

13TH SA SOCIETY FOR HUMAN GENETICS (SASHG) CONGRESS

5 - 8 April 2009
Spier Estate, Stellenbosch, South Africa

Final Programme and Book of Abstracts

<http://www.sashg2009.co.za>



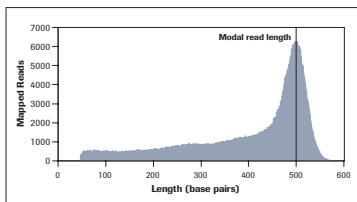


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WELCOME NOTE

Welcome from SASHG Conference Organising Committee Chair

To all delegates, invited speakers and sponsors

It is an honour to welcome you to the 13th South African Society for Human Genetics conference. We are hoping that it is lucky number 13 and that we will not experience any major mishaps!

I think that you will agree that Spier Estate in Stellenbosch is a magnificent setting for any conference but we are sure that it will be a major contributor to the success of our meeting. Our organising committee have put together a jam-packed, entertaining and informative program but we hope that you will find time in between to network and start new collaborations somewhere under the oak trees over a glass of Spier wine or juice.

We have 8 invited overseas plenary speakers who will share their latest research findings with us and to them I want to extend a special welcome and we look forward to your contribution to our conference.

On behalf of the organising committee I also want to thank all the sponsors (their names are listed in this booklet) because without them there would be no conference. Please take some time to visit the exhibition stands in the foyer as well as the Simonsberg, Stellenberg & Helderberg rooms to find out about their latest products and promotions, and to meet the people that you often just speak to telephonically- you might be able to negotiate a good deal on your next purchase!

Every evening there is a dinner and who can resist a sumptuous spitbraai, relaxing under the stars at Moyo or the 'boere hospitality' of Skilpadvlei - so be sure to reserve your place and you can worry about those extra kilos later! Also, the student monetary prizes will be awarded at the conference dinner on Tuesday evening at Skilpadvlei.

Finally, thank you to the members of the conference organising committee: Monique, Eileen, Greetje, Renate, Pedro, Louise, Valerie and Hanlie - you have been an excellent committee to work with and have made a difficult job easy. And to Christelle Snyman thank you for 'shouldering all the storms', the 'storms in a teacup' and for making sure that the books balanced!

So let's put Genetics to Practise!

Welcome! Welkom! Wamkelekile!

Soraya

NOTE FROM THE SASHG CHAIR

Dear Colleagues

It is a great pleasure and honour to welcome you to the 13th Congress of the SASHG at Spier.

Thanks to a wonderful response to the call for abstracts, the programme committee was able to put together a comprehensive programme that should appeal to everyone with an interest in human genetics, from clinicians and counselors to basic scientists. In line with the theme of the Congress, Genetics in Practice, we have a number of oral and poster presentations on genetic testing in various disorders, highlighted by plenary speakers who will share the EuroGentest experience with us.

As with previous congresses, this one will offer opportunities to share in the advancement of our knowledge, including observations and applications in the clinical setting, but also to discuss some of the ethical and social challenges that are facing us.

We are fortunate to have a number of distinguished international speakers who will add new perspectives to our discussions and we encourage all delegates to actively participate. On behalf of the Society, I want to take the opportunity to thank the Chair of the Organising Committee, Soraya Bardien-Kruger, and her team who worked hard to bring the congress to fruition. Their dedication and efforts are to the benefit of us all. I want to invite all of you to join in the congress with zest, to use the opportunity to learn, to share ideas and to build relationships with old and new acquaintances.

Finally, do not neglect to enjoy the very scenic environment that Spier offers to you!

Louise Warnich
SASHG Chair: 2007-2009

SECRETARIAT AND ORGANISING COMMITTEE

— Secretariat —

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— Organising Committee —

Alphabetical according to surname



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INVITED SPEAKERS

Alphabetical according to surname



DR. MICHAEL CHRISTIANSEN is Head of the Biochemical Markers Laboratory, Department of Clinical Biochemistry, Statens Serum Institut in Denmark. His areas of research include the genetic aetiology and pathogenetic mechanisms of cardiac diseases, prenatal screening for foetal chromosomal disease and adverse pregnancy outcome, and genetic variation in ion channels in multiple sclerosis.



PROF. CLARA GAFF is a Senior Genetic Counsellor at Genetic Health Services Victoria in Australia, Clinical Associate Professor in the Departments of Medicine and Paediatrics at the University of Melbourne, Honorary Secretary of the Human Genetics Society of Australasia and Regional Editorial Advisor to the Journal of Genetic Counselling.



PROF. ULF KRISTOFFERSSON is Head of the Department of Clinical Genetics at the University Hospital in Lund, Sweden, Head of the Swedish Clinical Cancer Genetic Group and member of the Ethical Delegation of the Swedish Society of Medicine. His interest in finding solutions to ethical and practical problems in provision of service in clinical genetics has led him to collaborate in several European projects, such as EUCROMIC where he was responsible for drafting pan-European guidelines for prenatal diagnosis, Gen Ed, CAGSE, EUROGAPPP, and is an expert in an OECD working group on quality assessment in molecular genetics.



PROF. IRMA NIPPERT is the Director, Womens' Health Research Unit, Universitätsklinikum Münster (UKM), Germany, affiliated with the Department of Human Genetics at the UKM, co-ordinator of the CAPABILITY project, and partner in the EuroGentest and EuroGenGuide. She has been conducting national and international research on the quality of genetic services and on the social and ethical impact of genetic testing, and has strong experience both on national and international levels on assessing genetic services provision.



DR. KATHRYN ROBSON is a Senior Scientist at the Weatherall Institute of Molecular Medicine, University of Oxford, UK. Her group investigates an iron overload disorder, haemochromatosis, with specific interest in increasing awareness, improving diagnosis and treatment of the condition. Her group also applies microarray analysis to investigate gene expression patterns in people with haemochromatosis. Dr Robson is also an Honorary Reader in Molecular Biology.



PROF. ERWIN SCHURR is a James McGill Professor of Human Genetics and Medicine at McGill University in Canada, a member of the Research Institute of the McGill University Health Centre, the Associate Director of the Centre for the Study of Host Resistance, a Chercheur National of the Fonds de Recherches en Santé du Québec and an International Research Scholar of the Howard Hughes Medical Institute. His research interest is the identification of host genetic factors predisposing to tuberculosis and leprosy.



PROF. HUGH WATKINS is the Field Marshal Alexander Professor of Cardiovascular Medicine at the University of Oxford, UK. He is Head of the Department of Cardiovascular Medicine and Honorary Consultant in Cardiology and General Medicine at the John Radcliffe Hospital, and Director of the British Heart Foundation Centre of Research Excellence, Oxford. His research focus is on the molecular basis of monogenic cardiomyopathies, the genetic causes of 'sudden cardiac death' syndromes and susceptibility genes for coronary artery disease.



PROF. MATTHEW WOOD is a University Lecturer in the Department of Physiology, Anatomy and Genetics and Fellow and Tutor in Medicine at Somerville College, University of Oxford, UK. The focus of his current research is on nucleic acid-based gene silencing and gene therapy in the nervous system and in muscle, specifically in the context of neurodegenerative disorders, motor neuron diseases and muscle disease.

PROGRAMME

13th SASHG Congress
Genetics in Practice
Spier, Stellenbosch
5-8 April 2009

— SUNDAY 5 April —

10:00 – 10:30 TEA / COFFEE

10:30 – 12:00 **CLINICAL GENETICS**

Chairperson: Arnold Christianson

10:30 BD Henderson Fabry Disease in South Africa

10:45 K Fieggen Bardet Biedl Syndrome. A Founder Mutation in
Black South Africans

11:00 DL Viljoen Fetal Alcohol Spectrum Disorders in Two Communities in the
Northern Cape

11:15 G de Jong Attitude of Parents Towards Puberty and Sexuality of Their
Cognitively Handicapped Children

11:30 PB Beighton Genetic Disorders on Oceanic Islands - St Helena and
Tristan da Cunha

11:45 RE Arendse Hereditary Bone Dysplasia with Pathological Fractures and Nodal
Osteoarthropathy

12:00 – 12:45 **PROBLEM CASE DISCUSSION**

Chairperson: Greetje de Jong

12:00 – 13:30 REGISTRATION

12:30 – 13:30 LUNCH

13:30 – 14:15 **OFFICIAL OPENING**

13:30 Louise Warnich, Chair: SASHG

13:35 Mr Mosibudi Mangena, Minister of Science and Technology

14:05 Soraya Bardien, Chair: Organising Committee

14:15 **CANCER GENETICS**

Chairperson: Pedro Fernandez

14:15 N van der Merwe Do Polymorphisms in the p53 Pathway Influence Breast Cancer
Risk in Afrikaner BRCA2 Mutation Carriers?

14:30 K Chatterjee FAS AND FAS Ligand Gene Polymorphisms in Susceptibility to
Herpes Simplex Virus-2 Infections but not in Cervical Cancer

14:45 NW McGregor HAMP as an Iron Regulator in Oesophageal Cancer?

15:00 PW Willem Molecular Cytogenetics of Cancer in the 21st Century and Its Clinical Relevance, Where To?

15:15 – 15:30 TEA / COFFEE

15:30 CARDIOVASCULAR GENETICS

Chairperson: Valerie Corfield

15:30 H Watkins Recent Insights into the Genetic Architecture of Coronary Disease Susceptibility

16:00 PA Brink Unusual Clinical Severity of Congenital Long-QT Syndrome (LQTS) Associated with the KCNQ1 A341V Mutation

16:15 R De Decker The Incidence of 22q11.2 Deletion Syndrome in Children Referred to a Tertiary Paediatric Cardiology Service: a Prospective Study

16:30 N Carstens Variants in Renin and Renin-Binding Protein Genes Modify Cardiac Hypertrophy in Hypertrophic Cardiomyopathy Patients, Independent of Blood Pressure

16:45 VA Corfield Gain-of-Function Mutation in *TRPM4* Cause Progressive Familial Heart Block Type I

19:00 OPENING FUNCTION – INFORMAL DINNER AT MOYO

— MONDAY 6 April —

07:00 – 08:30 Meeting of SASHG Committee

08:30 GENETICS IN PUBLIC HEALTH

Chairperson: Bertram Henderson

08:30 U Kristoffersson Building a National Cancer Genetics Service - what can be learnt from the Swedish experience?

09:00 I Nippert CAPABILITY Project

09:30 A Christianson Health Care Needs Assessment for Medical Genetic Services in Middle and Low Income Nations

09:45 L Geerts Lessons Learnt from a Local Audit of the Prenatal Diagnosis of Aneuploidy

10:00 MF Urban Prenatal Screening for Down Syndrome in the Peninsula Maternity and Neonatal Service: Past, Present and Future

10:15 – 10:45 TEA / COFFEE

10:45 NEUROGENETICS

Chairperson: Raj Ramesar

10:45 M Christiansen Mitochondrial Genetics and Cardiac Disease

11:15 DJ Morris-Rosendahl Molecular Genetic Analysis of Patients with Malformations of Cortical Development

- 11:30 CJ Kinnear Identification and Assessment of Novel Obsessive-Compulsive Disorder Candidate Genes Residing in Schizophrenia Susceptibility Loci
- 11:45 RJ Keyser Novel Deletion Variant Identified in the Promoter Region of the DJ-1 Gene in a Patient with Parkinson's Disease
- 12:00 RS Ramesar A Comparative Study of Two Dominant Retinal Degenerative Disorders on Chromosome 17
- 12:15 – 13:45 LUNCH AND POSTER VIEWING (BATCH A)
- 13:45 **GENETIC COUNSELLING**
Chairperson: Philip Venter
- 13:45 C Gaff What Defines a Genetic Counsellor?
- 14:15 JGR Kromberg Roles of Genetic Counsellors in South Africa
- 14:30 T Wessels "Do You Know Why You Are Here Today?" Genetic Counseling in an Antenatal Multicultural Setting
- 14:45 LJ Greenberg Delivery of an Ophthalmic Genetic Service Including a Telephone Counselling Model: a Bench-to-Bedside Review
- 15:00 C Penn Grandmothers as Gems of Genetic Wisdom: Exploring South African Traditional Beliefs About the Causes of Genetic Disorders
- 15:15 – 15:30 TEA / COFFEE
- 15:30 – 17:00 BI-ANNUAL GENERAL MEETING (EVERYONE INVITED)
ROUNDERS
- 19.00 SPIT BRAAI SPONSORED BY ROCHE AT WELGEVALLEN

— TUESDAY 7 April —

- 08:30 **COMPLEX DISORDERS I**
Chairperson: Michelle Ramsay
- 08:30 E Schurr Dissection of Mycobacterial Diseases by Host Genetic Analysis
- 09:00 EG Hoal Whole Genome Scans in Tuberculosis and Aspects of Population Admixture
- 09:15 M Moller Investigation of Chromosome 17 Candidate Genes in Susceptibility to TB in a South African Population
- 09:30 T McLellan Patterns of Variation in AIDS Restricting Genes in Black South Africans
- 09:45 M Collins Variants within the MMP3 Gene are Associated with Achilles Tendinopathy: Possible Interaction with the COL5A1 Gene
- 10:00 Z Lombard Analysis of Genetic Variation and Obesity-Related Traits in the Birth-to-Twenty Cohort Using the Illumina BeadXpress Genotyping Platform
- 10:15 – 10:45 TEA / COFFEE

10:45 COMPLEX DISORDERS II

Chairperson: Monique Zaahl

- | | | |
|-------|---------------|---|
| 10:45 | K Robson | Haemochromatosis |
| 11:15 | J Knezovich | The Effect of Alcohol on the Methylation Status of the H19 Imprinting Control Region in Mice |
| 11:30 | C van Heerden | Adapting the MeDIP Assay for Identification of Methylated DNA Sequences via Direct Sequencing |
| 11:45 | JM de Jager | Unravelling the PP13-Annexin II Complex in Pre-Eclampsia |
| 12:00 | M Venter | In Silico Promoter Models Facilitate Target Prediction in the Heme Biosynthetic Pathway |

12:15 – 13:45 LUNCH AND POSTER VIEWING (BATCH B)

13:45 GENE THERAPY AND SERVICE DELIVERY

Chairperson: Jacquie Greenberg

- | | | |
|-------|---------------|--|
| 13:45 | M Wood | Splice Correction Therapy for Duchenne Muscular Dystrophy |
| 14:15 | J Scholefield | Knockdown of the Disease-Causing Gene in South African Patients with SCA7 using Allele-Specific RNAi |
| 14:30 | F Essop | Trends in DNA Testing for Cystic Fibrosis at the Division of Human Genetics, NHLS, Johannesburg |
| 14:45 | N Chabibal | Severe Fetal Anomalies and Acceptance for Late Termination of Pregnancy Amongst Women Referred for 3rd Level Scan |
| 15:00 | CL Massyn | Genetic Service Delivery in the Eastern Cape |
| 15:15 | MED D'Amato | Design and Validation of a Highly Discriminatory 10-Locus Y-Chromosome STR Multiplex System for Forensic Application in South Africa |

15:30 – 16:00 TEA / COFFEE

16:00 FUTURE PERSPECTIVES ON HUMAN GENETICS

Chairperson: Soraya Bardien

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|-------|------------------|---|
| 16:00 | D Kumar | Genomic Perspectives of Evidence-Based Medicine |
| 16:30 | CM Aldous-Mycock | Genetics Curricula at four London Medical Schools |
| 16:45 | M Ramsay | Genetic Testing for Susceptibility to Complex Traits is Premature in South Africa |

19.00 CONFERENCE DINNER AT SKILPADVLEI

— WEDNESDAY 8 APRIL —

CHECKOUT AND DEPARTURE

POSTER SESSIONS: POSTER VIEWING DURING LUNCHEES AND TEA-BREAKS

Poster Batch A: up from Sunday noon to 14:00 Monday

Poster Batch B: up from 15:00 Monday to 17:00 Tuesday

■ Presenters are expected to be at their posters during the last 30 minutes of the lunch break

THANKS TO PARTICIPATING COMPANIES

THE ORGANISERS OF THE 13th SASHG CONFERENCE 2009 WOULD LIKE TO EXPRESS THEIR SINCERE APPRECIATION TO THE FOLLOWING COMPANIES FOR THEIR PARTICIPATION AND GENEROUS SUPPORT (*Alphabetical, at time of press*)

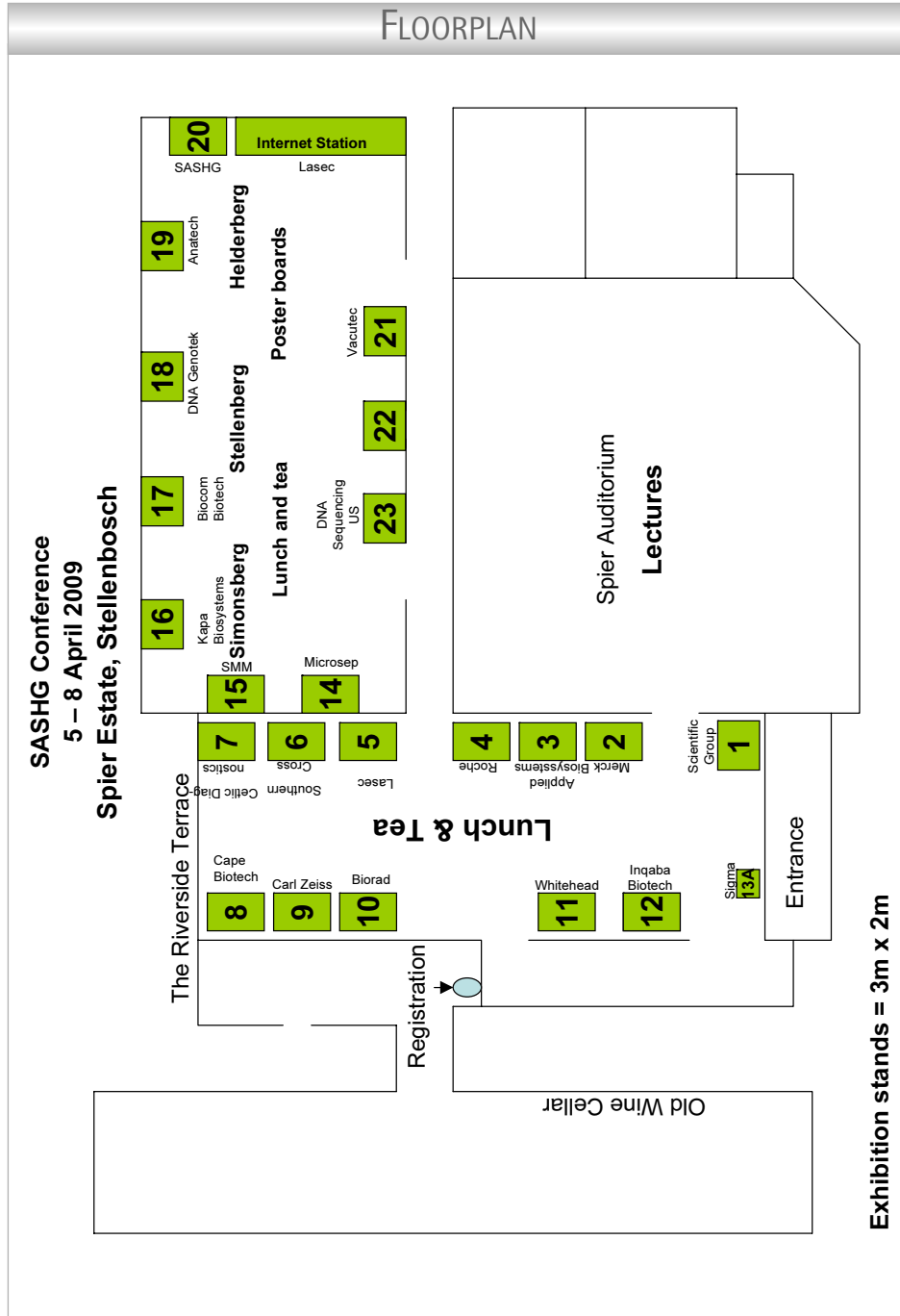
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| ■ DNA Genotek | ■ Vacutec |
| ■ DNA Sequencer US | ■ Whitehead Scientific |
| ■ Inqaba Biotech | ■ Support from the Faculty
of Health Sciences, University
of Stellenbosch is also
acknowledged |
| ■ Kapa Biosystems | |

STUDENT GRANT AWARDEES

SASHG 2009 Student grant awardees

Alphabetical by surname

- | | |
|------------------------|--------------------------|
| 1. Bosman, Marika | 11. Owens, Sarah |
| 2. Chatterjee, Koushik | 12. Pandor, Aisha-Bibi |
| 3. De Jager, Jomien | 13. Posthumus, Michael |
| 4. Drögemöller, Britt | 14. Strickland, Natalie |
| 5. Human, Veronique | 15. Truter, Erika |
| 6. Keyser, Rowena | 16. Van Dyk, Estresia |
| 7. Knezovich, Jaysen | 17. Van Heerden, Chrisna |
| 8. Leighton, Patricia | 18. Venter, Mauritz |
| 9. Mbongwa, Hlengiwe | 19. Vervalle, Jessica |
| 10. McGregor, Nathan | 20. Wright, Galen |
| | 21. Zandberg, Lizelle |



POSTER SCHEDULE

— MONDAY 6 April —

<i>Session</i>	<i>Poster No</i>	<i>Presenting Author</i>	<i>Title of Poster</i>
A	1	Urban M, Dr	Use of family history and pedigrees at Groote Schuur Hospital Outpatients Department
A	2	Glass M, Ms	Survey regarding the use of traditional medicines during pregnancy in women in Gauteng
A	3	Theron M, Prof	Chromosomal abnormalities in prenatal diagnosis
A	4	Olivier L, Ms	Implementation of a fetal alcohol spectrum disorders (FASD) Prevention Project in the Witzenberg district, Western Cape Province (2007-2009)
A	5	Chetty J, Ms	Retrospective chart review of referrals to the King Edward VIII clinic in Kwa- Zulu Natal from 1991-2007
A	6	Naidoo H, Ms	An analysis of the occurrence of Downs Syndrome at 3 hospitals in Durban
A	7	Wonkam A, Dr	Prenatal Diagnosis Represents A Point Of Entry Of Medical Genetics In Sub-Saharan Africa: Experience From Cameroon
A	8	Goliath R, Dr	A PCR based test for Fragile X syndrome: Validation
A	9	Noruzinia, Dr	Expansion In Fmr1 5' Utr Cgg Repeats Plus Recombination And Mosaicism In A Family With Fragile X Syndrome.
A	10	Owen EP, Dr	Pitfalls associated with diagnostic analysis of large genes using RT-PCR analysis.
A	11	Jano N, Ms	Aneuploidy FISH versus Karyotype
A	12	Kruger BJ, Ms	QF-PCR for the postnatal diagnosis of Down syndrome
A	13	Leighton PS, Ms	The Use of Family History and Pedigree
A	14	Myburgh M, Ms	Chromosome 18p+/18p(duplication) seen as a Normal Chromosome Variant
A	15	Oliver H, Ms	Unique mosaicism with trisomy 13, trisomy 18 and a normal cell line in a 17 year old female
A	16	Pfaffenzeller-Thom WM, Ms	Cytogenetics experience for the past 12 years and abnormalities detected during the year 2008
A	17	Ramjee H, Ms	A novel structural chromosome abnormality leading to a rare chromosomal disorder: Mosaic Trisomy 8 syndrome
A	18	Roberts LJ, Ms	Developing a molecular genetic diagnostic service founded on the use of the ABCR400 gene chip; lessons learnt.

<i>Session</i>	<i>Poster No</i>	<i>Presenting Author</i>	<i>Title of Poster</i>
A	19	Ruppelt T, Ms	More Than One Cause For Multiple Miscarriages
A	20	Sklar D, Ms	Infants requiring medical genetic assessment at birth: indications for referral and outcomes
A	21	Van der Merwe NC, Dr	Polymorphisms in DNA repair genes: potential modifiers of breast cancer risk in Afrikaner BRCA2 mutation carriers
A	22	Babb CL, Dr	11q25 cytogenetic aberrations in patients with Non-Hodgkin Lymphoma (NHL) and HIV
A	23	Owens SE, Dr	Genetic Modifiers of Age of Onset in Hereditary Nonpolyposis Colorectal Cancer in South Africa
A	24	Schnugh DS, Mr	Molecular Assessment Of Resistance To Tyrosine Kinase Inhibitor Therapy In South African Cml Patients
A	25	Adams DJ, Ms	A Novel Secondary Aberration in Chronic Myeloid Leukaemia Treated with Imatinib
A	26	Brady K, Ms	hMLH1 and hMSH2 mutation spectrum in 11 South African HNPCC families
A	27	Brown J, Ms	The rare, CML associated, variant BCR/ABL fusion transcript b2a3: implications for patient diagnosis, treatment and follow-up.
A	28	Bruwer Z, Ms	Personal Understanding of Predictive Test Result in South African Nonpolyposis Colorectal Cancer Families
A	29	De Klerk AH, Ms	Occurrence of double point BCR-ABL mutations in CML patients that are showing Gleevec resistance
A	30	Herd OJ, Ms	Simultaneous Amplification of the Her-2/neu Gene and Chromosome 17 Centromere in Breast Carcinoma Specimens
A	31	Theron M, Prof	The diagnostic and prognostic value of traditional cytogenetics in hematological malignancies
A	32	Moholisa R, Mr	Genetic polymorphisms in XPNPEP2 and ACE gene in hypertensive, ACE inhibitor (enalapril) induced angioedema patients.
A	33	Bloem LM, Ms	Sarcomeric modifiers of hypertrophy in hypertrophy cardiomyopathy
A	34	Parker M, Ms	Modifiers of Left Ventricular Hypertrophy in Hypertrophic Cardiomyopathy
A	35	Brink PA, Prof	Race and gender representation of hypertrophic cardiomyopathy or long QT syndrome cases in a South African research setting
A	36	Brink PA, Prof	Long QT syndrome type 1 (LQT1): Neural control of heart rate is a modifier of risk.

<i>Session</i>	<i>Poster No</i>	<i>Presenting Author</i>	<i>Title of Poster</i>
A	37	Carelse Tofa K, Ms	Variants in the energy metabolism gene, PDK4, are associated with increased cardiac muscle thickness in hypertrophic cardiomyopathy.
A	38	Swanepoel CC-A, Ms	HRM: a fast, cost-efficient approach to ab initio mutation-screening
A	39	Fourie Davies L-A, Ms	Longitudinal Study of the Neurodevelopmental Performance of Children Aged 5 with Fetal Alcohol Spectrum Disorder in Rsa Community
A	40	Baine F, Ms	Identification of a SNP for Allele-Specific Silencing of the Disease-Causing Gene In Sca1 Patients In South Africa
A	41	Arieff Z, Dr	Analysis of a SNP in the CNTNAP2 gene in an autistic and control South African population
A	42	Hemmings SMJ, Dr	Identification of SLITRK1 interactors: a means of identifying novel candidate genes for anxiety disorders
A	43	Botha M, Ms	Investigating polymorphisms in MAOA and MAOB and their role in Obsessive-compulsive disorder in the Afrikaner population
A	44	Frace SJ, Ms	Improving Knowledge, Evaluating Opinions, & Ascertaining Acceptance of Genetic Counseling for Bipolar Disorder: Responses in Us And India.

