Notes from the 19th Society for the Study of Behavioural Phenotypes (SSBP) International Research Symposium, University of Siena, 9-11th September 2016

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The conference was held at the University of Siena, Italy, founded in 1240, set right next to the Piazza del Campo, the square where the famous horse race, the Palio, is held twice a year. We were taken on a tour of the original Hospital of Santa Maria della Scala, opposite the beautiful Duomo. Of the 80 delegates 16 were from Australia. The medieval hill town was a delight to walk around.

Sakkubai Nadiu from Boston opened the conference with an account of Rett Syndrome; from discovery to treatment. The syndrome was named by Andreas Rett 50 years ago, and has now been found to be due in mutations in the X-linked MECP2 gene. Common features include lack of abnormality at birth, then substantially delayed growth in head circumference within the first few months of life and apathy, often mistaken as a sign of a 'good' baby. There is some early speech which is then lost, and some early purposeful hand use which is then lost, with purposeless or stereotypical hand movements common. Major features include microcephaly, seizures, respiratory anomalies, and autonomic dysfunction and severe intellectual disability. No one feature is invariable however. The severity is proportional to the pattern of X inactivation. 5% of patients are male who are generally much more severely affected. Most are de novo mutations, but there are occasional carriers with very skewed X inactivation who are mildly affected. The proportion of immature neurons is much higher, with MECP2 necessary for neuronal maturation. Neurons are small with a reduction of synapses by 50% and reduced dendritic arborization. Vision is preserved as ocular neurons do not use MECP2, and parents will observe that their children 'speak with their eyes'. There is an excess of both glutamate and glutamate responsive NMDA receptors, leading to excessive excitation and cell death, in children under 10 after which they appear to burn out, and these glutamate and NMDA receptor excesses are no longer seen. This centre conducted a trial of dextromethorphan, an NMDA antagonist, and found an improvement in language skills which reached significance. Other changes did not reach significance. Testing the outcomes of intervention in this population is compromised by the difficulty assessing changes in cognitive function at such low levels of ability.

Alessandra Renieri from Siena then spoke on the genomic complexity underlying Rett spectrum disorders. Her team identified the FOXG1-related Rett variant syndrome, which shows a shorter period of perinatal normalcy and more severe microcephaly. This gene is located on chromosome 14 and the sex ratio of affected individuals is therefore equal. Both this gene and MECP2 dysregulate GABA. Her team has investigated molecular mechanisms via the study of induced pluripotential stem cell (iPSC)-derived neurons, a technique involving cell cultivation of skin fibroblasts from affected individuals and the creation of stem cells, from which can be grown simple neural networks for detailed analysis. They found shared pathways in the neuronal damage produced by both genetic variants involving dysregulation of GABA and of genes producing the enzyme histone deacetylase 6 involved in the formation of microtubules. There are drugs currently under development that can target these abnormalities. The current clinical implications are unclear. The dysregulation of GABA suggests that carbamazepine should be avoided in Rett syndrome children under 10, after which the excess of glutamate no longer operates. The two speakers in subsequent discussions gave contradictory views about the safety of valproate in this population, one advocating it as inhibitory of GABA, the other concerned about its adverse effect on already dysfunctional mitochondria.

Dafin Muresanu from Romania presented on brain protection and recovery after stroke and traumatic

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brain injury, emphasising the need to take a whole brain approach. He described the very poor evidence base for many interventions aimed at promoting brain recovery, with unclear concepts and very poor study design. He emphasised that factors promoting immediate neuroprotection could work in contradictory ways from those which optimise long term recovery. He described the 'anti-correlation' common to many processes following an acute brain lesion, and the need to take an approach focused on large scale networks rather than molecules. There are many homeostatic mechanisms operating within endogenous neuromodulation, and interventions aimed at one process can have unforeseen consequences in upsetting this balance. Data support the idea that even a small lesion can trigger progressive disorganisation of axons even at a distance from the core site of injury, with possible mechanisms being widespread inflammation and dysfunction of neurovascular units. Promoting long term recovery via neuroplasticity and neurogenesis is currently a major field of endeavour in neurology.

This and the three subsequent talks, all by neurologists, served to highlight current foci of neurological research, but required significantly more basic knowledge than possessed by many conference delegates.

