the affected children presented with only ocular findings and no signs of systemic infection. The clinical features that should serve to differentiate these unusual infectious conditions from retinoblastoma will be stressed.

Friday, June 24 *0755 hours*

Franceschetti Lecture "The Marfan Syndrome"

Dr Irene Maumenee, Baltimore, USA

THE CLINICAL SPECTRUM OF POINT MUTATIONS IN THE NORRIE DISEASE GENE.
Lorenz B, Meindl A, Meitinger T; Regensburg/München, Germany.

Norrie's disease is an X-linked disorder characterised by bilateral blindness due to severe retinal dysplasia associated with mental deficiences in about half of the patients, and progressive hearing loss in about one third (Warburg 1961). The Norrie disease gene maps to the short arm of the X chromosome and codes for a protein with predicted structural homologies to growth factors such as TGFß and NGF, all having a characteristic cystine knot (Meitinger et al. 1993). Null alleles and cysteine mutations result in the classical ocular pathology present at or shortly after birth. Point mutations not disrupting the predicted structure of the protein may result in a milder phenotype. We have found two such mutations in two unrelated male patients (Lys 104 Glu, Arg 121 Gln), one with bilateral retinal folds, the other with a retinal fold in one eye and a progressive tractional detachment in the fellow eye. This is in accordance with data from a British family with X-linked exsudative vitreoretinopathy where the mutation (Leu 124 Phe) also causes a conservative amino acid exchange which does not disrupt the predicted structure of the protein (Chen et al 1993). We have found point mutations in two additional unrelated male patients not described so far, and presenting the full-blown ocular phenotype (Arg 74 Cys, Cys 95 Phe).. These mutations are predicted to destroy the cystine knot structure of the protein. Similar mutations affecting the cystine knot have been described previously (Berger et al. 1992, Meindl et al. 1993). The ocular phenotype is severe in all these cases. No correlation was found between the genotype and the incidence of extraocular changes in this latter patient group.

MUTATIONS IN THE ND GENE IN FAMILIES WITH NORRIE DISEASE

Danping Zhu, YingYing Li, and Irene Hussels Maumenee, Baltimore, Maryland.

Purpose. The Norrie Disease (ND) gene was analysed in 19 affected males and from 34 female members from 11 pedigrees with Norrie disease; among them two male fetuses. Methods. Polymerase chain reaction (PCR), single strand conformation polymorphism (SSCP) and DNA sequencing were performed. Results. Gene sequence alterations were found in nine out of 11 pedigrees. A microdeletion was detected in one family using Southern blot analysis with probe L1.28. Different size deletions were found in four pedigrees ranging from complete, partial, and 4bp to single base pair deletion of the ND cDNA. Three missense mutations, Leu62Pro, CysIloArg, and Arg121Gly of exon 3 in the carboxyl-terminal end of the mucin-like protein domain were detected. One pedigree had an xp11.4invq22 involving the Norrie disease region. No mutations have been found to date in two sporadic patients. Family studies demonstrated consegregation between the respective mutations and the disease. Eight carrier females from three pedigrees were identified. Subsequent research was done on a second group of 15 Norrie disease probands. To date, five more missense mutations and two insertions were detected. The combined mutation rate is 16 out of 25 or 64%. PCR, SSCP and direct sequencing are sensitive methods to detect mutations in Norrie disease.

DETECTION OF RB1 GERMLINE MUTATIONS IN RB PATIENTS BY SSCP

Zhang QJ, Minoda K; Ichihara, Japan; Guangzhou, China.

Retinoblastoma (RB) develops in hereditary and nonhereditary forms. Direct detection of the germline mutations of the hereditary form is of importance in presymptomatic prediction and genetic counseling. Small mutations of the RB1 gene in constitutional DNA are usually difficult to detect. We have used PCR and single-strand conformation polymorphism (SSCP) to screen for RB1 germline mutations in constitutional DNA, exon by exon, in patients with genetic RB. Oligonucleotide primers flanking all 27 exons and the promoter region of the RB1 gene were selected and synthesized. The PCR products range in size from 116 to 570bp and the majority can be analyzed directly without the need of restriction enzyme digestion. The amplified products were denatured to obtain single-strand DNA, electrophoresed in nondenaturing polyacrylamide gel, and visualized by silver staining. The mutations were revealed by aberrant electrophoretic migrational patterns. Four out of 16 genetic patients were found to have the mutations, one case in exon 20, other 3 in exon 18. The mutations were identified by sequence analysis. The PCR-SSCP technique is rapid and convenient, requires neither specific facilities nor isotope, and is especially useful in screening the mutations of non-familial genetic patients as well as their brothers and sisters and offspring. It might also be the easiest way to disclose unknown as well as known mutations.

RETINOBLASTOMA GENE MUTATION DETECTION.

Dunn JM, Mostachfi H, Jia Q, Dee G, Gallie BL; Eye Research Institute of Canada and Hospital for Sick Children, University of Toronto, Toronto, Canada.

For the majority of families with retinoblastoma, the only accurate way to predict which infants will be affected is to identify the mutation of the family. Linkage to the mutant chromosome is possible in only the 10% of families who have multiple members affected. However, identification of mutations requires study of the whole gene, since no "hot-spots" for mutation have been identified. Search of the mRNA is only possible if tumor tissue is available, since the mutant mRNA is not detectable in the presence of a normal allele. Therefore the promoter and each of 27 exons must be screened. This has been accomplished by the use of single-stranded conformation polymorphism and other methods to differentiate mutant from normal, but these methods have generally been labor-intensive.

In order to bring mutation identification into the clinical lab, we have developed a semi-automated procedure to find retinoblastoma gene mutations. Initially gene fragments are sized in multiplex PCR reactions and scored for as little as a single nucleotide variation in size on the A. L. F. DNA sequencer. This procedure has been quantitated to allow copy number of each exon to be determined, and detects exon deletions. About 50% of mutations are detected by this test and the gene fragment showing the variation is sequenced to confirm the abnormality. The samples that fail to show any abnormality are then systematically sequenced, using the A. L. F. automated sequencer. A small fraction of mutations involving translocation will be missed; these will be detected by fluorescent in-situ hybridization with retinoblastoma gene genomic probes. Once the mutation of the proband is discovered, other family members can be easily tested.

This strategy will allow us to efficiently identify retinoblastoma gene mutations and provide information to families that will be useful in predicting disease and preventing blindness. The technology and software developed for retinoblastoma mutation identification will be applicable to other genes.

Linkage disequilibrium studies confine the juvenile open angle glaucoma locus within a 5 cM interval on chromosome 1q21-31.

Raymond V[1], Plante M[2], Côté G[3], Anctil JL[3], Trope G[4], Héon E[4], Amyot M[5], Weissenbach J[6], Morissette J[2]. [1]Endocrinologie moléculaire et médecine génétique et moléculaire, CHUL, Québec, Qc, Canada; [2]Réseau de médecine génétique du Québec, CHUL; [3]Dépt. d'ophtalmologie, Hôpital du St.-Sacrement, Québec; [4]Dept. of ophthalmology, University of Toronto, Toronto, Ont; [5]Dépt. d'ophtalmologie, Hôpital Maisonneuve-Rosemont, Montréal, Qc; [6]Généthon, Evry, France.

A subclass of primary open angle glaucoma: juvenile open angle glaucoma (JOAG) is an hereditary disorder of unknown etiology that appears between the ages of three and forty. In 1968, we have described a very large French-Canadian family highly affected by JOAG (Côté et al. Canad. J. Ophthal. 3: 331, 1968). More than 200 members over 4 generations have been investigated; 138 were recruited for this study. Criteria for diagnosis include intraocular pressure ≥ 22 mm Hg and a characteristic optic disk damage and/or visual field impairement. The mode of JOAG inheritance was autosomal dominant and a founder effect was observed. The very large size of this familly allowed us to initiate our search to identify the disease-causing gene exploiting a linkage disequilibrium strategy. AFM microsatellites markers specific to chromosome 1q21-q31 (Gyapay et al. Nature Genet, in press) were selected since linkage of JOAG to this region was recently demonstrated in two Caucasian families (Nature Genet 4: 47, 1993 and Am J Hum Genet 54: 62, 1994). A characteristic haplotype composed of 18 microsatellite markers localized between D1S194 and D1S295 was recognized over a 17 cM interval in 41 affected individuals. Two critical recombination events have been identified on either side of the disease gene. These crossovers confine the JOAG gene locus within a 5 cM segment between two specific markers localized in this interval. The present results thus indicate that linkage disequilibrium is a very powerful approach for narrowing the JOAG locus region.

Supported by Le Réseau de médecine génétique du Québec, Formoeil-Oculus, Les Clubs Lions of the Québec District A-10 and the Medical Research Council of Canada.

FURTHER EVIDENCE FOR A LOCUS FOR AUTOSOMAL DOMINANT JUVENILE GLAUCOMA ON CHROMOSOME 1q AND EVIDENCE FOR GENETIC HETEROGENEITY.

Wiggs J[1], Haines J[2], Paglinauan C[1], Fine A[1], Sporn C[1], Lou D[1], [1]Department of Ophthalmology, New England Medical Center; [2]Molecular Neurogenetics Unit, Massachusetts General Hospital, Boston Massachusetts, USA.

Glaucoma is a term used to describe a group of disorders which have in common a characteristic degeneration of the optic nerve associated with typical visual field defects and usually associated with elevated intraocular pressure. Two percent of white Americans and 6-10% of black Americans are affected by the disease. Compelling data indicate that susceptibility to many types of glaucoma is inherited. Hereditary juvenile glaucoma is one form of glaucoma that develops in children and is inherited as an autosomal dominant trait with high penetrance. Using a single large Caucasian pedigree affected with autosomal dominant juvenile glaucoma, Sheffield *et al.* (Nature Genetics, 1993) discovered positive linkage to a group of markers that map to a 30 cM region on the long arm of chromosome 1 (1q21-q31). We have subsequently identified three unrelated Caucasian pedigrees affected with autosomal dominant juvenile glaucoma that also demonstrate linkage to this region on chromosome 1 with the highest combined LOD score of 5.73 at theta = 0 for marker D1S218. The identification of critical recombinant individuals in our three pedigrees has allowed us to further localize the disease gene to a 6 cM region between markers D1S445 and D1S452. In addition, we have identified several pedigrees which do not demonstrate linkage to chromosome 1q including a black family affected with autosomal dominant juvenile glaucoma that is indistinguishable clinically from the disorder affecting Caucasian pedigrees and two pedigrees affected with the pigmentary dispersion syndrome, a form of glaucoma that also affects the juvenile population and is also inherited as an autosomal dominant trait. These findings provide evidence for genetic heterogeneity in juvenile glaucoma.

ASYNCHRONOUS BILATERAL RETINOBLASTOMA: THE ST. JUDE CHILDREN'S RESEARCH HOSPITAL EXPERIENCE

Fontanesi J, Pratt CB, Meyer D, Elverbig J, Parham D, and Kaste SC; Memphis, TN, USA

Between 5/62 and 7/93, 172 children presented at St. Jude Children's Research Hospital for evaluation and/or treatment of retinoblastoma. Of these patients 65 presented with bilateral disease in the initial diagnosis and 107 patients had unilateral disease at the time of presentation. Of these 107 patients, 9 subsequently developed retinoblastoma in the unaffected eye. Age at first diagnosis ranged from 3 weeks to 24 months (median = 2 months), five of the nine patients have family history of retinoblastoma at the time of initial diagnosis. Time to development of second retinoblastoma was 1 to 61 months post diagnosis. Eight of the 9 patients were females. Treatment of the initial affected eye include enucleation (n=4), chemotherapy (n=3) and irradiation (n=6). Treatment of the second affected eye included irradiation in 6 patients, cryotherapy in 4 patients, and chemotherapy for 4 patients. No second affected eye diagnosed required enucleation. At last follow-up 15/19 eyes remain intact. There have been no documented cases of metastatic spread yet one patient developed second malignant neoplasm and died. This experience reinforces the need for close follow-up for patients diagnosed with retinoblastoma especially those with familial history of retinoblastoma who present with unilateral disease. This close follow-up has allowed for early diagnosis in treatment of the second affected retinoblastoma eye of which 9 of 9 have remained intact without evidence of disease. Recommendations based on this experience will be discussed.