— TUESDAY 7 April —

B	45	Erasmus S, Ms	Duchenne and Becker Muscular Dystrophy: Implications for at-risk Individuals
B	46	Loubser F, Ms	Genetic counselling experiences in delivery of genetic research results to patients affected with ABCA4 associated retinal degenerative disorders.
B	47	Macaulay S, Ms	The role of genetic counselling in the management of cystic fibrosis patients and their families
B	48	Mophosho M, Ms	Interactive dynamics of a mediated genetic counselling session
B	49	Schoeman M, Ms	Level of Genetic Knowledge and its Impact on Reproductive Choices and Risk Communication in Families with Cystic Fibrosis
B	50	Solomon GAE, Ms	Basic understanding of genetic concepts amongst isiXhosa-speaking caregivers of haemophilia patients
B	51	Van Wyk C, Ms	The practices, knowledge, and attitudes about common hereditary cancers: Survey of General Practitioners in Johannesburg
B	52	September A, Dr	The COL1A1 gene and anterior cruciate ligament ruptures

<i>Session</i>	<i>Poster No</i>	<i>Presenting Author</i>	<i>Title of Poster</i>
B	53	Posthumus M, Mr	The COL5A1 gene is associated with increased risk of anterior cruciate ligament rupture in female participants
B	54	Adams LA, Ms	Association of MC3R in tuberculosis susceptibility
B	55	Human VR, Ms	Characterization of the 5' Regulatory Region of the Cytochrome B Reductase 1 (Cybrd1) Gene of Oesophageal Cancer Patients
B	56	Kinsley N, Ms	Filaggrin gene variants in black South Africans - predisposing factor for atopic dermatitis
B	57	Vervalle JA, Ms	Iron metabolism and oesophageal cancer: an exploratory investigation into SLC40A1
B	58	Strickland NJ, Ms	Molecular-Genetic Analysis of Ceruloplasmin in Oesophageal Cancer
B	59	Truter E, Ms	Consolidation of tuberculosis candidate gene studies in a South African population
B	60	Arndt S, Ms	Glucocerebrosidase Gene Mutations in Black South Africans with Gaucher Disease
B	61	Van Dyk E, Ms	Effect of Ht1 Metabolites on NER And/Or BER Repair of DNA Damage
B	62	Van Dyk E, Ms	Demethylating Effects of PHPPA in Hereditary Tyrosinemia Type 1.
B	63	Zandberg L, Ms	Elevated 3-hydroxyisovaleric acid and trace amounts of 3-methylcrotonylglycine are not necessarily indicative of 3-methylcrotonylglycinuria
B	64	Wright GEB, Mr	Molecular genetic analysis of two genes in the schizophrenia-susceptibility locus on chromosome 22q in Xhosa schizophrenia patients
B	65	Drögemöller BI, Ms	Analysis of sequence diversity in the CYP2C19 gene in a unique South African population
B	66	Werely CJ, Mr	SNaPshot analysis of polymorphisms in the human NAT2 gene: analysing acetylation in MDR-TB patients
B	67	Carvill GL, Ms	Investigating Genomic Copy Number Variations in South African Patients with Mental Retardation (Mr)
B	68	Esterhuizen A, Ms	A high throughput screen for point mutations in the DMD gene.
B	69	Human H, Ms	Rapid Genetic Screening Method for Six Known Mutations Associated with Aminoglycoside-Induced Deafness
B	70	Meldau S, Ms	SMA in the Western Cape remains true to (geno) type.
B	71	Theron M, Prof	The diagnosis of genetic defects by conventional cytogenetics

<i>Session</i>	<i>Poster No</i>	<i>Presenting Author</i>	<i>Title of Poster</i>
B	72	Bosman M, Ms	Sequence Variants in the Lgals13 Gene: A Role in Abruption Placentae?
B	73	Pandor A, Ms	An Investigation into Protein Misfolding In Retinitis Pigmentosa 17 and other Dominant Retinal Disorders
B	74	De Milander L, Ms	The Interleukin-6, Serotonin Transporter and Monoamine Oxidase A Genes and Endurance Performance During the South African Ironman Triathlon
B	75	Baeyens AJC, Dr	Radiosensitivity of Lymphocytes in HIV Positive Individuals
B	76	Aron SL, Mr	Genome annotation of the 1.2Mb region on chromosome 8p22-p23.1 harbouring the gene for keratolytic winter erythema (KWE)
B	77	Swanson RV, Ms	Comparison of two methods to make pan-centromeric probes for assessing cellular damage from low doses of ionizing radiation
B	78	Van Heerden C, Ms	DNA Methylation Status of the P16 Promoter Sequence in Blood and Tumour Tissue
B	79	Hobbs A, Ms	Genetics of Keratolytic Winter Erythema
B	80	Appel M, Dr	Genetic testing directly from whole blood using KAPA Blood PCR Kits
B	81	Tucker R, Ms	Type III OI is the most prevalent OI Type in the Black population of KZN
B	82	Cole-Showers CL, Prof	Genetic variation and possible association among some Nigerian populations- reviewing their origin
B	83	Heitkamp IM, Ms	Variation in the 5' untranslated region of the ABCB1 multidrug resistance gene in the black South African population
B	84	Mbongwa HP, Ms	Characterisation Of The Sult1a1 Polymorphism In The South African Tswana Population Group
B	85	Rousseau J, Dr	HLA -A, -B, -C and -DRB1 allele and haplotype frequencies in the South African Xhosa-speaking population
B	86	Gentle NL, Ms	Genetic Variation in PSIP1 in Black South Africans
B	87	Frost JM, Ms	Differential gene expression of MMP-1, TIMP-1 and HGF in South African systemic sclerosis patients
B	88	Kumar D, Dr	Disorders of Genome Architecture: A New Class in the Taxonomy for Human Disease



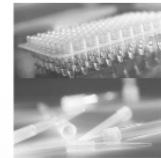
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Cape Biotech


To facilitate and invest in the development of a regionally focused, nationally integrated and world renowned Biotech economy, by focusing on select market offerings that create significant and sustainable economic and social value for South Africa.

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- Manage the risk of the investments in projects & portfolio
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CAPACITY DEVELOPMENT

- Via a set of ongoing interventions (business support, skills development, networking & knowledge dissemination events) that benefit the broader biotech



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- Nutraceuticals (Bio-processing)
- Vaccines (Combination & conjugated)
- Drug delivery
- Bio-prospecting (natural NCEs)




FOCUS AREAS

- Bio-entrepreneurial skills development
- Stakeholder network facilitation
- Cluster Facilitation
- Events & marketing
- Knowledge dissemination


FUNDAMENTAL RESEARCH



APPLIED RESEARCH



TECHNOLOGY & PROTOTYPE DEVELOPMENT



PRODUCT AND BUSINESS COMMERCIALISATION

Developing and supporting BIOTECHNOLOGY in the Western Cape

GENERAL INFORMATION

VENUE

The meeting will convene in the Auditorium at the Spier Estate's conference centre. The Clinical session on the morning of Sunday, 05 April 2009 will convene in the Amphitheatre Boardroom adjacent to the Spier Conference Centre.

REGISTRATION AND INFORMATION DESK

The registration and information desk will be situated in the foyer of the conference centre for the duration of the congress. The desk will be open for registration on Sunday, 05 April 2009 (12:00-14:00) and Monday, 06 April 2009 (08:00-08:30).

LANGUAGE

The official language of the congress will be English. No simultaneous translation services will be provided.

SPEAKER PRESENTATIONS

Speaker presentations must be handed in at the technical team in the Auditorium by LATEST the lunch / tea break prior to your scheduled time of presentation.

- All computer presentations must be checked for viruses and reach the technicians one hour before your presentation.
- Computer presentations must be in Windows Office XP or later versions and in Power Point.

ADMISSION BADGES

Congress badges should be worn by all participants at all times during the congress, whilst visiting the exhibition area and on all congress premises. Badges should also be worn when attending the events of the social programme. Only participants or accompanying persons wearing their congress badges will be admitted to the scientific sessions, the exhibition and the social events.

TOPICAL EXHIBITION

A topical exhibition will be located in the foyer in front of the auditorium, as well as the Simonsberg, Stellenberg and Helderberg rooms at Spier Conference Centre. Companies will exhibit their latest products and services in the field of Human Genetics and will be available to answer questions. You will have ample opportunity to visit the exhibition during refreshment breaks.

CREDIT CARDS

Major credit cards (American Express, Diners Club, MasterCard, and Visa) are accepted in most hotels, restaurants and shops. Conference registration fees need to be paid in advance, in cash local currency or local cheque. The conference centre does not offer credit card facilities.

FOREIGN EXCHANGE

The ABSA Bank in the Langenhoven Student Centre, University of Stellenbosch or in Plein Street, exchanges foreign currency and traveller's cheques, as well as the major banks in Cape Town and V&A Waterfront. Banks are open Mondays to Fridays, generally from 09:00-15:00 and on Saturdays from 09:00-11:00. Small amounts can also be exchanged at the reception of the Spier Village Hotel.

AIRPORT TRANSFERS

Airport transfers are for your own account and organisation. This can be booked at Minnaar Tours (approximately R170-00 one-way) at the following contact numbers:
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Delegates staying at The Spier Hotel can also organise transfers to and from the airport directly with the Traveldesk at Spier on tel: + 27 21 809 1911.

SOCIAL PROGRAMME

Sunday, 05 April 2009

The conference welcome function will take place at the superb Moyo Restaurant at Spier Estate at 19:00 for 19:30. The cost for attending this function is R50 per delegate and R300 for accompanying persons. Please note that you have to register to attend this function.

Monday, 06 April 2009

Delegates are cordially invited to attend a Spitbraai to be held at Welgevallen, Stellenbosch, sponsored by *Roche Diagnostics. This function is free for delegates while accompanying persons can attend at R150 per person. Please note that you had to register to attend this function.

Transport will be provided from the Spier conference centre to the spitbraai at Welgevallen on Monday 06 April 2009. The buses will depart from the conference centre at 17:15.

*Roche Applied Science: Innovating Genomic research by providing the complete genomic solution - LightCycler PCR, HRM, micro-arrays and high throughput sequencing. Visit www.roche-applied-science.com to find out more.

Tuesday, 07 April 2009

The conference will close on a high note with the Conference Dinner at the Skilpadvlei Wine Estate. The cost for attending this function is R50 for delegates and R375 for accompanying persons. Please note that you had to register to attend this function.

Transport will be provided from the Spier Conference Centre to the Conference Dinner at Skilpadvlei on Tuesday 07 April 2009. The buses will depart from the conference centre at 18:30.

CPD ACCREDITATION

The Congress has been accredited through Stellenbosch University. Please register for CPD credits on a daily basis at the Discovery Health stand.

ABSTRACTS: ORAL PRESENTATIONS

**Ordered according to programme*

MONDAY

— CLINICAL GENETICS —

Fabry disease in South Africa

PROF BD HENDERSON * (FPA/UFS), MR I SINCLAIR (NHLS/Wits), MS T LLOYD (Genzyme)

To assess the number of patients tested for, diagnosed with and treated for Fabry disease.

A retrospective analysis of laboratory and clinical records.

Fabry disease is seen across South Africa and needs to be included in the differential diagnosis of renal failure and certain forms of peripheral neuropathy.

Bardet Biedl Syndrome. A Founder Mutation in Black South Africans?

DR K FIEGGEN * (Division of Human Genetics, University of Cape Town), PROF E HEON (Department of Ophthalmology, University of Toronto), DR M URBAN (Division of Human Genetics, University of Cape Town), MS A ESTERHUIZEN (Division of Human Genetics, University of Cape Town), DR R GOLIATH (Division of Human Genetics, University of Cape Town)

Bardet Biedl syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder associated with retinopathy, intellectual disability, obesity, renal disease and genital abnormalities. The majority of our patients at Red Cross Children's Hospital are of indigenous Xhosa ethnicity. In a small cohort tested in collaboration with the University of Toronto, four children of Xhosa descent were found to be homozygous for the same BBS 10 mutation suggestive of a possible founder effect. The objective of this study was to describe the phenotypic expression of Bardet Biedl syndrome in our cohort of patients and to test an additional cohort for the same BBS 10 mutation. If confirmed to be a frequent cause of Bardet Biedl syndrome in our population, our intention would be to set up a diagnostic test for this particular mutation.

Retrospective review of records and prospective examination of affected individuals were performed to describe the phenotypic expression of BBS in a local cohort to allow correlation with genotyping.

PCR based molecular testing was performed in 12 individuals affected with BBS for a specific BBS 10 mutation.

Final conclusions can only be drawn once study completed.

Fetal Alcohol Spectrum Disorders in 2 communities in the Northern Cape Province

PROF DL VILJOEN * (FARR), MS L OLIVIER (FARR), PROF M URBAN (UCT),
PROF M CHERSICH (WHO), PROF C CHETTY (FARR), PROF L-A FOURIE (FARR)

To describe prevalence, characteristics and risk factors for fetal alcohol syndrome (FAS) and partial FAS among grade one children in the Northern Cape.

Design: Cross-sectional study using a two-tiered method for active ascertainment of FAS / partial FAS cases. This comprised screening of growth parameters, diagnostic assessment for screen-positive children using clinical and neuro-cognitive assessments, and maternal history of drinking during pregnancy. Mothers/care-givers of children with FAS were interviewed, as well as matched controls.

Setting: Primary schools in De Aar (8) and Upington (15).

Subjects: Grade One pupils in 2001 (De Aar, n = 536) and 2002 (Upington, n = 1299).

Outcome measures: FAS or partial FAS.

Results: Prevalence of FAS / partial FAS was high: 64/536 (119.4/1000, 95% CI = 93.2 – 149.9) in De Aar, and 97/1299 (74.7/1000, 95% CI = 61.0 – 90.3) in Upington. Overall, 67.2 per 1000 children (95% CI = 56.2 – 79.7) had full FAS features. Growth retardation was common: 66.6% (1181/1774) were underweight, 48.3% (858/1776) stunted and 15.1% had a head circumference <2S.D. for age. Interviews with cases and controls showed that mothers of children with FAS were less likely to have fulltime employment or have attended secondary school. These women also had lower body mass indices and about 80% currently smoked. Over two-thirds of all pregnancies were unplanned.

Nearly one in ten pupils has FAS / partial FAS, with the rate in De Aar the highest yet described in RSA. The epidemiological features described are important for designing essential preventive interventions.

Attitude of parents towards puberty and sexuality of their cognitively handicapped children

PROF G DE JONG * (div Molecular Biology and Human Genetics, University Stellenbosch Faculty of Health Sciences), DR PS STEYN (Dept Obstetrics and Gynaecology)

Test parental attitudes towards puberty and sexuality of their children with cognitive handicap and identify possible problem areas.

self-administered questionnaires were given to parents of these children at outreach clinics at LSEN schools and Down syndrome society meeting. Questionnaires were filled in anonymously after informed consent. Research assistant available for questions. Statistical analysis using SPSS vers16.

30 Children with Down Syndrome and 69 with other causes. Abilities and backgrounds varied. 44,6% had problems handling menstruation. A problem with sexual behaviour was reported in

12 of 95 cases. 68,8% of parents wanted their children to be on a contraceptive method. 46% of parents preferred sterilisation. 18,6% however would not mind if their children had offspring. A disturbing finding was the lack of knowledge of recurrence risk for offspring.

Puberty present parents of children with mental handicap with new decisions to take and sometimes problems to handle. Parents seem to need more advice and guidance.

Genetic Disorders on Oceanic Islands – St Helena and Tristan da Cunha

PROF PB BEIGHTON * (Division of Human Genetics, Faculty of Health Sciences, UCT)

To determine the presence and implications of genetic disorders on the Oceanic islands of St Helena and Tristan da Cunha.

Travel to the islands by boat, and examine affected families.

The following genetic conditions have been identified on these islands: St Helena: Christmas Disease (XL); HANO (AD); Retinitis Pigmentosa (AR); Albinism (AR); Familial Genu Valgum (AD); Acromesomelic Dysplasia (AR); Waardenburg Syndrome (AD); Symphalangism (AD); Foetal Alcohol Syndrome Tristan da Cunha: Retinitis Pigmentosa (AR); Nodal Osteoarthropathy (AD); Hypercholesterolaemia; Asthma.

Oceanic Islands have special genetic significance by virtue of their isolation, inaccessibility and well-defined population histories and demography. Heritable disorders on these islands are the consequence of the introduction of determinant genes, and in some instances, endogamy. The presence of specific genetic conditions has relevance for medical services. In the South African context, the islands of St Helena and Tristan da Cunha have special importance as tertiary medical services are provided in Cape Town. Other oceanic islands in the Southern hemisphere, notably Easter Island and Norfolk Island provide valuable insights into genetic epidemiology.

Hereditary Bone Dysplasia with Pathological Fractures and Nodal Osteoarthropathy

DR RE ARENDSE * (Tygerberg & Groote Schuur Hospitals), PROF PA BRINK (University of Stellenbosch), PROF PH BEIGHTON (University of Cape Town)

The aim of this paper is to present a previously undocumented hereditary bone dysplasia characterised by pathological fractures and nodal osteoarthropathy.

A 50 year old male and his 27 year old daughter both with multiple pathological fractures and nodal osteoarthropathy underwent clinical, biochemical, histopathological and radiological investigations. These were compared with other causes of a familial propensity to pathological fracturing. The Nosology and Classification of Skeletal Disorders too was referenced to determine if a combination of a fracturing tendency and nodal arthropathy had been reported previously.

The father had twenty healed fractures of the axial and appendicular skeleton, many of which had been surgically fixed prior to his first presentation to us. Fractures of the clavicles, thoracic cage and long bones of the arms and legs, had healed with malalignment and deformity. Healed

fractures were complicated by ankylosis of the cervical vertebrae and both elbows. He also had osteoarthritis of the hands with exuberant osteophytosis, and profound perceptive deafness. His general health was good, his intellect and facial features were normal, and his sclerae were white. The daughter had sustained seven fractures of the axial and appendicular skeleton. She had also experienced painful swelling of the fingers, which preceded progressive development of nodal osteoarthritis. Her hearing was normal. In both, biochemical and histopathological investigations yielded normal results. It was not possible to undertake molecular studies.

Pedigree data are consistent with autosomal dominant transmission and this disorder appears to be a previously undocumented heritable skeletal dysplasia.

— CANCER GENETICS —

Do polymorphisms in the p53 pathway influence breast cancer risk in Afrikaner BRCA2 mutation carriers?

DR NC VAN DER MERWE * (UFS & NHLS), MS B DAJEE (UFS), PROF G JOUBERT (UFS),
DR B VISSER (UFS)

The BRCA2 c.8162delG mutation confers a high risk for breast cancer (BC) in the Afrikaner, although variability in its development has been observed. This complicates genetic counselling and BC risk determination. Evidence has earmarked genes involved in the p53 pathway as potential candidate modifiers due to the functional loss of p53. Six polymorphisms in Tp53, WAF1 and MDM2 were selected and screened for in Afrikaner BRCA2 mutation carriers.

Sixty BRCA2 c.8162delG women were selected (of which 30 were affected with BC) and case matched with controls for age. All 120 participants were genotyped for polymorphisms within Tp53, WAF1 and MDM2 using PCR, RE, SSCP analysis and DNA sequencing.

Genotype analysis revealed no differences in gene frequencies between the controls and mutation carriers, but indicated disparities between the unaffected and affected participants. Construction of a Tp53 haplotype indicated that the 1 1 1 haplotype (representative of wild type alleles) was more frequent in the unaffected mutation carriers, whereas the 2 2 2 haplotype (all the variant alleles) was the most common in the affected BC mutation carriers. For WAF1, the majority of BC patients was heterozygous (46.7%) compared to only 20% for the unaffected cases ($P = 0.0244$). Homozygosity for the variant was associated with an increased risk among the affected BRCA2 mutation carriers (OR, 12 (95% CI, 1.78–512.97)).

Of the SNPs studied, the WAF1 variant was associated with an increased BC risk in the Afrikaner BRCA2 c.8162delG BC patients and could be a potential genetic risk modifier within this population group.

Fas and Fas Ligand Gene Polymorphisms in Susceptibility to Herpes Simplex Virus-2 Infections but not in Cervical Cancer Virus-2 infections but not in Cervical Cancer

MR K CHATTERJEE * (Medical Virology, Institute of Infectious Disease and Molecular Medicine, University of Cape Town), PROF U GYLLENSTEN (Departments of Genetics and Pathology, Uppsala University, Uppsala, Sweden), DR L VAN DER MERWE (Biostatistics Unit, Medical Research Council, Cape Town, Republic of South Africa), DR U GALAL (Biostatistics Unit, Medical Research Council,

Cape Town, Republic of South Africa), DR M HOFFMAN (School of Public Health and Family Medicine, University of Cape Town, Cape Town, RSA), PROF AL WILLIAMSON (Medical Virology, Institute of Infectious Disease and Molecular Medicine, University of Cape Town)

Herpes simplex virus type 2 (HSV-2) infections is a sexually transmitted disease and is also suggested as a co-factor for persistent human papillomavirus (HPV) infection leading to cervical cancer (CxCa). Fas and Fas-L genes play an important role in cell mediated immunity through the mechanism of apoptosis. Single nucleotide polymorphisms (SNPs) in those genes disrupt their ability of activation induced cell death leading to an unrestricted replication of the virus. These SNPs have been suggested to influence the susceptibility to several diseases including development of CxCa.

The aim of this study was to determine if CxCa and HSV-2 seropositivity was associated with FAS (-1377, -670) and FAS-L (844) SNPs in South African women.

The population consisted of 447 cervical cancer cases (106 blacks, 341 mixed ancestries) and 424 controls (101 blacks, 323 mixed ancestries) matched with their age group, ethnicity and urban/rural status. DNA was isolated and quantified. SNP genotyping was carried out and statistical analysis was done.

No significant association was found with CxCa and any of the polymorphisms. Analysing the data for non-CxCa controls, a highly significant association ($p=0.00020$) was found with Fas-1377A and HSV-2 infected individuals. The association was retained even after adjusting the data for ethnicity ($p=0.0290$). Haplotype analysis also showed significant association with Fas-1377 and HSV-2 positivity.

This is for the first time a genetic link of HSV-2 infection is being suggested. Our novel results suggest that Fas-1377 A/AA confer a susceptible effect to the infection with HSV-2.

HAMP as an Iron Regulator in Oesophageal Cancer?

MR NW MCGREGOR * (University of Stellenbosch, Department of Genetics),
DR MG ZAAHL (University of Stellenbosch, Department of Genetics)

The HAMP gene codes for the 25 amino acid protein, hepcidin, crucial to the regulation of the bodily iron status. Defects occurring within the promoter region of this gene may contribute to its dysregulation, subsequently resulting in an iron overload status. Iron overload has previously been identified as a risk factor in the development of various cancers.

Approximately 1500 bp of the 5'UTR of the HAMP gene was subjected to mutation analysis in order to identify promoter variants possibly affecting gene expression. Fifty Black, Xhosa-speaking South African individuals were screened against a cohort of 50 population-matched controls.

Four variants were identified within the screened promoter region: -153 C/T, -188 C/T, -429 G/T and -582 A/G; with the -429 G/T variant being of particular interest. The -429 G/T variant is found to disrupt a previously identified p53 response element required for the up-regulation of HAMP gene expression.

Variants occurring within the promoter region of the HAMP gene has been shown to alter gene

expression, provided they occur at points crucial to the transcriptional regulation of the gene. Dysregulation of the HAMP gene results in an increased uptake of dietary iron, which in turn could feed the proliferation of carcinogenic cells lines.

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Molecular cytogenetics of cancer in the 21st century and its clinical relevance, where to?

DR PW WILLEM * (Department of Hematology and Molecular Medicine, University of the Witwatersrand and the NHLS)

In parallel to the discovery of distinctive gene rearrangements associated with specific types of cancer and the development of target gene therapy, the field of molecular cancer cytogenetics has seen a steep increase in demand during the last 10 years likely to expand in the near future.

The objectives were two fold, applying molecular cytogenetic techniques for the diagnosis, prognosis, evaluation of response to treatment and follow up of patients with cancer, and identifying novel cancer genetic markers in regions of common rearrangements in leukemia, lymphoma and esophageal squamous cell carcinoma of high incidence in SA.

Over 6000 specialised tests were performed in 2008 in a diagnostic capacity. A combination of traditional cytogenetics, fluorescence in situ hybridization (FISH), quantitative reverse transcription PCR (RQ-PCR) and sequencing assays were performed to characterize patient's tumor and detect known target genes. In order to identify novel diagnostic/prognostic genetic markers, array CGH was applied on leukemia, lymphoma and ESCC cohorts for copy number analysis.

Significant differences in childhood leukemia associated translocations were observed between African and Caucasoid patients. Two diagnostic applications did stand out both for their increase in demand and their clinical relevance. The detection of BCR-ABL in chronic myeloid leukemia and of HER2 amplification in breast cancer, both genes alteration having a specific targeted therapy. Novel cancer markers were identified by CGH array including a 12p stem cell cluster in leukemia.

The field of molecular cancer cytogenetics is rapidly evolving toward a personalised approach that offers unique and tailor made therapeutic interventions opportunities.

— CARDIOVASCULAR GENETICS —

Unusual Clinical Severity of Congenital Long-QT Syndrome (LQTS) associated with the KCNQ1 A341V mutation

PROF PA BRINK * (University of Stellenbosch), PROF L CROTTI (University of Pavia), PROF A GOOSEN (University of Stellenbosch), PROF M HERADIEN (University of Stellenbosch), PROF A GEORGE (University of Venderbilt), PROF PJ SCHWARTZ (University of Pavia)

The reported life time risk of attacks, namely, syncope, cardiac arrest, or LQTS-related sudden cardiac death (SCD) is approximately 30%.

We compared the profile of persons with the KCNQ1 A341V mutation to other forms of LQTS and attempt to explain our findings by electrophysiological studies.

Mutation carriers (MC) genotyped were 166 and non mutation carriers (NMC) 154. The attack-pattern was compared to LQTS individuals in an international database. Mutant KCNQ1 plasmids

constructs were made with three mutations (A341V, G314S, and 543 del/ins) and expressed in Chinese hamster ovary cells line (CHO). Whole-cell currents (IKs) were measured by the patch-clamp technique.

A341V MCs are more symptomatic by age 40 years (79% versus 30%) and become symptomatic earlier (7.4 versus 13.9 years, both $P < 0.001$) compared to the international database. In functional studies the magnitude of IKs in the CHO cells co-expressing KCNQ1-A341V were reduced by 50%. Co-expression of a recessive LQTS mutant (543-del/ins) had no effect on IKs amplitude, whereas a strong dominant negative mutation (G314S) suppressed current by 70%. This demonstrates that KCNQ1-A341V behaves in a manner distinct from a pure loss-of-function allele as previously suggested.

KCNQ1-A341V is associated with unusually severe phenotype. A partial explanation is that it acts in a dominant negative way and is not purely haploinsufficient as reported. Subsequent studies in KCNQ1-A341V individuals from other ethnic backgrounds confirmed the severity to be intrinsic to the mutation and unlikely due to something specific in our population.

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The incidence of 22q11.2 deletion syndrome in children referred to a tertiary paediatric cardiology service: A prospective study

DR R DE DECKER * (Western Cape Paediatric Cardiology Service), MS Z BRUWER (Division of Human Genetics, University of Cape Town), PROF M SCHOEMAN (Division of Human Genetics, University of Cape Town), DR J LAWRENSON (Western Cape Paediatric Cardiology Service), DR L ZUHLKE (Western Cape Paediatric Cardiology Service), MS G SCHUTTE (National Health Laboratory Services)

1. To determine the exact incidence of the 22q11.2 deletion syndrome in a cohort of patients with congenital heart disease presenting to a tertiary cardiology referral centre.
2. To assess the utility of an international scoring system for the clinical recognition of the 22q11.2 deletion syndrome in patients with congenital heart disease.

All "new patients" with a significant congenital cardiac lesion presenting to the cardiology service at the Red Cross Children's Hospital were assessed for recruitment to the study. Once consent was obtained, all children were tested for the 22q11.2 deletion by the standard TUPLE1 fluorescent-in-situ-hybridisation (FISH) probe.

All children were assigned a clinical "O score" at presentation, based on the presence of 8 phenotypic hallmarks and used to estimate the indication for TUPLE1 FISH testing. A score of 2 or more suggests the need for FISH testing.

Since March 2008, to date 142 patients have been recruited and FISH tested; of these, 125 (88%) FISH tests have been reported, and 6 (4.8%) have been found to carry the deletion. The mean O score of these 4 positive patients on presentation was only 2.7 (range 2-3). This study suggests that the 22q11.2 deletion is almost 3 times as common in a referred cardiac population than previously anticipated. Possible reasons for this disparity will be discussed. Clinical suspicion must remain high to ensure that the diagnosis is not missed in these patients. The utility of a phenotypic scoring system in our patient population is unconvincing and requires review.

