Update on Neurogenetics

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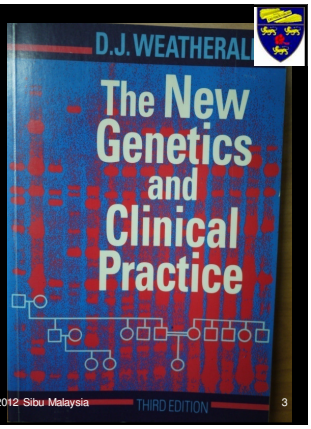
Update on Neurogenetics

- Personalised Medicine and the beginning of 'Personal Genomics'
- Update on technology
- Recent understanding on disease mechanisms
  - Copy number variations
  - Epigenetics
  - MicroRNA
- Challenges and opportunities

New Genetics

- The majority of 'genetic' patients in a paediatric clinic are in search of a unifying diagnosis.
- In the coming decade, the issues raised by families will be different in view of recent developments in 'New Genetics'.
- Management of genetic disorders had changed in the past 30 years



The end of 'New Genetics' and the beginning of 'Personal Genomics'

- Classical or Mendelian genetics
  - Modes of inheritance
  - Recurrence risk
  - Chromosome and abnormalities
  - The concept of one gene - one enzyme
- What is the role of the clinician?

Personal Genomics

- Moving in the second decade of the 21st century
- Clinic scenario 1 (Direct-to-consumer testing)
  - *I had my child tested for xxx disease using a mouth swab kit and sent to a genetic laboratory overseas. I found the company from the internet. The results showed a complex disease. They said our geneticists can interpret the results for us.*
- Scenario 2 (Genetic susceptibility testing)
  - *Healthy adults asking: "What are my risks based on family history? Based on the 'genome scan'? What options can I take to have a healthy child?"*
- Scenario 3 ('Retail genetics')
  - *Can you advise me on the proper tests (or medication) for my child's genetic condition? Can I buy it from the website?*

A. Understanding family tree

- Can be used to record medical conditions
- Concisely record family relationships
- Can assist in identifying people at risk of a genetic condition

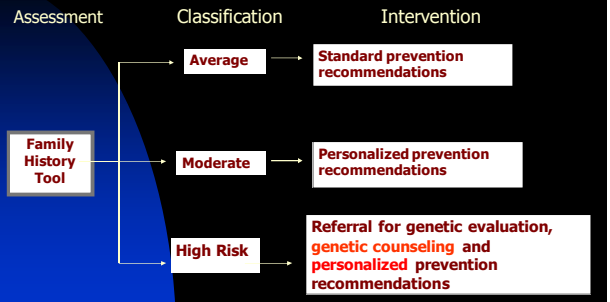
Center for Disease Control, USA: Family History Public Health Initiative (2002)

Why focus on family history?

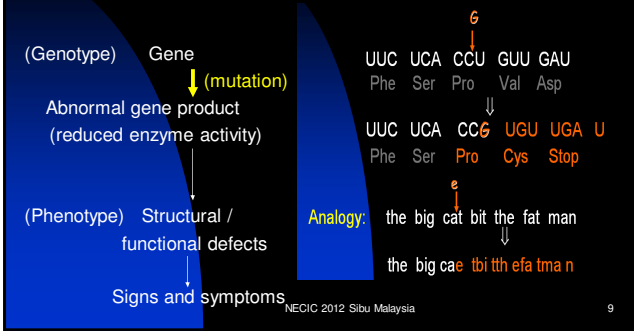
- ✓Family history is a risk factor for many common (multifactorial) diseases.
- ✓Family history is underutilised in preventive medicine
- ✓Widespread use of pedigrees in public domain
- ✓Current strategies not working (diet, exercise, smoking)

Family history is an independent risk factor for most chronic diseases of public health significance

Using family history for disease prevention



B. Genetics: Genotype and phenotype correlation



C. Pathogenesis of genetic diseases

Mutant gene
Mutant mRNA
Mutant protein
Metabolic / Structural dysfunction
Clinical phenotype
Affected family member(s)