THE TOPOGRAPHY AND AGE OF RETINOBLASTOMA TUMORS

Abramson DH, Gombos DS: New York, United States

Purpose: To study the intraocular geographic location and timing of intraocular retinoblastoma tumor foci.
Methods: Retrospective analysis of tumors in 556 bilateral retinoblastoma eyes diagnosed by us between 1960 and 1985.
Results: 409 eyes (72%) had more than one tumor. There was no correlation between age at diagnosis, sex, laterality, nasal vs. temporal location or superior vs. inferior location. There was a direct correlation between age of tumor presentation and retinal topography. At birth, tumors could be found in any zone of the retina. Tumors in the macula presented at a younger age while peripheral tumors were detected at a later age
($p<0.001$) (macular tumors were diagnosed at an average of 5.6 months and did not present after 15.5 months of age). Once retinoblastoma was diagnosed in one eye it never subsequently developed a new retinoblastoma focus in either macula. Age at diagnosis of the first tumor was inversely related to family history ($p<0.028$). When the retinal surface area of each zone was calculated, it correlated wtih the percent of tumors in that zone.

FREQUENT LOSS OF BOTH COPIES OF A VNTR FROM WITHIN INTRON 16 OF THE RB1 GENE IN RETINOBLASTOMA

Mastrangelo D., Bruni S., Hadjistilianou T., Ormando R., Squitieri N. and Frezzotti R.; Siena, Italy

VNTR regions are informative genetic markers for linkage mapping and individual identification. Some authors have recently developed a procedure for enzymatic amplification of a VNTR located in the intron 16 of the human retinoblastoma (RB1) gene. The core repeat of this VNTR is about 50bp in length and 11 different alleles, ranging from 650 and 1800bp have been detected.
By using this marker in the genetic characterization of our RB families, we have been able to identify loss of heterozygosity in one tumor but the most frequent finding has been the loss of both copies of this VNTR in the tumor cells.
This finding, that to our knowledge has never been reported before, deserves further explanation and its relationship with the loss of heterozygosity for other informative markers located within the RB1 gene should be better understood.
A series of relevant cases showing loss of both copies of this VNTR in tumor cells is illustrated and discussed in detail.

DIFFERENTIAL DIAGNOSIS OF PRIMARY MICROCYSTS OF THE CORNEAL EPITHELIUM

Lisch W, Lisch C; Hanau Germany

Under the term " epithelial microcysts of the cornea" we understand either pellucid or greyish - white changes. The cysts are round, oval, tubular, or clavate in appearance and can develop alone, in contact with each other, or in clumps. Primary microcysts of the corneal epithelium make their initial appearance in 4 diseases in particular:

1. The recovery state of traumatic corneal erosion with pellucid cysts
2. Epithelial basement membrane dystrophy with pellucid and/or greyish cysts
3. Meesmann's dystrophy with pellucid cysts
4. A newly - identified microcystic dystrophy (Lisch,W et al: Am J.Ophthalmol 114:35,1992)with pellucid cysts

Over the past 5 years, we have examined 30 patients with epithelial basement membrane dystrophy, 8 patients with Meesmanns's dystrophy and 9 patients with the New dystrophy. The back-lit slit-lamp examination represent an essential step for the differentiation of the various types of pellucid microcysts and against other epithelial diseases. For example, the whorled corneal opacities of Fabry's disease invariably discloses by retroillumination ultrafine dots, in contrast to the microcysts of the newly-identified whorled microcystic dystrophy.

Our clinical study demonstrates, that each unclear corneal opacity must be examined in retroillumination to evaluate the correct diagnosis.

X-LINKED MEGALOCORNEA - A FOUR GENERATION PEDIGREE

Evans AR, Levin AV: Toronto, Canada

Simple megalocornea (unassociated with other ocular anomalies) is usually inherited in an autosomal dominant fashion. An X-linked form of this condition has been described with close linkage to DXS87 and DXS94 has been demonstrated. We report a four generation pedigree with X-linked megalocornea in which the affected males had corneal diameters measuring between 13.75 and 14.75 mm. The two youngest members of the family had previously undergone surgery for presumed congenital glaucoma, the correct diagnosis only being established after a maternal uncle attended for genetic/family studies. The absence of many classic features of megalocornea in infancy and early childhood may lead to misdiagnosis.

MICROFIBRILLAR ABNORMALITIES IN ECTOPIA LENTIS.

Kielty CM, Charles SJ, Child AH, Shuttleworth CA; Manchester, UK.

Simple (isolated) ectopia lentis and Marfan syndrome are the most common causes of hereditary lens dislocation, associated with abnormalities of the suspensory ligament of the lens (zonule). The glycoprotein fibrillin is a key structural component of the zonule in the form of microfibrils. Mutations in the fibrillin gene (FBN1) have been found in Marfan syndrome patients, but little is known about the effects that these mutations have upon fibrillin structure and microfibril assembly. A new technique for isolating intact microfibrils from fibroblast cell culture has recently enabled abnormalities of microfibril assembly in patients with Marfan syndrome to be visualised by rotary shadowing electron microscopy.

In this presentation we describe gross microfibrillar abnormalities in two unrelated cell lines from patients with simple ectopia lentis using the same technique. In both cases, fibrillin assemblies are characterised by beaded periodicities extending substantially beyond the normal range and poorly organised interbead domains. These abnormalities are distinct from those characterised from Marfan cell lines to date. The observations suggest that ectopia lentis may in some cases reflect impaired microfibrillar capacity to extend and retract in response to lens movement.

Characterisation of abnormalities of microfibrillar assembly will be extremely useful in the correlation of phenotype and genotype in conditions associated with ectopia lentis.

Severe

USHER'S SYNDROME IN CONGENITIALLY DEAF CHILDREN. Marilyn B. Mets, M.D., Nancy Young, M.D. and Arlene Pass, B.S., Northwestern University Medical School, Chicago, IL. COOK COUNTY

Usher's syndrome is an autosomal recessive disorder that includes a sensorineural hearing deficit of varying severity, which is usually nonprogressive and a progressive visual loss that is "retinitis pigmentosis-like". Associated vestibular abnormalities, ataxia, mental retardation, and psychoses, have also been described.

We have screened 69 deaf children (ages ranged from 6 months to 9 years) for Usher's syndrome. The patients were seen initially for possible hearing deficit. Standard otologic and audiologic (behavioral, and in most cases auditory brain response) testing methods revealed previously undiagnosed, bilateral, sensorineural hearing loss ~~that ranged from moderate to~~ profound. All children then had full ophthalmologic examinations, including: vision, anterior segment examination, pupillary examination, cycloplegic retinoscopy, and indirect ophthalmoscopy. Photopic, photopic flicker and scotopic (blue and white) electroretinograms (ERG) were then performed with contact lens electrodes and Gansfeld stimulation, adhering to standard protocol as described by Fishman, et al. ~~Eight~~ 5 children showed definitively abnormal ERGs and were given the diagnosis of Usher's syndrome. The ERGs of ten children were approaching the lower limits of our normal range and these patients were requested to have a repeat testing in two years. The ERGs were normal in 51 patients.

The prevalence of Usher's syndrome among children who are born deaf has been considered to range from 3-6%. Our prevalence is 11.6%.

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RELATION OF VISUAL LOSS TO NEUROLOGIC DYSFUNCTION AND NEW OCULAR FINDINGS IN 23 PATIENTS WITH BATTEN'S DISEASE

Salama H, Maumenee IH, Naidu S, Traboulsi EI; Baltimore, USA

We review the ocular findings in 23 patients with late infantile or juvenile NCL (Batten's disease). 10M and 13F patients ranged in age at onset of visual loss from 1 to 7 years (mean=4.8years). Neurologic dysfunction preceded visual loss in 12 patients by an average of 1.4 yrs, concurred with it in 2 patients, and followed it by an average of 5 yrs in 5 patients; 1 patient had no neurologic symptoms 6 yrs following severe visual loss; 1 patient had normal ocular evaluation, including an ERG and 20/20 vision at age 4.5 years; and in 2 patients the onset of neurologic deterioration was not clear when severe visual loss was evident. One patient had 20/50 vision at age 4 years and 2 had 20/200 vision at ages 5 and 7 years respectively . Vision ranged from HM to NLP in all remaining patients. Visual loss in most patients was rapid, deteriorating to HM or NLP over a period of months. 3 older patients had cataracts at age 17, 21 and 25 years. One patient had a cornea verticillata at age 21. Conjunctival biopsies were diagnostic in 16 patients and agreed with skin and or rectal biopsies in 5 patients. Blindness is a major complication in this group of disorders where patients are surviving longer because of improved clinical care. Older patients may develop lenticular or corneal opacities.

CATARACT FORMATION IN SPIELMEYER-VOGT

Bateman JB, Philippart M; Los Angeles, California, USA

The presenting symptom of Spielmeyer-Vogt, an autosomal recessive ceroid lipofuscinosis, is usually reduced visual acuity in late childhood due to macular degeneration. A generalized retinal and neurologic degeneration progresses. Diagnosis is made on the basis of electron microscopy of lymphocytes or biopsy of conjunctiva or skin; fingerprint inclusions are found in multiple cell types. We reviewed the records of 11 patients with Spielmeyer-Vogt and confirmed retinal degeneration in all. Cataracts which have not been reported previously in Spielmeyer-Vogt occurred in 5 patients and ranged from posterior subcapsular opacities to totally opaque lenses. Significant keratoconus was found in one. In addition to the previously reported retinal degeneration, the ophthalmologic clinical features of Spielmeyer-Vogt include premature cataract formation and keratoconus.

IS THERE A ROLE FOR CHEMOTHERAPY IN THE MANAGEMENT OF INTRAOCULAR RETINOBLASTOMA?

Kingston JE, Hungerford JL, Plowman PN; St Bartholomew's Hospital, London, UK

There is approximately a one in three chance of retaining an eye with Reese-Ellsworth grade V disease treated by external beam irradiation alone. The aim of our study was to see whether the addition of chemotherapy to conventional external beam radiotherapy could improve the visual outcome of children with extensive bilateral retinoblastoma. Between March 1989 and February 1993, thirteen patients with advanced bilateral retinoblastoma were given two courses of chemotherapy (Vincristine, carboplatin and etoposide) prior to external beam irradiation. The more recently diagnosed patients received a further two courses of chemotherapy following completion of radiotherapy. All tumours decreased in size by more than 50% in at least one dimension (usually height) following two courses of chemotherapy. Reduction in elevation of the tumour was found to be the most significant measurement relating to change in volume of the tumour. To date, 18 of the 26 eyes have been retained with a median follow-up of 36 months. Two children had one eye enucleated as a primary, planned procedure. Subsequently, six other eyes have required enucleation, two for the development of glaucoma, one for poor response and three for recurrent disease at 13, 20 and 30 months. From our previous experience with external beam irradiation alone, by this stage, only nine eyes would have been salvaged. Although the addition of chemotherapy to radiotherapy has improved the potential for vision, longer follow-up is required to assess the quality of that vision.

VP 16 AND CARBOPLATIN IN EXTRA-OCULAR RETINOBLASTOMA (ERB) : A STUDY OF THE SOCIETE FRANCAISE D'ONCOLOGIE PEDIATRIQUE.

Doz F[1]., Neuenschwander S., Bouffet E., Plantaz D., Gentet J.C., Méchinaud-Lacroix F., J.P. Vannier, J.M. Zucker [1]Paris, France

ERB still remains a high risk disease.Our aim was to investigate the response rate obtained with the combination of VP 16 and Carboplatin.Between january 1988 and decembre 1993, 19 patients (pts) (14 males, 5 females) with ERB were included.The median age was 35 months (m)(range : 9-125 m).Median interval between the diagnosis of retinoblastoma and ERB was 14 m (range : 0-122 m).Site of ERB was orbital disease (5 pts), combined orbital and bone or bone marrow disease (3 pts), metastatic disease outside the central nervous system (CNS) (7 pts) or CNS disease (4 pts).VP 16 and Carboplatin were both administered day (d) 1 to d5 at a respective dosage of 100 and 160 mg/sqm/d. Evaluation of activity of the drug combination was performed after two courses.**Results**:The response rate was 84% (16/19): complete response (8), partial response (8), progressive disease (2), stable disease (1).Hematologic toxicity was evaluable in the 38 courses.Plateletts transfusions were used in 11 courses ; there was no severe hemorrhage.Erythrocytes transfusions were used in 16 courses.Grade IV neutropenia was observed in 16 courses and hospital admission for fever associated to a neutropenia was necessary in 9 courses.No otoxicity and no significant decrease of glomerular clearance was observed in the 12 evaluable pts.

Conclusions : 1- Combination of VP 16 and Carboplatin is highly effective in ERB. 2- This high response rate is encouraging to use this drug combination as adjuvant chemotherapy after enucleation when indicated. 3- Long term follow-up nevertheless must be the rule because this chemotherapy regimen might be involved in increasing the risk of second tumor in retinoblastoma pts.(This work was supported by the grant n°6498 of the Association pour la Recherche sur le Cancer.)