Variants in Renin and renin-binding protein genes modify cardiac hypertrophy in hypertrophic cardiomyopathy patients, independent of blood pressure

MS N CARSTENS * (MRC Centre for Molecular and Cellular Biology, University of Stellenbosch), PROF L VAN DER MERWE (Biostatistics Unit, Medical Research Council of South Africa), DR M REVERA (Department of Cardiology, IRCCS San Matteo Hospital, Pavia, Italy), DR M HERADIEN (Department of Medicine, University of Stellenbosch Health Sciences Faculty, Tygerberg), PROF PA BRINK (Department of Medicine, University of Stellenbosch Health Sciences Faculty, Tygerberg), MS A GOOSEN (Department of Medicine, University of Stellenbosch Health Sciences Faculty, Tygerberg), PROF JC MOOLMAN-SMOOK (MRC Centre for Molecular and Cellular Biology, University of Stellenbosch)

Hypertrophic cardiomyopathy (HCM), an inherited primary cardiac disorder mostly caused by defective sarcomeric proteins, is considered a model for studying left ventricular hypertrophy (LVH). The disease manifests extreme variability in the degree and pattern of LVH, even in HCM patients with the same causal mutation. The clinical phenotype of HCM can therefore be viewed as a product of sarcomere dysfunction and additional genetic modifiers. Components of the renin-angiotensin-aldosterone system (RAAS) are plausible candidate modifiers because of their effect on blood pressure and their direct hypertrophic effect on cardiomyocytes. The renin section of the RAAS pathway has not previously been investigated in HCM.

Here we investigated Single Nucleotide Polymorphisms (SNPs) in the renin (REN) renin-binding protein (RENBP) genes for association with cardiac hypertrophy traits, in 353 genetically and echocardiographically affected and unaffected family members, belonging to 22 HCM families with HCM founder mutations.

We found evidence for association of all three SNPs in RENBP and three of four SNPs in REN with one or more hypertrophic traits, including echocardiographically determined left ventricular mass and other wall-thickness measurements, independent of blood pressure and other known hypertrophy covariates ($p < 0.05$).

We demonstrate for the first time that variations in the REN and RENBP genes modulate hypertrophy in HCM, independent of blood pressure. Both these genes are plausible modifiers for hypertrophy due to their functions within the RAAS: renin is a rate-limiting component of the RAAS as it controls the initial conversion of angiotensinogen to angiotensin I, while renin-binding protein inhibits renin activity.

Gain-of-function mutation in TRPM4 cause progressive familial heart block type I

PROF VA CORFIELD * (University of Stellenbosch), MR M KRUSE (Centre for Molecular Neurobiology Hamburg, Institute for Neural Signal Transduction), MR E SCHULZE-BAHR (University Hospital Münster), MR A BECKMANN (Centre for Molecular Neurobiology Hamburg, Institute for Neural Signal Transduction), PROF PA BRINK (University of Stellenbosch), PROF O PONGS (Centre for Molecular Neurobiology Hamburg, Institute for Neural Signal Transduction)

Progressive familial heart block type I (PFHBI) a dominantly inherited cardiac bundle-branch disorder with high risk of complete heart block has been mapped to C19q13.

The disease-causing interval was further reduced through high resolution mapping and the causal gene within the interval identified through mutational and functional analysis.

Markers D19S1059 and D19S604 (4.3cM apart; ~ 0.5 Mb) defined a disease-causing interval with +/- 25 genes. Of these TRPM4, a member of the TRP (transient receptor potential)-channel gene-family was an attractive candidate. TRPM4 forms a widely expressed Ca²⁺- and voltage-activated, non-selective cation channel. In PFHBI a missense mutation is present in exon one changes Glu7 to lysine. In a cellular in vitro expression system wild-type and mutant (E7K) TRPM4b channels displayed similar gating parameters with respect to ion selectivity, Ca²⁺- and voltage-dependent activation, and single-channel conductance. In contrast, tissue-culture cells transfected with TRPM4b E7K consistently exhibited higher TRPM4b-current densities (40.3 ± 5.2 pA/pF, n=15, s.e.m.) than controls (21.7 ± 3.2 pA/pF, n = 16, s.e.m.). Immunofluorescence studies with tagged wild-type and mutant TRPM4b channel support the view that increase in TRPM4b E7K-current density is due to an increase of channel density in the plasma membrane.

We conclude that i) PFHBI is associated with a mutation in the TRPM4 gene and ii) the phenotype of this cardiac disorder is correlated with a dominant gain-of-function in TRPM4b-channel function. The relation between a gain in TRPM4b-channel activity and conduction block is under investigation.

— GENETICS IN PUBLIC HEALTH —

Health Care Needs Assessment for Medical Genetic Services in Middle and Low Income Nations

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Background: Consequent on improving health, education and infrastructure many middle- and some low-income nations have experienced epidemiological transition resulting in congenital and common complex disorders becoming health care priorities. The WHO [2000] recommended that developing countries implementing medical genetic services should produce a 'country report', in essence a strategic health care plan. The first component of such a plan is a health care needs assessment (HCNA). In the 1970s industrialised nations standardised the process of HCNA. The approach was never applied to medical genetic services. Experience from advising middle-income nations on developing their nascent medical genetic services indicates they are not undertaking HCNA. A major impediment is the lack of a documented approach.

Objectives: To develop an evidence-based tool for HCNA for medical genetic services in middle- and low-income nations.

Outcome: CAPABILITY, an EU funded Specific Support Action investigating the transfer of medical genetic knowledge and technology to developing nations, has formalised a HCNA for medical genetic services that middle- and low-income nations can utilise for their 'country reports'. Key

elements of the HNCA are the development of strategic aims; an evaluation of existing services and the environment in which they function; a review of epidemiology, effective interventions, opinions of consumers and professionals, available resources and possible constraints. Analysis of these enables the production of a HCNA.

This approach will assist nations with emerging economies to marshal and allocate their limited resources when developing medical genetic services

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Lessons learnt from a local audit of the prenatal diagnosis of aneuploidy.

DR L GEERTS * (Tygerberg Hospital)

Termination of aneuploid pregnancies is legal in South Africa. A selection process is utilized to identify pregnancies at high risk. Maternal age is often the only selection criterion used but ultrasound (US) findings can assist in the risk assessment.

Study Aim Assess the contribution of an integrated US risk assessment to the prenatal diagnosis of aneuploidy.

Retrospective study, including all aneuploidies detected perinatally in the Human Genetics Laboratory of Tygerberg Hospital (1.1.2003 to 31.12.2005) matched with prenatal US findings.

Classic aneuploidies made up 92% of abnormal perinatal karyotypes (124/135) and classic trisomies 84% (114). Only 63 women underwent an US examination and 33% of perinatal samples (45) were prenatal (41 with an abnormal US and all with a high risk result). Of the 130 clinically relevant aneuploidies, 60 underwent US assessment (46.2%), with a high risk result in 91.7% (55/60), US abnormalities in 90% (54/60) and invasive testing in 75% (45/60).

The false negative rate for our screening strategy, combining age and US findings, was 7.9% (5/63) (6.7% (4/60) when 47,xxx, xyy and xxy were excluded). The sensitivity was much higher than screening by maternal age alone (93.3% versus 43.2%, $p=0.00000$, OR 18.36 (95CI 5.80-64.53; RR 2.16 (95CI 1.71-2.72)). Lack of US assessment was reason in 80% of the 90 postnatal diagnoses. Failure of detection was mainly caused by lack of assessment and not its failure. Increased access to US services will improve the prenatal diagnosis of chromosomal abnormalities.

Prenatal screening for Down syndrome in the Peninsula Maternity and Neonatal Service: Past, Present and Future

DR MF URBAN * (Division of Human Genetics, UCT), MS P LEIGHTON (Medical student, UCT)

Advanced maternal age (AMA) remains the primary method for prenatal screening of Down syndrome in our service, despite increased use of ultrasound-based screening methods. Our study aimed to assess past and current effectiveness of prenatal screening for Down syndrome based on maternal age, and discuss implications.

Analysis of cytogenetic laboratory database 1975–2005 (stratified into 5 year periods) to compare rates of prenatal and postnatal rates of laboratory diagnosis of Down syndrome over time.

Of 5832 amniocenteses documented in the time period, 4300 were for AMA. Preliminary analysis indicates that frequency of amniocentesis for AMA peaked at 1071 for the period 1981–85 and has progressively declined since, with 367 amniocenteses for AMA in the period 2001–05. Conversely, there has been a progressive increase in amniocenteses for fetal abnormalities detected on ultrasound. Prenatal diagnosis of Down syndrome has remained relatively constant at 13–17 per 5-year period, with an increasing proportion based on ultrasound abnormalities. Cytogenetically confirmed postnatal diagnoses of Down syndrome were obtained from the same database, and have been relatively steady at 203–268 per 5-year period. Rates of amniocentesis uptake have only recently been monitored: among 209 women counseled for AMA in 2008, 64 (29%) accepted amniocentesis.

Only a small proportion of Down syndrome cases are diagnosed prenatally. There has been a reduction in prenatal diagnostic tests done for AMA, and uptake of amniocentesis is relative low. Implications for prenatal screening programs will be discussed.

— NEUROGENETICS —

Molecular genetic analysis of patients with malformations of cortical development

PROF DJ MORRIS-ROSENDAHL * (Institute of Human Genetics, University of Freiburg)

Malformations of cortical development (MCDs) are abnormalities of the cerebral cortex that arise as a consequence of an interruption in the formation of the cortical plate.

A large number of MCDs have been described, each with characteristic pathological, clinical, and imaging features; most patients have severe psychomotor retardation and epilepsy. Mutations in 11 genes are currently known to cause disorders of neuronal migration. Our aim is to clarify genotype-phenotype relationships for these disorders, as well as to identify new genes involved in cortical development.

Clinical data including brain magnetic resonance images (MRI) have been collected for over 300 patients with various malformations of cortical development. Comprehensive mutation analysis has been performed in all patients using cytogenetics, DNA sequencing, quantitative PCR, MLPA and comparative genome hybridisation (CGH).

Comparison of brain MRIs in patients with classical lissencephaly and mutations in the LIS1, DCX and TUBA1A genes has refined the genotype-phenotype correlation, and shed light on the effects of the type of mutation at the protein level. The relationship between genotype and phenotype in patients with cobblestone dysplasias

(Type 2 Lissencephaly) and mutations in the POMT1, POMT2, POMGNT1, and FKTN genes is unclear and awaits the description of additional patients. CGH and whole genome linkage analysis has revealed four additional loci harbouring genes for classical lissencephaly, polymicrogyria, microcephaly and cerebellar hypoplasia.

Extensive genetic analysis of patients with MCDs is aiding the classification of such disorders, increasing the effectiveness of genetic testing and counseling, and providing a resource for the identification of further genes involved in cortical development.

Identification and assessment of novel obsessive-compulsive disorder candidate genes residing in schizophrenia susceptibility loci.

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Obsessive-compulsive disorder (OCD) is a debilitating psychiatric disorder for which the underlying molecular aetiology remains unclear. Family studies have suggested that OCD may be caused by a complex interaction of environmental and genetic factors. In order to identify the genetic factors, most studies have focused on a limited set of candidate genes. Thus there is a need to identify and assess novel OCD candidate genes. One method of identifying novel OCD candidate genes is by utilising knowledge of diseases with phenomenological overlap with OCD, which lend themselves to better genetic dissection through linkage analysis, such as schizophrenia, which manifests some overlap in both symptoms and brain circuits with OCD. In the present investigation, schizophrenia susceptibility loci were searched to identify novel OCD candidate genes.

All genes residing in each of the selected loci were individually analysed using a battery of bioinformatic techniques in order to assess their potential candidature for OCD susceptibility. As proof of principle, 10 credible OCD candidate genes from 10 of these loci were assessed for their potential role in the aetiology of OCD by case-control association studies in a cohort of Afrikaner OCD patients and control individuals.

The C allele of the SYN3/-631C>G polymorphism, additively, and the C allele of the DLX6int1C/T polymorphism, dominantly, increased susceptibility to OCD in both an original and a combined replication sample, while interaction between these two polymorphisms also significantly influenced susceptibility ($p=0.038$).

These associations are exciting as they may point to novel mechanisms involved in OCD development.

Novel deletion variant identified in the promoter region of the DJ-1 gene in a patient with Parkinson's disease

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Parkinson's disease (PD), a devastating neurodegenerative illness, is due to a selective loss of dopaminergic neurons in the substantia nigra. PD-causing mutations have been identified in the DJ-1 gene resulting predominantly in loss-of-protein function. Oxidative stress has been implicated in the pathogenesis of central nervous system damage in neurodegenerative diseases. Recent studies suggest that DJ-1 forms part of the neuronal cellular defence mechanism against oxidative insults, due to its ability to undergo self-oxidation. We aimed to investigate the

functional significance of a 16bp DJ-1 deletion which spans the transcription start site and is situated 93bp from a Sp1 site.

The DJ-1 promoter region, containing the sequence flanking the 16bp deletion, was cloned into a pGL4.10-Basic luciferase-reporter vector and transfected into HEK293 and neuronal BE(2)-M17 cells. The effect of the deletion on promoter activity was assayed using a dual-luciferase reporter system. Promoter activity under hydrogen peroxide-induced oxidative stress conditions was also investigated.

The deletion caused a reduction in luciferase activity of approximately 47% in HEK cells and 60% in M17 cells compared to the wild-type, indicating the importance of the 16bp sequence in transcription regulation. Activity of both constructs was upregulated during oxidative stress, with the deletion variant responding more effectively.

This is the first report of a functional DJ-1 variant, which has the potential to influence transcript stability or translation efficiency due to its location. Further studies are necessary to confirm whether this DJ-1 promoter variant also confers a risk for the disorder as has been previously shown for other PD-causing genes.

A comparative study of two dominant retinal degenerative disorders on chromosome 17

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To identify the biological basis of disease in two clinically indistinguishable autosomal dominant forms of retinitis pigmentosa (adRP). The RP17 form of adRP is caused by a R14W mutation in the Carbonic Anhydrase IV (CA4) gene on chromosome 17q. Constructs of mutant (R14W) and wildtype versions of the CAIV gene were made and various tagged forms of these constructs were investigated in COS7 and HEK293 cells. The RP13 form of adRP is caused by a mutation in the spliceosomal component, PRP8. A genome-wide expression survey of splicing was conducted using the Affymetrix® Exon 1.0 ST microarray. The CA4 gene (RP17) is expressed in the endothelial cells of the choriocapilaris; whereas the PRP8 protein is universally expressed. Our cell biological work shows mis-folding, mis-trafficking of mutant CA4, and raised markers of apoptosis in mutant cell lines. The expression data on the RP13 gene has shown that a large number of genes are mis-spliced as a result of a defective PRP8 gene; it would seem that there is a global downstream effect resulting in biological compromise.

Although clinically indistinguishable, these two forms of adRP, are the result of totally disparate pathological mechanisms. RP13 reflects only a retinal phenotype, despite a biological aberrancy that is universally expressed in the body. The mutant RP17 gene (CA4) on the other hand, is localised in the choriocapilaris, and results in death of endothelial cells, and deterioration of the vascular supply, resulting in ischemia-related photoreceptor death.

— GENETICS COUNSELLING —

Roles of Genetic Counsellors in South Africa

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The genetic counselling profession is developing but no research on the roles genetic counsellors play has been carried out locally. The objective was to investigate the roles genetic counsellors play in South Africa.

Roles were investigated by means of: interviews with genetic counsellors using an interview schedule; data from their log-books; and departmental statistics, for the period 1 January 2006 to 30 September 2008. The sample includes all registered counsellors (22) who agree to volunteer (8 in Johannesburg, 2 in Cape Town, to date).

During the period, 5285 (Johannesburg figures only) clients were counselled (about 37% by counsellors and 63% by clinical geneticists) in Gauteng state hospitals (75%), private (19%), or outreach (6%) clinics. The main role of the 10 counsellors was counselling for monogenic, polygenic and chromosome disorders and prenatal diagnosis. About 120 clients were seen per counsellor, annually, for about 50 different diagnoses, and cancer genetic counselling was increasing. Counsellors also had an educational role and gave about 5 genetics lectures each, annually, to health professionals and the lay public. They received good supervision (~40 sessions a year) and in-service training and participated in research. Marketing roles, particularly to other health professionals, were increasing. Administrative roles included being on intake duty and letter-writing to referring doctors. The profession is seen as becoming a more high profile and widely accepted one, valued in both private and state practice, in future.

The roles of genetic counsellors are extensive and the profession is growing, but their expertise and service could be better utilised.

Do you know why you are here today? Genetic Counselling in an antenatal multicultural setting

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Research studies on the genetic counselling (GC) process in multicultural settings are limited. Little is therefore understood about the impact of diverse cultures and languages, social inequalities and poor health status on this process. Research in this area is being conducted and preliminary findings on the interactive dynamics in prenatal genetic diagnosis sessions for AMA, specifically participant's expectations, are presented here.

Using qualitative methodology, the required data were obtained from GC sessions. Sessions were in English and were video recorded, transcribed and analysed using a combination of thematic

content and discourse analysis. At the time of abstract submission the data from 15 sessions (15 participants, six counsellors) were analysed. Data collection is ongoing.

It was found that there is a standard structured format in conducting the sessions. Establishing the women's expectation was done at the beginning of the session (1–36sec). The women's access to the clinic was through a referral from another health care provider; about half of the women (7/15) did not know why they were referred and they did not know what to expect from the sessions. The counsellors used different strategies to deal with aligning their and the women's agenda.

Establishing a counsellees expectation of the GC session is critical. This sets the tone and has a major impact on the "success" of GC, and in prenatal sessions it influences the women's ability to make an informed decision. As facilitators and inhibitors in the process were identified, the results from this study can be used to improve practice.

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Delivery of an ophthalmic genetic service including a telephone counselling model:

A bench-to-bedside review

PROF LJ GREENBERG * (UCT), MS LJ ROBERTS (UCT), MS M SCHOEMAN (UCT),
MS Z BRUWER (UCT), MS K LOGGENBERG (UCT), MS F LOUBSER (UCT)

This review describes some of the challenges experienced in the translation of molecular genetic diagnostic results to clinical care and highlights the role of telegenetics in delivering an effective ophthalmic genetic clinical service in southern Africa.

Over a three year period, more than 60 molecular genetic test results were delivered by a genetic counsellor or genetic counsellor intern to individuals and families with a history of inherited forms of blindness, following genetic mutation screening using commercially available gene chips. The protocol for the delivery of test results was established by a multi-disciplinary team which includes genetic counsellors, genetic nursing sisters, medical geneticists, medical scientists and a clinical coordinator.

Locally validated genetic test results were delivered to patients/families with a variety of clinical diagnoses including Stargardt Disease, autosomal recessive Retinitis Pigmentosa, Macular Degeneration, Cone-Rod dystrophy or Lebers Congenital Amaurosis. There were four telephonic counselling sessions which will be highlighted in this review.

Commercially available microarray chips are effective for mutation screening. Once all the mutations identified are verified, carefully worded, meaningful written reports should accompany the personal delivery of genetic test results by an appropriately trained and experienced genetic counsellor. Given the size of South Africa and the scarcity of resources, the role of telegenetics in building the bridge from bench-to-bedside in ophthalmic genetics translational medicine is indisputable.

Grandmothers as gems of genetic wisdom: exploring South African traditional beliefs about the causes of genetic disorders

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Genetic counselling is a relatively new field in South Africa. Counselling models from developed, western contexts have been implemented in the South African context but these are not necessarily appropriate given our multicultural heritage and unique disease profile. Limited research exists which investigates cultural perceptions surrounding genetic syndromes and conditions. Prior research suggests intergenerational differences regarding models of causation which influence treatment-seeking paths. This pilot study therefore aimed to explore South African traditional beliefs regarding common childhood genetic disorders and congenital conditions.

Three focus groups were conducted with fifteen grandmothers from different cultural backgrounds in an urban community in Gauteng, in their home languages. Questions related to the role of the grandmother in the community, traditional beliefs regarding the causes of and cures for various genetic disorders, traditional explanations of heredity and community responses to genetic disorders. The focus groups were video recorded and the discussions transcribed and translated. Themes were extracted from the data using qualitative methods of thematic content analysis.

Preliminary results indicate a variety of cultural explanations for the causes of childhood genetic disorders and different community responses to children with genetic disorders. These causes can be classified into categories of ancestral reprisal, witchcraft and sorcery, mechanical explanations, social causes and religious attributions.

The results of this study have implications for genetic counselling practice in South Africa, which needs to take cognisance of traditional beliefs which may conflict with western counselling models.

TUESDAY

— COMPLEX DISORDERS I —

Whole Genome Scans in Tuberculosis and Aspects of Population Admixture

PROF EG HOAL * (Stellenbosch University)

Despite the large environmental component of tuberculosis (TB), the central role of the genetic makeup of the host is becoming clearer. The host genetic component impacts on susceptibility to disease progression, severity of disease, length of time on chemotherapeutic drugs before cure is effected, and on sensitivity to these drugs. Our aim is to identify these genes.

The progression in research methods from association studies to whole genome scans, very large case-control studies and whole genome association studies has been rapid in the last few years, due to technological advances. Population admixture is a new aspect to be exploited in furthering our understanding of the genetic loci crucial to disease risk, if the risk alleles are differentially

distributed between ancestral populations, which then enables admixture mapping studies. The population studied is the high-TB burden South African Coloured population.

All of the above methods were employed in the search for susceptibility genes to TB, and the genes identified were tested and in many cases confirmed to be involved. These genes often represented unexpected candidates in the disease, such as MC3R and CTSZ.

Genome-wide approaches have delivered a number of reproducible candidate genes in the search for determinants of susceptibility to tuberculosis, and new technologies are expected to continue and improve this approach.

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Investigation of chromosome 17 candidate genes in susceptibility to TB in a South African population

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Chromosome 17 is known to contain tuberculosis (TB) susceptibility genes. Polymorphisms in two of these genes, namely NOS2A and CCL2, have been associated with TB in various populations. To investigate a possible association of gene variants with TB in the South African Coloured population we used case-control association studies.

Genotyping of 10 polymorphisms from NOS2A and CCL2 was done in over 800 TB cases and controls by the amplification mutation refraction system, the SNaPshot genotyping method®, TaqMan® SNP genotyping assays or the SNPlex Genotyping System™. Haplotypes and linkage disequilibrium in the candidate genes were also investigated.

We found a significant association between TB and two haplotypes, containing the functional rs9282799 and rs8078340 SNPs, in the NOS2A promoter. The T allele of rs8078340, found in the haplotype overrepresented in cases ($p=0.015$, $p_c=0.038$, $OR=1.4$, $95\%CI [1.1-1.8]$), was previously shown to decrease the quantity of DNA-protein complex bound as well as the duration of binding and may decrease nitric oxide (NO) production. The C allele of rs8078340 was present in the haplotype more frequent in controls ($p=0.011$, $p_c=0.029$, $OR=1.4$, $95\%CI [1.1-1.8]$). In the single-point analysis of NOS2A, rs2779249 (previously associated with TB in Brazilians) and the functional rs8078340 were nominally associated with disease. No association was found between any of the other SNPs or haplotypes studied and TB.

This study presents evidence that haplotypes in the NOS2A promoter influence susceptibility to TB and confirms the importance of NO production in the disease.

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Patterns of variation in AIDS restricting genes in black South Africans

PROF T MCLELLAN * (University of the Witwatersrand)

Host response to HIV infection and treatment can influence disease outcome. The greater genetic diversity of African populations, together with a different history of infectious disease, suggest that variation in AIDS restricting genes could be different and therefore informative for the design of novel therapies.

We have sequenced parts of 10 genes that have been shown to influence HIV infection: APOBEC3G, TLR2, ABCB1, Haptoglobin, PSIP1, Interleukin-4, Interleukin-10, Tetherin, KIF3C and TRIM5. Two genes involved in response to antiretroviral therapy, Cytochrome P4502B6 and ABCB1, have also been examined. The upstream noncoding regions and some key coding regions were sequenced. Genotypic data were collected for several hundred individuals representative of black South Africans in Johannesburg.

One third of the variable sites found have not been detected previously. The population frequency of novel alleles ranged up to 15 %. Some regions had no variation. Two genes each had several insertion/deletion polymorphisms. There are significant differences in allele frequencies between this population and others. Over half of the variable sites are known only from African populations. The phase of haplotypic associations is the opposite of that found in European populations.

The results confirm high levels of genetic diversity and low levels of linkage disequilibrium known in African populations. Ongoing studies are evaluating functional effects of the variation on levels of gene expression, response to antiretroviral therapy, and rate of disease progression. High levels of variation indicate that genetic associations established in other populations should be applied to African populations with great caution.

Variants within the MMP3 gene are associated with achilles tendinopathy: possible interaction with the COL5A1 gene

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Sequence variation within the COL5A1 and TNC genes are known to associate with Achilles Tendinopathy. The primary aim of this case-control genetic association study was to investigate whether variants within the matrix metalloproteinase 3 (MMP3) gene also contributed to both Achilles tendinopathy and Achilles tendon rupture in a Caucasian population. A secondary aim was to establish whether variants within the MMP3 gene interacted with COL5A1 rs12722 variant to raise risk of these pathologies.

One-hundred and fourteen subjects with symptoms of Achilles tendon pathology and 98 healthy controls were genotyped for MMP3 variants rs679620, rs591058 and rs650108.

As single markers, significant associations were found between the GG genotype of rs679620 (OR=2.5, 95% CI 1.2-4.90, P=0.010), the CC genotype of rs591058 (OR=2.3, 95% CI 1.1-4.50, P=0.023) and the AA genotype of rs650108 (OR=4.9, 95% CI 1.0-24.1, P=0.043) and risk of Achilles tendinopathy. The ATG haplotype (rs679620, rs591058, and rs650108) was under-

represented in the tendinopathy group when compared to the control group (41% vs 53%, $P=0.038$). Finally, the G allele of rs679620 and the T allele of COL5A1 rs12722 significantly interacted to raise risk of AT ($P=0.006$). No associations were found between any of the MMP3 markers and Achilles tendon rupture.

Variants within the MMP3 gene are associated with Achilles tendinopathy. Furthermore, the MMP3 gene variant rs679620 and the COL5A1 marker rs12733 interact to modify the risk of tendinopathy. These data further support a genetic contribution to a common sports related injury.

Analysis of genetic variation and obesity-related traits in the Birth-to-Twenty cohort using the Illumina BeadXpress genotyping platform

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Heritability studies have demonstrated a genotype-phenotype correlation in obesity, with whole-genome association studies linking several genes and regions to obesity risk. The Birth-to-Twenty project is the largest and longest running study of child and adolescent health and development in Africa, and one of the few large-scale longitudinal studies in the world.

Over 400 participants from the Birth-to-Twenty cohort will be evaluated for genetic variation within a selection of candidate genes previously shown to be related to obesity. Genes were selected based on a literature review and SNPs were chosen based on previously reported associations and linkage disequilibrium in an African population. In addition, a selection of ancestry informative markers was included to assess population structure. Genotyping will be performed using the GoldenGate assay on the Illumina BeadXpress genotyping platform geared at low- to medium-throughput genotyping projects. It allows the simultaneous analysis of multiples of 96 SNPs, up to 384, in a single sample. 13 genes, including FTO, MC4R, LEP and NPY2R, were selected and represented by 64 SNPs. 18 Ancestry informative SNPs were chosen. The assay was evaluated computationally by Illumina.

Association of increased body-mass index, hip circumference and weight with genetic variants will be evaluated. Ancestry informative markers will be analysed using STRUCTURE, a model-based clustering method that utilizes multi-locus genotype data to infer population structure. This pilot study aims to demonstrate the utility of the BeadXpress platform in association studies.

— COMPLEX DISORDERS II —

The Effect of Alcohol on the Methylation Status of the H19 Imprinting Control Region in Mice

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The H19-Igf2 imprinted locus is critical in foetal development and is regulated by the methylation-specific CTCF binding protein which binds the H19 imprinting control region (ICR).

Alcohol reduces global DNA methylation which may affect gene expression. The effect of alcohol on the DNA methylation of the H19 ICR was examined in mouse sperm and in their offspring.

Male mice were gavaged with ethanol or sucrose. DNA was extracted from sperm of treated males and tail biopsies from offspring. Samples were bisulfite modified and the H19 ICR amplified using nested PCR. The ICR was analysed quantitatively via pyrosequencing.

No significant difference was observed in sperm samples of treated males. A significant reduction in DNA methylation at the first ($p=0.0055$) and second ($p=0.0091$) CTCF binding sites of the ICR in the offspring of ethanol treated sires was observed. A lower growth rate trend was observed in female and male offspring sired by ethanol treated males. At day 35, male offspring sired by ethanol treated males had a significantly lower growth rate ($p=0.0442$) than controls.