D. Genetic diseases 2012: management

Mutant gene	Transplantation / gene therapy
Mutant mRNA	Modulation of gene expression
Mutant protein	Protein replacement
Metabolic dysfunction	Disease specific compensation (diet modification; pharmacological)
Clinical phenotype	Medical or surgical intervention, patient education
The family	Genetic counselling; carrier screening, presymptomatic testing
Community / society	Public awareness; preventive/public health steps

Genetic counselling and beyond

- Genetic counselling is defined as a communication process that deals with the human problems associated with the occurrence or risk of occurrence of a genetic disorder in a family.
- The aim is not to reduce the incidence of genetic diseases in the community.
- Assist clients, patients and families to make informed choices and to cope with their difficulties
- Non-directive; allows individuals or couples to make the best choice for themselves

From genetics to genomics…

- On June 26, 2000, it was announced that a working draft of the entire human genome DNA sequence has been completed. Chromosome 22 was the first to be fully sequenced.
- On April 14, 2003, the completed human genome sequence was announced.
- 2003 - represents the 50th year of description of the DNA double helix by Watson and Crick.

From genetics to genomics…

- Genetics vs genomics: the main difference is that genetics scrutinizes the functioning and composition of the single gene whereas genomics addresses all genes and their inter-relationships in order to identify their combined influence on the growth and development of the organism

Genomics: susceptibility factor

- Over 98% of the human genome does not code for proteins.
- These non-coding regions include:
  - Gene regulatory sequences
  - Single nucleotide variants or polymorphisms (SNPs)
- SNPs are used to assess a person's susceptibility to disease and response to drug treatments.
- Many "adult" diseases such as cancer, coronary heart disease, diabetes, infertility and psychiatric illnesses are included. Imply a role for environmental factors

Clinicians in the genomics era: A DNA test for every disease?

Limitations of DNA mutation analysis:

- Ethical concerns – mutation studies should not be performed in children unless there are important medical consequences or individuals without genetic counselling
- Availability and cost of the tests - many DNA tests are done on a research basis; not for diagnostic purposes
- A negative molecular result does not exclude a diagnosis
- Novel DNA variants or polymorphism may be mistakenly regarded as pathogenic by those not familiar with genetics
- Sensitivity of mutation detection is different for various techniques.

Personalised Medicine – The Gap (1)

- Emerging data from recent research showed public perception towards non-specific genomics personalised medicine has been muted
- Personal genomics provide mainly risks and probabilities.
- Patients, families want an accurate diagnosis for their ailments, information on reproductive risks, prognosis and their offspring's health.
- They need empathy and support from the healthcare providers. The doctor-patient relationship is still vital, now made even more important with the emergence of personalised medicine.

Khoury MJ et al. The Scientific Foundation for Personal Genomics. Genet Med 2009; 11(8): 559-567

Personalised Medicine – The Gap (2)

- For most genomic applications, direct evidence about the effectiveness and value of testing is rarely available from randomised clinical trials (RCT).
- Personal genomics applications used mainly in symptomatic patients rather than asymptomatic population at large (main target group for personalised medicine)
- Clinical validity (CV) and utility (CU) of personal genomics and the balance of benefits and harms must be evidence-based and subjected to further research and RCTs, as practised in all fields of medicine.

Khoury MJ et al. The Scientific Foundation for Personal Genomics. Genet Med 2009; 11(8): 559-567

Therapeutic possibilities

- The promise of 'gene therapy'
  - Replace mutant gene with modified normal gene e.g. plasmid DNA in viral vector
  - Exon skipping e.g. antisense oligonucleotide
  - Suppress STOP codon e.g. pharmacological agents

Problems with gene therapy

- The number of protein variants outnumbers the number of coding genes. One gene may affect expression of other genes. E.g. dystrophin gene mutation downregulates 327 other genes but upregulates 77 genes.
- Insertional mutagenesis. E.g. apparent successful gene therapy for SCID but 2 / 9 patients died later of T-cell acute leukaemia due to activation of an adjacent oncogene.
- Immunological / toxicity issues. E.g. Gene therapy for urea cycle defect using viral vector associated with mortality.
- Targeted delivery of the normal gene copy to all affected tissues, including brain and heart may not be successful.