RESPONSE OF CHEMOTHERAPY (CT) IN EXTRA OCULAR RETINOBLASTOMA (RB)

Antoneli, CBG; Marceno, SR; Lopes, LF; Epelman, S; Novaes, PE; Erwenne, C; Saba, LM; Bianchi, A. Sao Paulo - Brazil.

From Jan 86 to Aug 93, 46 consecutive patients with extraocular (E.O.) RB were referred to our Institution. In patients with bilateral disease we considered the stage of the more extensively affected eye. Mean age at onset of symptoms was 17,6 mo and mean time of referral was 11 mo. 24 female and 22 male. Unilateral tumors in 36 patients and bilateral in 10. 4 patients presented microscopic involvement of scleral emissaries (I), 22 microscopic involvement of the cut end of optic nerve (II), 15 with orbital disease in the biopsy (III), 3 with CNS disease with brain mass or spinal fluid with positive tumor cell (IV) and 2 with blood born or lymphatic metastase (V). In the first period (January 86 to August 91) 34 patients were treated with CDDP + VM26 + I.T. MTX and external Rxt 45 Gy. Patients classified as I (r patients) received VCR and CTX 10 times each 21 days and no radiation therapy. In the second period (Dec 91 to Aug 93), 6 patients received IFO + VP16 x 3 as a window. External radiation therapy 45 Gy and I.T. MTX (except stage I) 2 patients stage IV received the meduloblastomas protocol.

An educational and alert program to address the problem of late referral is the main topic in developing countries.

In our preliminary results, the current approach with more aggressive combined CT is responsible for improvement in the treatment of patients with advanced RB.

ADJUVANT CHEMOTHERAPY FOLLOWING ENUCLEATION FOR RETINOBLASTOMA IN CHILDREN WITH ADVERSE HISTOLOGICAL FEATURES

Kingston JE, Hungerford JL; St Bartholomew's Hospital, London, UK

A proportion of children with retinoblastoma develop metastatic disease. Risk factors include extensive invasion of the optic nerve, choroid and sclera. During the period 1970 to 1985, 378 children newly diagnosed with retinoblastoma were seen in the Ocular Oncology Unit at St Bartholomew's Hospital. Twenty-two children had adverse histological features, of whom, 14 (63%) developed metastatic disease and died. In order to try to prevent the development of metastatic disease in such patients, more recently diagnosed children with adverse histological features have been given adjuvant chemotherapy. Between January 1986 and March 1993, a further 257 new cases of retinoblastoma were seen in our institution. Of these, 16 children with adverse histological features were given adjuvant chemotherapy using a platinum-based regimen. None of these 16 children has developed metastatic disease and all are alive and well with a median follow up of four years. Therefore, adjuvant chemotherapy is beneficial for the majority of children with adverse histological factors and we believe it would be unethical to do a randomised trial of chemotherapy versus no chemotherapy in such patients.

REGRESSION PATTERNS FOLLOWING CHEMOTHERAPY AS A PRIMARY TREATMENT OF INTRAOCULAR RETINOBLASTOMA

FREZZOTTI R., HADJISTILIANOU T., GRAGNOLI A.; SIENA, ITALY

The use of chemotherapy for intraocular retinoblastoma has gained interest during the last years since the tumor appears to respond to various chemotherapeutic drugs (cyclophosphamide, vincristine, carboplatin and etoposide), but the indications are not yet clearly established.

In our personal experience chemotherapy presents the following advantages:

1. to avoid radiotherapy (particularly in hereditary cases at high risk for " second tumors").
2. to shrink and reduce the tumor mass to allow a conservative treatment (even in advanced cases).
3. to use lower photocoagulative energies and consequently eleminate photocoagulation " over dosage " side effects, sparing vision.

The use of chemotherapy as a primary treatment of intraocular retinoblastoma has allowed us to study the regression patterns following this conservative approach. In none of the cases we treated, chemotherapy alone brought to a complete remission.

After the first 3 cycles of chemotherapy the regression patterns we observed were type III for big tumors (areas of cottage cheese and fish-flesh) and type 0 (complete disappearance of the tumor) for small ones.

We discuss the protocols we used, dosage and side effects and the followup of our series of cases.

CHEMOREDUCTION OF INTRAOCULAR RETINOBLASTOMA.

Murphree AL, Steele D, Malogolowkin M, Sato J; Los Angeles, USA.

We have significantly reduced intraocular tumor volume in each of 15 patients with bilateral retinoblastoma using one or two monthly cycles of VP16 (100 mg/M^2/day) days 1, 2 and 3, carboplatin (560 mg/M^2) day 1and vincristine (1.5 mg/M^2) days 1, 8 and 15. In no case has there been failure to respond. We have found this drug combination useful in 2 clinical settings: (1) in the absence of vitreous seeding even when total retinal detachment is present. The reduction in tumor volume has been sufficient to allow curative local therapy (cryo, plaque or thermochemotherapy) and (2) in the presence of vitreous seeding 2 monthly cycles both preceding and following external beam radiotherapy shows promise. Primary chemoreduction may allow the elimination of external beam radiotherapy in certain genetically predisposed children.

The triple drug protocol can be modified slightly by the addition of laser hyperthermia. We have used chemothermoreduction in the occasional tumor which failed to respond adequately or recurred following thermochemotherapy.

CYCLOSPORIN-MODULATED CHEMOTHERAPY USED WITH FOCAL THERAPY: A NEW APPROACH TO RETINOBLASTOMA.

H.S.L. Chan, G. DeBoer, G. Koren, J.J. Thiessen, P.S. Thorner, E. Giesbrecht, G. Haddad, Z. Verjee, V. Ling, and B.L. Gallie; Hospital for Sick Children, Toronto-Bayview Regional Cancer Center, Ontario Cancer Institute, Faculty of Pharmacy, University of Toronto, Toronto, Canada.

The conventional treatment for bilateral retinoblastoma is irradiation of one or both eyes, associated with the severe complication of second malignancy. Increased expression at diagnosis of P-glycoprotein, the anticancer drug efflux transporter, confers multidrug resistance and may explain why chemotherapy has generally failed to control intraocular tumor growth. Cyclosporin A can counteract P-glycoprotein *in vitro*. We compared results of chemotherapy with and without cyclosporin A, combined with subsequent destruction of residual tumor using 532 and 1064 nm laser, for effectiveness in controlling intraocular retinoblastoma. In a phase I-II study, cyclosporin A doses have been increased from 4 mg/kg to 33 mg/kg. Favorable initial responses (12/16 vs 7/18) are more frequent (P=0.045), and actuarial relapse-free rate is higher (80% vs 39%) with cyclosporin than without. Median followup of cyclosporin patients is still short (1.5 years) compared to historic controls (3.6 years). Since relapses in the control groups virtually all occur by one year, and all cyclosporin patients are currently off therapy and most have been followed beyond one year, the present responses are likely to be durable. Improved responses correlate with both higher mean ($P = 0.019$) and higher cumulative CSA doses ($P = 0.019$) in individual RB patients, as do relapse-free rates ($P = 0.031$ and $P = 0.028$). We conclude that cyclosporin may improve response to chemotherapy, which together with focal therapy may cure retinoblastoma without the use of radiation.

CORNEAL TOPOGRAPHY IN DOWN SYNDROME

Weiser BA, Stein RM, Levin AV; Toronto, Canada

Keratoconus has been shown to occur in up to 6 - 8% of patients with Down Syndrome. Computerized corneal topography has revealed subclinical keratoconus in relatives of patients with keratoconus without Down syndrome which suggests autosomal dominant transmission with variable expressivity and penetrance. We used the EyeSys Computer video photokeratoscope to study corneal topography in 28 eyes of 15 children with Down syndrome. We found a significantly higher incidence of steeper corneas in our patients compared to previously reported keratometry readings in normal children. These topographic abnormalities, with relative inferior steepness consistent with subclinical keratoconus may be indications of early keratoconus development or a clinically unapparent varient. These clinical findings support a link between keratoconus and chromosome 21. This study is the first to use the video photokeratoscope to study patients with Down syndrome.

A SIBLING WITH MESENCHYMAL DYSGENESIS OF THE OCULAR ANTERIOR SEGMENT.

IKEDA K, SHIRAI S, MAJIMA A; Nagoya, Japan

Neural crest-derived mesenchymal cells have been generally acknowledged to make a major contribution to the tissues of corneal endothelium and storma, storma of iris, stroma of ciliary body, and trabecular meshwork. Therefor, congenital anomalies of the anterior segment such as congenital glaucoma, posterior embryotoxon, Axenfeld-Rieger syndrome, Peters' anomaly and sclerocornea have been believed to provide possible evidence of abnormalities in the migrations of neural crest cells.

We encountered a sibling, the proband referred to our clinic for examination of congenital corneal opacity in both eyes, who was diagnosed as having Peters' anomaly. Her elder sister came to our clinic for family examination and was diagnosed as having congenital glaucoma in both eyes. It was clinically assumed that these two diseases fall within the same spectrum based on abnormalities in the migrations of neural crest cells.

VIDEOKERATOGRAPHY IN FAMILY PEDIGREE ANALYSIS OF FAMILIAL KERATOCONUS.

Bhatt S[1], Bahri S[1], Maureen Lundergan[2], Rabinowitz Y[1]; Cedars Sinai Medical Center, Los angeles[1], University of Utah, Salt Lake City, Utah[2].

Purpose: To demonstate the utility of videokeratographs (VK) in constructing family pedigrees and determining modes of heredity in familial keratoconus (KC).

Methods: We constucted a family pedigree with 234 family members spanning 4 generations of a proband with KC. 26 family members were examined clinically and with VK using the Topographic Modelling System. VK from each patient were compared to a set of VK derived from 400 normal eyes, to identify patterns typical for 'early KC'.All Vk were analysed with a computer software program Conecare TM which generates VK indices descriptive of the KC phenotype: K (central steepening) and I-S values (inferior - superior dioptric power asymmetry) and compared to the VK indices derived from the 400 normal control eyes.

Results: Of the 26 patients examined 13 were affected and 13 were normal. Of the 13 affected, 8 were affected by clinical examination, 5 patients were clinically normal but had VK patterns suggesting 'early KC'. The 16 normal patients had VK patterns and indices within 2 SD of the normal controls.

Conclusions: VK are useful in family pedigree analysis of KC. Using VK in this family, we demonstrated an autosomal dominant mode of inheritance with variable expressivity.

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CONGENITAL ALACRIMA: ASSESSMENT OF AN AUTOSOMAL DOMINANT PEDIGREE.

Charles SJ, Lloyd IC, Clayton-Smith J, Tullo AB; Manchester, UK.

Congenital alacrima (deficient tearing) is a rare condition usually associated with systemic conditions such as anhydrotic ectodermal dysplasia and familial dysautonomia. There are very few reports of isolated congenital alacrima.

We report a pedigree with 4 persons in 3 generations affected by deficient lacrimation from infancy. Male-to-male transmission was present suggesting autosomal dominant inheritance. Other associated ocular features were photophobia, blepharophimosis, lower lid hypotrichosis and punctate corneal erosions. Salivation was normal. Some cases suffered from asthma but there were no other associated systemic abnormalities.

CORNEAL DEGENERATION IN SEVERE TURNER'S SYNDROME WITH A PECULIAR KARYOTYPE: A SIGN OF SYNDROME OR A NEW MUTATION?

C Fiore, A. Cianchetti, P. Giovenali, S. Ignagni, A. Calabro. Perugia, Italy.

We report the case of a 28-year-old woman who presented a mesomelic dwarfism with hypoplastic forearms and legs; dorsal-lumbar scoliosis; primitive amenorrhea and severe sexual immaturity; mental deficiency and opacification of corneal anterior strata with neovascularization located on the third anterior part of the stroma. The karyotype is: 45, XO/46, X del (pter → p11). The cellular lines are represented by 50% each. The maternal karyotype is 46,XX/45, XO (the line 45/XX is 10%). The patient underwent penetrating keratoplasty on one eye. The results of histologic examinations are described.