Although no significant difference in sperm DNA methylation was observed, decreased methylation profiles were evident at the H19 CTCF binding sites and reduced growth rates observed in offspring sired by ethanol treated males. These data suggest that excessive prenatal ethanol exposure has the ability to negatively influence the epigenetic state of developmentally significant imprinted genes in sired offspring, which correlates with reduced postnatal growth.

Adapting the MeDIP assay for identification of methylated DNA sequences via direct sequencing

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Hypermethylation of gene promoters mediates epigenetic gene silencing, and it has been shown to be altered during development and the lifetime of mammals, resulting in several malignancies, including tumorigenesis. Methylated DNA immunoprecipitation (MeDIP) is an immunocapturing approach commonly followed in microarray analyses. This is, however, an expensive and exclusive technology, while direct sequencing is readily available and accessible. The aim of this study was to adapt the current MeDIP protocols for subsequent identification of hypermethylated DNA regions via sequencing.

The MeDIP assay is based on the recognition of 5-methylcytidine (5mC) by an antibody associated with an IgG-coated magnetic bead for subsequent isolation. We used genomic DNA isolated from cultured HepG2 cells to standardize the protocol, which was then applied to samples treated with various reagents which influence DNA methylation, including demethylating agents, methylating agents and a glutathione depletor. After isolation of the methylated DNA fragments, they were ligated into a blunt-cloning vector and transformed into competent E. coli cells. Colony PCR was performed and the amplified fragments were sequenced. The methylated DNA fragments present in the cultured HepG2 cells were successfully isolated and ligated into an appropriate vector for sequencing.

This new application for the MeDIP method enables general molecular laboratories, without access to microarray technology, to identify hypermethylated DNA sequences involved in various pathologies fast and cost-effectively.

Unravelling the PP13-Annexin II complex in Pre-eclampsia

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Reduced levels of PP13 early in pregnancy are associated with a higher incidence of pre-eclampsia in later gestation. PP13 and Annexin II have been co-localised to the brush border membrane of syncytiotrophoblasts, and form a complex which is transported to the maternal circulation. We hypothesise that genetic alteration in the gene encoding Annexin II (ANXA2) may underlie the reduced PP13 levels.

We report the screening of 6 of the 13 ANXA2 exons in a cohort comprising 77 pre-eclampsia maternal-fetal pairs and 50 healthy control individuals. The various conformations identified on Multiphor SSCP/HD gels were subjected to automated sequencing, and subsequently to REA, to confirm the genotypes.

Seven novel SNPs were identified, including intronic: IVS7+38 (C/T), IVS6-17 (A/G) [rs12898604], IVS8-5 (C/T), IVS9+49 (C/T), IVS12-30 (A/G), IVS12-24 (C/G) and exonic Ser96Gly (G/A), Thr97Ala (A/G) and Gly15 (C/T) [rs12442554] loci. Significant association was demonstrated at two loci: Ser96Gly was identified in 4% of the controls, but was absent in the maternal cohort ($p=0.012$), implying a protective effect or reduced pre-eclampsia risk. Marginal association ($p=0.03$) was demonstrated at intronic IVS8-5 C/T locus in the fetal cohort (4%), but was absent in the controls.

This pilot study provides evidence for genetic alteration in the ANXA2 gene, which could impact on binding with PP13. Intronic splicing and coding variant analyses and their potential effects on gene expression, protein functionality and interaction with PP13 should be included in follow-up studies.

In silico promoter models facilitate target prediction in the heme biosynthetic pathway.

DR M VENTER * (Stellenbosch University, Department of Genetics), MR SE ROBERTSON (Stellenbosch University, Department of Biochemistry), DR A LOUW (Stellenbosch University, Department of Biochemistry), PROF L WARNICH (Stellenbosch University, Department of Genetics)

Elucidation of gene regulatory complexity holds much promise towards aiding therapeutic interventions in medical research. With special emphasis on the promoter, accurate analyses of cis-motif architecture combined with integrative in silico modelling might serve as a more refined approach for prediction and study of regulatory targets and major regulators governing transcriptional control.

To demonstrate this approach, we used a combination of specific computational tools i) comparative sequence conservation analysis (rVISTA), ii) probabilistic detection method, expectation maximization (MEME) and iii) transcription factor (TF) binding site databases combined with first round functional analyses to construct in silico promoter models of the heme biosynthetic pathway enzymes. Heme is a major regulator in several cellular functions and impaired biosynthesis of heme lead to several hematological disorders, aging and tissue degeneration.

We discovered highly conserved Alu-repeat elements with specific nuclear hormone receptor direct repeat (DR) cis-elements that reside within the promoters. Initial promoter-reporter analysis supports in silico predicted cis/trans interactions and provides proof of concept.

We thus suggest that in silico promoter models could be used for the prediction of transcription factors and "master"-regulators governing transcriptional control and that an integrative strategy could serve as a diligence assessment for a more refined experimental design.

— GENE THERAPY AND SERVICE DELIVERY —

Knockdown of the disease-causing gene in South African patients with SCA7 using allele-specific RNAi

MS J SCHOLEFIELD * (UCT; University of Oxford), PROF J GREENBERG (UCT), PROF M WEINBERG (Wits), DR P ARBUTHNOT (Wits), DR A ABDELGANY (University of Oxford), DR M WOOD (University of Oxford)

The ability of RNA interference (RNAi) to knock-down gene expression in a sequence-specific manner has been exploited in numerous disease models. Since RNAi can be abolished by the introduction of nucleotide mismatches, mutant transcripts can be selectively targeted in a dominantly inherited disorder. In South Africa, a founder effect exists in the polyglutamine disease, spinocerebellar ataxia 7 (SCA7), such that over half of the patients with SCA7 are heterozygous for an exonic single nucleotide polymorphism (SNP). Thus, the objective was to use RNAi-based mechanisms to specifically knock-down the disease-causing gene in SCA7 in an in vitro model.

Using this SNP to discriminate between mutant and wild-type transcripts, expressed short hairpin RNA (shRNA) cassettes were designed, cloned and tested in various hemi- and heterozygous cellular assays in triplicate. Counting of cellular aggregates was performed with the most selective hairpin using fluorescence microscopy. Finally, the same hairpin was re-designed to mimic a predictably safer microRNA (miRNA)-based structure, and tested in the aforementioned cellular assays.

Significant selectivity ($p < 0.05$) can be achieved by placing the mismatch 3' to the centre of the guide strand. Introduction of the selective shRNA in a heterozygous model restores the wild-type protein to its native expression pattern. Cloning of the selective shRNA into a more natural miRNA-based structure demonstrates remarkably similar selectivity at the same position.

Our data has implications not only for mechanistic aspects of RNAi, but also provides a platform to investigate an in vivo RNAi-based therapy for SCA7, specific to South African patients.

Trends in DNA testing for cystic fibrosis at the division of human genetics, NHLS, Johannesburg

MS F ESSOP * (NHLS), PROF M RAMSAY (NHLS), PROF A KRAUSE (NHLS)

Cystic fibrosis (CF) is a common autosomal recessive disorder which is present in all South African populations. It is characterized by pancreatic insufficiency, chronic lung disease, elevated sweat chloride levels and a number of other features. The clinical presentation and severity is variable. CF is caused by mutations in the CFTR gene, with more than 1600 mutations identified. DNA testing for CF at the Division of Human Genetics, NHLS Johannesburg is offered routinely. Trends in cystic fibrosis (CF) DNA testing were investigated over a period of 5 years (2002–2006).

White, coloured and Indian patients are tested for 30 common CF-causing mutations. Black patients are tested for the common black mutation, 3120+1G>A.

An increasing number of CF tests are performed annually (78 in 2002 up to >100 in 2006). Approximately 73% of tests during 2002–2006 were referred to confirm a clinical suspicion of CF. From the ethnic distribution of referrals it was noted that 57% were white, 26% black, 11% coloured and the rest are not known. An average of 77% of referrals were from the public sector of which 70% were from Gauteng. Approximately 23% of referrals were received from the private sector. Since the introduction of genetic counsellors at the CF clinics, an increase in the number of referrals has been noted. Two CF-causing mutations were identified in ~30.5% of white patients, ~10.7% had 1 mutation identified and 58.8% had no mutation identified. 18 prenatal investigations were undertaken.

There is an increasing demand for CF diagnostic, carrier and prenatal testing.

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Severe fetal anomalies and acceptance for late termination of pregnancy amongst women referred for 3rd level scan

MS N CHABILAL * (Fetal Medicine Unit, Inkosi Albert Luthuli Central Hospital), DR L GOVENDER (Fetal Medicine Specialist, Dept of Obstetric & Gynaecology, Nelson Mandela School of Medicine, UKZN.), PROF WS WINSHIP (Associate Professor; Dept. of Paediatrics, Nelson Mandela School of Medicine, UKZN)

To examine the correlation between the types of fetal anomalies and acceptance for late termination of pregnancy (TOP) amongst women referred for 3rd level scan in KZN.

Data was extracted from the medical records of all women seen at the Fetal Medicine Unit at Inkosi Albert Luthuli Central Hospital during the period August 2003–November 2008 with the diagnosis of severe congenital abnormalities detected after 24 weeks gestation. Five independent categories of severe congenital abnormalities were analysed in relation to women's choices regarding acceptance for late TOP.

A total of 2190 women had a level 3 scan during the study period. Severe congenital abnormalities were detected in 244 women after 24 weeks gestation. Isolated central nervous system abnormalities were detected in 95/244 (39.5%) women, of which 69/244 (73%) accepted TOP.

There were 42 (17%) women in each of the category of neural tube defects and chromosomal abnormalities, of which 27/42 (64%) and 24/42 (57.1%) women accepted TOP respectively. Forty seven of 57 (81.3%) of women with multiple congenital abnormalities and 5/6 (83%) women with major twin abnormalities accepted late TOP. The overall acceptance for late TOP was 173/244 (71%).

The majority of women seen at our unit come from a low socio-economic background with strong cultural beliefs. Our study shows a high acceptance rate for late TOP. Possible reasons for women's changing attitudes toward late TOP will be presented.

Genetic Service Delivery in the Eastern Cape

MS CL MASSYN * (Cytogenetics Unit, Pathcare, St Georges Hospital, Port Elizabeth)

Discussion regarding Genetic Service Delivery in the Eastern Cape.

Although a "Human Genetics Policy Guideline" was published by the DOH in 2001, which set standards by which Medical Genetics should be governed, these guidelines, in most instances, due to a lack of staff or infrastructure fail to be realistic.

Further to the workshop of the SASHG held in Cape Town in March, 2006 on "Priorities in Service Delivery in Medical Genetics", this presentation focuses on the GSD in the Eastern Cape.

There is in reality very often a huge gap between the guidelines and the reality of Medical Genetics in practice.

The lack of involvement by the Provincial Health Department and the near absence of genetic services in all areas except the Western Cape and Gauteng are factors which hinder the development of GSD.

This presentation shall entail a brief description of the GSD in the Eastern Cape which includes predominantly the two cities of East London and Port Elizabeth and vast rural areas.

Discussion with involved parties and research.

A strong clinical component in identifying and referring of patients from community clinics is sorely lacking.

Future need: A Regional Service who has been involved with the training of community nurses who is able to converse in the local language and who may work alongside the diagnostic laboratory to provide a Genetic Service not only to the private sector but also to all public hospitals and the rural clinics.

Design and validation of a highly discriminatory 10-locus Y-chromosome STR multiplex system for forensic application in South Africa

DR MED D'AMATO * (Forensic DNA Lab, UWC), PROF VB BAJIC (SANBI, UWC),

DR MB BENJEDDOU (Forensic DNA Lab, UWC), PROF SD DAVISON (Forensic DNA Lab, UWC)

The Y-chromosome STRs (short tandem repeat markers) are routinely utilized in the resolution of forensic casework related to sexual assault. For this, the forensic community has adopted a set of eleven (core) Y-STRs that are incorporated in all commercial diagnostic systems. The Forensic DNA Lab at the UWC have studied Y-STR polymorphisms in South African populations and identified low levels of diversity and discrimination capacity for many Y-STR loci that are incorporated in commercial human identification systems. This finding highlights the limited applicability of the known commercial systems in the resolution of forensic casework in South Africa. To overcome this limitation, we designed a Y-STR 10-plex system that shows higher discriminatory capacity than all known commercial systems.

We genotyped a total of 283 individuals from three different populations groups (English Caucasian, Asian Indian and Xhosa) at a total of 44 Y-STR loci, including all known commercial

Y-STRs and additional novel loci. We selected the minimum number of loci that achieve the highest discriminatory capacity.

A total of 10 Y-STR loci were arranged in a single multiplex that was successfully subjected to validation exercises.

We designed a multiplex with 10 Y-STR loci that outperforms the known Y-chromosome STRs commercial human identification systems in its discriminatory capacity to be applied in forensic casework in South Africa.

— FUTURE PERSPECTIVES ON HUMAN GENETICS —

Genomic perspectives of evidence-based medicine

DR D KUMAR * (Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK)

To study the genomic basis of evidence-based medicine.

Review of literature.

Clinicians, public health practitioners, health commissioners/providers and planners, politicians and public seek formal 'evidence' in approving and funding any form of health care provision. Essentially 'evidence-based medicine' [EBM] aims at the conscientious, explicit and judicious use of the current best evidence in making decisions about the individualised patient care. Human and medical genetics research has contributed enormously in the clinical care of patients and families. Clinical genetics offers truly evidence-based health care in dealing with a number of inherited disorders that requires the accurate diagnosis and factual information on the molecular pathology and phenotypic correlations. Since the completion of the human genome project rapid accumulation of huge data on genomics, scientists and physicians are excited on the prospect of 'personalized health care'.

Translational research in human genetics and genomics has led to developing powerful tools for clinical diagnosis, assessing individual's genomic profile for disease prediction and prevention, high-throughput genome-wide screening for predisposition and/or protection to complex medical conditions, and discovery and development of new drugs and vaccines. Key examples include cascade genetic testing in cystic fibrosis, familial hypercholesterolemia, inherited cardiomyopathies and channelopathies and familial breast/ovarian cancers. The first decade of the new millennium now witnesses the transition to the 'genomic medicine'.

The practice of medicine, including health promotion and prevention of disease, stands now at a wide open road as the scientific and medical community embraces itself with the rapidly expanding and revolutionising field of translational genomic research.

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Genetics Curricula at four London Medical Schools

DR CM ALDOUS-MYCOCK * (UKZN)

To investigate the undergraduate and post-graduate genetics curricula at four London Medical Schools in order to inform curriculum change at the Nelson R Mandela School of Medicine.

Structured interviews were conducted with curriculum experts and genetics lecturers at Imperial College, St George's, University College London and King's College medical schools in London.

The four different medical schools offered genetics at different levels for different health care professionals.

These four medical schools offer genetics education at an undergraduate level because the discipline is becoming more relevant to the practice of medicine. In order to improve the undergraduate curriculum at the Nelson R Mandela School of Medicine it is important to take into consideration the observations made at other universities.

Genetic testing for susceptibility to complex traits is premature in South Africa

PROF M RAMSAY * (Division of Human Genetics, National Health Laboratory Service and University of the Witwatersrand)

South Africa is home to people with diverse origins spanning the continents of Africa, Europe and Asia. They bring a history of genetic variation that has arisen over thousands of years under different environmental conditions. How do we assess the appropriateness of DNA screening for genetic susceptibility to disease in South Africa?

A literature survey examines the scientific validity of potential tests for non-communicable diseases (like diabetes and cardiovascular disease) and pharmacogenetic traits.

Studies on South African populations are limited, restricting our knowledge of genetic susceptibility markers. Several studies have demonstrated that African populations, in particular, have different genetic variants which predispose them to disease. Some ubiquitous susceptibility alleles have different frequencies in Africans affecting the utility of genetic tests. With rare exceptions, the predictive value of individual variants is questionable and should be viewed in the context of wider genetic variation, which may be population or even individual specific.

The clinical validity of genetic susceptibility tests in a South African context can only be evaluated with sufficient local research that will enable objective assessment of sensitivity and specificity. Clinical utility is of critical importance to testing in a public health setting and in health insurance based on a cross-subsidisation model. Genetic tests for susceptibility to disease, drug metabolism rates and adverse drug events must be validated for the people to whom they are being offered. How do we ensure appropriate and timely introduction of tests in South Africa?

ABSTRACTS: POSTER PRESENTATIONS

**Ordered according to poster schedule*

A1

Use of family history and pedigrees at Groote Schuur Hospital Outpatients Department

DR M URBAN * (Division of Human Genetics, UCT), DR O LASKOV (Division of Human Genetics, UCT)

Family history (FH) is valuable in identifying genetic disorders, and in stratifying risk for common and potentially preventable multi-factorial diseases (MD's). This study aimed to review FH-taking in adults attending Groote Schuur Hospital and assess screening practices for common MD's, focusing mostly on some cancers, and the 'metabolic syndrome' group of conditions.

Retrospective review of medical records randomly selected from those attending specialist outpatient clinics in a one week period. Records of 50 patients were assessed. Patients aged over 50yrs were excluded following a pilot study.

Of 50 records, covering a total of 104 clinics, 36 (72% of records) had evidence of FH-taking by at least one clinic they attended, 3 (6%) were in the form of pedigrees and 13 (26%) adequately explored potential genetic causes of the condition. The study compared screening for FH and environmental risk factors of MD's, with 32 (64%) cases having evidence of screening for environmental factors and 23 (46%) cases having evidence of screening for FH (chi-square = 0,11), of which 12 (52%) had sufficient information to stratify risk of developing MD's.

FH was obtained in the majority of cases but only a minority were adequate to the condition being managed. Pedigrees were rarely used. Screening for risk of MD's was suboptimal, although there was a trend for environmental risk factors to be better collected than FH. Inability to stratify risk for common MD's on the basis of FH has implications where potentially modifiable risk factors play a role in the aetiology of MD's.

A2

Survey regarding the use of traditional medicines during pregnancy in women in Gauteng

MS M GLASS * (NHLS and Wits), MS J LAMPRET (NHLS), MS T ZWANE (MRC)

To establish whether women use traditional medicine during pregnancy;

To determine the ages, ethnic groups and socio-economic status of those women who do use traditional medicine, at what stage of pregnancy they use them, and to clarify the reasons for which women use traditional medicines

The study design was a survey, using a questionnaire, conducted by a research nurse from the Medical Research Council and the NHLS. Questions included demographics, pregnancy history, and use of traditional and western medication. Sample consisted of 150 pregnant women attending antenatal clinics at the three academic hospitals in Gauteng.

12% (5) of the women used traditional medicine during the first trimester of pregnancy; 42% (18) during the second trimester; 33% (14) during the 3rd trimester; 2% (1) during 1st and 2nd trimester and 12% (5) during all three trimesters; Reasons for the use included "easing the pain of labour", to "protect the pregnancy", and to "protect the baby from evil spirits".

Women use traditional medicine at all stages of pregnancy and for a variety of reasons. It was not possible however, to determine whether they shared all the information about their use of traditional medicine during the pregnancy.

A3

Chromosomal abnormalities in prenatal diagnosis

PROF M THERON * (UFS & NHLS), MS TB NTHANGASE (NHLS), MS IZ SPIES (NHLS)

Chromosomal abnormalities are the leading cause of spontaneous abortions, accounting for approximately 50% of clinically recognized early pregnancy losses. It is estimated that a minimum of 10 – 15% of conceptions have a chromosomal abnormality, and at least 95% of these are lost before term. Conventional cytogenetic analysis of miscarriages indicates that about 50% of these abnormalities are trisomies, 20% are monosomies, 15% are triploids and the remainder consists of various structural abnormalities.

During the period January to July 2008 eighty-three amniotic fluids samples were referred for prenatal screening by conventional cytogenetics. The ages of women who underwent an amniocentesis range from 33 to 44 years. Amniotic fluids were set up as long time in vitro cell cultures, harvested, stained for G-banding and metaphase spreads were analyzed and karyotyped.

Various numerical abnormalities, 47,XX,+21 or 47,XY,+21 or 47,XXX or 47,XX,+mar and structural abnormalities, 46,XY,9qh+ or 46,XX,inv(9)(p13q22.3) were identified.

Six percent of all samples revealed a chromosomal abnormality (5/83). The most common aneuploid condition compatible with survival to term is trisomy 21. Monosomy X is a frequent cause of pregnancy loss with only about 1% of 45,X conceptuses surviving to term. Sex chromosome aneuploidies compatible with survival in most cases include trisomy X. Pericentric inversions are a structural chromosomal variant that occurs frequently and usually has no phenotypic effect. However infertility, miscarriages and/or chromosomal imbalanced offspring can be observed in carriers. An extra euchromatic G band in the long arm of chromosome 9 is a well recognized normal variant.

A4

Implementation of a fetal alcohol spectrum disorders (FASD) Prevention Project in the Witzenberg district, Western Cape Province (2007-2009)

MS L OLIVIER * (Farr), DR S MARAIS (MRC), MS E JORDAAN (MRC), PROF DL VILJOEN (FARR)

Principle investigators: Prof Denis Viljoen (FARR), Dr Sandra Marais (MRC)

Project Coordinator: Leana Olivier (FARR)

Fetal alcohol syndrome (FAS) and its variants (FASD) are the consequence of maternal alcohol abuse during pregnancy and common causes of mental retardation, growth disturbances and

behavioral anomalies. Common maternal risk factors for FASD are binge drinking, smoking, poor nutrition, low socio-economic status, poor education and concomitant health issues such as HIV/AIDS and tuberculosis.

Populations of The Witzenberg District have many of the above. Although no formal epidemiological study has previously been undertaken, FASD is known to occur with a high frequency particularly in under-privileged communities. The burden of disease of FASD is thus probably considerable and 'risk' factors associated with the disorder need to be evaluated.

The objectives were to:

- Develop a FASD prevention model for the Departments of Health and Social Development to incorporate in their day-to-day service packages;
- Assist pregnant women not to drink during pregnancy;
- Assess the babies born to these mothers for FASD, thereby monitoring and evaluating the impact of the project/model.

Methodology:

- 192 pregnant women were recruited before 20 weeks of gestation.
- The women were engaged in brief motivational interviewing sessions with 2 field workers.
- Babies born to these mothers were assessed for FASD at approximately 9 month of age.

Study results: Not available as yet as the last clinical session is on 10 December 2008. Will be available at the time of Conference.

A5

Retrospective chart review of referrals to the King Edward VIII clinic in Kwa-Zulu Natal from 1991-2007

MS J CHETTY * (UKZN), DR CM ALDOUS-MYCOCK (UKZN), DR H RAMDHANI (UKZN), DR N HENRIQUES (UKZN), PROF W WINSHIP (UKZN), PROF E KORMUTH (UKZN)

An investigation into the incidences of various congenital disorders presented at the King Edward VIII Pediatric Genetics Clinic from 1992 to present. The investigation is important to inform genetic counselling which is currently limited in KwaZulu Natal.

A sample of files from all the patient records from 1992 to present were scrutinised in order to establish the incidences of chromosomal, Mendelian inherited, multigenic (quantitative) and acquired disorders.

Down syndrome is the most common chromosomal disorder. A unique form of Osteo Imperfecta which is recessively inherited is found in the KZN population and foetal alcohol syndrome is the most commonly occurring teratogenic cause of congenital abnormality.

The KZN population shadows other areas within South Africa as well as the world in terms of the frequencies of some congenital disorders. However, there are some anomalies which need to be further investigated.

A6***An analysis of the occurrence of Down's Syndrome at 3 hospitals in Durban***

MS H NAIDOO * (UKZN), DR C ALDOUS-MYCOCK (UKZN), DR H RAMDHANI (UKZN),
DR N HENRIQUES (UKZN), PROF W WINSHIP (UKZN), PROF E KORMUTH (UKZN)

Seventy one DS cases from King Edward VIII, Inkosi Albert Luthuli and Prince Mshiyeni hospitals were analysed in order to determine the trend for maternal age, age of patient at diagnosis and provision of screening tests and counseling services.

Retrospective chart reviews were undertaken for the most recent patients seen at the various genetic clinics. A medical chart checklist was designed adhering to guidelines of the American Academy of Pediatrics. The relevant data was captured and manipulated to show trends for maternal ages; patient age at diagnosis; patients' gender and weight at diagnosis, the percentage of patients screened for hearing, vision, thyroid function, cervical spine and cardiac defects and the percentage of patients offered antenatal counselling, referred to support groups and counselled on diagnosis and prognosis postnatal.

Most mothers of DS infants in this study are in their thirties. There were more mothers with DS infants below the age of 35 (40 out of 70) than older than 35 years (30 out of 70). Most children (34 out of 72) with DS are diagnosed at the infancy stage. Screening for cardiac defects was shown to occur most frequently, with screening for cervical spine structure and function, screening for hearing and vision and screening for thyroid function occurring in some cases. A significant number of parents studied, were counselled on diagnosis, however few were referred to support groups.

Better genetic counselling services and support for parents of DS children are required at the hospitals where this study was undertaken.

A7***Prenatal diagnosis represents a point of entry of medical genetics in Sub-Saharan Africa: experience from Cameroon***

DR A WONKAM * (2Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon), DR AK NJAMNSHI (2Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon), DR FF ANGWAFO (2Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon), DR MA MORRIS (Department of genetic medicine and development, Geneva University, Geneva, Switzerland)

To enquire about knowledge of medical students, Cameroonian physicians and parents with affected sickle cell anemia (SCA) children, concerning medical genetics; and attitudes towards prenatal diagnosis (PND) and medical abortion

Semi-structured questions survey:

Medical students and physicians: Respondents rates were 50.5 % (n=101), 47.5% (n=95) and 36.7% (n=110), for pre-clinical, clinical medical students and physicians, respectively. Their awareness of DNA diagnosis was poor: respectively, 0.0, 2.2 and 1.2% for SCA. The principle of PND was found to be acceptable by the majority (96.9, 96.7, and 98.1%); and that of medical

abortion increased with the level of medical education (62.6, 74.7 and 90.7%). In all the three groups, the acceptance of medical abortion "if the respondent's own child was affected" was lower for SCA than Down syndrome (22.4 vs. 40.2%, 10.8 vs. 29.3% and 36.1 vs. 70.4%).

Parents with affected sickle cell anemia children:

The 121 respondents were mostly Christians (89.9%) and females (74.4%). The role of both parents in the transmission of SCA was acknowledged by 87.8 % of respondents. Up to 94% of respondents would request PND for SCA, and 63.2 % would opt for medical abortion of an affected SCA-pregnancy. The acceptance of medical abortion for SCD significantly increased with the level of the parent's education ($P<0.01$), male gender ($P<0.01$) and Christian religion ($P<0.05$).

The data suggest a poor knowledge of genetic tests amongst medical students and physicians. Medical students, physicians and parents appear to accept the principle of medical genetics involving counseling, PND and in some cases medical abortion.

A8

A PCR based test for Fragile X syndrome : Validation

DR R GOLIATH * (NHLS/UCT), MS A ESTERHUIZEN (NHLS/UCT)

Fragile X (FRAX A) is a common request for genetic testing. A significant proportion of these reports are only issued after carrying out Southern blot analysis as PCR analysis is inconclusive in homozygous normal female samples and large expansions in affected subjects cannot be detected by standard PCR methods.

Abbott Molecular have recently released a PCR based kit for Fragile X syndrome diagnosis. The kit is designed to reduce the burden of Southern blot testing by reliably PCR amplifying repeat alleles much larger than those possible using 'in house' methods. This study focuses on validating the kit in our laboratory towards incorporating the test into the current diagnostic service offering.

The study will involve:

- testing a set of reference control samples,
- retrospective blind testing and
- prospective unblind testing representative of routine referrals.

The kit has been tested in 13 centres and allele sizing shown to be precise; 95% of the normal distribution of the estimated allele size of the sequenced reference control alleles lied within the quoted range of +/-1 repeat.