Personalised Medicine

- The failure of gene therapy has forced genetic research to re-focus back on basic issues – the study of multiple gene effects as well as to associate specific variations with clinical disease phenotypes.
- This has resulted in many new findings - epigenetics, micro ribonucleic acids (RNAs) and copy number variations (CNVs)
- New approaches such as genome-wide association studies (GWAS) ,microarray analyses and low cost sequencing technology - personal genomics has arrived

Pharmacogenomics

- Antiepileptic therapies are associated with a high incidence of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Carbamazepine (CBZ)-SJS/TEN is strongly associated with the HLA-B*1502 in Han Chinese
- Oxcarbazepine (OXC), phenytoin (PHT) and lamotrigine (LTG),which possess an aromatic ring as CBZ, when causing SJS/TEN, share a common risk allele.
- Research suggests that aromatic AEDs, including CBZ, OXC and PHT, should be avoided in the B*1502 carrier and caution should also be exercised for LTG.

Hung SI et al. Common risk allele in aromatic antiepileptic-drug induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese. Pharmacogenomics 2010 Mar;11(3):349-56

Pharmacogenomics

- The CYP2C9 and VKORDC1 genes were implicated in in warfarin and vitamin K metabolism and genetic variants were associated with bleeding complications.
- However, without well-designed large clinical trials, it is uncertain if genotyping to determine warfarin dosing could reduce adverse effects or improve health outcomes.
- Studies validated the role of pharmacogenomics but uptake of these tests were limited.

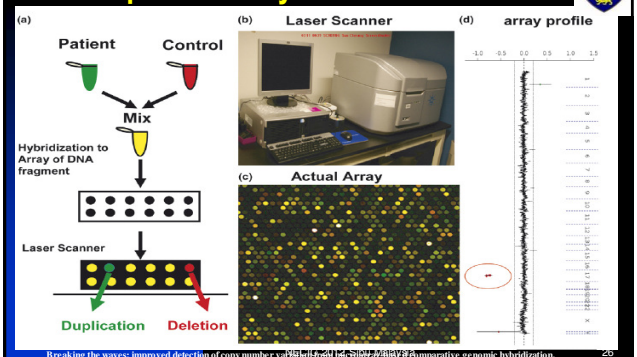
Shurin SB, Nabel EG. Pharmacogenomics – ready for prime time. NEJM 2008; 358: 1061-3.

Array comparative genomics hybridisation (aCGH)

- aCGH also known as chromosomal microarray (CMA) or molecular karyotyping.
- aCGH compares the DNA content from 2 differentially labelled genomes: the patient and the control.
- The two genomes are co-hybridised into a slide which cloned DNA fragments are immobilised (arrays).
- The array is able to detect DNA copy number changes at multiple loci in a genome in one test.
- These copy number changes may include deletions, duplications or amplifications.

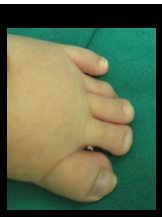
Shaffer LG et al. Targetted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases. J Pediatrics 2006;149:98-102.

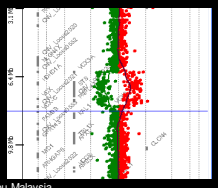
Principles of array CGH



Breaking the waves: improved detection of copy number variation from microarray-based comparative genomic hybridization. *Marioni JCME* Genome Biol. 2007;8:R228.