PRENATAL DIAGNOSIS OF RETINAL DETACHMENT IN THE WALKER-WARBURG SYNDROME

Levin A, Chitayat D, Toi A, Michaud J, Babul R, Summers A, Blaser S; Toronto, Canada

Prenatal detection of retinal detachment by ultrasound may represent the presence of a true retinal detachment or congenital nonattachment of dysplastic retina. We report a case of prenatal detection of Walker-Warburg syndrome based on antenatal ultrasound abnormalities including hydrocephalus and retinal detachment. Post-partum, this live born male showed severe hydrocephalus, pachygyria, dysplastic nonattached retina, and a variant of Peters anomaly with iridocorneal strands and a defect in Descemet membrane in an otherwise clear cornea. In the absence of clear genetic linkage studies or gene identification, prenatal diagnosis of this autosomal recessive condition becomes particularly important.

OCULAR ABNORMALITIES IN THE PRENATAL ULTRASONOGRAPHIC DELINEATION OF GENETIC DISORDERS

Toi A, Chitayat D, Babul R, Van Allen M, Kent N, Levin AV; Toronto, Canada

With the improvement in fetal ultrasound resolution, examination of the ocular structures has become an important tool in prenatal identification of genetic disorders. We report on the prenatal detection of ocular abnormalities in five fetuses resulting in the delineation of the fetal condition.

Case 1: A fetal U/S done at 20.9 weeks gestation revealed a single central eye, proboscis, and a single cerebral ventricle with unilobar brain. The findings were confirmed on fetal autopsy.

Case 2: Fetal U/S done at 25.8 weeks gestation revealed right orbital and cerebral cyst with hypoplasia of the maxilla and nose. The prenatal diagnosis was Delleman syndrome. Fetal autopsy showed features consistent with this syndrome but an apparently new genetic disorder cannot be ruled out.

Case 3: A fetal U/S done at 22 weeks gestation on a primigravida woman whose husband has Crouzon syndrome revealed proptosis with anterior position of the eyelids and hypertelorism consistent with Crouzon syndrome. The diagnosis was confirmed postnatally.

Case 4: Fetal U/S done at 18 weeks gestation revealed absent eyes, absent cranium with brain tissue floating in the amniotic fluid and cleft upper lip. A diagnosis of amniotic band sequence was confirmed postnatally.

Case 5: Fetal U/S done at 24 weeks gestation revealed anophthalmia and severe right microphthalmia. There was a single cerebral ventricle. The findings were consistent with semilobar holoprosencephaly and this was confirmed on fetal autopsy.

FAMILIALITY IN AGE RELATED MACULAR DEGENERATION.

Bhatt S, Warren C, Raffel L, Rotter J, Nesburn A, Kenney MC.

Age related macular degeneration is a major cause of blindness in developed countries. It still remains a very poorly understood disorder. Both environmental and genetic factors have been implicated in its pathogenesis, but none have been firmly established. This study was undertaken to assess the familiality of this condition.

Study participants from an office based ophthalmologist's practice were interviewed for their occupational, smoking, sun exposure and family histories. 27 probands with age related macular degeneration (ARMD) and 17 age and sex matched controls were included in this study. In the ARMD group, 22 % (6 / 27) of probands reported a positive family history of ARMD; 10 of the102 first degree relatives (parents, siblings, offsprings) over age 60 were reported to be affected. In the control group, none of the 50 first degree relatives had a history of ARMD. This difference is statistically significant (p value = 0.031). In addition, 11 % of the ARMD probands (3/27) gave a history of a second degree relative (grandparent, aunt, uncle) affected with ARMD, compared to none known among the relatives of controls.

We conclude that familiality plays an important role in the pathogenesis of age related macular degeneration. Identification of familiality is a necessary first step in identifying the pattern of inheritance of this highly prevalent and debilitating condition.

(Supported in part by the Dr. Henry and Lilian Nesburn research program in macular degeneration.)

EVALUATION OF ACTIVITY OF FUNCTIONALITY OF COCHLEAR OUTER HAIR CELLS IN PATIENTS WITH RETINITIS PIGMENTOSA AND IN THEIR RELATIVES.

Fiore C., C. Cagini, P. Menduno, G. Ricci, E. Molini, A. Pennacchi, Simoncelli C. Perugia, Italy.

The hearing function was studied in 44 patients affected by Retinitis Pigmentosa (R.P.) and in 31 of their relatives. 30 patients and 25 relatives showed bilateral normal hearing when examined with traditional audiometric methods. In these normal hearing patients and in normal hearing relatives Evoked Otoacoustic Emissions (EOEs) were studied. The EOEs offer a unique opportunity to measure objectively the function of the ear outer hair cells. The data obtained with the EOEs were statistically compared with those obtained from a homogeneous control-group of normal subjects. In normal hearing subjects with R.P. and in normal hearing relatives the amplitude, the intensity and the frequency values of the EOEs were statistically lower than those of control subjects. The EOEs recorded in 18 normal hearing RP patients and in 6 normal hearing relatives appeared to be markedly damaged. Thus, a subclinical alteration of the Organ of Corti was found in 60 % of the patients affected by Retinitis Pigmentosa and in 24 % of their relatives. The alteration of cochlear outer hair cells in a high percentage of patients with RP and in some of their relatives seems to corroborate the hypothesis that, in many cases, Retinitis Pigmentosa may be due to a structural anomaly of the ciliated cells.

SEVERE INFANTILE CARDIOMYOPATHY AND INFANTILE CONE-ROD DEGENERATION: ALSTRÖM SYNDROME.

De Becker I, Tremblay F, LaRoche R, Shea S, Nanton M, Ludman M. Halifax, Canada.

Alström syndrome is a rare autosomal recessive disorder (MIM 203800) resembling Bardet-Biedl because of the combination of obesity, retinal degeneration, and diabetes mellitus However, patients with Alström syndrome have a very early onset cone-rod degeneration during the first few months of life, as opposed to the later onset of a rod-cone degeneration in Bardet-Biedl. Alström patients do not have polydactyly or hypogonadism, and they develop sensorineural deafness. This disorder is unusually frequent among the French Acadians in Nova Scotia, Canada.

We present 3 patients who first came to medical attention because of a severe infantile cardiomyopathy. Several weeks later, they were diagnosed has having a severe cone-rod dystrophy and were found to be above the 97th percentile for their weight. Sufficiently long follow-up in two of these three patients lead to a firm diagnosis of Alström syndrome, and all three patients were found to be part of an extended kindred with Alström disease.

The combination of severe infantile cardiomyopathy followed within months by a diagnosis of severe cone-rod degeneration should evoke the diagnosis of Alström disease. The clinical features of cardiac failure, retinal degeneration, sensorineural hearing loss, and diabetes mellitus suggest that the mitochondrial metabolism may be at fault in these patients.

STICKLER SYNDROME: CORRELATION BETWEEN VITREO RETINAL PHENOTYPES AND LINKAGE TO COL 2A1.

Snead MP, Payne SJ, Barton DE, Yates JRW, Al Imara L, Pope FM, Scott JD.

This study examines the linkage of two vitreo retinal phenotypic subgroups of Stickler syndrome to COL 2A1. A total of 97 affected patients from 24 pedigrees were examined. This is the largest published series of Stickler syndrome patients to date and all have undergone full clinical and ophthalmological examination by a single investigator (MPS). A new clinical classification is proposed based on vitreo retinal phenotype. All patients demonstrating the congenital vitreous anomaly have been designated Stickler syndrome Type 1 and those without the congenital vitreous anomaly as Stickler syndrome Type 2 patients. There were 69 affected patients from 20 unrelated Type 1 pedigrees and 28 affected patients from 4 unrelated Type 2 pedigrees. Using two markers, Stickler syndrome Type 1 pedigrees showed complete linkage to COL 2A1 with a maximum lod score of 12.33, at zero recombination. Linkage to COL 2A1 was excluded in the two Type 2 pedigrees that were informative. From these data it appears that this new clinical classification is a useful first step in resolving the genetic heterogeneity in this condition.

DEVELOPMENT OF A YAC CONTIG IN THE MINIMAL REGION OF X-LINKED CSNB IN Xp11.

Boycott, KM, Gratton, KJ, Moore, BJ, *Johnson, D, Bech-Hansen, NT; Calgary, Canada and *St Louis, USA.

X-linked congenital stationary night blindness (CSNB1) is a disorder that includes impairment of night vision, myopia, reduced visual acuity, and congenital nystagmus. Electroretinography reveals a marked reduction of the b-wave suggesting that individuals with CSNB1 have a defect in the bipolar layer of the retina. The CSNB1 locus has been mapped to a 7 cM region on Xp11.23-Xp11.22, bounded centromerically by DXS988 and telomerically by DXS426. Using radiation and conventional hybrids a detailed map of new and published STSs has been generated for the minimal region of CSNB1. Primer sequences for twenty-three sequence tagged sites were used to isolate YAC clones from the CEPH, mega CEPH, and X-chromosome-specific YAC libraries by PCR-based screening. The YACs were characterized for STS overlaps and assembled into six contigs across this region. YACs that cover the gaps between these contigs are being sought by PCR-based screening with primer pairs derived from the sequences of the YAC end-clones. End-clone analysis has demonstrated chimerism in as many as half of the YACs.

Screening of fetal and adult retinal and placental cDNA libraries using selected YACs and the direct selection method is being undertaken to identify genes which map within the CSNB1 minimal region. Such genes would be candidate genes for CSNB1.

This work has been funded by the RP Research Foundation. KMB is the recipient of the RP Research Foundation Cook-McCann Studentship.

RFLP ANALYSIS OF CHOROIDEREMIA IS COMPLICATED BY QUESTIONABLE PATERNITY. Sutherland J, Musarella M, Popovich B and Ray P; Toronto, Canada.

Prior to the isolation of the choroideremia gene, a woman whose father, paternal uncle and two paternally related first cousins were affected with choroideremia sought genetic counselling about her reproductive options. Her ophthalmological examination was normal however in X-linked conditions, all of the daughters of an affected male, are carriers each having a 25% risk of an affected child. Rather than opting to terminate all male pregnancies, of which 50% would be unaffected sons, she chose to undergo a family linkage study to distinguish which X-chromosome was paternal in origin and thus carried the choroideremia mutation. Prenatal diagnosis could then be offered based on paternal X-chromosome exclusion. Blood samples were obtained from the woman, her 2 sisters, their mother and her nephew with the expectation that markers (RFLPs) would identify the paternal X-chromosome. Molecular analysis was not consistent with the pedigree. With hesitation, the mother indicated that all three of her daughters were products of "mixed" artificial insemination (AI) (mixing sperm from father with an unknown donor). Subsequent molecular identity testing indicated that all three sisters had different paternally derived X-chromosomes and therefore different biological fathers. Since none of the daughters shared any alleles with their affected cousin, it is likely that their father was not their biological father, eliminating their risk of having an affected son. This molecular testing could have been avoided with thorough genetic counselling which included a discussion of the importance of accurate paternity.

CLINICOPATHOLOGIC CORRELATION OF RETINOBLASTOMA SUCCESSFULLY TREATED WI
TEMIC CHEMOTHERAPY.

Greenwald MJ, Strauss LC, O'Grady RB; Chicago, USA

There has been little documentation of the effectiveness of systemic chemotherapy management of intraocular retinoblastoma. We report a case in which complete regressi large tumor was confirmed pathologically after treatment only with intravenously admin drugs.

A 7-month-old boy with sporadic bilateral retinoblastoma had multiple small to mod large lesions with vitreous seeding in the right eye, and a solitary mass with total detachment in the left eye. Initial treatment consisted of 6 monthly cycles of carboplatin a 16. Within 3 months the left eye's tumor regressed to a calcific mass, and all subretina resorbed. At age 26 months, this eye, which never recovered useful vision, was enuc because of inability to control with cryotherapy a moderate-sized but rapidly growing recently arisen *de novo* at some distance from the original mass.

Histopathologic examination of the globe demonstrated a large fibro-calcific mass in the i temporal quadrant of the retina. No cells identifiable as retinoblasts were found on mu sections. In contrast, the smaller discrete active tumor whose growth prompted enucleatio composed mainly of undifferentiated retinoblasts with numerous mitoses.

The right eye showed an encouraging initial response to chemotherapy, but subsequ required external beam radiation because of recurrent implantation from the vitreous. months after completion of treatment this eye appears to be free of active disease, with visual function.

TREATMENT OF VITREOUS SEEDS OF RETINOBLASTOMA RECURRENT AFTER RADIOTHERAPY USING VITREOUS INJECTION OF MELPHALAN COMBINED WITH OCULAR HYPERTHERMIA

Kaneko A, Inomata M, Ueda M, Tanabe J; Tokyo, Japan

Retinoblastoma with vitreous seeds is one of the most difficult problems to be solved for successful conservative treatment. Radiotherapy is sometimes effective in treating vitreous seeds. However vitreous seeds recurring after radiotherapy were almost impossible to cure without enucleation of the eyeball. We have reported that retinoblastoma is very sensitive to melphalan and hyperthermia increases that sensitivity. Because of the results of this basic research, vitreous injection of melphalan followed by ocular hyperthermia was tried to save 8 eyes with unilateral retinoblastoma with recurrent vitreous seeds after radiotherapy from 1990 to 1993. The success rate of this treatment was 88%. No orbital recurrence was found. This treatment is safe and effective for the preservation of the eyeballs with vitreous seeds recurrent after radiotherapy.