The findings of the validation study for our centre will be presented. As well as an outline of the strategy for incorporation into the diagnostic service in Cape Town

A9

Expansion in FMR1 5' UTR CGG repeats plus recombination and mosaicism in a family with fragile x syndrome

DR NORUZINIA * (-), MR KEYHANEH (-), MS ZOLFAGHARI (-), MS FATEHMANESH (-), MS ROSTAMI (-), PROF SHAFEGHATI (-)

Fragile X syndrome is caused by an expanded CGG repeat (>200 units, full mutation) at the 5' end of the FMR1 gene, which is associated with methylation of a CpG island upstream of the FMR1 gene and down regulation of the transcription. The association between the rate of fragile x symptoms revealing and the FMR1 repeat number (41> CGGn< 200) has been widely investigated in recent world studies.

In this article we present a family with several male members with mental retardation corresponding to a heritable disease with X-linked recessive inheritance. The phenotype corresponded to Fragile X syndrome. However the phenotypes were different in the two patients. We developed four PCR based methods to detect full mutation in these patients. This method confirmed Fragile X syndrome in these patients. However in the patient with milder phenotype a mosaic form of full and permutation was detected that corresponded to clinical findings.

STR marker analysis showed the presence of different STR alleles in these two patients. This finding could be explained by occurrence of a recombination in the region. This is the first case of mosaic Fragile X syndrome reported in an Iranian patient.

In this case report we present several PCR based triplet repeat mutation detection techniques which can be implemented in molecular laboratories and can reduce the demand for southern blot analysis which remain the gold standard in Fragile X diagnosis.

A10

Pitfalls associated with diagnostic analysis of large genes using RT-PCR analysis

DR EP OWEN * (Chempath UCT/NHLS Cape Town), MS C EDWARDS (Dept Medical Biochemistry and Immunology, Cardiff University), MR AD THOMAS (Dept Medical Biochemistry and Immunology, Cardiff University), PROF HE HENDERSON (Chempath UCT/NHLS, Cape Town), PROF BP MORGAN (Dept Medical Biochemistry and Immunology, Cardiff University), DR A ORREN (Dept Medical Biochemistry and Immunology, Cardiff University)

Introduction: Complete deficiency of C5D was diagnosed in a young South African Xhosa woman after two attacks of Neisseria meningitidis meningitis. She was treated with bicillin injections as prophylaxis and has been observed for twelve years. Her serum lacked C5 dependent haemolytic activity.

Methods: Mutations in the C5 gene were investigated using genomic DNA and cDNA.

Results: Sequencing of all 41 exons and promoter regions of the C5 gene revealed a known heterozygous nonsense mutation 55C>T (Q19X) in exon 1. A second heterozygous mutation (novel) 754G>A (A252T) was identified within exon 7, five bp from the intron 7 boundary which may

cause splicing problems. In genes containing numerous exons the preferred method is to investigate the mRNA using RT-PCR. Sequencing of the cDNA only complicated the issue. Initial results revealed homozygous 55C>T, no 754G>A mutation, a homozygous absence of exon 6 and the presence of exon 7. Further investigations in both normals and the patient revealed several unknown rare mRNA species that contained either inserts or deletions within the 5'end of the mRNA.

Conclusion: In patients where the mutant mRNA species may be degraded rapidly the rare mRNA species become more apparent allowing PCR of cDNA to amplify one or several species at the same time. It is assumed that many of these rare mRNA species are non-functional. The C5 gene contains 41 exons making routine analysis using genomic DNA impractical. Analysis of C5 mRNA via RT-PCR revealed several mRNA species containing insertions and deletions making diagnosis of C5D using RT-PCR complicated.

A11

Aneuploidy FISH versus Karyotype

MS N JANO * (NHLS Groote Schuur Hospital/ UCT Department of Human Genetics),
MS GJ SCHUTTE (NHLS Groote Schuur Hospital/ UCT Department of Human Genetics),
DR M URBAN (NHLS UCT Department of Human Genetics)

The aim was to assess the accuracy and utility of aneuploidy FISH in amniocentesis samples. Results were grouped according to reason for referral, to determine whether the aneuploidy FISH could be used as a stand alone test for antenatal diagnosis of Down Syndrome.

The results of 250 samples from the NHLS / UCT Cytogenetic database for antenatal FISH studies was reviewed. Both the Vysis and the Q Biogene FISH probes were used during the reviewed period where locus specific probes were used for chromosomes 13 and 21. Karyotypes and FISH results were compared.

In 116 women referred for advanced maternal age or other risk for Down syndrome, 6 (5%) abnormalities were detected – all trisomy 21. In 132 women referred for fetal congenital abnormalities detected on ultrasound, 25 (19%) aneuploid results were detected on FISH. These included trisomy 18 (14), trisomy 13 (2), trisomy 21 (5) and polyploid (4). In addition, 5 structural chromosome abnormalities that were only detected on karyotype.

Aneuploidy testing with stand-alone QF-PCR is an increasingly accepted practice for cases in which screening indicates increased risk of Down syndrome. In addition, published evidence shows that aneuploidy FISH testing has similar accuracy to QF-PCR. Our results indicate that stand-alone aneuploidy FISH may be sufficient in cases referred for risk for Down syndrome, although the sample is not large enough to be definitive. Bloody samples have not precluded detection of abnormal FISH results, although some have apparent mosaicism, probably due to maternal contamination.

A12

QF-PCR for the postnatal diagnosis of Down syndrome

MS BJ KRUGER * (NHLS & WITS), MS J LAMPRET (WITS capability),
PROF A CHRISTIANSON (NHLS & WITS)

This retrospective study aimed to validate the hypothesis that quantitative fluorescent polymerase chain reaction (QF-PCR) is a more feasible means of testing for postnatal Down syndrome (DS) than chromosome analysis, where there are limited human and financial resources in public health services in South Africa.

Data were collected on 642 chromosome analyses done for postnatal testing of DS from January 2007 to May 2008. Samples included those where the request was for DS confirmation (563 samples) or the result revealed this as the diagnosis, but was not the initial request (79 samples). Information relevant to the clinical diagnosis and cytogenetic result was obtained.

In the 563 DS requests, this diagnosis was confirmed in 307 samples (54.5%) and 185 (32.9%) had normal chromosome results. The remaining 71 analyses were unsuccessful (67 samples, 11.9%) or had a different chromosome abnormality (4 samples, 0.7%). Of the unsuccessful analyses 30 were due to the sample being clotted, infected, an insufficient amount or arriving late.

QF-PCR is a feasible technique to replace cytogenetic analysis as the standard diagnostic technique in confirming postnatal DS.

It was evident that as many as 32.9% of chromosome analyses done for DS confirmation were normal. For the 54.5% where DS was confirmed, QF-PCR may now be used as a valid alternative.

QF-PCR may also obtain results from samples unsuitable for chromosome analysis.

A13

The Use of Family History and Pedigree

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The family history (FH) is an important element in the practice of medicine, with a role in diagnosis, screening, determining genetic risk, assessing the need for genetic counselling and considering reproductive options. The pedigree provides a standard nomenclature and structure for recording and interpreting FH, and it allows genetic inheritance patterns to be determined at a glance. Paediatric guidelines recommend that all patients should have a FH taken, the extent of which depends on diagnoses considered. The study aimed to assess frequency, methods and adequacy of FH-taking in various paediatric medical and surgical disciplines at Red Cross Children's Hospital.

Medical notes of 51 outpatients attending a total of 133 outpatient clinics were reviewed. Each case was classified by a medical geneticist, based on both presenting features and final diagnosis, as to genetic nature of the condition and standardised criteria for an adequate FH were created for each category.

There was evidence of a FH in 37 (73%) of cases. Of 28 patients requiring specific consideration of genetic or chromosomal conditions, only 14 (50%) had an adequate FH documented. A pedigree was present in only 8 (16%) cases, and a three-generation FH in 6 (12%).

We conclude that documentation of FH-taking is suboptimal in a local paediatric tertiary-care outpatient setting, and that the pedigree is under-utilised. We recommend the use of a pedigree as opposed to lengthy written notes, regular family history updates, and genetic education of health professionals.

A14

Chromosome 18ph+/18p(duplication) seen as a Normal Chromosome Variant

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We aim to present 2 families where an abnormal chromosome 18p was found in both the normal parent and abnormal child of the parent. Chromosomes from the patients and from their parents were studied using high resolution G-Banding, C-Banding, and FISH studies.

C-Banding indicated an extended 18ph+ region in both patients and one of their parents. One patient was referred from Red Cross Hospital and the other from Tygerberg Hospital. The families appear unrelated, and each patient has a different clinical presentation. The parents that carry the abnormal 18, each present with a normal phenotype. The phenotype of each patient appears unrelated to the extra material on chromosome 18p.

The literature cites this rare phenomenon as a normal variant which has previously been found in parent to child transmission, with normal phenotypes in both parent and child. Care should be taken when interpreting unusual karyotypic results, especially when found antenatally.

A15

Unique mosaicism with trisomy 13, trisomy 18 and a normal cell line in a 17 year old female

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Re-evaluation of a 17 year female patient with features of Trisomy 13 and unusual mosaicism.

BACKGROUND: A newborn female baby presenting with features of Trisomy 13 was referred for cytogenetic studies. Chromosome analyses confirmed the clinical findings. Mosaicism was not detected. Parental chromosome analyses were normal. Six years later the patient was reassessed after developing pigmentary streaks on the skin.

The chromosome complement revealed an unusual mosaicism with three cell lines, including two autosomal trisomies 13 and 18 in addition to a normal cell line.

DISCUSSION: The long survival of this individual with mosaicism of two autosomal trisomies 13 and 18 is unique. It is of scientific interest to reassess this unusual chromosomal abnormality.

A16

Cytogenetics experience for the past 12 years and abnormalities detected during the year 2008
MS WM PFAFFENZELLER-THOM * (NHLS)

The objective of this study was to document the Braamfontein NHLS Cytogenetics laboratory's experience during the last 12 years.

Conventional Cytogenetics on banded chromosomes as established in the nineteen seventies, and it was supplemented by FISH towards the end of 1996. QF-PCR was introduced in 2002 and continues to be used in parallel with conventional chromosome studies.

In 1996 the total number of tests on lymphocytes was 1524, this gradually increased to 2400 by 2008. Between 35 and 50 abnormal karyotypes are currently detected per month. Down syndrome was the most common abnormality detected (289 cases) followed by Trisomy 18 (45), Trisomy 13 (31), Turner syndrome (25), translocations (16) and other (58), which includes deletions, duplications, inversions, four ways translocation, trisomy 22 and marker chromosomes. Thirty three microdeletions were detected by means of FISH during the year. The Lab has experienced a big exodus of well trained staff, making it hard to keep to the expected turn around times. As a consequence, it has recently been decided that all samples from individuals with a diagnosis of possible Down syndrome will first be screened by QF-PCR.

Future, new approaches such as chip array technology will further reduce the need for arduous microscope work.

A17

***A novel structural chromosome abnormality leading to a rare chromosomal disorder:
Mosaic Trisomy 8 syndrome***
MS H RAMJEE * (NHLS), MS W THOM (NHLS)

The objective of this study is the identification of a novel structural chromosome abnormality leading to a rare disorder: Mosaic Trisomy 8 syndrome

A male patient aged 1yr 7months with delayed milestones and dysmorphic features presented at a nearby hospital and subsequently referred for chromosome studies. The patient's blood sample was processed and a karyotype was done

The karyotype revealed a mosaic pattern consisting of two cell lines. The first line revealed 45,XY,idelic(8;8)(p23)-8 and the 2nd cell line 46,XY,idelic(8;8)(p23).

These results suggest the presence of an isodicentric chromosome 8 has led to mosaic trisomy 8 syndrome. This is the first time such a particular karyotype has been reported in the literature. Molecular cytogenetic techniques such as FISH need to be applied to reveal the actual breakpoints

of the isodicentric chromosome 8. This information can thereby aid to detect any possible carriers of the structural abnormality as well as confirm the diagnosis of affected individuals and also can be used in prenatal diagnosis.

A18

Developing a molecular genetic diagnostic service founded on the use of the ABCR400 gene chip; lessons learnt

MS LJ ROBERTS * (UCT), PROF R.S RAMESAR (UCT), PROF J GREENBERG (UCT)

The ABCR400 microarray from Asper Biotech (Estonia) currently tests for 513 sequence variants in the ABCA4 gene. Mutations in ABCA4 are known to cause a range of autosomal recessive retinal degenerative disorders. The aim of this research was to assess the clinical utility of ABCR400 mutation screening in patients with ABCA4-associated retinopathies (AARs), and ultimately apply this information in the development of a molecular genetic diagnostic service.

178 probands with AARs were screened with the microarray. Diagnostic assays were designed to verify all mutations identified in individuals in whom at least two causative mutations were found. Mutations were verified in these probands, and wherever possible co-segregation analysis was performed in additional family members.

ABCR400 screening identified a total of 83 different ABCA4 mutations in the South African cohort, with seven common mutations occurring at higher frequencies. Two or more disease-associated mutations were found in 99/178 probands. After verification, however, several family members could not benefit from genetic result delivery due to either the confounding effect of three mutations or the incorrect identification of variations.

The ABCR400 microarray is a useful screening tool, however all mutations should be verified and, wherever possible, investigated in other family members. Validated ABCR400 results provide an unequivocal molecular diagnosis, allowing family members to be offered diagnostic, predictive, carrier and prenatal testing. The identification of common mutations in the South African cohort may also be exploited, with targeted testing possibly reducing costs in the future.

A19

More Than One Cause For Multiple Miscarriages

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A female patient presented with four previous early miscarriages but no postmortem examinations had been performed. On investigation, it was found that she (and also her mother) had protein S deficiency which was considered to be the cause of the multiple miscarriages.

At a subsequent pregnancy, antenatal ultrasound revealed fetal abnormalities. The couple declined amniocentesis. On newborn examination, multiple congenital abnormalities were present and the karyotype showed a marker chromosome. The mother was found to be a carrier of an 11;22 trans-

location and the final karyotype in the infant was 47,XX,+der(22)t(11;22)(q23;q11.2)mat, consistent with a diagnosis of Emanuel syndrome.

The 11;22 translocation detected in the mother is the only known recurrent, non-Robertsonian translocation in humans. The chromosomal imbalance in Emanuel syndrome is a supernumerary derivative chromosome 22, and there may in addition be a balanced (11;22) translocation. The majority of recurrent rearrangements take place within 22q11.2 suggesting genomic instability related to the structure of the chromosome 22q11.2 region.

Although the multiple miscarriages were initially ascribed to protein S deficiency, the maternal balanced translocation may in fact be the cause. Offspring produced from the 11;22 translocation can have a normal karyotype or the balanced translocation. Unbalanced forms of the translocation are not found in viable offspring and result in miscarriages. Emanuel syndrome can be inherited from the t(11;22) as a result of a 3:1 malsegregation in meiosis I with a supernumerary derivative chromosome 22. The risk for this outcome is 3,7% for the female carrier and <0,7% for the male carrier.

A20

Infants requiring medical genetic assessment at birth: indications for referral and outcomes

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The study was conducted in the secondary and tertiary hospitals of the Peninsula Maternal and Neonatal Service (PMNS). In our service, medical genetic assessments are requested at or near birth if the neonatal team suspects a genetic condition requiring early management decisions, or with difficult diagnostic, prognostic and counselling implications. We aimed to assess indications for assessment and follow-up outcomes of liveborn infants for whom an early medical genetic assessment was requested.

The study sample comprised all newborn infants in the PMNS receiving a neonatal medical genetics assessment from 2005–2007. A retrospective review was conducted of the genetic callout records and routine hospital data to determine diagnosis and outcome.

A total of 161 newborn infants received a medical genetic assessment in the study period. Follow-up outcomes included: 18 (11%) deaths and presumed deaths (in known lethal conditions), 102 (63%) attended genetic follow-up, 14 (9%) attended other paediatric follow-up and 27 (17%) lost to follow-up. In a small subset genetic follow-up was not deemed necessary.

Early newborn assessments are regularly requested, on approximately a weekly basis, but represent only a fraction of expected serious birth defects within the PMNS. Outcomes of assessed patients will be discussed in relation to diagnostic categories. In total, there was no documented genetic follow-up in 59 (37%) patients, and unmet needs for genetic care and counselling will be assessed in this group.

A21

Polymorphisms in DNA repair genes: potential modifiers of breast cancer risk in Afrikaner BRCA2 mutation carriers

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Penetrance of the BRCA2 c.8162delG mutation varies within Afrikaner families. Polymorphisms in the DNA repair genes are currently targeted as potential modifiers of breast cancer. The aim was to investigate SNPs within XRCC1, XRCC3, XPD, RAD51, and BARD1, in an attempt to elucidate the difference in penetrance of this founder mutation.

Sixty BRCA2 c.8162delG women were selected (of which 30 were affected with BC) and case matched with controls for age. All 120 participants were genotyped for polymorphisms within these genes using PCR, RE, SSCP analysis and DNA sequencing.

Genotype analysis revealed no informative results for XRCC1 and XRCC3. Differences ($P < 0.05$) were observed between the control and mutation positive groups for Lys751Gln in XPD and Cys557Ser in BARD1. The majority of participants (controls & mutation carriers) were either heterozygous (45% & 56.7%) or homozygous (45% & 41.7%) for the variant Gln allele in XPD. For BARD1, the majority of participants (controls & mutation carriers) were homozygous (98.3% & 91.7%) for the ancestral Cys allele, with the Ser allele present in only 1.7% and 8.3% respectively. No disparities were observed between the mutation carriers. Although the majority of mutation carriers (73.3% of affected vs 90% of unaffecteds) were homozygous for the ancestral G allele in RAD51, more BC patients were heterozygous for the variant (26.7% vs 6.7%) ($P > 0.05$).

Mutation carriers exhibiting variant alleles for XPD and BARD1 have an increased risk for BC. None of the SNPs explained the variance in penetrance, although the SNP in RAD51 showed potential.

A22

11q25 cytogenetic aberrations in patients with Non-Hodgkin Lymphoma (NHL) and HIV

DR CL BABB * (NHLS), DR P WILLEM (NHLS and University of Witwatersrand)

Individuals with HIV infection are at an increased risk of developing lymphoma's and often have a poorer prognosis than their HIV negative counter parts. We wanted to assess the cytogenetic profile of HIV positive lymphoma and compare them to HIV negative lymphoma patients.

Reports of bone marrow samples submitted for diagnosis of lymphoma disease were reviewed, retrospectively. Cases that had unique aberrations seen by karyotyping were noted along with additional clinical information.

Karyotypes with unique aberrations were identified in a number of cases. Three cases (two HIV positive and one of unknown HIV status) were of particular interest as they, strikingly, involved the same breakpoint, 11q25. They included a $t(1;11)(q25-32)(q25)$,

a $t(6;11)(q22;q25)$ and $add(11)(q25)$. The translocations were the sole aberrations found in those patients whereas the other had an extensive number of aberrations.

The increasing number of HIV associated lymphoma in South Africa is a concern, especially given the fact that these patients have a poorer prognosis than HIV negative lymphoma patients.

Although the effect of the unique aberrations described here are still unknown, it is vital that they are recorded, followed up and characterised. In particular the 11q25 breakpoint, seen here in HIV positive individuals, is of interest. This region contains a recently identified tumour suppressor gene (TSG), OPCML. Mapping the breakpoints could potentially verify the importance of this newly identified TSG, which could have important prognostic value and potentially identify drug targets.

A23

Genetic Modifiers of Age of Onset in Hereditary Nonpolyposis Colorectal Cancer in South Africa

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Colorectal cancer (CRC) is the 2nd leading cause of cancer-related deaths in the U.S. and the 3rd leading cause in South Africa, with both genetic and environmental factors. Hereditary nonpolyposis colorectal cancer (HNPCC) is an inherited disorder caused by mutations in DNA mismatch repair (MMR) genes. Individuals with HNPCC have an 80% lifetime risk of CRC but the age of onset is highly variable due to additional genetic and environmental factors. We hypothesize that in individuals predisposed to HNPCC with a primary mutation in a MMR gene, one or more secondary mutations act to influence the age of CRC onset. This project aims to identify these genetic modifiers.

A South African cohort of 273 HNPCC individuals harboring primary mutations in MMR genes MLH1, MSH2, MSH6 will be genotyped for selected polymorphisms in 8 candidate genes (ATRX, GCLC, CYP1A1, EPHX1, IGF1, RAD51, CHEK2, and OGG1). Modifier effects for each SNP will be tested both individually and haplotypically for association to age of onset. Epistatic interactions between potential modifiers will be assessed, including gender-specific effects.

When the effect of gender on age of onset was investigated, SNP rs3088074 in ATRX was significantly associated ($p = 0.023$) with age of onset in individuals carrying the c.1528C>T mutation in MLH1. Analysis for the remaining SNPs is underway and results will be presented.

Results will directly translate into improved clinical management of CRC in South Africa, including risk stratification based on genetic testing for modifier polymorphisms, especially important for a resource-poor country.

A24

Molecular Assessment of Resistance to Tyrosine Kinase Inhibitor Therapy in South African CML Patients

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Determine the incidence and characterize point mutations in the tyrosine kinase and p-loop domains of the ABL oncogene in South African chronic myeloid leukemia (CML) patients on tyrosine kinase inhibitor (TKI) therapy.

Point mutations in the kinase and p-loop domain of ABL are the main cause of resistance to Gleevec and other TKI therapy. Patients' blood samples were selected on the basis of an increased level of BCR/ABL determined by real time quantitative PCR or on failure to obtain a major molecular response (MMR) within a year. RNA was extracted and ABL was sequenced using published protocol. Ninety-five specimens were sequenced.

Out of the 95 specimens, 34 mutations were detected in 30 patients. The most frequent mutations were the T315I (7.37%), E255K (4.2%) and M244V (4.2%). The T315I and E255K mutations both cause full resistance to Gleevec. The rate of mutations detected in TKI resistant patients were comparable to previously published data, with the exception of the M244V, which was observed at a higher rate (4.2%). 65% percent of patients had developed resistance within two years of commencing treatment.

The tyrosine kinase inhibitor Gleevec has revolutionized the treatment of CML. However owing to the inherent genetic instability in CML point mutations causing resistance tend to develop with time. The frequency for mutations observed in this study was by in large on par with previously published data. The high number of patients that rapidly developed resistance may suggest poor compliance or incorrect dosage of therapy.

A25

A Novel Secondary Aberration in Chronic Myeloid Leukaemia Treated with Imatinib

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We report a case of chronic myeloid leukaemia (CML) in which cytogenetic analysis revealed a 46,XY,t(9;22;2)(q34;q11.2;q13)der(7)t(1;7)(q21;q36) karyotype in 60% of the metaphases analysed.

We retrospectively reviewed bone marrow biopsy, conventional cytogenetic and FISH results for this patient.

Cytogenetic investigation of the patient at diagnosis showed the complex variant translocation t(9;22;2)(q34;q11.2;q13). He was started on Imatinib 6 years after the initial diagnosis – his bone marrow showed the disease was in the chronic stable phase and FISH demonstrated his cells to be 98% positive for the BCR/ABL fusion gene.

8 months after commencing Imatinib treatment his marrow demonstrated a minor cytogenetic response (40% Ph positive). After 30 months on treatment the percentage of Philadelphia positive cells had increased to 92% and by 42 months to 100% with 60% of the cells demonstrating a secondary aberration: der(7)t(1;7)(q21;q36). The result of this translocation is a partial trisomy of chromosome 1q.

It has been postulated that genes residing on chromosome 1q may be involved in the acute transformation of CML, as the extra copy of 1q may be central to the creation of a proliferative advantage. Advances in genetic study have raised the question: could the translocated region on the partner chromosome of the 1q be associated with the phenotype of leukaemia clones when blast crisis occurs? Of interest will be the Phenotype of the blast cells when blastic transformation occurs – as chromosome 7 abnormalities are typically associated with the myeloid lineage.

A26

hMLH1 and hMSH2 mutation spectrum in 11 South African HNPCC families

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Hereditary non-polyposis colorectal cancer (HNPCC) is the most common form of familial colorectal cancer. Germline mutations in the DNA mismatch repair genes, the majority of which occur in hMLH1 and hMSH2, underlie HNPCC. Mutation detection facilitates genetic testing to identify at-risk relatives for participation in surveillance and management programmes. This study served to further characterise the largely undefined mutation spectrum in South African HNPCC families.

Eleven South African families, who fulfilled either the Amsterdam II criteria or the revised Bethesda guidelines, were investigated. All exons and exon-intron boundaries of the hMLH1 and hMSH2 genes were screened for mutations using single stranded conformation polymorphism/heteroduplex analysis (SSCP/HA) in combination with MDE gel electrophoresis/silver staining. Large rearrangements of both genes were investigated by multiplex ligation dependent probe amplification (MLPA).

Two pathogenic germline mutations were detected, the novel c.885-1G>A in hMLH1 and a previously reported frameshift c.227_228delAG (p.Gln76GlnfsX5) in hMSH2. The splice site mutation was detected in a Caucasian family whereas the frameshift was detected in a Black family. Interestingly, both these families complied with the revised Bethesda guidelines for HNPCC. A polymorphic missense mutation in hMLH1 and a number of benign polymorphic intronic variants were also identified.

This constitutes the first report of these germline mutations in the South African population. The identification of the mutations now allows for predictive testing of unaffected family members, who can benefit from prevention programmes.

A27

The rare, CML associated, variant BCR/ABL fusion transcript b2a3: implications for patient diagnosis, treatment and follow-up

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Chronic Myeloid leukemia is a clonal myeloproliferative disorder characterized by the Philadelphia translocation t(9;22), which results in the BCR-ABL fusion gene. The resulting chimeric protein is a constitutively active tyrosine kinase, which induces malignancy. The bcr/abl fusion protein can vary in size depending on the location of the breakpoint in the BCR gene and, more rarely in the ABL gene. Few sporadic cases

(<5% of cases) have been reported with a variant resulting from the fusion of BCR exon 2 to ABL exon a3 (b2a3). There is conflicting literature regarding the effect of this variant on patient outcome. Here we present a young male patient diagnosed with CML who has the b2a3 variant.

He presented clinically as a classic CML but responded poorly to treatment.

The objectives were to confirm the presence of the variant fusion in this patient for correct diagnosis and follow up.

Quantitative PCR was negative while FISH was positive for the BCR-ABL fusion. Various primers were tested for both sequencing and RT-PCR experiments in an attempt to isolate a variant.

A transcript was characterized as the rare b2a3 variant. This case highlights the necessity to adapt genetic testing to correctly diagnose patients and to establish a tailor made molecular test to follow up response to therapy.

These results contribute to the literature regarding response to therapy for these rare bcr/abl fusion transcripts. Two of the previously reported cases showed a good response to tyrosine kinase inhibitor treatment, while this young patient has in fact responded poorly.

A28

Personal Understanding of Predictive Test Result in South African Nonpolyposis Colorectal Cancer Families

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Services providing predictive genetic testing for Hereditary Non-polyposis Colorectal Cancer have been available in South Africa (SA), since 1994. Currently, over 600 individuals have received genetic test results and 259 live with the knowledge of a hereditary predisposition to cancer. This study attempted to improve the genetic counselling practice in SA by gaining an understanding of the personal interpretation of genetic information received in such families.

Using qualitative research, in-depth interviews were conducted with ten individuals {all mutation-positive and asymptomatic of colorectal cancer (CRC)} subsequent to the disclosure of their predictive test (PT) result. The use of personal interviews elicited rich descriptions of each individual's subjective understanding of the information received during genetic counselling, specifically the comprehension of the PT result.

Results showed that none of the individuals involved in the study reported a perceived risk identical to the genetic risk communicated to them during counselling. Interpretation of risk was influenced by the knowledge derived from a personal experience of the familial cancer in a relative and binary reasoning. In addition interpretation was influenced by the fact that the participants were already involved in a screening programme, and had adjusted their risk due to the high efficacy of the colonoscopy, in terms of CRC prevention. Potential anxiety-provoking experiences were highlighted and could form the basis of recognising where psychological support and counselling should more actively be offered.

It is anticipated that counselling strategies which expand upon an individual's experiential knowledge of the disease may enhance effective communication of genetic risk.

A29

Occurrence of double point BCR-ABL mutations in CML patients that are showing Gleevec resistance

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To determine the frequency of double point mutations in the kinase and P-loop domain of the ABL oncogene in a cohort of South African CML patients on Gleevec therapy, that are showing resistance to treatment.