Patient : Developmental delay and dysmorphism





Microarray technology

Microarray technology - validated

- The International Standard Cytogenomic Array Consortium (ISCA), conducted a literature review of 33 studies, including 21,698 patients tested with aCGH, and compared aCGH to G-banded karyotyping.
- They found that aCGH consistently has a diagnostic yield of 15 to 20%, compared to approximately 5% with G-banded karyotyping - higher sensitivity for submicroscopic copy number variations.
- Consensus statement: microarray is a first tier test for individuals with developmental disabilities or congenital anomalies

Miller D et al. Consensus Statement: Chromosomal microarray is a first tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genetics 2010

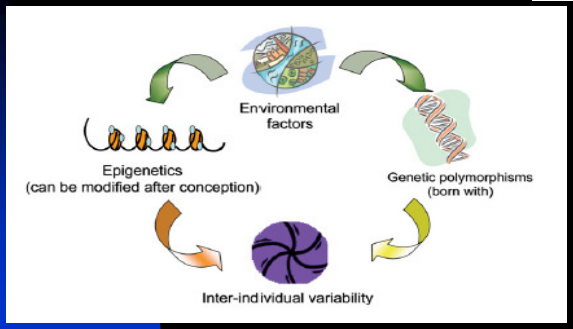
Microarray technology - limitations

- Offers a higher diagnostic yield (15-20%) but majority of patients may not be helpful. Also, diagnosis ≠ cure.
- Interpretation of results require expertise
- Unable to detect:
  - Balanced chromosomal rearrangements
  - Low-level mosaicism
- G-banded karyotype should be continued for:
  - Obvious chromosomal conditions e.g trisomies
  - Family history of chromosomal rearrangement
  - History of multiple miscarriages
- Cost issues

Epigenetics

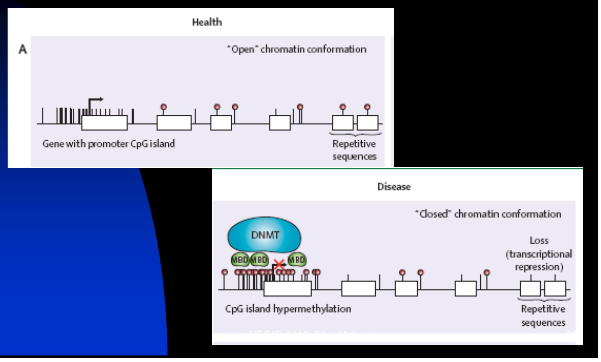
- **Epigenetics** is the study of inherited changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence, hence the name *epi-* (Greek: over,above) *genetics.*
- These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations.
- However, there is no change in the underlying DNA sequence of the organism; instead, non-genetic factors cause the organism's genes to behave (or "express themselves") differently.

Epigenetics



Jones PA, Baylin SB. Cell. 2007;128:682-92.

Epigenetics – health and disease



Childhood neurological conditions as an epigenetic disorder

- Many childhood neurological conditions can be explained through differential methylation and modification of histones
- Epigenetic modifications are reversible and are linked to potential targets for drug treatment.
- The enzymes that carry out DNA methylation are DNA methyltransferases (DNMTs) and these can be inhibited
- Histone acetyltransferases (HATs) carry out histone modifications, which can be reverted by histone deacetylases (HDACs).

Urdinguio R et al. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies Lancet Neurol 2009.