THERMOCHEMOTHERAPY FOR RETINOBLASTOMA: PROTOCOL, INSTRUMENTATION AND OUTCOME.

Murphree AL, Steele D, Malogolowkin M, Sato J; Los Angeles, USA.

In 1990, as an alternative to external beam radiotherapy, we introduced the concept of focal thermochemotherapy for the management of localized posterior pole tumor(s) in the second eye of a child under age 6 months. We have now treated more than 80 individual tumors with focal thermochemotherapy and have an overall tumor control in excess of 75%. Tumors which respond best are 6 DD or less in greatest diameter and are located in the posterior pole. The treatment protocol consists of Carboplatin (560mg/M^2) given on day 1 followed within 1-4 hours by transpupillary diode laser hyperthermia (continuous mode, 300-700 mw out of fiber, 0.8 to 2.0 mm spot, for 20-30 minutes per individual tumor). The hyperthermia is repeated once between days 4 and 7. The monthly cycle (chemo, laser, laser) is repeated for at least 3 months. The diode laser and microscope delivery system designed especially for thermochemotherapy are available internationally from Iris Medical.

Thermochemotherapy allows successful management of small posterior pole tumors in the young bilaterally affected child and may prevent the use of external beam radiotherapy. Focal thermochemotherapy is not indicated in the presence of vitreous seeding.

Doz F., Peter M., Schleiermacher G., Vielh P., Putterman M., Validire P., Desjardins L., Dufier J.L., Zucker J.M., Delattre O.
Paris, France

N-myc amplification, loss of heterozygosity (LOH) in 1p and DNA index in tumor specimen of 45 patients (pts) with retinoblastoma

Biological criteria might be helpful for therapeutic decision in retinoblastoma. Since retinoblastoma as neuroblastoma is an embryonal neuro-ectodermal tumor we looked for criteria described as bad prognosis factors in neuroblastoma : n-myc amplification, 1p deletion and DNA index.

N-myc copy number was evaluated in 45 tumor samples (45 pts) by spot blot procedure. N-myc amplification was observed only once.

Analysis of LOH 1p was performed by PCR analysis in 43 tumors samples (42 pts).1p deletion was observed in 9 pts(4 eyes, 1 orbit and 4 metastases). 1p deletion was more frequent in metastases (4/8) than in eye or orbit disease (5/35) ($p<0.05$).

DNA index was evaluated by flow cytometry in 30 pts : tumors were diploid in 25 cases, multiploid in 1 case, aneuploid in 4 cases.

More patients with more follow-up are necessary to assess a possible prognostic value of these parameters in retinoblastoma and their relationship with stage and differenciation.

DNA TESTING IN RETINOBLASTOMA FAMILIES IS COST EFFECTIVE.
Mackey DA1, Maumenee IH2. 1 Melbourne, Australia; 2 Baltimore, USA

DNA testing in retinoblastoma (RB) has progressed since the sequencing of the retinoblastoma gene. The use of intragenic polymorphic sites allows accurate linkage analysis of affected individuals, tumour and relatives. Similarly RB gene sequencing allows identification of the site of the mutation and the precise identification of carriers within families. The use of such powerful information allows clinicians to avoid unnecessary examinations under anaesthesia (EUA). Aside from the obvious benefits of avoiding unnecessary EUAs and anxiety, DNA testing is less expensive than the cost of multiple examinations.

THE POLYMERASE CHAIN REACTION AND ITS USEFULNESS IN THE GENETIC CHARACTERIZATION OF RETINOBLASTOMA PATIENTS: A TOOL FOR CLINICAL LABORATORY.

MASTRANGELO D., HADJISTILIANOU T., FREZZOTTI P., SQUITIERI N. and FREZZOTTI R. ; SIENA , ITALY

The Polymerase Chain Reaction (PCR) is an easy and widely diffused molecular technique which allows the manipulation of large amounts of DNA starting from a relatively low quantity of template. Sequence polymorphisms from within intron/exon 1 and 17 of the RB1 gene can be analyzed by coupling the PCR and enzymatic digestion of the amplified sequences with BamHI and XbaI respectively. Futhermore, by using appropiate oligonucleotide primers, variable number of tandem repeats (VNTR) sequences, from introns 17 and 20 of the RB1 gene, can be amplified and directly analyzed by polyacrylamide gel electrophoresis. Moreover a number of variable and highly polymorphic dinucleotide repeats (Microsatellites) from within chromosome 13 and the Rb1 gene, have been recently discovered and completely characterized. Finally, by the use of appropriate oligonucleotide primers and PCR, it is possible to amplify all the 27 exons of the RB1 gene that can be further analyzed by semiautomated SSCP for mutation screening. All the above mentioned markers and analytical methods make the PCR approach to the diagnosis and characterization of RB families, a pontentially invaluable tool extremely useful for clinical laboratories. A few examples of these tools and techniques are shown and their implications for clinical laboratory are discussed.

TRILATERAL RETINOBLASTOMA INCIDENCE AND OUTCOME: A DECADE OF EXPERIENCE

Blach LE, McCormick B, Abramson DH, Ellsworth RM: New York, United States

Purpose: The literature for children with trilateral retinoblastoma (TRB) is made of anecdotal case reports, rather than population-based studies. This report examines the incidence and outcome of TRB in children treated for retinoblastoma (RB). A group of patients who are at highest risk for the development of TRB is defined.

Methods and Materials: Between 1979 and 1990, 117 children were treated with external beam radiation therapy for RB, (97/117, bilateral). Median follow-up time was 68 months. The median age at diagnosis was 7 months.

Results: Six cases of TRB were identified. The incidence of TRB in children with bilateral RB was 6% (6/97) and 10% in those with a family history of RB. The median age at diagnosis of RB for children with TRB was 3 months, younger than the median age of the entire retinoblastoma group. In all cases, the pineal region was excluded from the radiotherapy fields. Treatment for the TRB consisted of craniospinal axis radiation therapy and chemotherapy in 3 patients, chemotherapy alone in 2, and no treatment in one. All patients died from this disease. Overall, of the 117 children treated at our institution for RB over the last decade, 12 have died. TRB was the major cause of death, accounting for 50% (6/12) of deaths.

Conclusion: Trilateral retinoblastoma is a major and under-appreciated cause of mortality in the first 5 years after the diagnosis of bilateral retinoblastoma. A more aggressive approach toward screening a defined population of childhood retinoblastoma survivors may be warranted.

MULTIDISCIPLINARY MANAGEMENT OF TRILATERAL RETINOBLASTOMA

Roarty JD, Trese MT, Ravinindrath V, Latin P, Slovis TL; Detroit, United States

An eight month old female with a family history of retinoblastoma presented with bilateral ocular tumors and a non-calcified pineal tumor. The patient was initially treated with chemotherapy. Once off chemotherapy, ocular tumor recurred. Over a three year period, treatment included cryotherapy, argon laser, brachytherapy, external beam and enucleation. The pineal tumor has not recurred. The patient is presented as an unusual case of trilateral retinoblastoma with a non-calcified pineal tumor and to emphasize the usefulness of a multidisciplinary approach to retinoblastoma.

ORBITAL VOLUME OF LONG-TERM SURVIVORS OF RETINOBLASTOMA TREATED WITH ORBITAL IRRADIATION.

Kaste, SC, Crom DB, Fontanesi J, Pratt, CB; Memphis, TN, USA.

Sequelae of orbital irradiation in children 5 years of age are not well described. We present our findings of orbital growth based upon computed tomographic (CT) volume measurements ($\triangle V$) with comparison between anterior and lateral irradiation (XRT) fields; XRT dose levels; age at treatment <vs> 1 retinoblastoma (RB) (n=16 globes).

Four children were treated with 3000 to 4220 cGY lateral field XRT: 2 received bilateral XRT; 1 unilateral XRT; 1 unilateral and contralateral anterior XRT. Median $\triangle V=5.1cm^3$ (range 2.6-14.6cm^3).

Seven patients were treated with 2800 to 4400 cGY anterior XRT. Median $\triangle V=5cm^3$ (range, 0-29.2cm^3).

In all 4 children > 1 year of age treated with unilateral XRT, the irradiated orbit was the smaller (median $\triangle V=11.3cm^3$, range 4.4-23.1cm^3). In 4 of 8 children < 1 year of age (7 with bilateral disease treated with unilateral enucleation and contralateral XRT; 1 with unilateral disease treated with unilateral XRT), the irradiated orbit was the larger (median $\triangle V=4.7cm^3$, range 0-29.2cm^3).

Six globes received 2501 to 3600 cGY XRT with the resultant median $\triangle V=4.7cm^3$ (range, 0-29.2cm^3). The irradiated globe was the larger in 3 of these patients. Eight received 3600 cGY with median $\triangle V=5.4cm^3$ (range, 1.3-23.1cm^3) with 1 having the irradiated side larger.

Conclusion: Bony orbital development in long-term survivors of RB is influenced by therapy and patient age at therapy. The median $\triangle V$ in all subgroups was approximately $5cm^3$ though the ranges varied widely. The final outcome appears to be multifactorial. With a larger series, determination of those factor combinations yielding the greatest growth disturbance may be determined.

ORBITAL DEVELOPMENT IN LONG-TERM SURVIVORS OF RETINOBLASTOMA

Kaste SC, Crom DB, Fontanesi J, Mounce KG, Elfervig J, Meyer D, Pratt CB; Memphis, TN, USA.

Long-term sequelae of therapy for retinoblastoma (RB) have not been well described. We therefore, undertook to evaluate orbital growth in long-term survivors of RB and assess the effects of enucleation, irradiation therapy (XRT), and patient age at time of therapy.

Orbital volumes of 24 patients (unilateral, n=11; bilateral, n=13) were calculated from orbital CT studies. Patients were stratified by age at diagnosis and therapeutic regimen (enucleation vs XRT). All enucleated globes were replaced by a prosthesis.

Median orbital volume differences (ΔV) were: enucleation without XRT, $\Delta V = 6.7$ cm with the enucleated orbit smaller than the uneffected orbit; 7 of 12 patients with bilateral disease, unilateral enucleation and contralateral XRT, $\Delta V = 6.4$ cm^3 with the irradiated side smaller; bilateral XRT for bilateral disease (n=2) resulted in $\Delta V = 5$ cm^3 and 2.6 cm^3.

All children treated at $\geq$ 1 year of age for bilateral disease with unilateral enucleation and contralateral irradiation, developed a smaller orbit on the irradiated side ($\Delta V = 22.5$ cm^3. Half of patients similarly treated at < 1 year of age developed a smaller orbit on the irradiated side and half on the enucleated side ($\Delta V = 4.7$ cm^3).

Enucleation and irradiation both result in abnormal orbital development. The severity of growth disturbance varies with patient age at treatment and type of treatment. Prosthetic globes have improved orbital growth and facial symmetry.

SECOND MALIGNANT NEOPLASMS IN PATIENTS WITH RETINOBLASTOMA: ST. JUDE CHILDREN'S RESEARCH HOSPITAL EXPERIENCE

Fontanesi J, Pratt CB, Parham D, and Meyer D; Memphis,TN, USA

Between 5/62 and 7/93, 172 children with preliminary diagnosis of retinoblastoma were evaluated for treatment at St. Jude Children's Research Hospital. Of these 165 patients, 65 patients presented with bilateral retinoblastoma, 107 patients presented with unilateral retinoblastoma. During follow-up which ranged from 2 to 350 months (median = 175 months), 6 children (3.9%) developed second malignant neoplasm. All patients presented with bilateral disease and two patients had familial history of retinoblastoma. Four patients developed osteogenic sarcoma within this irradiated volume, one child developed a basal cell carcinoma in the temporal region within the irradiation field and one patient was diagnosed with a lower extremity Ewing sarcoma. Those patients with second malignant neoplasms within the irradiated field presented 125 to 194 months post irradiation. Initial treatment dose ranged between 32 and 43.76 Gy. Three patients were treated with anterior field irradiation, two patients had anterior and lateral field irradiation, one patient received lateral field irradiation.