Peripheral blood samples from 95 CML patients were analyzed. Resistance to treatment was suspected on the basis of increasing levels of BCR-ABL using real-time quantitative PCR results, or failure to obtain a major molecular response within a year. Following direct sequencing of the P-loop domain, any amino acid substitutions were determined using a known Genbank database, which detailed any known mutations and their resultant sensitivity to known treatments.

In total, 30 of the 95 patients had detectable point mutations within the P-loop of ABL. Three patients had double point mutations, which conferred either partial or full resistance to Gleevec. Second generation tyrosine kinase inhibitors were either fully sensitive or partially sensitive/resistant. In one of these patients, M244V and E255K mutations were observed, conferring partial resistance and full resistance to Gleevec respectively. However, the M244V mutation was dominant over the E255K mutation.

Out of studies performed to date, very few patients with double mutations have been identified. In the aforementioned patient, each mutation confers different patterns of drug resistance, the clinical significance of these double mutations has yet to be elucidated. As mutations in the P-loop are associated with a poor prognosis, revised treatment strategies, which may include combination tyrosine kinase inhibitor therapy, may prove beneficial in treating CML patients with double mutations.

A30

Simultaneous Amplification of the Her-2/neu Gene and Chromosome 17 Centromere in Breast Carcinoma Specimens

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The National Cancer Registry's most recent report states Breast Cancer as the most common cancer amongst South African women. Her-2/neu is a proto-oncogene, amplified in approximately 20-30% of Breast Cancers. It is a popular molecular marker because amplification is associated with poor prognosis and can predict response to treatments such as Herceptin. We tested the Her-2/neu status of 588 samples using Fluorescent in Situ Hybridisation (FISH).

This was done with the PathVysion kit (Vysis, Inc.) that includes a probe for the Her-2/neu locus (17q12, SpectrumOrange) and a control probe on CEP17 (SpectrumGreen). A Her2/Cep17 ratio of more than two was considered positive.

Of the 588 samples, 292 (49%) were positive for Her-2/neu amplification. 13 (2%) of these samples were found to be ambiguous. Ambiguous cases presented with clusters of innumerable red and green signals, where the number of green (CEP17) signals were too numerous to be accounted for by chromosome 17 aneusomy.

The hypothesis is that in these instances the CEP17 control is being included in the Her-2/neu amplicon, resulting in a calculated Her-2/CEP17 ratio that is low, indicating non-amplification, however it is possible that the Her-2/neu gene is amplified. We have identified a gene, ACTG1, on chromosome 17 that may act as a superior control. ACTG1 is highly conserved, does not appear to play a role in Breast Cancer, and is situated far enough from Her2 to be excluded from the hypothesised amplicon. We show our first results with a novel control that accounts for the large Her2/neu gene amplicon.

A31

The diagnostic and prognostic value of traditional cytogenetics in hematological malignancies

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Hematological malignancies are caused by various numerical and structural chromosomal abnormalities. The aim of this study was to investigate the diagnostic and prognostic importance of cytogenetic analysis in hematological cancers.

Six patients with a clinically-defined diagnosis of hematological cancer were referred for routine cytogenetic screening. Two adult patients diagnosed with CML, a sixteen year old patient with ALL, two teenagers with AML and a child with Burkitt's lymphoma were investigated. Bone marrow specimens were set up as short-term cell cultures, harvested and stained by the G-banding method. Metaphase spreads were analyzed and karyotyped.

Various numerical and structural abnormalities were observed. Hyperploid cell lines (4/6) and translocations (5/6) were common. Both CML patients presented with a hyperdiploid chromosome number and the presence of translocations t(7;9) and the so-called Philadelphia chromosome in one case and t(8;22) in the other. A translocation between chromosomes 1 and 19 was implicated as pathogenic in a pediatric ALL case. Both AML cases presented with a tetraploid cell line, while one also harbor t(8;21). An pediatric patient with Burkitt's lymphoma presented with t(8;14).

The Philadelphia chromosome, the hallmark of CML was present in one of the two CML patients. The ALL patient presented with t(1;19), causative in 30-40% of all ALL cases. Translocation t(8;21) underlying the AML phenotype in almost 30% of FAB-M2 cases was identified in one of the AML patients. Burkitt's lymphoma was caused by t(8;14), the hallmark of this malignancy. Traditional Cytogenetics proved to be a valuable diagnostic and prognostic tool in hematological malignancies.

A32

Genetic polymorphisms in XPNPEP2 and ACE gene in hypertensive, ACE inhibitor (enalapril) induced angioedema patients

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Introduction: Angiotensin converting enzyme (ACE) inhibitors are an important class of drugs widely used by more than 40 million patients for hypertension, congestive cardiac failure and diabetes. Severe side-effects include angioedema induced by activation of bradykinin which is significantly more common in Black and Coloured South Africans than in the Caucasian population. The C-2399A SNP XPNPEP2 gene which encodes membrane bound Aminopeptidase P (APP) enzyme has been linked to angioedema¹.

Methods: Patients with a history of angioedema and controls on enalapril were recruited from the hypertension clinic at GSH/Victoria hospital. Samples were taken for genetic analysis, and ACE, APP and bradykinin analyses. All patients gave informed consent and the study was approved by the UCT Research Ethics Committee.

Results: Preliminary results show 17% of patients (n=141) had a history of angioedema. A-2399 allele frequency in XPNPEP2 was 23% in patients with angioedema and 31% in controls. There were also no significant differences between females and males, or ethnic groups. ACE-ID polymorphisms, which affect ACE activity, showed increased frequency of the D allele (75% vs 53%) in black patients with angioedema compared to controls.

Conclusion: Initial results indicate no link between the C-2399A SNP in the XPNPEP2 gene and angioedema. The majority of black patients with angioedema had ID and DD genotypes giving a higher D allele frequency, which may be associated with angioedema.

Reference: Qing LD, et al., A variant in XPNPEP2 is associated with angioedema induced by angiotensin I-converting enzyme inhibitors. *Am J Hum Genet*, 2005. 77: p. 617-626.

A33

Sarcomeric modifiers of hypertrophy in hypertrophy cardiomyopathy

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Hypertrophic cardiomyopathy is an autosomal dominantly inherited cardiac disorder characterized by myocyte disarray, fibrosis, an increased risk of sudden cardiac death and left ventricular hypertrophy. HCM thus serves as a model for the investigation of LVH, a symptom that is the strongest predictor of morbidity and mortality after age itself. Interestingly, the degree and pattern of LVH in HCM patients shows a large degree of variation even in patients with the same HCM-causing mutation, indicating that additional factors, environmental and genetic, influence the extent of hypertrophy that develops in this condition. Candidate genetic modifiers include

genes encoding proteins involved in contractility of the cardiac sarcomere, as well as sarcomere-associated metabolic enzymes involved with control of energy homeostasis. Muscle-type kinase (MM-CK), a member of the creatine kinase isoenzyme family is one such enzyme, which interacts with the M-band of the sarcomere and there functions as ATP-regenerator. Due to its role in cellular energetics MM-CK was considered a plausible candidate modifier.

This study investigated SNPs in the creatine kinase, muscle (CKM) gene. A total of 227 individuals, belonging to 22 HCM families with known founder HCM-causing mutations, were clinically evaluated by echocardiography and their DNA genotyped using Taqman technology.

Two of the CKM gene SNPs investigated indicated association with a hypertrophic trait, independent of blood pressure.

The data suggests that CKM plays a role in augmenting the extent of hypertrophy in HCM. The research thus offers insight into the factors which modify hypertrophy and highlights the potential for future therapeutic intervention studies.

A34

Modifiers of Left Ventricular Hypertrophy in Hypertrophic Cardiomyopathy

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To test positional candidate genes for association with variation in left ventricular hypertrophy (LVH) in families with hypertrophic cardiomyopathy (HCM). The positional candidate genes were identified by genome-wide mapping in the Oxford Family Blood Pressure (HTO) Study and, in this study, HCM has been used as a model for LVH. The most promising candidate gene, from a region on chromosome 10 with a LOD score >2 , was selected for investigation. This gene, GHITM, encoding a Growth Hormone Inducible Transmembrane protein, is highly expressed in the heart and is likely to be involved in growth hormone signalling and energy metabolism.

Our unique cohort of 267 South African individuals consisted of known HCM founder gene mutation carriers and their relatives. Continuous measures of LVH were assessed by echocardiography. SNaPshot was employed for single nucleotide polymorphism (SNP) genotyping of 8 haplotype tagging SNPs across this gene. Family-based tests of genetic association were performed by Quantitative Transmission Disequilibrium Test (QTDT) analysis of the genotypic and phenotypic data.

Despite a modest cohort size, preliminary results showed evidence of association between a single SNP (c2306321g) in GHITM and a range of different measures of LVH including cumulative wall thickness (CWT) score and interventricular septum (IVS) ($p < 0.005$).

Our data suggest that GHITM may be a novel genetic modifier of LVH. The validity of these results remains to be established by replication in an independent cohort.

A35

Race and gender representation of hypertrophic cardiomyopathy or long QT syndrome cases in a South African research setting

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We researched hypertrophic cardiomyopathy (HCM) and long QT syndrome (LQTS) as models for studying the pathophysiology of arrhythmias and hypertrophy, and in the process we have had the opportunity to compare local disease profiles with global patterns.

We trawled our database entries to identify all cases of heart muscle and arrhythmic disease. Index cases were separated from the rest of their family members, segregating for the relevant disease, so that numbers were not biased by family size, and analysed the race and gender composition.

The majority of HCM index cases ($n = 90$, 51.1%) were of mixed ancestry (MA), with white Caucasian ancestry following closely behind with 74 (42.0%) and with only a few black African ($n = 9$, 5.1%) or Indian/Asian ($n = 3$, 1.7%) cases seen or referred. LQTS index cases were almost exclusively white Caucasian ($n = 36$, 88%), four (9.8%) of MA, one (2.4%) of Indian/Asian and none of black African descent. Race demographics did not fit the national demographics for South Africa as a whole. In contrast, in both groups, gender biases (slightly more male than female HCM cases, and a 0.4 ratio of males to females in LQTS) previously reported elsewhere appeared to be replicated in our database.

Genetic bias is an unlikely explanation for the skewed demographics in our database; a more likely explanation relates to missed opportunities to diagnose, missed diagnoses and misdiagnoses, as well as the real population drainage of our referral centre.

A36

Long QT syndrome type 1 (LQT1): Neural control of heart rate is a modifier of risk.

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Some long QT syndrome (LQTS) patients experience life-threatening attacks of cardiac arrhythmias, whereas others, inexplicably, remain asymptomatic. We previously showed that relatively low heart rates equated with lower risk. Here we tested the hypothesis that differences in autonomic responses modify severity of LQT1 in the case of KCNQ1 A341V mutations.

Mutation carriers (MCs) were stratified into those who had major cardiac events (symptomatic) and an asymptomatic group. The groups were compared with respect to resting heart rate (HR) and to baroreflex sensitivity (BRS).

In 56 MCs, mean HR was lower among asymptomatic patients ($p < 0.05$). Among MCs with a QT interval corrected for heart rate < 500 ms, those in the lower HR tertile were less likely to have suffered cardiac events ($p < 0.02$). The BRS was lower among asymptomatic than symptomatic MCs (11.8 ± 3.5 ms/mm Hg vs. 20.1 ± 10.9 ms/mm Hg, $p < 0.05$). A BRS in the lower tertile

was associated with a lower probability of being symptomatic ($p < 0.05$). The MCs in the lower tertile for both HR and BRS were less frequently symptomatic than MCs with different patterns (20% vs. 76%, $p < 0.05$).

Lower resting HR and BRS ("blunted") are protective in LQT1. Paradoxically, in post myocardial infarction (MI) subjects lower BRS responses were associated with increased risk of sudden cardiac death. We identified a physiological risk modifier showed that different arrhythmogenic substrates (LQT1 vs. post MI) react differently to autonomic influences.

A37

Variants in the energy metabolism gene, PDK4, are associated with increased cardiac muscle thickness in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is an autosomal dominantly inherited cardiac disorder primarily characterized by left ventricular hypertrophy (LVH) and is considered a model for the investigation of LVH, in itself the strongest predictor of morbidity and mortality after age. In HCM patients, the degree and pattern of LVH is highly variable even in patients with the same HCM-causing mutation, indicating that additional factors, environmental and/or genetic, influence the extent of hypertrophy that develops in this condition.

Switches in myocardial substrate utilization and energy production rates are known to occur in many cardiomyopathies, including HCM. Thus, candidate genetic modifiers in HCM also include genes encoding metabolic enzymes involved in the control of energy homeostasis. The pyruvate dehydrogenase kinase isoform 4 (PDK4) gene is a member of the protein kinase family that encodes a mitochondrial protein crucial for energy metabolism. Due to its role in cellular energetics, PDK4 was considered a plausible candidate modifier of hypertrophy in HCM.

This study investigated two SNPs in the PDK4 gene. A total of 227 individuals, belonging to 22 HCM families with known founder HCM-causing mutations, were genotyped using validated Taqman assays.

Both these SNPs investigated indicated association with at least one hypertrophic trait, independent of hypertrophy covariates (eg. rs2073978 vs. maximum interventricular septal thickness: $p=0.027$).

The data suggests that PDK4 plays a role in augmenting the extent of hypertrophy in HCM. The research thus offers insight into the factors which modify hypertrophy and highlights the potential for future therapeutic intervention studies by means of metabolic modulation.

A38

HRM: a fast, cost-efficient approach to ab initio mutation-screening

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Hypertrophic cardiomyopathy (HCM) is a genetically heterogeneous disease caused by more than 400 mutations in 11 sarcomeric and 3 non-sarcomeric proteins. Because of this allelic heterogeneity, the development of efficient and cost-effective mutation-screening protocols is paramount to identification of "private" mutations, in order to offer DNA-based testing to family members of index cases. While capillary electrophoresis single-stranded conformation polymorphism analysis (CE-SSCP) has been shown to be a sensitive screening method, it is a time-consuming and costly technique. We aimed to assess the usefulness of high resolution melt analysis, a new approach which uses thermal melt properties of DNA to generate characteristic melt curves, as an alternative method for ab initio mutation screening.

We screened 18 South African HCM patients for mutations in the MYBPC3 gene, using an exon-by-exon approach, with CE-SSCP on an ABI Prism™ 3100 genetic analyzer at temperatures of 180C, 300C and 350C. Samples displaying aberrant conformers were sequenced bidirectionally. Thereafter, we also screened all exons with HRM on the Rotor-gene™ 6000 and compared the sensitivity of variant detection, time and cost of CE-SSCP and HRM.

Although only synonymous variants, but no disease-causing mutations, were found, HRM showed good correlation with CE-SSCP sensitivity in flagging samples with nucleotide variation. Moreover, HRM offered analysis was more cost-effective and time-efficient than CE-SSCP.

We conclude that HRM offers a viable alternative method for ab initio mutation screening which is particularly useful for diseases with high allelic heterogeneity.

A39

Longitudinal Study of the Neurodevelopmental Performance of Children Aged 5 with Fetal Alcohol Spectrum Disorder in RSA Community

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Epidemiology studies in Northern Cape Province have shown a FASD prevalence of 12% amongst school-going children. In a recent study on 394 infants, 45 (11%) were diagnosed as FASD. This study follows the neurodevelopment of these infants, now aged 5 years, over three developmental stages, comparing the developmental delay between FASD & Non FASD cases. METHODS: 130 children (aged 5) with a FASD (n=26) or a Non FASD (n= 104) diagnosis were assessed using the Griffiths Mental Developmental Scale. Developmental abilities were assessed over the 4 domains: at 7-12, 17-29 months and 5 yr.

RESULTS: All children regardless of their diagnosis performed lower at their 17-29 month assessment than when compared to their 7-12 month assessment. Some form of developmental

delay was present amongst all children during infancy with a FASD diagnosis. During infancy even children with a Non-FASD diagnosis tended to show a falloff in their neurodevelopmental performance at their second assessment. Preliminary data from the 5-year-old developmental assessment suggests that children with FASD show more delays across all developmental subscales than children with Non FASD. Further analysis may prove that specific developmental subscales implicated during infancy due to a FASD diagnosis predict developmental delay at 5 years of age.

CONCLUSIONS: These findings illustrate that neurodevelopmental delay amongst infants is more evident at a later developmental assessment. This study adds to understanding the longitudinal neurodevelopment of children through infancy to childhood. The results identify the need for the creation of an effective developmental assessment tool specific to poor, underprivileged communities.

A40

Identification of a SNP for allele-specific silencing of the disease-causing gene in SCA1 patients in South Africa

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Spinocerebellar ataxia 1 (SCA1) is an incurable progressive neurodegenerative disorder. As an inherited disease caused by a dominant negative effect, SCA1 is a natural target for RNA interference (RNAi)-based therapies since knocking down the mutated gene (ATXN1) should prevent or delay the development of disease symptoms.

Using microsatellite analysis, a previous study performed in South Africa reported two distinct founder events in SCA1 families of Mixed Ancestry from the Western Cape. The main objective of the present study was to confirm the existence of the two founder events using a more informative SNP-based haplotype; and in addition to identify any appropriate single nucleotide difference between the mutant transcript of ATXN1 and its normal counterpart that could be targeted for allele-specific gene-silencing.

Four SNPs were selected in and around the ATXN1 gene for the haplotype study. Using restriction enzyme digests, Single Stranded Conformation Polymorphism analysis and DNA sequencing, 43 individuals from six different known SCA1 families were genotyped at each of the four loci. A SNP-based haplotype was constructed which confirmed the existence of the two reported founder events. Furthermore, two SNPs with greater than 50% heterozygosity in the SCA1 patient population were identified.

The molecular genetic information obtained from the founder events in the SCA1 families of Mixed Ancestry may be extrapolated to other population groups. More importantly, the two identified heterozygous SNPs will be useful in the development of an allele-specific RNAi-based therapy for SCA1 patients in South Africa.

A41

Analysis of a SNP in the CNTNAP2 gene in an autistic and control South African population

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Autism or "classic autism" is a neurobiological developmental disorder characterized by a triad of features, namely, difficulties in social interaction; repetitive behaviours and difficulties in communication and language development. This study aimed to investigate the allelic frequency and genotype of a single nucleotide polymorphism (SNP), rs7794745 in the gene encoding CNTNAP2 in a normal and an autistic South African population.

The study was conducted with 100 Caucasoid male samples: 50 control and 50 autistic. DNA was extracted from the samples, amplified by conventional PCR and sequenced to determine the genotype of the individuals.

The results showed that the T/T genotype was 16% more common in normal individuals compared to autistic individuals; the A/A genotype was 12% more common in autistic individuals compared to autistic individuals; and the A/T genotype was approximately the same for both groups. A 14% difference in the allele distribution was also observed between the normal and autistic individuals. Chi Square tests were performed to compare the genotype and allele frequencies of the affected with the control populations. A chi square of 4.61 ($p=0.1; df=2$) was calculated for the genotype data which indicates that there is no statistically significant link between genotype and autism.

Future studies should include both genders, family studies, other SA ethnic populations and other candidate SNPs.

A42

Identification of SLITRK1 interactors: a means of identifying novel candidate genes for anxiety disorders

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The gene encoding Slit and trk like family member 1 (SLITRK1) has been strongly implicated as a susceptibility gene for anxiety disorders. Although cumulative data suggest that SLITRK1 plays an important role in neurodevelopment, and in particular, neurite outgrowth, the precise function of the SLITRK1 protein has yet to be elucidated. The function of a protein or domain can be characterised by its interactions with other proteins. Identifying ligands of proteins that are known to be involved in anxiety disorders represents an important means of elucidating the network of interactions that may play a role in the development of such disorders. In addition, genes encoding such ligands can be investigated as novel candidate genes in case-control association analyses.

A bait containing the LRR domains from the SLITRK1 protein was used to identify potential SLITRK1 interactors using yeast two-hybrid methodology.

A yeast two-hybrid screen of a human foetal brain cDNA library was completed, and potential interacting ligands are currently being validated and characterised.

Numerous ligands interacting with the leucine-rich repeat (LRR) domains of SLITRK1 were identified. Once verified and validated, these ligands can serve as novel candidate genes that may be investigated for their association with anxiety disorders.

A43

Investigating polymorphisms in MAOA and MAOB and their role in Obsessive-compulsive disorder in the Afrikaner population

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Obsessive-compulsive disorder (OCD) is a common and debilitating psychiatric disorder that has an immense impact on the emotional, occupational and social functioning of affected individuals. Although the underlying molecular aetiology of this disorder remains largely unclear, several lines of evidence suggest that it may be caused by the complex interplay numerous genetic and environmental factors. The focus of this investigation was on the role of the Monoamine A and B (MAOA and MAOB) gene-cluster in OCD susceptibility, since their associated proteins are involved in the degradation of neurotransmitters previously found to play a role in OCD pathophysiology.

Seven tagging SNPs and a MAOA promoter region variable number of tandem repeats polymorphism were analysed in 88 OCD patients and 101 control subjects in the genetically homogeneous South African Afrikaner population by means of case-control association studies. The study sample was stratified according to gender since the genes under investigation are located on the X-chromosome, and because of possible sexual dimorphism in OCD.

One single nucleotide polymorphism (rs5905449) was significantly associated with OCD ($p = 0.035$ in the male cohort) however, no statistically significant allelic or genotypic associations were detected for the other variants investigated. Haplotype analyses yielded no statistically significant results, and none of the markers showed a significant association with severity or age of onset of OCD.

Our data provide suggestive evidence for an association between polymorphisms in MAOA and MOAB and an increased risk for developing OCD, warranting further investigation in a larger Afrikaner cohort.

A44

Improving knowledge, evaluating opinions, & ascertaining acceptance of genetic counseling for bipolar disorder: Responses in US and India.

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Bipolar disorder (BPD) is a serious mood disorder that affects about 1% of the population of the United States. Twin, family, and adoption studies have shown evidence for a genetic component of BPD, but monozygotic twin concordance is less than one, indicating that BPD is a multifactorial disorder. First-degree relatives of an individual with BPD have approximately a 3-15% risk of developing BPD because of shared genes and environment. No strong susceptibility loci for BPD have been located, although some areas of interest are currently being evaluated. With increasing genetic information, demand for genetic counselling for BPD and other psychiatric disorders is increasing.

This study was conducted with two populations, one in the US (Pittsburgh) and one in India (Ram Manohar Lohia (RML) Hospital New Delhi). Using anonymous surveys and/or a semi-structured interview for individuals with BPD and their first-degree relatives, the knowledge, opinions, and acceptance of genetic counselling in this population have been studied. The Health Belief Model was used to assess current health beliefs relating to BPD. Additionally, using a brief educational session, the effect of education on knowledge and health beliefs was assessed.

Data show that the perceived severity of BPD and perceived barriers of testing were high in both populations. Data show that the perceived susceptibility, benefit, and knowledge of BPD in affected individuals were higher in the US population than in the Indian population.

There is a demand for genetic counseling for BPD and other psychiatric disorders in these populations.

B45

Duchenne and Becker Muscular Dystrophy: Implications for at-risk Individuals

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Duchenne muscular dystrophy(DMD) and Becker muscular dystrophy(BMD) are severe X-linked recessive, degenerative neuromuscular diseases. The risk of being a carrier of DMD/BMD has a psychosocial impact on individuals and affects their requests for testing and reproductive choices.

This study is the first South African study to investigate the implication of DMD/BMD for at-risk relatives.

The objective of the study is to investigate the behaviour of different risk groups regarding genetic testing, genetic counselling and reproduction.

The study is retrospective and data are being obtained by reviewing genetic counselling files at the National Health Laboratory Service and the University of the Witwatersrand. The sample consists of 86 files from 1995 to 2008 including all the families with affected individuals. Subjects include the mothers, sisters, aunts and cousins of DMD/BMD affected individuals as well as the daughters of BMD affected individuals, of reproductive age (15-49years).

Subjects are divided according to their assigned reproductive risks: low (0-9%), intermediate (10-24%) and high (>25%). The data will be analysed using descriptive comparison and chi-squared analysis.

A pilot study on 5 patient files with 20 at-risk individuals showed that the low and intermediate risk groups did not seek genetic counselling or carrier testing and that 62.5% had children. The high risk group was more aware of their risk, 41.67% attended genetic counselling, 33.3% pursued carrier testing and 66.67% of individuals had children.

The pilot study suggests that reproductive risks affect individual's decisions regarding genetic counselling and testing but to a lesser extent their reproductive decisions.

B46

Genetic counselling experiences in delivery of genetic research results to patients affected with ABCA4 associated retinal degenerative disorders

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To describe genetic counselling experiences in the translation of ABCA4 molecular genetic research results into meaningful information for patients affected with ABCA4 associated retinal degenerative disorders (RDD).

Patients with a clinical diagnosis of Stargardt Disease, Retinitis Pigmentosa, Macular Degeneration or Cone-Rod dystrophy and a family history concordant with autosomal recessive inheritance were selected from the UCT RDD cohort. Some participants gave blood samples for research as much as 18 years ago without receiving pre-test genetic counselling. DNA samples were sent for genetic mutation screening in Estonia using the Asper Ophthalmics commercially available gene chip. Research results were validated locally and subsequently delivered to 58 participants by registered genetic counsellors. Challenges relating to genetic counselling within the context of a research programme were identified.

The implications of the identification of disease-causing mutations for the participant and family members plus the availability of genetic testing (diagnostic testing for affected siblings or carrier testing for unaffected family members or spouses) were explained to the index patient. The following outcomes were identified: multiplicity of clinical labels for the diagnosis; expectations of results by relatives; misunderstanding of recurrence risk; identification of more than 2 mutations; carrier testing of spouses and delivery of results to affected children.

These findings, their impact and potential efficacy in development of protocols for delivery of research results will be discussed.

B47***The role of genetic counselling in the management of cystic fibrosis patients and their families***

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Cystic fibrosis (CF) is a common genetic condition and yet genetic counselling services seem underutilised by affected families. The study objectives were to determine who utilises the genetic counselling services and why; to estimate the number of at-risk relatives per family; to ascertain how many relatives had mutation testing and genetic counselling; and to assess what impact new genetic counselling services at the Johannesburg Hospital CF Clinics, during 2006, had on the referrals to and uptake of the service.

The study was retrospective and file-based. The files of 153 families seen for genetic counselling for CF from 1990 to 2006 were obtained and, using a data collection sheet, relevant data were sought in order to achieve the study objectives.

The majority of counselees were parents of CF probands (35%). Relatives at high risk (125%) of being CF carriers formed only 13% of all counselees. Most individuals attended genetic counselling to gather information. On average, six families received genetic counselling per year from 1990 to 2005, whereas in 2006, 58 families were seen. Referrals increased nearly tenfold during 2006, compared to prior years. In 140 unrelated families, 1991 relatives at high risk were identified. Only 11% of these relatives had mutation testing and 8% received genetic counselling.

Uptake of the genetic counselling service is greater when it is integrated into CF management clinics. The low uptake of mutation testing and genetic counselling by at-risk relatives suggests that educating individuals regarding their risks and available testing must improve.

B48***Interactive dynamics of a mediated genetic counselling session***

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In South Africa there are 11 official languages. As health care providers are mostly proficient in only one or two languages, many health care interactions are mediated by a third person. This is also a feature of Genetic Counselling (GC) sessions where in most instances, ad hoc mediators are used to assist the genetic counsellor. Little is understood about this process. As part of a larger ongoing project, we present a transcribed interview of a prenatal GC session (in English) which was mediated by a nurse (first language Afrikaans) in the participant's language, Sepedi.

Using qualitative methodology, the GC session was video recorded, transcribed and analysed using a hybrid analytical approach which included conversation analysis and discourse analysis. The interactive dynamics are described focusing on the use of scientific language in conveying concepts.

During the session, scientific terminology was used to explain complex concepts, such as inheritance, chromosomes and risks. For most of the terminology there were no Sepedi translations

that could be used by the mediator. The mediator had difficulty conveying the messages to the participant and this appeared to affect the goals and effectiveness of the GC session.

The findings confirm that in multilingual interactions the interpreter's role is complex. Assessing the participants understanding and decision-making are affected. The finding has implications for practice and highlights the need for policies, posts and appropriate training.

B49

Level of genetic knowledge and its impact on reproductive choices and risk communication in families with cystic fibrosis

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The Red Cross Children's Hospital (RCCH) has a weekly cystic fibrosis (CF) clinic, attended by children with CF from all over the Western Cape. The aims of this study were to determine the level of genetic knowledge of parents with a child with CF; to determine the impact of the birth of a child with CF upon subsequent reproductive choices and to investigate family communication about genetic risk.