Epigenetics - therapies

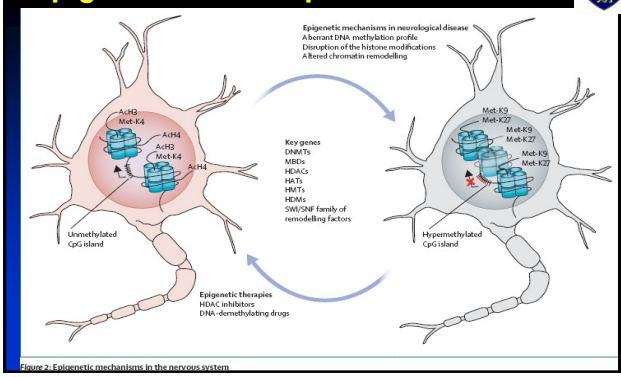


Figure 2: Epigenetic mechanisms in the nervous system

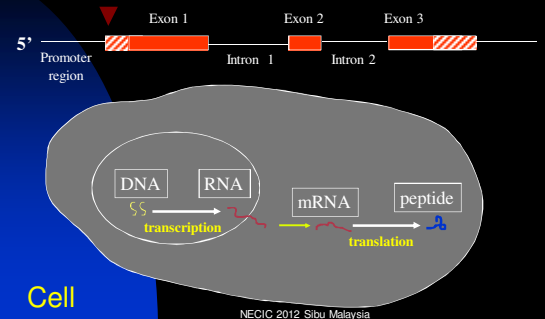
Epigenetics – drug targets

	Type of drug	Model tested	Effect
Adrenoleukodystrophy			
Phenylbutyrate	HDAC inhibitor	Wistar rats and primary cultures	Restoration of peroxisome proliferation[78]
Alzheimer's disease			
AZA	DNA-methylation inhibitor	CEC cells	Restoration of NEP mRNA expression levels
SAHA	HDAC inhibitor	HDAC-overexpressing mice	Synapse number increase and memory facilitation
Phenylbutyrate, trichostatin A	HDAC inhibitors	CK-p25 transgenic mice	Reinstated learning behavior and re-established long-term memories
Epilepsy			
Valproic acid	HDAC inhibitor	Common human therapy	Enhance GABAergic function
Friedreich's ataxia			
SAHA, HDACI 106, HDACI 4b, oxanflatin	HDAC inhibitors	FRDA lymphoblasts (GAA-TCC expanded)	Restoration of FXN expression[79,80]
HDACI 106	HDAC inhibitor	KIKI mice (GAA knock-in)	Restoration of FXN expression and, partially, general mRNA expression[81]
Huntington's disease			
Sodium butyrate, SAHA	HDAC inhibitors	Httex1p polyQ expanded flies	Blockage of neurodegeneration and restoration of intracellular transport impairment
SAHA, trichostatin A	HDAC inhibitors	Striatal cells derived from Hdh(Q)109 mice	Restoration of intracellular transport impairment
Phenylbutyrate	HDAC inhibitor	R6/2 and 82Q HD mice	Neuroprotection and survival increase
SMA			
M344, oxamflatin, romidepsin, SAHA, scriptaid	HDAC inhibitors	SMA human fibroblasts	Overexpression of SMN2 (SMN1 paralogue)[82]
Sodium butyrate	HDAC inhibitors	SMA mice	Activation of SMN pathway and extension of lifespan[83]

RNA interference

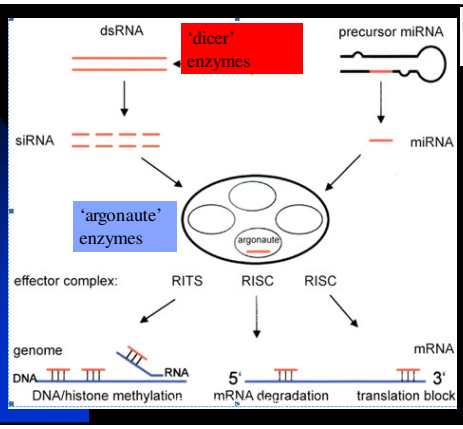
- In 2006, Andrew Fire and Craig Mello shared the Nobel Prize in Physiology or Medicine for their work on RNA interference which they published in 1998.
  - Fire A, Mello CC et al Potent and specific genetic interference by double stranded RNA in C elegans. Nature. 1998 Feb 19;391(6669):806-11
- **RNA interference** (**RNAi**) is a system within living cells that determines which genes are active and how active
- Therapeutic strategies are developed based on RNA interference
  - Matzke MA, Matzke AJM (2004) Planting the Seeds of a New Paradigm. PLoS Biol 2(5): e133

Central dogma of molecular biology



RNA interference

- Two types of small RNA molecules – microRNA (miRNA) and small interfering (siRNA)
- The *'dicer'* enzymes produce siRNA from double-stranded RNA and mature miRNA from precursor miRNA.
- miRNA or siRNA is bound to an '*argonaute'* enzyme and an effector complex is formed, either a RISC (RNA-induced silencing complex) or RITS (RNA-induced transcriptional silencing) complex.
- RITS affects the rate of transcription by histone and DNA methylation, whereas RISC degrades mRNA to prevent it from being translated.



RNA interference as a therapeutic strategy

Suppression of disease-causing alleles or elimination of the mutant gene products is a potential approach to treatment of intractable diseases caused by dominate-negative alleles.