At last follow-up 4/6 patients have died of a second malignant neoplasm. The cumulative risk for development of SMN for patients with bilateral disease is 22% at 25 years. The specifics of the treatments associated with these second malignancies and reasons for the lower than expected incidence of second malignant neoplasm will be discussed.

NON-OCULAR TUMOURS IN PATIENTS TREATED WITH MEGAVOLTAGE EXTERNAL BEAM IRRADIATION FOR RETINOBLASTOMA.

Imhof S.M., Moll A.C., Mourits M.Ph., Hofman P., Schipper J., Tan K.E.W.P.; Amsterdam, The Netherlands

From 1972 to 1990, 102 patients with retinoblastoma were treated with megavoltage external beam irradiation in a D-shaped field. Ten patients were excluded because of loss to follow-up. Eighty-one patients suffered a hereditary retinoblastoma, 11 a non-hereditary retinoblastoma. In total 13 patients (all hereditary) developed a secondary primary non-ocular tumour or orbital recurrence. Of the 10 secondary tumours, 7 died and 3 are still alive. Three patients died of orbital recurrence or metastases. We will discuss the effect of external beam irradiation on the incidence of non-ocular tumours.

RHABDOMYOSARCOMA AND RETINOBLASTOMA IN A NINE YEAR OLD CHILD WITH RING CHOROMOSOME 13 - A CASE OF THE SECOND TUMOUR FIRST.

Elder JE, McKenzie JD; Melbourne, Australia

A nine year old girl was found to have bilateral retinoblastoma. She has a past history of embryonal rhabdomyosarcoma of the bladder diagnosed at nine months of age.

At the time she was found to have the following chromosome constitution: 46, XX, r(13) (p11q34)/46,XX, r del(13) (p11q14)

It would appear that this child has a deletion of the retinoblastoma gene and developed a non-ocular tumour prior to the retinoblastoma.

The late development of the retinoblastoma stresses the need for the longterm ophthalmic follow-up of patients with mutations in or deletions of the retinoblastoma gene.

RGDS PEPTIDE INTERFERES WITH THE ATTACHMENT AND SPREADING OF RETINOBLASTOMA CELLS IN VITRO.

Sasabe T, Suwa Y, Kiritoshi A, Kishida K; Osaka Japan

(Introduction): Arg-Glu-Asp-Ser (RGDS) peptide, one of the cell binding domains of fibronectin is known to interfere with the metastasis of malignant melanoma in animal experiments and to inhibit the attachment of malignant melanoma cells in vitro. In the present study the effect of the RGDS peptide on the attachment of retinoblastoma cells was investigated in vitro. (Methods): The retinoblastoma cell used were WERI-m cells originated from WERI-RB1 cells, which grow anchrage dependently and form some dendritic processes. $3x10^5$ of WERI-m cells were seeded in each well of a 6-well culture plate. The cells were cultured with MEM supplemented with 15% FCS under a humidified atmosphere (5% CO_2 and 95% air). After 5 days, the medium was replaced with new one containing the RGDS peptide at a concentration of 1mg/ml. Then, the alteration of morphology was obseved under a microscope and both floating cells and attached cells were counted. (Results): Before the addition of the RGDS peptide, WERI-m cells attached to the bottom of the culture dish, forming dendritic processes. After one hour, the processes of the cells became blunt, and within the 6 hours they disappered and the cells became round. After 24 hours, most of the cells were free-floating. (conclusion): RGDS peptide showed the inhibitory activity on the attachment of retinoblastoma cells, indicating that the RGDS can be used for prevention of metastasis of retinoblastoma.

OUTCOME FOR FIVE PATIENTS WITH CONSTITUTIONAL 13q CHROMOSOMAL ABNORMALITIES AND RETINOBLASTOMA. Pratt CB, Raimondi SC, Kaste S, Crom DB, Meyer D, Elfervig J. Memphis, TN, USA.

Five of 179 children with retinoblastoma treated at St. Jude Children's Research Hospital between 1962 and 1994 have had constitutional abnormalities involving chromosome 13. Patients were 3-16 months of age (median = 11 months) at diagnosis. Three had bilateral disease. All tumors were unifocal, two children had neither globe enucleated, two had unilateral enucleations, and one had both globes enucleated. Two had bilateral irradiation and two had unilateral irradiation. Three received vincristine-cyclophosphamide chemotherapy. Four patients survived; one died of pneumonia 4 months after diagnosis. The 4 surviving patients had been followed for 1.5-22 years (median=19+ years), and currently reside with their families. With aging, all patients have demonstrated profound mental retardation. The three older survivors have received special education, and one is too young for education outside his home. All patients have had abnormal facies, one with hypertelorism. Two patients had microcephaly, two had cervical ribs, three had metaphyseal abnormalities. All patients had abnormal phalangies - three had tubular fingers, two overlapping toes. The prognosis for survival of patients with constitutional abnormalities of chromosome 13 is good, while the prognosis for their societal contributions is poor.

MUTATION FREQUENCY AND NUMBER OF RETINOBLASTS

Tan, K.E.W.P., Moll, A.C., Imhof, S.M.; Amsterdam, The Netherlands

It is generally accepted that a tumour develops after a number of oncogenic mutations have taken place in a single cell. The number and the exact nature of the mutations, and the number of cells capable of malignant transformation, are not known. Estimates of the numbers of target cells and the mutation rate can only be arrived at in a very indirect manner.

In retinoblastoma however the situation is completely different. It is an established fact that two mutations are necessary in the 13th chromosome. Besides, retinoblastoma exists in two forms: a hereditary one in which only one additional mutation is necessary, and a sporadic form for which two mutations are needed. Because the incidences of both forms are known, this enables us to calculate both the mutation rate and the number of target cells (retinoblasts).

RETINOMA ASSOCIATED WITH VITREOUS SEEDING

Lueder GT, Gallie BL, Heon E

Retinomas are benign tumors initiated by mutations of the retinoblastoma gene. Diagnosis of retinoma is based on a characteristic clinical appearance. Malignant transformation of retinoma to retinoblastoma has been reported in three patients. These tumors were all associated with vitreous seeding. We have followed and documented with photographs two patients with retinomas which are associated with vitreous seeding, but which have remained stable for eight and thirty-two years. Therefore, vitreous seeding alone does not signify malignant transformation of a retinoma.

WORK UP OF NEW RETINOBLASTOMA PATIENTS.

Budning AS, Fucillo L, DaSilva L, Gallie BL; Toronto, Canada.

Work-up of the patient with suspected retinoblastoma requires alertness on the part of the primary physician, with quick referral to the ophthalmologist for definitive diagnosis and treatment. The faster the treatment is begun the better the prognosis.

A flow diagram and check list have been developed which is used as our protocol for the management of retinoblastoma children. This incorporates a cascade of events as follows: An urgent CT scan allows clinical and radiological correlation to confirm the presence of retinoblastoma and exclude extraocular disease. Blood for genetic analysis is collected from baby and family. Surgical consent is followed by examination under anaesthesia, bone marrow and lumbar puncture to look for systemic spread, and oncology consultation. When needed, enucleation of the worst eye, laser treatment, cryotherapy, and possible portacath insertion for chemotherapy are preformed under the same general anaesthetic. Tumor from an enucleated eye is placed directly in cell culture medium for genetic analysis. Follow-up for the patient is then arranged as required by disease status.

This protocol is particularly useful for efficient patient management, teaching, and assures optimal treatment, including application of appropriate molecular tests.

TREATMENT OF RETINOBLASTOMA AT HOSPITAL GARRAHAN IN ARGENTINA. Schvartzman E., Fandiño A., Raslawski E., Dávila M., Chantada G., Manzitti J. Buenos Aires. Argentina.

From Aug 1988 to Dec 1992, 95 patients with Retinoblastoma were admitted (87 eligible). Mean age was 22 m (0-104). 41 patients (47%) had bilateral Retinoblastoma. Mean follow up was 40 ± 17 months (13-70m). Grabowski and Abramson's clinicopathological system was used for staging. Results and therapy were the following.

Stage	n	(%)	Therapy	Alive
Ia	24	27.5	Enucleation (en)	24
Ib	4	4.5	En	4
Ic	11	12.6	En	10
IIa1	2	2.2	En-Chemotherapy(Ch)(VAdrC)	2
IIa2	1	1.1	En-Ch(VAdrC)	1
IIb1	12	13.7	En-Ch(VAdrC)	12
IIb2	14	16.1	En-Ch(VAdrC)+Triple i.t. + Orbital Radiotherapy	11
III	6	6.8	En-Ch(VAdrC+CDDP+VP16)+ Triple i.t.+Cranial Radiotherapy	0
IV	2	2.2	The same therapy	0

Special patients: 8 patients with bilateral Retinoblastoma had both eyes treated conservatively (Alive 8). 3 patients with malignant preauricular adenopathy were treated as stage III plus radiotherapy of the involved lymph nodes (Alive 3). All patients died of progressive disease except for one who had a secondary ANLL. We conclude that intraocular Retinoblastoma, even with choroid invasion can be treated without chemotherapy. An aggressive therapy of IIb2 patients improved our previous results however, stages III and IV were resistant to conventional therapy. Patients with malignant preauricular adenopathy are curable with an aggressive approach.

LOCAL THERAPY OF BILATERAL RETINOBLASTOMA AT THE HOSPITAL GARRAHAN, ARGENTINA.

Manzitti j, Fandiño A, Raslawski E, Dávila MT, Chantada G, Schvartzman E; Buenos Aires, Argentina.

From Aug 1987 to Dec 1992, 41 patients with Bilateral Retinoblastoma were admitted (38 eligible). Mean age was 12 months (1-53) and mean follow up was 43±21 m (13-70). Essens' prognostic classification was used for ophthalmological evaluation. Eight patients underwent conservative therapy for both eyes and all remain disease free (mean follow-up 36.7 months). In 5 patients bilateral enucleation was undertaken initially. In the remaining 25 patients the most affected eye was removed. The initial therapy of the remaining 41 eyes (Essen 1=12, 2=8, 3=14, 4=7) included: radiotherapy in 29 eyes (local control=18), cryo and/or photocoagulation in 9 (local control=1) and two eyes with both modalities (local control=0). One patient died before the treatment of the remaining eye was completed. Further local therapy included: radiotherapy 7, cryo±photocoagulation=8, enucleation=4 and plaque radiotherapy 2. Local control was ultimately achieved in all surviving patients. Three patients died of progressive disease, whereas 1 died because of a second malignancy (ANLL). Overall survival was 90%.

We conclude that conservative therapy with bilateral visual preservation is feasible in our setting, however, most patients present with advanced disease. This therapy proved to be effective in avoiding bilateral enucleation without jeopardizing survival.

TRENDS IN RETINOBLASTOMA MANAGEMENT 1950-1991.

Niksarli K, Abramson DH, Ellsworth RM, Servodidio CA; New York, United States

Purpose: A retrospective analysis of 1501 retinoblastoma (RB) patients treated at The Ophthalmic Oncology Center at The New York Hospital, between the years 1950-63 (479 patients, "Interval I"). 1964-77 (629 patients, "Interval II") and 1978-91 (393 patients, "Interval III") was undertaken to determine changes involving presenting features of the patients and the enucleation frequencies as a means to compare the trend in preservation of vision over the three time intervals.

Methods: Data was collected and analyzed for gender, age at diagnosis, laterality, family history and enucleation status.

Results: In all three intervals, age at diagnosis was younger for unilateral (U) vs. bilateral (B) patients (P<0.001), and for patients with family history (FH) of RB vs. those with no FH (P<0,001). Over the three intervals, there was no significant change in the ages at dianosis among the U or B patients, as well as patients with and without FH of RB (P<0.001). Occurance of disease and laterality was equally distributed between the two genders. For all patients, total (97%, 90%, 76%) and bilteral (25%, 15%, 7%) enucleations decreased in Intervals II and III (P 1&2<0.001*) and unilateral enucleations (72%, 76%, 69%) decreased in Interval III (P 2<0.05). Patients with B-RB underwent progressively fewer enucleations (97%, 91%, 70%; P 1<0.01, P 2<0.001), and those with U-RB did so for Interval II (98%, 90%, 84%; P 1<0.01, P 2>0.1). B had significantly fewer enucleations than U in Interval III (70% vs. 84%; P<0.01). U without FH had decreasing rates of enucleation (99%, 92%,86%, P 1<0.01, P 2>0.05) from Interval I to II, B with FH (94%, 69%, 33%; P 1&2<0.001) over the entire time period, and B without FH (97%, 95%, 82%; P 1>0.1, P 2<0.001) from Intervals II and III, patients with FH had fewer enucleation than those with no FH (69% vs 95%, 33% vs 82%; P 1&2<0.001 and over the entire time period, all patients with FH combined (92%, 64%,34%, P 1&2<0.001), had more significantly decreasing rates of enucleation than all patients without FH (98%, 94%, 84%, P 1<0.01, P 2<0.001).