A qualitative approach was selected as it aims to understand and provide descriptions that portray the richness and complexity of ordinary events from the participants' perspective. Ten semi-structured interviews were conducted with parents who had a child with CF.

The participants in this study generally had a flawed understanding of the genetics of CF. The level of understanding was identified as being related to socioeconomic status. The birth of a child with CF had a major impact on subsequent reproductive decisions. Risk information was not readily disseminated in most families. A lack of genetic knowledge was found to be the main barrier to risk communication.

The findings of this study will help healthcare professionals involved in the CF clinic to address gaps and misconceptions in parents' knowledge and to understand barriers to risk communication in these families. The service delivered at RCCH CF clinic may be improved by having a genetic counsellor as a member of the team. A genetic counsellor could play an important role in facilitating knowledge gain; dissemination of risk information; assisting in reproductive decision-making; and in providing psychosocial support.

B50

Basic understanding of genetic concepts amongst isiXhosa-speaking caregivers of haemophilia patients

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Haemophilia A and B are X-linked recessive inherited, life-long chronic bleeding disorders. An understanding of the intrinsic knowledge and basic understanding of genetic inheritance is central

to provide effective, culturally sensitive genetic counselling to fully inform carriers and their partners of the implications for a prospective child. The objective of this study was to explore the level of understanding of basic genetic concepts among isiXhosa-speaking caregivers of haemophilia patients, to develop a more comprehensive understanding of the needs of this study group and to develop appropriate health promotion information.

An exploratory qualitative research design was adopted. Participants included first-language Xhosa speaking mothers or caregivers of haemophilia patients residing in townships near Cape Town. Qualitative data was generated from transcribed and translated audio-records of ten semi-structured interviews, conducted in Xhosa by an interpreter, as well as from participant observation by the investigator.

Results suggest that the participants have a very limited understanding of the clinical management, genetic inheritance and physiological causes of haemophilia and remain unsure as to the implications of this condition. While treatment and care by health care service providers is fully accepted, several participants believe that the root of the problem can only be found by pursuing traditional methods.

Participants are deeply affected by their child's condition, they feel overwhelmed and often frightened and find their lives transformed. Awareness, by all role players, of cultural beliefs and of how illness is interpreted is essential to help improve communication between health care service providers and isiXhosa speakers.

B51

The practices, knowledge, and attitudes about common hereditary cancers: Survey of General Practitioners in Johannesburg

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About 5-10% of all cancers are due to inherited mutations. In affected families "at risk" individuals may benefit from genetic counselling and predictive testing. General practitioners (GPs) therefore need to identify patients and families at risk and refer them appropriately.

To assess the practices, knowledge and attitudes of GPs with regards to cancer genetics.

An exploratory research design was chosen. An existing questionnaire was used, piloted and mailed to 196 GPs. The 61 GPs who responded constituted the final sample. Data were analysed using descriptive statistics.

Participants spend, on average, 45 hours per week in practice seeing about 110 patients. Forty five (67%) GPs had a family history of cancer. The GPs used several cancer screening procedures, but obtained limited information on cancer history from their patients. Fifteen (25%) GPs assessed patients' risks for cancer susceptibility and 22 (36%) referred patients to other facilities for assessment and testing. Thirty two (52%) GPs were aware of genetic testing facilities. Most were

not familiar with the genetic counselling facilities available, but felt patients should have counselling before testing. Attitudes towards genetic testing were positive. Overall 53 (87%) GPs were interest in learning about cancer genetics (services) and expected to play an appropriate active role in the future.

The GPs in this study had limited knowledge about inherited cancers and they did not participate actively in genetic management. Education of GPs about basic cancer genetic concepts and services is needed so that they are better equipped to identify and refer at risk families.

B52

The COL1A1 gene and anterior cruciate ligament ruptures

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Anterior cruciate ligament (ACL) ruptures have been reported as the most severe injury sustained in a sporting population. Although various intrinsic and extrinsic risk factors have been identified, the exact aetiology is not yet fully understood. The Sp-1 binding site polymorphism within the COL1A1 gene, which encodes for the alpha1 chain of type I collagen, has been shown to be associated with cruciate ligament ruptures in a Swedish population. The aim of the study is to investigate if the Sp-1 binding site polymorphism within the COL1A1 gene is also associated with increased risk for ACL rupture in a South African population.

One hundred and seventeen Caucasian participants with surgically diagnosed ACL ruptures, as well as 130 Caucasian physically active controls (CON) without any history of previous ligament or tendon injuries were recruited. All participants were genotyped for the functional Sp1-binding site polymorphism (G/T; rs1800012) within the COL1A1 gene.

The rare TT genotype was significantly ($p=0.031$; OR 12.3; 95% CI 0.7 to 220.4) under-represented in the ACL group (5% vs 0%).

In conclusion, although no significant differences in genotype or allele distributions between the groups were observed, it is interesting to note that the rare TT genotype was absent in subjects with ACL ruptures. Although this observation should be interpreted with caution due to the low frequency of the TT genotype, the results are similar to what was observed in a Swedish population. Further research is required to evaluate the possible protective role of this genotype in sustaining an ACL rupture.

B53

The COL5A1 gene is associated with increased risk of anterior cruciate ligament rupture in female participants

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Anterior cruciate ligament (ACL) ruptures, especially to young female athletes are a cause of major concern in the sports medicine fraternity. It has been suggested that certain individuals may be genetically predisposed to ACL rupture. The gene that encodes for the alpha1 chain of type I collagen (COL1A1) is the only gene which has been shown to be associated with increased risk of cruciate ligament rupture. The COL5A1 gene, which encodes for the alpha1 chain of type V collagen, has been shown to be associated with Achilles tendinopathy, another common soft tissue injury. The objective of this study is to investigate if the COL5A1 gene is also associated with increased risk of ACL rupture.

129 Caucasian participants with surgically diagnosed ACL ruptures and 216 physically active control participants (CON) without any history of ACL injury were genotyped for the BstUI (SNP rs12722) and DpnII (SNP rs13946) restriction fragment length polymorphisms (RFLP) within the 3'-UTR of the COL5A1 gene.

There was a significant difference in the BstUI RFLP genotype frequency between the ACL and CON group among the females, but not males. The CC genotype in the female participants was significantly over-represented in the ACL group compared to the controls (27.4% vs 5.6%, OR= 6.6; 95% CI 1.5 to 29.7; P=0.006). There were no DpnII RFLP genotype differences between the ACL and CON groups.

The COL5A1 BstUI RFLP is associated with ACL rupture in females. Females with a CC genotype are 6.6 times less likely to rupture their ACL.

B54

Association of MC3R in tuberculosis susceptibility

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Tuberculosis is a disease like few others with complex host/pathogen/environment interactions. Genetics has been shown to play a role in susceptibility to this disease. An association of a polymorphisms in melanocortin receptor 3 (MC3R), rs3827103, was found following a genome wide association study conducted in a number of African populations. A role for MC3R has previously been reported in energy metabolism, inflammation and cardiovascular regulation and defects of this receptor have been shown to be associated with severe obesity.

An unmatched case-control study was conducting in a South African Coloured population from metropolitan Cape Town to test the reported association of polymorphisms in MC3R with

tuberculosis susceptibility. Four SNPs were successfully genotyped and p-values were obtained using either χ^2 or Fischer exact test where appropriate.

One SNP, rs6127698, was found to have a significant association with the most common allele, in this case T, being transmitted more frequently in cases than in controls ($p = 0.0043$, OR = 1.491 and 95% CI [1.166-1.907]). This association remained significant even after correcting for multiple testing. Rs6127698 is located in the promoter region of the MC3R gene. While the same polymorphism was not implicated in both studies, the two SNPs were found to be in LD.

This study supports the findings that polymorphisms in MC3R may be associated to tuberculosis susceptibility. Further studies are still required to determine the functional effects of these SNPs on the activity and expression of the melanocortin-3-receptor.

B55

Characterization of the 5' Regulatory Region of the Cytochrome B Reductase 1 (Cybrd1) Gene of Oesophageal Cancer Patients

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CYBRD1 is a membrane protein responsible for the reduction of ferric to ferrous iron, enabling dietary iron absorption into the enterocytes. The precise mechanisms whereby this happens is still unknown and there is still little information on the mechanisms regulating CYBRD1 transcription. Promoter variants identified in this study may create or disrupt transcription factor binding sites (TFBS), promoting or impairing the negative feedback regulatory circuit existing between iron levels and CYBRD1 expression. The 5' regulatory region of the CYBRD1 gene of oesophageal cancer (OC) patients will be characterized to identify a possible correlation between promoter variants and gene expression.

The 5' regulatory region (2100bp) of 110 Black Xhosa-speaking OC patients and 100 population-matched control individuals was characterized by PCR, HEX-SSCP analysis and restriction enzyme analysis, where appropriate, for the identification of known and/or novel variants. Statistically significant differences were determined by the Fisher exact test and/or chi squared analysis, with a $P < 0.05$ regarded as statistically significant.

Variants identified include -1844C/G, -1834G/A, and the G(T)8G(T)nG(T)nG(T)9 repeat. Predictive functional analyses using bioinformatic tools indicated that the presence of these variants affected TFBS of transcription factors playing a role in iron homeostasis, haematopoiesis and liver regulation.

In silico analysis is merely a predictive tool highlighting putative binding motifs. Until the transcriptional regulation of the gene has been clarified, we can only speculate that these TFs have a role in the regulation of CYBRD1. Further analysis will include luciferase reporter gene assays to identify promoter variants that could affect gene expression.

B56***Filaggrin gene variants in black South Africans - predisposing factor for atopic***

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The aim of this study is to investigate variation at the FLG locus in the black South African population and its role as a predisposing factor for atopic dermatitis (AD).

Forty-three black patients with atopic dermatitis as well as a black control group of seventeen were investigated. PCR followed by RFLP analysis was carried out to determine the presence of two previously described FLG variants associated with AD in European populations. In addition, DNA sequencing of all samples provided SNP data that were used to generate inferred haplotypes.

The FLG mutations R501X and 2282del4 were not present in black AD patients. SNP based haplotypes spanning a region of the FLG gene were generated. An exact test confirmed that differences observed between black AD patients and the control group was statistically significant. Two haplotypes were overrepresented, one in the patient and one in the control group.

The two common European FLG mutations, R501X and 2282del4, were not present in black patients.

To date, no causal FLG mutation was identified in black South African AD patients. Linkage disequilibrium was observed for some SNPs across the FLG locus, supporting its role in the AD disease process in black South Africans. The haplotype frequencies differed significantly between the two groups suggesting that this locus is linked to a predisposing variant. The sample size used was too small for these observations to be conclusive, but important in elucidating the presence of this variant.

B57***Iron metabolism and oesophageal cancer: an exploratory investigation into SLC40A1***

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Solute carrier family 40 member 1 (SLC40A1) is a protein responsible for the iron transport across the apical membrane of enterocytes as well as iron export from tissue cells. Iron regulation is crucial in the human body as iron is required for enzyme synthesis, however excess iron promotes the formation of reactive oxygen species, linking iron overload to cancer. We investigated the 5'UTR of the SLC40A1 gene to identify promoter variants that could potentially be involved in gene expression regulation.

A Black South African Oesophageal cancer patient cohort (n=45), and population matched control cohort (n=50) was subjected to PCR amplification of overlapping promoter region fragments of SLC40A1. Subsequently, PCR products were subjected to heteroduplex single-stranded conformational polymorphism (HEX-SSCP) analysis for mutation detection. Variation was confirmed with bi-directional semi-automated DNA sequencing analysis.

The significance of variants was determined by estimating allele and genotype frequencies and determining probability values using chi-square analysis. The mutational analysis identified previously described variants: -1461 T/C, -1399G/A, -1355G/C, -750G/A and -23A/G; as well as

one novel variant: -1470C/T. No statistically significant associations were identified; however, bioinformatic analysis demonstrated abolishment and creation of various transcription factor binding sites, which is of importance for gene regulation.

Although no statistically significant associations were identified, allele and genotype frequencies for the variants identified in the Black South African population have been documented. Bioinformatic results are in the process of being confirmed by luciferase reporter gene constructs. These results would add to better understanding of SLC40A1 promoter regulation and its involvement in iron regulation.

B58

Molecular-Genetic Analysis of Ceruloplasmin in Oesophageal Cancer

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Oesophageal Cancer (OC) is a disease characterised by the development of malignant tumours in the epithelial cells lining the oesophagus. It demonstrates marked ethnic variation, with squamous cell carcinoma (SCC) being more prevalent in the Black population and adenocarcinoma (ADC) occurring more often in Caucasians. The aetiology of this complex disease has been attributed to a variety of factors, including an excess of iron (resulting in increased tumourigenesis), oesophageal injury and inflammation (due in part to Barrett's oesophagus and smoking, amongst others). The aim of this study was to determine if genetic variations identified in the Ceruloplasmin (CP) gene (implicated in iron homeostasis) contribute in any way to OC pathogenesis or susceptibility.

The study cohort consisted of 96 unrelated OC patients from the Black Xhosa-speaking South African population and 88 population-matched control individuals. The promoter and coding regions of the CP gene were analysed for DNA sequence variation using the polymerase chain reaction (PCR), heteroduplex single-strand conformation polymorphism (HEX-SSCP) analysis, restriction fragment length polymorphism (RFLP) analysis and semi-automated bidirectional DNA sequencing analysis.

Fourteen previously described and four novel variants were identified. Statistical analysis revealed that two of the novel variants were significantly associated with OC in this study; the promoter variant 5'UTR-308G/A (P=0.012) and the exonic variant G633 (P=0.0003).

This is the first study to examine CP with respect to OC in the Black South African population. As such, these findings should serve to further our understanding of the relationship between iron metabolism and disease pathogenesis, specifically OC.

B59

Consolidation of tuberculosis candidate gene studies in a South African population

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Human genetic susceptibility to tuberculosis (TB) includes environmental influences and the

interplay of host genetic factors. Several susceptibility genes have been identified in studies across the globe, but there is little indication of the relative impact of these susceptibility alleles on disease. The aim was to assess possible gene-gene interactions.

Case-control association studies were done using a South African population to investigate association of 11 polymorphisms in 9 genes previously identified as being involved in the progress of TB infection to disease. A total of 505 TB cases and 320 healthy controls were genotyped by various methods including ARMS-PCR, RFLP, REA and electrophoresis on ABI. Regression models were constructed.

Significant associations were found between 5 of the genes tested and TB, and the effect sizes and relative contribution of the various genes to TB in this uniform group could be shown. The sample size and extent of environmental information was not large enough to determine gene-environment interactions. In a few cases associations that had been demonstrated previously in a smaller sample, were not significantly associated with TB. Nine significant gene-gene interactions were found.

Genotyping the same individuals for a large number of the "usual suspects" in TB susceptibility enables direct comparison of the gene effects. The effect of each individual polymorphism, even when significant, is usually small. At the point when the majority of susceptibility genes for human TB have been identified, it will be possible to conclude whether true gene-gene interaction is present, or whether their effects are additive.

B60

Glucocerebrosidase Gene Mutations in Black South Africans with Gaucher Disease

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This study was aimed at elucidating the molecular cause of Gaucher Disease (GD), the most common Lysosomal Storage Disorder, in the black South African population. The identification of 1 common disease causing allele, exclusively found in black GD patients, prompted us to investigate the haplotype background in order to see if this variant was of single origin.

We studied nineteen unrelated black GD patients and identified 37/38 disease causing variants. The methodology applied to obtain the results included an enzyme assay, PCR, RFLP, DNA sequencing and Pyrosequencing.

Deletion c.222-224delTAC (p.T36del) was found in 17/38 (0.45) alleles and 8/38 (0.21) alleles were identified as the recombinant allele RecNcil. The remaining 12/38 disease causing mutations were missense and nonsense mutations of which three are novel. The mutation profile in black patients showed noticeably low genotypic heterogeneity, with 7/19 (0.37) compound heterozygote individuals for p.T36del and RecNcil. The carrier frequency for the p.T36del mutation in the general population was determined as 1/66. One haplotype spanning a 200kb region 5' of the GBA gene shows complete LD with the frequently observed p.T36del variant, supporting a single origin hypothesis for this allele.

The GBA mutation profile in black South African Gaucher Disease patients shows low complexity with a founder mutation, p.T36del, occurring at a frequency of 0.45. Recombinant allele RecNcil is also common at a frequency of 0.21. Our data are compatible with a single origin of the p.T36del mutation in the black SA population. Contrary to expectation, no p.T36del homozygote patient was identified.

B61

Effect of HT1 metabolites on NER and/or BER repair of DNA damage

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Hereditary Tyrosinemia Type 1 (HT1) is an autosomal recessive disorder of tyrosine catabolism caused by a defective fumarylacetoacetate hydrolase enzyme. We previously suggested that the main effect of one of the minor accumulating metabolites, p-hydroxyphenylpyruvic acid (pHPPA), is the long term impairment of the DNA repair machinery. In this study we investigated the effect of pHPPA and succinylacetone (SA) on the excision repair pathways in HepG2 cells.

The comet assay (single cell gel electrophoresis) has been modified to measure the ability of a sub-cellular extract of HepG2 cells to carry out the initial incision step of DNA repair (BER and NER). Gel embedded nucleoids from HepG2 cells pre-exposed to MMS or Benzo[a]pyrene were incubated with cell extracts from HepG2 cells exposed for 24 hours to SA or pHPPA. The repair capacity of the extracts is derived from the rate at which incisions are introduced and the subsequent increase in tail DNA.

MMS pre-treated nucleoids exposed to cell extract from pHPPA treated HepG2 cells showed a significant ($p < 0.05$) decrease in tail DNA compared to untreated HepG2 cell extract, whereas Benzo[a]pyrene treated nucleoids showed no significant difference compared to controls. Also, no significant difference in tail DNA was seen in MMS or Benzo[a]pyrene nucleoids exposed to SA treated HepG2 cell extracts.

Exposure of HepG2 cells to SA seems to have no effect on DNA repair via base or nucleotide excision repair. However, exposure to pHPPA appears to affect the repair enzymes of BER but not NER.

B62

Demethylating effects of PHPPA in hereditary Tyrosinemia Type 1

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Hereditary Tyrosinemia Type 1 (HT1) is a disorder of tyrosine catabolism caused by a defective fumarylacetoacetate hydrolase enzyme. We previously showed that p-hydroxyphenylpyruvic acid (pHPPA), causes DNA damage, although the specific mechanism remains unsolved. Since the development of hepatocarcinoma (HCC) is characteristic of the chronic form of HT1 and global hypomethylation has been linked with the development of HCC, hypomethylation may form part of mechanism underlying the damaging effects of pHPPA. The aim of this study was to determine if pHPPA causes any epigenetic alterations, which could be implicated in the development of HCC.

The standard comet assay was adapted to measure DNA methylation levels. pHPPA exposed gel-embedded nucleoids were incubated with methylation sensitive restriction enzymes Hpa II and Msp I, and changes in tail DNA were calculated. The cytosine extension method based on the selective use of Hpa II and Msp I were then used to determine the level of radio active cytosine incorporation and the absolute methylation percentage was calculated.

Initial genotoxicity experiments with the comet assay indicated a change in the DNA methylation status following treatment of isolated liver cells with pHPPA. Subsequent Cytosine Extension assays confirmed global demethylation at lower concentrations of pHPPA while concentrations higher than 100uM produced inconclusive results since these concentrations resulted in greatly decreased DNA yield.

In conclusion, pHPPA alters the DNA methylation status of mammalian cells via global demethylation. We therefore propose that accumulation of this metabolite in HT1 could contribute to genomic instability and therefore the development of hepatocarcinoma.

B63

Elevated 3-hydroxyisovaleric acid and trace amounts of 3-methylcrotonylglycine are not necessarily indicative of 3-methylcrotonylglycinuria

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Persistent elevated levels of urinary 3-hydroxyisovaleric acid, 3-hydroxyisovalrylcarnitine 3-methylcrotonylglycine acid and 3-methylcrotonic acid, in the absence of other markers that suggest combined carboxylase deficiency, usually indicate 3-methylcrotonyl-CoA carboxylase (MCC) deficiency, an autosomal recessive disorder of leucine catabolism. MCC activity in homogenates of cultured skin fibroblasts of MCC deficient patients is usually less than 2% of control values, patients with MCC activity between 4 and 12% of normal have been reported.

Here we present the first evidence that elevated urinary 3-hydroxyisovaleric acid levels and trace amounts of 3-methylcrotonylglycine are not necessarily indicative of 3-methylcrotonyl-CoA carboxylase deficiency. Four members of a family, two adults and two infants, with no known consanguinity all excrete high levels of 3-hydroxyisovaleric acid and trace amounts of 3-methylcrotonylglycine.

One of the adults, proband NWU001, presented with a 20 year history of acute chronic fatigue and muscle weakness. The other three probands (NWU002, NWU003, NWU004) are asymptomatic. The MCC activity of the two adult probands (NWU001, NWU002) were determined and found to be normal. No mutations associated with MCC deficiency were detected in the open reading frames (ORF) of the two subunits that constitute MCC of NWU001 and NWU002. However one heterozygous SNP was identified in

MCCA of proband NWU001 and a heterozygous splice variant were observed in MCCB of NWU002.

We conclude that high levels of urinary 3-hydroxyisovaleric acid levels and trace amounts of 3-methylcrotonylglycine are probably caused by an as yet unidentified inheritable genetic defect.

B64

Molecular genetic analysis of two genes in the schizophrenia-susceptibility locus on chromosome 22q in Xhosa schizophrenia patients

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Chromosome 22q has been associated with schizophrenia in numerous studies and harbours two important genes for psychiatric genetic research – CYP2D6 and COMT. Here we describe a comprehensive approach to elucidate the relevance of variants in these genes in Xhosa schizophrenia patients.

Genomic DNA was extracted from 50 Xhosa schizophrenia patients and 50 healthy Xhosa control individuals. The spectrum of common DNA sequence variation in the CYP2D6 gene and distal COMT promoter was determined by sequencing 15 controls and 15 schizophrenia samples respectively. Candidate polymorphisms were selected by means of bioinformatic analysis and the literature and typed in the entire cohort for association analysis.

Sequencing data revealed the presence of > 50 polymorphisms in CYP2D6 as well as 11 polymorphisms in the COMT promoter. A rapid genotyping protocol was then utilised to type over 30 polymorphisms in the two genes. A high frequency of the CYP2D6*5 deletion allele was observed in the case (0.20) and control groups (0.17) and a novel CYP2D6 allele was identified in the schizophrenia cohort. COMT SNP, rs737865, was found at a significantly higher frequency in the controls than patients ($P = 0.001$) and could be associated with schizophrenia-risk reduction.

The Xhosa population has a unique genetic makeup and studies such as these determine whether candidate schizophrenia genes are associated with the disorder in this population. The data generated suggest that these two genes warrant further research and should also be considered when designing pharmacogenetic treatment plans for Xhosa schizophrenia patients.

B65

Analysis of sequence diversity in the CYP2C19 gene in a unique South African population

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This study was aimed at determining sequence variation present in the CYP2C19 gene and surrounding untranslated regions (UTR) of interest, within the Xhosa population.

Comparative sequence analysis was utilised to identify regions in the 5' and 3' UTRs of CYP2C19 exhibiting high homology to other related UTRs. Semi-automated DNA sequence analysis was subsequently performed to identify variants in the UTRs of interest, as well as the coding regions, in 15 Xhosa individuals. Haplotype and various in silico analyses were performed to prioritise the variants identified for further RFLP genotyping in an additional 85 individuals.

This study revealed the presence of 27 variants, of which five are novel. Among the variants observed, were the previously characterised CYP2C19*2, CYP2C19*9, CYP2C19*15 and CYP2C19*17 alleles. These alleles were observed at a frequency of 0.205, 0.085, 0.091 and 0.097 respectively, with the first three reportedly occurring at lower frequencies in Caucasians. Furthermore, all these alleles, except CYP2C19*15, have previously been shown to influence enzyme activity. The novel variants include one non-synonymous SNP located in exon 7, observed at a frequency of 0.005 and two occurring in perfect linkage disequilibrium (LD) with the CYP2C19*15 variants.

Our data demonstrate that the Xhosa population exhibits a unique genetic profile that could influence the outcome of drug therapy in this population. By utilizing this information in combination with other pharmacogenetic data, this study is anticipated to contribute to optimising therapeutic treatment within the South African context.

B66

SNaPshot analysis of polymorphisms in the human NAT2 gene: analysing acetylation in MDR-TB patients

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The NAT2 gene inactivates Isoniazid, which confounds the efficacy of anti-TB treatment. We investigated the acetylation status in TB patients via a multi-plex technique, to test the suitability of this analysis for the rapid determination of the acetylation status.

The SNaPshot method utilizes a primer extension reaction to incorporate fluorescently-labelled dideoxynucleotides at each targeted SNP in a multiplex reaction.

These targeted SNPs were also analysed via the standard PCR-RFLP technique, to test for concordance between methods.

The infecting Mtb strains were classified by IS6110-DNA fingerprinting. Secondly, the drug sensitivity profiles for these strains were established by both phenotypic and genotypic analyses.

The SNaPshot method facilitated the analysis of multiple targets in the samples, in a high throughput setup. This method also discriminated between homo- and heterozygosity at each SNP position. Secondly there was complete concordance of results obtained with the SNaPshot and PCR-RFLP techniques.

These techniques independently segregated individuals into Fast, Intermediate and Slow acetylators.

The strain characterisation indicates that the infecting strains only belonged to two separate clades. The majority of these strains were resistant to both Isoniazid and Rifampicin, the primary antibiotics used in TB treatment regimens.

The SNaPshot technique allowed a substantial saving in operational time, thereby facilitating the rapid genotypic analysis for acetylation status, which is an improvement on the standard PCR-RFLP method. However the viability and suitability of this technique is hampered by the cross reactivity between primers.

B67

Investigating genomic copy number variations in South African patients with mental retardation (MR)

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Mental retardation has a prevalence of 2-3% within the general population with a substantial proportion being attributable to genetic factors. It has been well established that gross chromosomal aberrations commonly underlie the etiology of the disorder, with prevalence estimates ranging from 12-24%. These aberrations have traditionally been detected by implementing a host of cytogenetic techniques. However, subtle chromosomal aberrations often go undetected owing to the limited resolution of these methods.

In this pilot study DNA from 30 patients with MR and a range of additional clinical features were investigated for subtle chromosomal aberrations using microarray technologies. The platform used was the 250K Nsp Affymetrix SNP array and data analysis was performed using the CNAG software.

A total of 94 copy number variations (CNVs) were observed, clearly indicating the polymorphic nature of CNVs and the high incidence of these polymorphisms within the population. One known disease-causing deletion and two novel putative X chromosomal disease-causing aberrations (one deletion and one duplication) were identified. The 8Mb X-chromosomal deletion exhibits incomplete penetrance within the family.

The finding of a large X chromosomal deletion exhibiting incomplete penetrance has important implications in the interpretation of the disease-causing nature of copy number variations. Additionally, this finding has ramifications in the genetic counselling of individuals who carry these aberrations. Finally, this pilot study demonstrates the superior capabilities of microarray technologies to detect subtle chromosomal rearrangements and highlights the importance of incorporating this technology within the diagnostic protocols offered to patients with MR.

B68

A high throughput screen for point mutations in the DMD gene

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Duchenne Muscular Dystrophy (DMD) is a severe, X-linked, recessive muscle-wasting disorder affecting 1 in 3 500 live male births worldwide.

The disease progression sees most patients wheelchair-bound before the age of 12. Death generally occurs before the third decade of life, with no treatment available to date.

Large deletions or duplications of one or more exons in the dystrophin gene are found in approximately 70% of DMD patients, with small deletions/insertions or point-mutations making up the remaining 30%. These small (point) mutations occur randomly across the gene and mostly result in premature termination codons (PTCs) and severe phenotypes. PTC124 is a new investigational drug, which has been shown to promote ribosomal read-through of premature but not normal termination codons, thus forcing completion of translation and protein synthesis. It therefore, along with its minimal toxicity profile, appears to offer therapeutic potential for patients with DMD due to PTC's and other genetic disorders where therapeutic options are limited or absent.