Difficult challenge lies in the translation of this technology to the CNS

Davidson B et al. Molecular medicine for the brain: silencing of disease genes with RNA interference . Lancet Neurol 2004

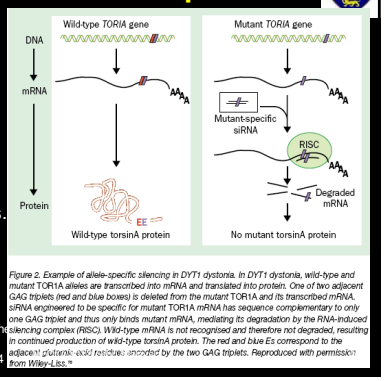
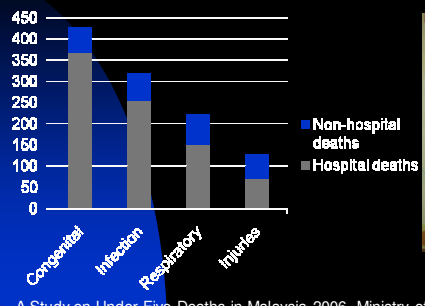


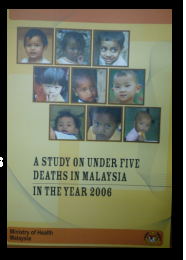
Figure 2. Example of allele-specific silencing in DYT1 dystonia. In DYT1 dystonia, wild-type and mutant TOR1A alleles are transcribed into mRNA and translated into protein. One of two adjacent GAG triplets (red and blue boxes) is deleted from the mutant TOR1A and its transcribed mRNA. siRNA engineered to be specific for mutant TOR1A mRNA has sequence complementary to only one GAG triplet and thus only binds mutant mRNA, mediating its degradation by the RNA-induced silencing complex (RISC). Wild-type mRNA is not recognised and therefore not degraded, resulting in continued production of wild-type torsinA protein. The red and blue Es correspond to the adjacent glutamic-acid residues encoded by the two GAG triplets. Reproduced with permission from Wiley-Liss.™

Genetic testing: not the answer for all

- Genetic testing should be preceded by genetic counselling.
- Ethical concerns that mutation studies should not be performed in children or minors unless there are important medical consequences.
- Many genetic tests are often done on a research basis and are not meant for diagnostic purposes.
- A 'negative' molecular result does not exclude a diagnosis.
- A novel DNA variant or polymorphism may be mistakenly regarded as pathogenic
- Biotechnology is rapidly evolving and the sensitivity of mutation detection may vary with different techniques used

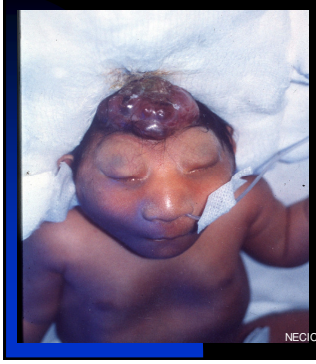
Cause of death in Malaysian children (< 5 years)

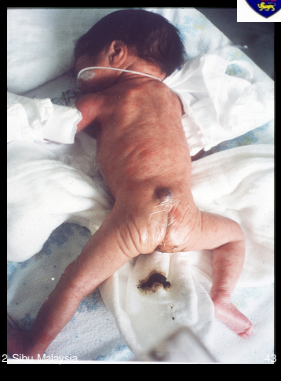




- A Study on Under Five Deaths in Malaysia 2006, Ministry of Health Malaysia

Neural tube defects





Support for patients and families

- Stigmatisation and discrimination
- Achieving equitable access to services
- Insurance issues
- Difficulties with early interventional programs
- Ethical, legal, social and religious issues

Summary

- Both suitable curative and preventive aspects be utilised to reduce the impact of neurogenetic diseases.
- Genetic counselling should remain the mainstay of all genetic services
- More clinical research into neurogenetic conditions is required in Malaysia.
- Personal genomics should be subjected to clinical trials
- Empowering at-risk families and individuals should be a priority

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Thank you