* P 1 refers to P value from Interval I to II, P 2 refers to P value from Interval II to III.

ASSESSMENT OF RETINOBLASTOMA VIABILITY WITH DOPPLER FLOW ULTRASONOGRAPHY

Roarty JD, Slovis TL, Trese MT, Ravinindrath V, Latin P; Detroit, United States
Children's Hospital of Michigan Ocular Oncology Group

One of the difficulties in treating retinoblastoma is objectively establishing tumor viability. Doppler flow ultrasonography assesses blood flow to a tissue and has been used successfully to determine tissue viability in transplant situations. Eight patients with retinoblastoma have been evaluated prospectively, six patients seen prior to any therapy. Three patients presented with unilateral disease and five with bilateral disease. Five patients have been followed with ultrasonography while treated with chemotherapy, irradiation or cryotherapy. Six eyes required enucleation.

Doppler flow ultrasonography demonstrated a decrease in tumor blood flow consistent with clinical regression in five patients. Three eyes were evaluated histopathologically. In two of three eyes, histopathology showed diffuse gliosis and small areas of calcifications with no viable cells. Histopathologic evaluation revealed viable tumor cells in the face of clinical regression in one case. While Doppler flow ultrasonography demonstrates blood flow decrease consistent with clinical regression, clinical nor ultrasonographic examination give absolute assurance of tumor death. Discussed are the flow characteristics of retinoblastoma on presentation with treatment.

AMBULATORY ANESTHESIA FOR RETINOBLASTOMA: THE NEW YORK HOSPITAL EXPERIENCE 1980-1993

Susman D, Shah N, Turner L, Dinner, M, Abramson DH, Ellsworth RM; New York, United States

We have performed ambulatory anesthesia on more than 3,000 children between 1980 and 1993. Our technique has changed over theyears and we will describe our present technique which requires no pre-operative medications, no intravenous lines and no injections, but utilizes parents for induction with Nitrous Oxide/Halothane mixtures delivered through the Hudson mask. Anesthesia times varied from 5 to 80 minutes.The advantages of this technique include a clear face mask with built in head strap, a low silhouette of the mask, easy acceptance by the patient, family and examining ophthalmologist and the low cost of the procedure. Retrospective analysis demonstrated that there were no recovery room or overnight admissions. There were no cases where endotracheal intubation was performed. Nausea and vomiting occurred in 1.2% of cases and 0.5% of cases required positive pressure. Pre-anesthesia examination by the anesthesiologist was performed on the day of anesthesia on all cases. Despite the fact that the children were dilated with 10% Phenylephrine no cases of hypertensive crisis were noted.

PERSISTENT HYPERPLASTIC PRIMARY VITREOUS: IMAGING FINDINGS WITH PATHOLOGIC CORRELATES

Kaste, SC, Jenkins JJ, Meyer D, Fontanesi J. Pratt CB.

Resistant hyperplastic primary vitreous (PHPV) is the most common lesion to simulate retinoblastoma (RB). PHPV and RB both present as unilateral or bilateral leukocoria in infancy. PHPV is usually associated with microphthaemia and lacks calcifications with the mass though usually occurs sporadically, it may be associated with trisomy syndromes 13, 15, 18, and 21; fetal alcohol syndrome; fetal hydantoin syndrome; and midline congenital cranial defects.

We present imaging pathologic, and clinical findings of this rare entity in five patients (n=6 globes).

OCULAR AND SKELETAL ELECTROMYOGRAPHY IN PATIENTS AFFECTED WITH EXTERNAL CONGENITAL OPHTHALMOPLEGIA AND THEIR UNAFFECTED FAMILY MEMBERS.

Magli A, de Berardinis T, Vastarella P, D'Esposito F, Santoro L, Perretti A.

Studies aiming to clarify the etiophatogenesis of the External Congenital Ophthalmoplegia by means of electromyographic research have been carried out on a total of 46 affected patients and their unaffected family members. The electromyographic examination performed in skeletal and ocular muscles, shows a frequent existence of neurogenic lesions of the skeleton and ocular muscles. The presence of such alternations, not only in the affected subjects, but also in some apparently healthy family members, suggests and incomplete gene penetrance associated with a variable expression.

PSEUDOXANTHOMA ELASTICUM

Tijmes NT, Dorp van DB; Amsterdam, the Netherlands.

We want to report on two pedigrees with pseudoxanthoma elasticum (PXE). Although the mode of inheritance seems to be autosomal recessive, in three families there are people with ocular involvement in two subsequent generations. For this reason the question remains if the condition in these families is autosomal dominant with variability in expression or autosomal recessive with signs in carriers.

Another interesting phenomenon was that one of the affected females showed signs of retinitis pigmentosa: pigment clumping along vessels in addition to angioid streaks. Also the ERG was strongly decreased. Although pigment clumping may be a sign of PXE, it is difficult to decide whether in this case this is a manifestation of PXE; or if it exists as a seperate entity as Tapetoretinal degeneration (TRD).

A PEDIGREE OF PRESUMED X-LINKED FAMILIAL EXUDATIVE VITREORETINOPATHY.
MAJIMA A, Mizuno S; Nagoya, Japan, EGUCHI K; Hakodate, Japan.

The proband of this pedigree and two of his grandsons had already been diagnosed as having familial exudative vitreoretinopathy (FEVR) by one of the authors (EK). When the 5th grandson of the proband underwent fundus examination soon after birth, pathological changes similar to retinopathy of prematurity stage 3 were observed in both eyes. Although photocoagulation therapy was performed immediately, the right eye had a dragged disc appearance and the left eye a retinal fold when he was referred to Nagoya City University Hospital at the age of 6 months. We found evidence of a presumed X-linked inheritance in this pedigree after examining three generations. Although the hereditary trait of FEVR has been generally thought to be autosomal dominant, a few pedigrees of X-linked FEVR have recently been reported. FEVR may have genetic heterogeneity similar to retinitis pigmentosa.

We propose diagnostic criteria for FEVR which somewhat modify those established in Japan by Ohkubo et al. in 1987.

CLINICAL AND GENETIC FEATURES OF X-LINKED RETINOSCHISIS

George NDL, Moore AT, Yates JRW, Payne SJ, Barton DE; Cambridge, UK.

X-linked retinoschisis is thought to be the commonest juvenile macular dystrophy. Although there is electrophysiological evidence of widespread involvement of the inner retina, the fundus appearance is variable and in some patients where the schisis is confined to the fovea, the diagnosis is often delayed or overlooked. This study was undertaken to evaluate the clinical findings in affected males and obligate heterozygotes and to refine the disease locus on Xp22.

Members of 17 apparently unrelated pedigrees, including 55 affected males and 35 obligate carriers have been examined by a single ophthalmologist (NDLG). All affected males had macular abnormalities, the commonest being foveal schisis (78%). Peripheral schisis was present in 70% and a variety of other retinal abnormalities were seen. There were no pathognomonic features of the carrier state.

Results from typing of the first 7 families confirms linkage to markers in Xp22 (RS vs DXS207: $Z_{max}=10.0$; $\theta_{max}=0.03$) with no evidence of heterogeneity. Flanking recombinants map between DXS1052 and DXS1053. Results of typing of the remaining families using DXS207, DXS1053, DXS999, DXS443, DXS365, and DXS1052 will be presented.

DELETION MAPPING OF EFFECTS OF THE DUCHENNE MUSCULAR DYSTROPHY GENE ON THE ELECTRORETINOGRAM. Pillers DM, Sigesmund DA, Tremblay F, DeBecker I, Dooley JM, Riddell DC, Bunger MK, Musarella MA, Ray PN, Weleber RG; Portland, Oregon, U.S.A., Toronto, Halifax, Canada.

An abnormal scotopic electroretinogram (ERG) in a boy with a contiguous gene deletion syndrome that includes the Duchenne muscular dystrophy gene (DMD) led us to study Duchenne and Becker muscular dystrophy patients to determine whether this was a consistent ERG phenotype. Over fifty patients have been studied. The characteristic abnormality of the scotopic ERG is a reduced amplitude b-wave. Mapping of the deletions and correlating them with the patients' ERG phenotypes has led us to make observations regarding the positional effect of the deletion on the severity of the abormalities. Deletions range from involving a single exon to deletion of the entire Duchenne locus. The majority of deletions are clustered in the central "hot-spot" region of the gene. These patients consistently show reduced amplitude b-waves. Patients with deletions limited to the 3' end of the gene are predominantly those with the contiguous gene deletion syndrome of complex glycerol kinase deficiency. Thus, their deletions originate within the Duchenne gene but extend further telomeric to include other loci, namely glycerol kinase (GK) and congenital adrenal hypoplasia (AHC). These 3' deletion patients show consistent abnormalities of the ERG b-wave, which is usually markedly reduced in amplitude. In addition to their ERG abnormalities, these patients also may demonstrate ocular hypopigmentation, strabismus, ametropia, astigmatism, nystagmus, and night blindness. The ocular phenotype of the patients with only Duchenne or Becker muscular dystrophy, however, does not vary significantly from that of the normal population range. This leads us to propose that other genetic sequences, or pleiotropic effects of one of the known loci (GK, AHC) lying within the extent of the deletions on Xp21 may be responsible for the additional eye findings. Patients with deletions limited to the 5' portion of the Duchenne locus have a more variable phenotype. Although many have reduced scotopic b-waves, the range of findings includes individuals who have normal ERGs. We propose that this variablility of the abnormalities of the ERG as a function of the position of the deletion within the DMD gene reflects differential expression of dystrophin isoforms within retina.

CORRELATION OF ELECTRORETINOGRAM FINDINGS WITH MOLECULAR ANALYSIS IN THE DUCHENNE MUSCULAR DYSTROPHY PHENOTYPE.

De Becker I, Tremblay F, Riddell DC, Dooley JM. Halifax, Canada.

We studied 15 consecutive patients with the Duchenne muscular dystrophy (DMD) phenotype. Each patient was asked to undergo an ophthalmological examination, an electroretinogram (ERG), and donate a bloodsample for molecular diagnosis. All 15 patients had a normal ophthalmic examination. Electroretinography was successful in 14/15 patients. The ERG tracings were normal in 7 patients, abnormal in seven, and unreliable in one. Blood for molecular analysis was obtained in 12/15 patients. In the 7 patients with a normal ERG, 5 underwent molecular analysis, and in these five, no deletion was detected in the dystrophin gene. In the 7 patients with an abnormal ERG, 6 had molecular analysis available, and all 6 were found to have a deletion. Our results suggest that patients with a classical DMD phenotype are genetically heterogeneous, and that this heterogeneity is reflected in the ERG. Patients with DMD phenotype and a normal ERG may have an as yet undetected mutation in the dystrophin gene that does not affect the retinal isoforms. They could also have an intact dystrophin gene but an altered promoter. Lastly, it is possible that both the dystrophin gene and promoter are intact, and that the anomaly lies in a dystrophin-related protein.

DUCHENNE MUSCULAR DYSTROPHY (Xp21) AND CONGENITAL STATIONARY NIGHT BLINDNESS(Xp11.3): SIMILARITIES AND DIFFERENCES OF TWO DISTINCT RETINAL CONDITIONS.

Tremblay F, De Becker I, Riddell DC, Dooley JM; Halifax, Canada.

Patients with Duchenne muscular dystrophy (DMD; Xp21 deletion) and with Complex glycerolkynase deficiency (CGKD), a continuous gene deletion syndrome which involves in a telomeric direction the DMD gene itself, have recently been reported to have electroretinographic results showing weak rod-related responses along with a negative configuration of the bright-flash response. Qualitatively, this ERG pattern is similar to the one found in either forms of Congenital Stationary Night Blindness (complete (cCSNB) and incomplete (iCSNB) forms). We carried out a quantitative analysis on the four groups of patient (total of 30 patients: 6 DMD; 10cCSNB; 13 iCSNB and 1 CGKD), with the same ERG protocol applied in all of them. The statistical comparison reveals that the four groups can be distinguished on the basis of their cone-related activity. Patients with DMD and CGKD have three well-defined oscillatory potentials (OPs) but in CGKD all three present with subnormal amplitudes. In patients with CSNB, only the fourth OP could be identified. In iCSNB, no OPs could be delineated from the background noise. The rod-related activities and the bright-flash responses were not different in any of these groups and were overall, sharing the same general characteristics. This study clearly show that patients with DMD and CGKD have a retinal condition distinct from the one in CSNB and iCSNB.