This project will focus on setting up a cost effective, high-throughput screening technology in an attempt to identify DMD patients carrying nonsense mutations thereby establishing their eligibility for PTC124 treatment.

The DMD gene coding region will be screened for mutations using the high resolution melting curve analysis(hrMCA), followed by direct sequencing. The results will be presented.

Identification of small/point mutations other than those resulting in PTCs, will be valuable to the patient and the clinician when considering other forms of therapy, genetic or otherwise.

B69

Rapid genetic screening method for six known mutations associated with aminoglycoside-induced deafness

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Aminoglycoside antibiotics, used in the treatment of bacterial infections such as tuberculosis, can result in irreversible ototoxic side effects.

Multi-drug resistant tuberculosis (MDR-TB) incidence is increasing in South Africa (SA) and as these

patients receive aminoglycosides as part of their treatment they are therefore at risk of developing aminoglycoside-induced deafness. We aimed to develop a rapid, cost effective screening test to determine the frequency of the six mutations (A1555G, C1494T, T1095C, 961delT+insC(n), A827G, T1291C), associated with hypersensitivity to aminoglycosides, in a group of MDR-TB patients and the background population.

A multiplex SNaPshot technique was used to detect these mutations in 101 MDR-TB patients from Brooklyn Chest Hospital and 360 controls from three SA ethnic groups.

The A1555G mutation was found in 0.9% of the Black controls. The A827G mutation was observed in 1% of the MDR-TB patients. The 961delT+insC(n) mutation was found in unexpectedly high frequencies in the control samples. For all 3 subpopulations this variant was found at a frequency of more than 1%. The C1494T, T1095C and T1291C mutations were not detected in any study participants.

These results indicate that 961delT+insC(n) is not pathogenic and is a common polymorphism. The high frequency of A1555G detected in the Black population is alarming since this group has a high MDR-TB incidence. Implementation of this rapid detection method in South Africa has the potential to identify individuals or population groups at increased risk of developing aminoglycoside-induced deafness and can therefore prevent hearing loss in these susceptible individuals.

B70

SMA in the Western Cape remains true to (geno) type

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Black South Africans (n=92) from the Northern parts of the country are reported to have a genetic cause for SMA that differs significantly from other populations of the world and from their counterparts in the Western Cape who all (n=27) show conformity with the universal genotype. This is a confusing situation and poses problems for genetic counselling.

SMA is diagnosed by PCR analysis of exons 7 and 8 of the SMN1 gene. Worldwide, >90% of SMA patients are shown to be homozygous for a deletion of exon 7 of SMN1.

In the Western Cape, 100% of SMA patients have shown a deletion of exon 7 of the SMN1 gene; White (n = 5); Mixed ancestry (n = 29); Black (n = 27). All patients, regardless of ancestry, with features consistent with the international inclusion criteria for SMA were confirmed to have the deletion. The remaining patients who were negative for the deletion (n = 39), screened as part of a neuromuscular "work-up", all had exclusion features and subsequent alternative diagnoses confirmed.

The Western Cape shows: 1) No discord in the detection of the deletion mutation in children who clinically comply with the international inclusion criteria. 2) No patients with the deletion mutation have had facial weakness. 3) No clinically diagnosed patients have been found not to manifest the exon 7 deletion mutation. Genetic counselling in the Western Cape will continue in

compliance with international guidelines that the genetic screen provides >95% specificity and sensitivity for detecting SMA for all ancestries.

B71

The diagnosis of genetic defects by conventional cytogenetics

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Chromosome analysis remains one of the most commonly performed diagnostic genetic tests, being offered for a wide variety of indications in obstetrics and gynecology, pediatrics and oncology. At cytological level, banded chromosomes show a constant and similar pattern in clinically healthy individuals. The aim of this study was to investigate the diagnostic value of conventional cytogenetic analysis in genetic defects.

Five patients with suspecting genetic defects were referred for routine cytogenetic analysis. Peripheral blood samples were set up as cell cultures using RPMI 1640 medium, fetal calf serum, phytohaemagglutinin as stimulant and an antibiotic. Specimens were cultured for 72 hours, harvested and stained by the G-banding method. Metaphase spreads were then analyzed and karyotyped by using the Cytovision system.

A 7-month old baby with abnormal features and possible Down syndrome was diagnosed with Wolf-Hirschhorn syndrome caused by a deletion of the short arms of chromosome 4 (del(4p)). The clinical diagnosis of Down syndrome was confirmed in two neo-natals, one with a translocation 46,XX,t(21q;21q) and one with a derivative 46,XX,der(14q;21q),+21. A 19-year old patient, presented with primary amenorrhea and delayed puberty was diagnosed as Turner syndromes based on the presence of an iso-chromosome 46,X,i(Xq). A deletion of the long arms of chromosome X, 46,X,del(Xq) in a 24-year old patient leads to the diagnosis of Turner syndrome.

Conventional cytogenetics is a proven and valuable tool in the diagnosis, genetic counseling, treatment and management of patients with a genetic defect.

B72

Sequence variants in the LGALS13 Gene: A role in Abruptio Placentae?

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Obstetric haemorrhage accounts for 13.4% of maternal deaths in South Africa. One of the most common causes of obstetric haemorrhage before labour is abruptio placentae, the premature separation of the placenta. Placental protein 13 (PP13) has been implicated in embryogenesis, specifically, implantation and placentation. Decreased first trimester PP13 levels have been identified in pre-eclampsia and intrauterine growth restriction (IUGR). The aim of this study was to investigate the role of the LGALS13 gene in patients with abruptio placentae, in an attempt to broaden our understanding of this devastating condition.

The study cohort comprised 195 Black and Coloured patients (maternal-fetal pairs) and 336 ethnically matched controls. The LGALS13 gene was amplified by polymerase chain reaction (PCR) and

screened by Multiphor SSCP/HD gel electrophoresis. Conformational variants were characterized by automated sequencing and restriction enzyme analysis.

Seven sequence variants were identified in this study, of which four were novel. In the Coloured population, statistical significance was observed at the following loci: -98A/C (rs3764843) ($p=0.036$), IVS2 -36G/A ($p=0.043$) and the 'hotspot' ($p=0.038$), a haplotype comprising 6 single nucleotide polymorphisms within an 11bp region.

The three LGALS13 sequence variants highlighted in this study may, by their position within the gene, affect regulation of gene expression, splicing and protein folding. The Luciferase reporter gene assay and RNA analyses are underway in order to substantiate these findings.

B73

An Investigation Into Protein Misfolding In Retinitis Pigmentosa 17 And Other Dominant Retinal Disorders

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Several sporadic and genetic diseases are caused by protein misfolding. In an autosomal dominant form of retinitis pigmentosa (RP17), a mutation in carbonic anhydrase IV (CAIV) has been identified as the cause of disease. CAIV catalyzes the reversible hydration of carbon dioxide and water to bicarbonate. It is strongly expressed in the choriocapillaris of the human eye. It has been suggested that the R14W mutation in CAIV causes accumulation of the enzyme in the endoplasmic reticulum (ER) of choriocapillaris cells, leading to ER stress and apoptosis.

We have cloned mutant and wildtype CA IV signal peptide and full length nucleotide sequences into fluorescent vectors to study protein trafficking. Cells were also transfected with CAIV, and antibody staining used to localise the protein. We have used fluorescence microscopy, protein expression studies, and FACS analysis to determine whether differences exist in processing of the wildtype and mutant proteins.

The mutant form of the protein is retained within the ER. Expression studies show that mutant cells have a large amount of the unprocessed, immature form of the protein. Apoptosis assays demonstrate that apoptosis occurs in Cos-7 and HT-1080 cells transfected with the mutant, but not the wildtype form of the protein.

Our results show that the R14W mutant form of CAIV is retained within the ER, resulting in apoptosis. This apoptotic effect may be able to be reversed using siRNA specifically targeted to the mutant form of the enzyme, leading towards therapy for this form of blindness, and possibly other misfolding diseases.

B74

The Interleukin-6, Serotonin Transporter and Monoamine Oxidase A Genes and Endurance Performance During the South African Ironman Triathlon

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Previous studies have identified an association with the angiotensin converting enzyme (ACE) I allele, bradykinin beta 2 receptor (BDKRB2) -9 allele and nitric oxide synthase 3 (NOS3) GG genotype and performance in the Ironman triathlon. The products of these gene variants are believed to alter physiological and biochemical processes locally within the skeletal muscle favouring endurance athletic ability. There is growing evidence that the serotonergic system and circulating IL-6 levels are involved in mediating endurance capacity. Investigators have demonstrated that recombinant human IL-6 administration and serotonergic neurotransmission manipulation, with 5-HTT and MAO-A inhibitors, prior to exercise can alter running performance. The aim of this study was to investigate possible associations of functional polymorphisms within the IL-6, 5-HTT and MAO-A genes with endurance performance of Ironman triathletes.

Four hundred and sixty eight male Caucasian triathletes that completed the 2000 and/or 2001 South African Ironman Triathlon and 200 healthy Caucasian male controls were genotyped for the -174 IL-6 G/C, 5-HTT 40 bp insertion/deletion and 30 bp VNTR MAO-A gene polymorphisms.

There were no significant associations between any of the genotype and allele distribution frequencies within the triathlete and control groups.

In conclusion, there were no direct association between the IL-6 -174 G/C, 5-HTT 44bp insertion/deletion and MAO-A 30 bp VNTR gene polymorphisms and endurance performance in the 2000 and/or 2001 South African Ironman Triathlons.

B75

Radiosensitivity of Lymphocytes in HIV Positive Individuals

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Radiosensitivity in relation to HIV status is an essential area of research for radiation applications in South Africa, as the prevalence of HIV infection is high. Possible change in radiosensitivity to ionizing radiation with HIV status has significant implications for both radiation workers and radiation therapy patients. The aim of this study is to investigate the radiosensitivity of white blood cells of individuals who are HIV positive, and compare it with that of a group of HIV negative individuals.

Blood samples were collected from HIV infected donors as well as from HIV negative individuals. Lymphocytes were exposed in vitro to doses of 6 MV X-rays ranging from 0 to 4 Gy. Blood samples are cultured for 70h and Cytochalasin B is added at 24 hours. After 3 days the yields of binucleated lymphoblasts and micronuclei are counted using fluorescence microscopy. Micronuclei

frequency is a measure of chromosomal damage and is quantified in at least 500 cells per sample. Un-irradiated control samples from each donor are also analysed. Micronuclei frequencies were consistently higher in blood cells from HIV positive individuals than those observed in blood cells from HIV negative donors.

Dose response curves show a clear linear-quadratic shape for both sets of donors indicating that residual radiation damage has been successfully quantified in all samples. It can be stated that HIV positive donors exhibit significantly higher micronuclei frequencies than uninfected donors, and a clear increase in radiosensitivity has therefore been established for these patients.

B76

Genome annotation of the 1.2Mb region on chromosome 8p22-p23.1 harbouring the gene for keratolytic winter erythema (KWE)

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Keratolytic winter erythema (KWE) is an autosomal dominant disorder that manifests in the form of erythema and hyperkeratosis of the palmar-plantar regions and has been linked to chromosome 8p between markers D8S1759 and D8S552. The objective of this study was to examine the critical region for highly conserved coding and non-coding regions and copy number variants (CNV) and determine if these regions may play a role in the molecular aetiology of the disease. Highly conserved regions were identified based on sequence conservation across closely and distantly related organisms. These regions were further analysed for functional signatures and motifs. In addition a CGH tiling array (384K Nimblegen) was done across the region to identify CNVs. Ten related samples from four families were tested of which 5 were from affected and 5 from unaffected individuals.

Multi-species sequence alignment revealed six regions that showed a high level of conservation above an 80% threshold. Of these one is a known open reading frame C8orf13 and another is a recently annotated gene DUB3. Further analysis of DUB3 revealed that it was a likely candidate gene for KWE. The remaining four highly conserved regions are non-coding and analysis revealed no functional motifs. Two CNVs located upstream of two candidate genes were identified in three of the affected individuals and are being further investigated.

Analysis of highly conserved regions revealed a previously uncharacterized gene, DUB3 as a likely candidate for KWE. In addition, possible copy number variants within the KWE critical region were identified and are candidates for further investigations.

B77

Comparison of two methods to make pan-centromeric probes for assessing cellular damage from low doses of ionizing radiation

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As part of a Bio-Monitoring project - using an automated microscope that is funded by the

Flemish Interuniversity Council (VLIR) - new methods are investigated to quantify residual radiation damage. Micronuclei, small nuclear fragments in the cytoplasm of interphase cells, are the result of breaks in the DNA. Almost all micronuclei induced by radiation are centromeric negative. The pan-centromeric probe can be used to differentiate between micronuclei induced by low doses of radiation and that from the background - micronuclei found in normal unexposed individuals. We want to compare the application of pan-centromeric probes generated using different methods in detecting centromeric negative micronuclei.

The first probe utilises a human alphoid DNA sequence clone, p82H, which is extracted from bacterial colonies through plasmid extraction. The second probe is a PCR-product produced from human DNA that has been targeted by designed primers. The probes are labelled via standard nick translation using the same procedure. Fluorescent In Situ Hybridisation (FISH) is performed with the probes on normal metaphase slides. The probes are then used on healthy male and female controls, in conjunction with the micronucleus assay.

The probes are compared to ensure all centromeres are targeted. The advantages and disadvantages are listed for each method. Background levels of micronuclei are determined in healthy males and females, and the probe characterises them as centromere-negative and centromere-positive.

The probes can be used to determine clastogenic and aneugenic events that occur as induced or spontaneous events in individuals in future cytogenetic studies.

B78

DNA Methylation Status of the P16 Promoter Sequence in Blood and Tumour Tissue

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DNA methylation is an epigenetic alteration with an essential gene transcription regulation function, and has been shown to be a common alteration at the root of several malignancies. Aberrant CpG island methylation in tumour-suppressor gene promoters causes gene silencing, and contributes to tumorigenesis. In this study the authors established and optimized the Methylation-specific PCR assay (MSP), and analysed the p16 promoter methylation status of 23 individuals using DNA isolated from whole blood, plasma and paraffin-embedded tumour tissue.

This assay is based on the sodium-bisulfite treatment induced differences between methylated and unmethylated DNA. The treatment converts unmethylated cytosines to uracils, while methylated cytosines remain unchanged, resulting in two different DNA sequences. Using two sequence-specific PCR primers sets, one can then distinguish between the methylated and/or unmethylated nature of the target sequence. The MSP assay was established and applied successfully to determine the methylation status of the p16 promoter sequence for 13 control individuals and 10 clinically diagnosed breast cancer patients.

The results obtained showed that for the control individuals all the p16 promoters were unmethylated for both the genomic DNA and the free-circulating DNA. The p16 promoter

methylation status of the patient samples was diverse, and both methylated- and unmethylated-specific products were observed for the tumour DNA sample.

This study confirms the applicability of the MSP assay to measure methylation status in various sample types, including genomic DNA, free-circulating DNA and DNA isolated from paraffin-embedded tumour tissue, and the assay has several potential applications in the South-African environment.

B79

Genetics of keratolytic winter erythema

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The identification and characterization of the causative gene for keratolytic winter erythema (KWE), by examining plausible positional candidate genes.

Seven families were genotyped for nine microsatellite markers in a haplotype analysis study that aimed at confirming and possibly refining the KWE critical region. The candidate genes within the KWE critical region were prioritized according to function and the DNA and RNA of the four most likely candidate genes was sequenced in affected and unaffected individuals using BigDye chemistry and analyzed using Lasergene software. Palmar skin biopsies were obtained from KWE affected individuals and unrelated, unaffected controls for the quantitative analysis of CTSB and FDFT1 gene expression. The expression profiles of these genes in affected and non-affected skin was analyzed.

The KWE region was confirmed to be between markers D8S1759 and D8Stet, a region roughly 1.5 cM in size, larger than previously thought. This region contains 8 known protein coding genes, 3 open reading frames, 5 genes encoding beta defensin precursors and 2 genes encoding zinc finger motifs. The most likely candidate genes in this region were CTSB, FDFT1, DUB-3 and the open reading frame, C8orf49. Although many novel genetic variants were identified within these genes, none segregated with the disease. The expression studies are in progress.

No causative mutations were identified in the positional candidate genes analysed in this study. The screening methods did not extend to the entire intronic and promoter sequences. Expression analysis results may shed light on the involvement of these genes with the pathology of KWE.

B80

Genetic testing directly from whole blood using KAPA Blood PCR Kits

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High-throughput PCR-based genetic testing directly from blood has not been feasible with wild-type DNA polymerases due to the presence of multiple PCR inhibitors in whole blood. The objective of this study was to determine whether standard genetic tests can be successfully performed with the novel, engineered KAPA Blood DNA Polymerase, without any pretreatment of blood samples or DNA isolation.

Human EDTA blood (stored at 4°C for 72 years, or at room temperature on Guthrie cards or FTA Elute cards) was used directly in 10 - 25 µL PCRs, using KAPA Blood PCR Mix A or B. Three assay types used routinely in genetic testing were assessed, namely (i) SNP detection by DNA sequencing of PCR products, (ii) RFLP analysis of amplified fragments to distinguish heterozygotes from homozygotes, and (iii) commercial multiplex STR systems used in paternity testing. Results obtained with standard protocols, using genomic DNA purified from blood as template, were compared with those obtained with KAPA Blood PCR Kits.

All three assays were easily converted and results obtained with KAPA Blood PCR Kits were comparable in quality to those obtained with the standard protocol. In most cases, no post-PCR treatment of reactions containing whole blood, other than centrifugation, was required.

The ability to amplify DNA fragments directly from whole EDTA blood with KAPA Blood PCR Kits offer significant advantages for high-throughput genetic testing. These include reduced costs, turnaround times and the risk of sample cross-contamination associated with the need for DNA extraction.

B81

Type III OI is the most prevalent OI Type in the Black population of KZN

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Osteogenesis imperfecta is a genetic disorder characterised mainly by fragility of the bones and low bone mass. To date, these disorders have been classified into seven different types. The aim of this study was firstly to confirm that Type III OI is indeed the most prevalent OI type in the Black South African population and secondly to determine whether there has been progress in patients treated with the bisphosphonate disodium pamidronate in terms of number of fractures, bone pain and developmental milestones.

Patient records from 1992 up until the present date at the King Edward VIII Genetic Clinic were scrutinized. Electronic records at IALCH Ward B1 East (Paediatric Endocrinology) were searched using a computer system. A sample size of 25 OI patient records was used, 16 from KEH and 9 from IALCH.

Type III is the most prevalent OI type in the Black South African population. Nine patients are currently on bisphosphonate treatment: four records include information on improvement, three patients had not been on treatment long enough yet. No information for the remaining records were given.

Doctors need to be aware of this disorder and the symptoms other than fractures. This would lead to earlier diagnosis and earlier commencement of treatment. Bisphosphonate treatment improves the quality of life of OI patients in the Black population.

B82

Genetic variation and possible association among some Nigerian populations- reviewing their origin

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The Nigerian population of over 140 million is multi-ethnic and multi-lingual. Three historically unique but geographically related groups (the Yorubas, Igbos and Ijaws) were assessed for their genetic distinctions and possible convergence to establish the association among them though they all belong to the Niger-Congo language group.

Buccal swabs for DNA extraction and quantification were obtained from 1012 male subjects from the 3 Nigerian populations and 2 others of the Nilo-Saharan and Afro-Asiatic languages respectively. Their maternal and paternal ancestries were traced using their mitochondrial, autosomal and sex DNAs. The analyses involved the PCR amplification of specific loci on both the Y-chromosome and mtDNA for both length and sequence polymorphisms respectively. Autosomal and Y-STRs (Short Tandem Repeat markers) and SNPs (Single Nucleotide Polymorphisms) as well as mitochondrial HVR I & II (Hyper Variable Regions I and II) were assessed.

This is essentially a research proposal and provisional results will be available by the time of the conference. AMOVA (Analysis of Molecular Variance) and Mantel's test will be reported.

We shall be able to explain the correlate among genetic, linguistic and geographical distances of these 3 Nigerian populations.

B83

Variation in the 5' untranslated region of the ABCB1 multidrug resistance gene in the black South African population

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The multidrug resistance gene 1 (MDR1 or ABCB1) C3435T polymorphism has been the subject of extensive but focused research, attempting to determine the cause for the functional variation seen in the protein P-glycoprotein (P-gp). The upstream region, containing regulatory elements for gene transcription, has not been well researched. ABCB1 has two promoter regions, one upstream of exon 1, and the other upstream of exon 3, where the transcriptional start site is found.

We sequenced both these regions in the Bantu-speaking southern Africans and genotyped the known T-129C polymorphism. A number of bioinformatic tools were used to analyze both upstream regions. Each region was analyzed with the major and minor alleles at each polymorphism and the results compared.

Sequencing revealed higher levels of variation in both regions than had previously been recorded. Sequencing also confirmed the presence of the known T-129C polymorphism, and genotyped we found the minor allele to be more frequent than in other populations. The bioinformatic analysis showed differences in the promoter regions in terms of transcription factor binding sites, promoter prediction and motif identification in the presence of different alleles.

We conclude that the upstream regions of the ABCB1 gene could vary functionally with different alleles and could provide insight into the nature of the transcription of the gene and possibly help in understanding the variability in the expression of P-gp.

B84

Characterisation of the SULT1A1 Polymorphism in the South African Tswana Population Group

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Sulphation by the SULT enzymes is important in mammalian chemical detoxification mechanisms. Polymorphisms in the SULT1A1 gene occurs in various population groups. The key question is: do such polymorphisms have an effect on the detoxification ability of humans?

The profile of this polymorphism (PCR-RFLP) and the copy number of this gene in selected individuals (Fluorescence-based Quantitative Multiplex PCR) in a selected Tswana population group (the PURE-group) in the North West Province of South Africa was investigated.

SULT1A1 genotype was assessed in 1867 DNA samples. The mutant allele SULT1A1*2 was detected at a frequency of 0.32. We also demonstrate the allele frequency of SULT1A1 in 453 samples which showed 1 to ~5 copies. 0.66% of the subjects contained a single copy and 89.18% of the subjects had three or more copies. Using the sample of size 1867 we calculated the 95% simultaneous confidence intervals for the true population probabilities, and also estimate the achieved coverage probability using a parametric bootstrap procedure. From these bioinformatics analyses we conclude that the estimated probabilities are accurate in the sense that the half-width of the intervals was not more than our prescribed accuracy distance, and the bootstrap estimated coverage (0.957) is indeed larger than the prescribed nominal level (0.95).

The mutant frequency presence seems to be similar to that of previous studies done on the Caucasian populations. Also, the number of copies of three or more is significantly higher when compared to the Caucasian-American and African-American populations.

B85

HLA -A, -B, -C and -DRB1 allele and haplotype frequencies in the South African Xhosa-speaking population

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The distribution of HLA alleles at both class I and II loci in the Xhosa-speaking population and the detailed analysis of the haplotypic relationships between alleles of different loci was determined. Sequencing-based typing was used to identify human leukocyte antigen (HLA) - A, B, C and DRB1 alleles from 92 unrelated black South African Xhosa-speaking individuals. The data was analysed using PyPop (Python for population genetics, <http://www.pyPop.org>).

The number of distinct alleles identified at each locus were as follows; 33 for HLA-A, 37 for HLA-B, 22 for HLA-C and 29 for HLA-DRB1. Allele frequencies were obtained by direct counting and allele frequencies at each HLA locus were evaluated for deviations from Hardy-Weinberg. The action of balancing, rather than directed, selection was inferred at all 4 loci and was significant for the HLA-C locus using the Ewens-Watterson test of homozygosity. Two and three locus haplotypes were estimated using the expectation maximization algorithm.

As in other sub-Saharan populations there was remarkable allelic and haplotypic diversity in the Xhosa-speaking South Africans who comprise 25% of the Black population. This finding presents practical challenges for the design of T-cell vaccines and for finding HLA-matched unrelated donors for patients needing an allogeneic transplant.

B86

Genetic Variation in PSIP1 in Black South Africans

MS NL GENTLE * (Wits University), PROF T MCLELLAN (Wits University)

PSIP1 encodes LEDGF/p75, which stably associates with the core domain of HIV-1 integrase via a highly-conserved integrase binding domain located in its C-terminal. Through this interaction, the protein tethers HIV-1 integrase to chromosomes at sites of high LEDGF/p75-mediated transcription. The aim of this investigation was to identify and characterize genetic variation within PSIP1 in black South Africans, in order to establish whether this variation exerts an influence on an individual's susceptibility to HIV infection and/or rate of disease progression.

PCR assays were designed to amplify intronic regions within the DNA-binding and integrase-binding domains, as well as the upstream non-coding region of PSIP1. Twenty samples from each region were sequenced to identify variation. Selected polymorphisms were then genotyped using allele-specific PCR, RFLP-PCR and pyrosequencing assays.

Three novel insertion-deletion and seven single nucleotide polymorphisms (SNP) were identified. Four of the SNPs had been recorded previously, while the six other polymorphisms had not and appear to be unique to our population. Analysis of the genotyping data revealed that data collected for all but one of the genotyped sites did not deviate from Hardy-Weinberg equilibrium and minor allele frequencies were found to range between 0.09 and 0.35.

The level of polymorphism within this gene is relatively high – particularly with regard to the presence of insertion-deletion polymorphisms – and many of these polymorphisms are apparently restricted to African populations. This suggests the possibility that this variation may influence gene expression in a regulatory fashion and could, therefore, influence infection by HIV.

B87

Differential gene expression of MMP-1, TIMP-1 and HGF in South African systemic sclerosis patients

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PROF M RAMSAY (National Health Laboratory Service and University of the Witwatersrand)

In systemic sclerosis there is an increase in fibrosis of the skin. Matrix metalloproteinase-1 (MMP-1) and hepatocyte growth factor (HGF) both degrade collagen whilst tissue inhibitor of metalloproteinases-1 (TIMP-1) is the antagonist of MMP-1. The primary objective of this study is to examine the expression of these genes in skin samples from systemic sclerosis patients. The second objective is to correlate the expression data to detailed clinical information from the patients.

Two 4mm skin biopsies from 17 patients were used, one from clinically affected skin (lateral forearm) and the other from unaffected skin (back). RNA was extracted and reverse transcribed

before doing relative quantification on real time PCR. Ratios of the target gene expression relative to the endogenous control, the housekeeping gene, GAPDH were calculated and statistical analysis performed.

Data for TIMP-1 suggests that the gene has increased expression in the patients when compared to the controls. In the majority of patients the lateral forearm had increased expression of the gene compared to the back sample. Preliminary statistical analysis suggests that the differences in TIMP-1 expression is significant, both when the patients are compared to the controls and when comparing the forearm and back samples. Work on HGF and MMP-1 is still in progress.

Relative quantification analyses of TIMP-1 in controls and patients have shown that the gene has significant increased expression in patient samples, especially in the forearm sample. This result is consistent with the hypothesis that increased TIMP-1 expression will lead to reduced collagen breakdown in these patients.

B88

Disorders of Genome Architecture: A new class in the taxonomy for human disease

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To review the molecular mechanisms in genomic disorders.

Clinical cases review and literature search.

Genetic diseases are recognized to be one of the major categories of human disease. Traditionally genetic diseases are subdivided into chromosomal (numerical or structural aberrations), monogenic or Mendelian diseases, multifactorial / polygenic complex diseases and mitochondrial genetic disorders. A number of complex disorders and multiple malformation syndromes do not conform to the conventional inheritance patterns and mechanisms are often complex and unique. Examples include submicroscopic microdeletions or microduplications, triple repeat disorders, epigenetic disorders due to imprinting, defective transcription or translation due to abnormal RNA patterning and pathogenic association with single nucleotide polymorphisms (SNPs) and copy number variations (CNVs). Among these several apparently monogenic disorders result from non-allelic homologous recombination (NAHR) associated with the presence low copy number repeats (LCNRs) on either side of the critical locus or gene cluster. The term 'disorders of genome architecture' is alternatively used to highlight these disorders, for example Charcot-Marie-Tooth type IA (CMT1A), Smith-Magenis syndrome, Neurofibromatosis type 1 and many more with an assigned OMIM number.

Disorders of the genome architecture (GENOMIC DISORDERS) represent a new class within the taxonomy of human disease. This group includes a significant number of complex disorders including multiple malformations, unexplained developmental delay and unusual and extended phenotypes in some Mendelian disorders.

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