FURTHER MAPPING OF X-LINKED CONGENITAL STATIONARY NIGHT BLINDNESS IN REGION Xp11.3-p11.22.

Bech-Hansen NT, Boycott KM, Field LL, Gratton KJ and Pearce WG; Calgary and Edmonton, Canada.

Clinical features of X-linked congenital stationary night blindness (CSNB) include night blindness, reduced visual acuity, congenital nystagmus, and myopia. Tests of visual function are characterizd by a 'negative' b wave on electroretinography and impaired rod function on dark-adaptometry. These findings are consistant with the underlying defect of CSNB being in the bipolar layer of the retina.

A number of studies have shown that a locus for CSNB lies in the proximal region of the short arm of the X chromosome. We have performed linkage analysis on families with clinical findings of CSNB using genetic markers spanning the Xp11.3-Xp11.22 region. Significant linkage was found between a CSNB locus and markers across this X chromosome interval and a minimal region limited by MAOB and DXS988/DXS1000 has been defined. In one family (P060) in which the CSNB phenotype is similar to that of Åland Island Eye disease, we have observed a crossover which suggests that the CSNB gene in this particular family lies proximal to DXS426, and defines a minimal region spanning an estimated distance of 5-7cM. Using radiation and convential hybrids, several new genetic markers and expressed sequences, some representing candidate genes for CSNB, have been mapped to the minimal region of the CSNB locus.

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Xth Symposium of the

INTERNATIONAL SOCIETY FOR GENETIC EYE DISEASE

&

VIIth Symposium of the

RETINOBLASTOMA SOCIETY

GENETIC TESTING FOR HEREDITARY EYE DISEASES

DNA DIAGNOSIS OF RB

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. . . early detection . . .

. . . identifying disease causing mutations . . .

. . . clinical service . . .

Introduction:

Most Retinoblastoma (RB) tumors arise as a result of new mutations and, therefore, are not diagnosed until the tumor is large, which usually results in enucleation. On the other hand, early detection of tumor formation allows clinicians to save the eye. The early detection of tumors in at-risk family members requires repeated retinal examinations under anesthetic (EUA). Clearly, a test that identifies family members who carry the disease-causing mutation would eliminate all the unnecessary EUAs.

Genetic counselling based on DNA tests has usually been restricted to families with multiple affected members. This is primarily due to the time and expense involved in developing and executing an accurate method to detect mutations. Visible Genetics Inc. (VGI) is currently developing a strategy to detect mutations that initiate RB. The tests are performed on DNA isolated from the patient's blood. Only blood from bilateral or familial RB patients and unilateral RB tumors will be tested. VGI does not currently test isolated unilateral RB patients' blood because 85% will not carry a mutation. We are currently evaluating the performance of this strategy and, therefore, all samples are treated as research material and tested at no charge. VGI expects to identify disease-causing mutations in more than 95% of families.

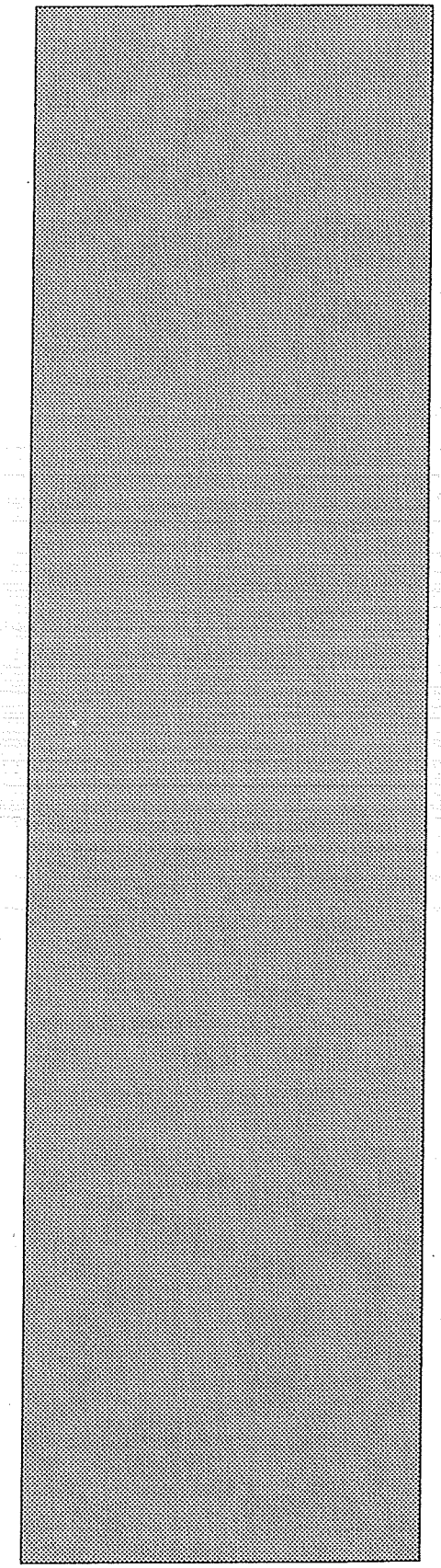
Upon completion of the evaluation, VGI will offer RB tests as a clinical service. The cost of these tests is being calculated during the evaluation period. We intend to price the first affected member separately from at-risk family members, since the greatest cost is incurred during the initial identification of the family's mutation.

Sample preparation for families with bilateral or familial RB:

These tests require a blood sample from at least one affected member of the family; we also suggest that blood from the parents and siblings accompany the sample. When possible, draw 10 mls (2-5 mls for infants and small children) of venous blood from each individual. Please use yellow-top ACD tubes for the blood collection. If possible, a fresh tumor sample would contribute to the detection process. The tumor sample must arrive sealed in a sterile culture tube in tissue culture media such as RPMI. These samples should be sent at room temperature by overnight delivery and be accompanied by a separate completed test request for each sample sent. These forms are available upon request by fax at 416-369-5126.

Sample preparation for families with a unilateral RB:

VGI requires a fresh tumor sample in order to effectively determine the risk to these families. The tumor sample must arrive sealed in a sterile culture tube in tissue culture media such as RPMI. We also request a blood sample from the affected member. These samples should be sent at room temperature by overnight delivery and be accompanied by a completed test request form. These forms are available upon request by fax at 416-369-5126.



For further information, please contact:

Dr. James Dunn
399 Bathurst Street
Toronto, Ontario
M5T 2S8

Tel: (416) 369-5181
Fax: (416) 369-5126

Visible Genetics Inc.

We would be pleased to provide you with more information about Visible Genetics Inc. Check where applicable:

I would like more information about:

Diagnostic DNA Sequencing ____

Retinoblastoma Test ____

Other (please specify) ____

Name: _____

Address: _____

Suite: _____ City: _____

Prov./State: _____ Postal/Zip Code: _____

Telephone: _____ Fax: _____

Please return this form by mail or by fax to:

Visible Genetics Inc.
2 First Canadian Place
The Exchange Tower
Suite 2810, PO Box 47
Toronto, Ontario M5X 1A9

Fax: (416) 369-5126
Tel: (416) 603-2981

Eye Research Institute of Canada

Laboratory Requisition Form

Family Name: _____ Date (yr/mo/dy): _____

First Name: _____

Date of Birth: _____

Sex(M/F): _____

Mother's Name: _____

Father's Name: _____ Condition/Syndrome:

_____

Street _____

City, Prov/State _____ Clinical Status (Bilateral/Unilateral/Normal/Unknown)

Postal/Zip Code _____ _____

Phone # _____

Name of Proband: _____ Relationship to Proband:
(Affected member of family)

Proband: ___
Mother: ___
Father: ___
Sister: ___
Brother: ___
Maternal Aunt: ___
Paternal Aunt: ___
Maternal Uncle: ___
Paternal Uncle: ___
Maternal 1st Cousin: ___
Paternal 1st Cousin: ___
Other please specify:

Sample type sent: (eg. tumour, ACD tubes)

_____

Health # (Ontario Residents): _____

Referring Physician: _____

Address:

_____

_____

_____

_____

Telephone: _____

Please attach pedigree

Completed form must accompany sample

Send samples to:

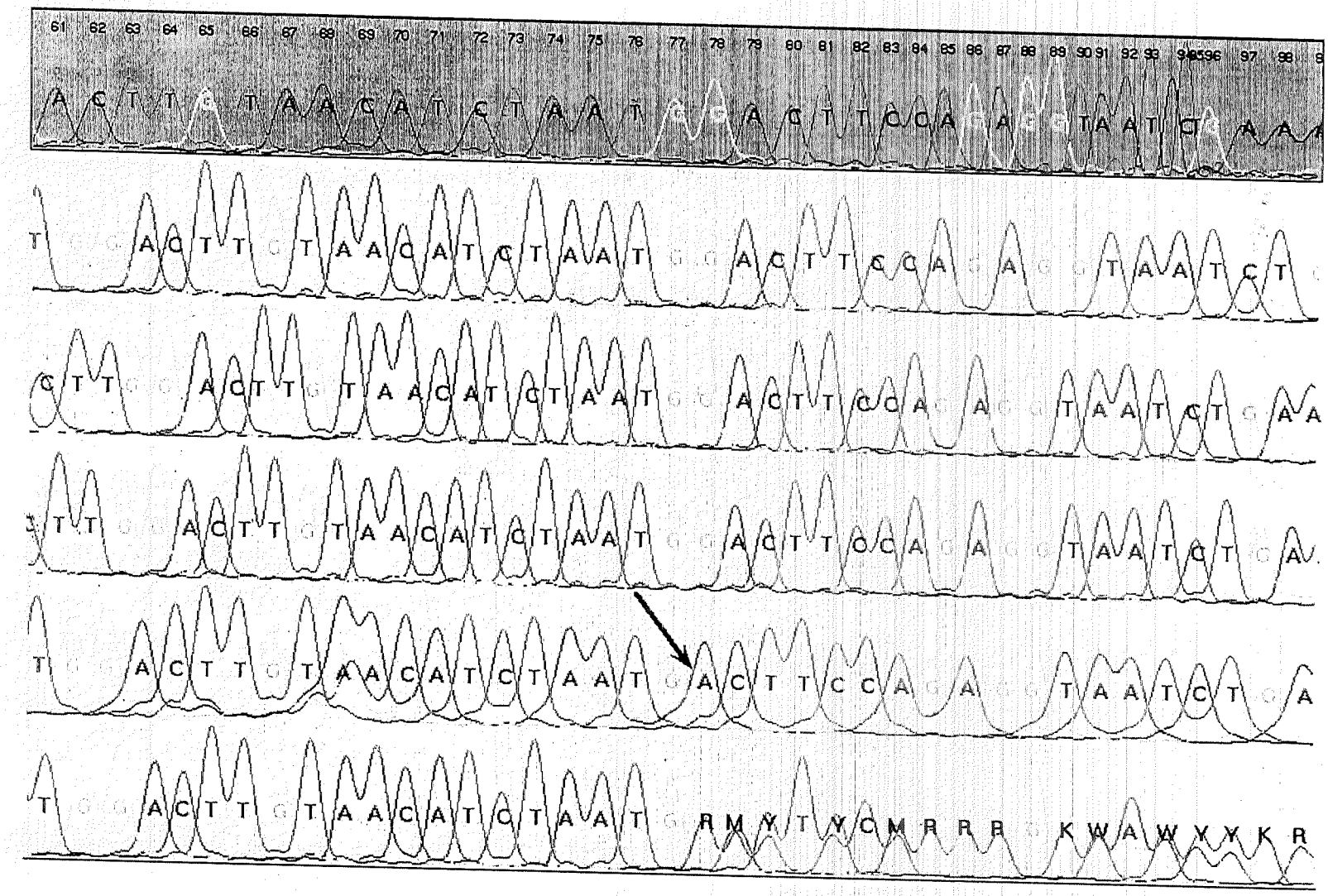
Qi Jia/Daisy Du
E.R.I.C.
Toronto Hospital, Western Division
399 Bathurst Street
Toronto, Ontario M5T 2S8
Tel: (416) 369-5181
Fax: (416) 369-5126

If report sent previously on other family members:

Molecular Kindred #: _____

Kindred Name: _____

Please call one week after sending sample to ensure that it was received.



1 bp DELETION LEADS TO
STOP CODON IN EXON 10

T G G A C T T C C A G A G | G T A A T C T G NORMAL
T G A C T T C C A G A G | G T A A T C T G A MUTANT

EXON 9 INTRON

Visible Genetics Inc.