Antonio Giorgio from Siena spoke on brain connectivity during development, the study of which has been immeasurably facilitated by the development of non-invasive imaging including functional MRI, especially in the resting state, and diffusion tensor imaging. Structural connectivity precedes functional connectivity although the process is probably bi-directional.

Massimo Filippi from Milan spoke on the 'vegetarian brain', describing the differences in regional brain activation to relevant stimuli between omnivores and a group comprised of vegetarians and vegans. Vegans for example activated the fusiform gyrus to faces of animals more than to faces of people, whereas omnivores did not activate this facial recognition gyrus on presentation of pictures of faces of animals. Pictures of suffering humans or animals evoked different responses depending on whether the subject experienced empathy versus cognitive understanding. While there were some differences between vegans and vegetarians, the overarching conclusion appears to be that both anthropomorphise animals to an extent and mount more empathic responses to animals than do omnivores.

Stefano Cappa from Pavia spoke on brain connectivity in neurodegenerative diseases. He described the idea of excessive attribution of functions to specific cortical areas as akin to a modern phrenology, and emphasised that the reality in most degenerative disease is one of large scale dysfunction in cognitive networks. The process of degeneration starts well before presentation and diagnosis. The old model of specific histopathology as described in Alzheimer's, Picks and motor neurone diseases is a late stage phenomenon. Investigations into functional impairment provide far more information when compared to normals than do investigations into those without any remnant of the relevant skill. Behaviourally disturbed patients will not tolerate MRI scanning especially when this is combined with an expectation of task performance. Brief scanning of resting states however can provide valuable information, with evidence that resting state deficits parallel those deficits seen during specific task

performance. He highlighted the concept of biological reserve as underpinning the variability in age onset of many diseases. There is early evidence for example that individuals with language variant Alzheimer's have a history of delayed childhood language acquisition, creating later vulnerability.

David Zee from Johns Hopkins gave a very informative account of eye movements in degenerative disorders, incorporating compelling reasons to study saccades, i.e. the rapid eye movements we make to scan the environment. Accurate localisation of brain lesions according to the type of disorder of saccades can lead to early diagnosis. He listed many reasons to study them, including that various saccadopathies are signatures of a number of degenerative diseases such as Parkinson's or Huntington's, that they can be studied in a wide range of animals including zebra fish, and that their formation is dependent on healthy functioning of a wide number of brain functions, including the cerebellum, brain stem, basal ganglia and cerebral hemispheres. He noted that nystagmus is common in various neurodevelopmental disorders, is poorly studied, and may be secondary to ion channelopathies.

Andreas Chiocchetti from Frankfurt described the complex genetic architecture of autism spectrum disorders. Little real progress has been made. There are no biomarkers and no models of pathogenesis. Greatly differing genetics can produce a similar phenotype, and the mechanisms underlying this remain unknown. Most inheritance appears to rely on either multiple common variants acting together, or is unknown. The prevalence of ASD is now estimated at 1%, with the risk of ASD in a sibling of a proband being 20%. Perhaps 3-10% can be explained by rare or de novo variants. Genetic patterns appear to differ between those with ASD with and without ID.

Terry Naerland from Oslo spoke on autism symptoms and gender ratios across different disorders, focusing on the role of aetiology and the degree of ID. This team is analysing ASD symptoms in different groups, including idiopathic ASD, Down syndrome, Angelman's and Smith-Magenis syndromes, and Fragile X. They note that the range in ratio of male to female subjects is 2 to 7:1. They are investigating the connection between gender and ASD symptoms.

Stephan Huijbregts from Leiden discussed executive functioning and the hypodopaminergic state in adults with PKU. In Europe this disorder has an incidence of 1:10,000. Degrees of severity occur. Elevated phenylalanine levels block the blood brain barrier and stop other neurotransmitter precursors entering the brain. Phenylalanine also directly damages white matter. Dietary treatment must be started before one month of age. Executive function is damaged much more than IQ, and the extent of damage depends on the serum level of phenylalanine. Problems compound with the expectation of performance of simultaneous tasks of executive functioning (EF). His study found a direct link between low levels of dopamine availability and EF, but further contributions were probably made by low levels of serotonin and white matter damage. Stimulant medications may therefore be helpful in PKU. The presence of a hypodopaminergic state raises the question of whether patients will be more vulnerable to the development of Parkinsonism. He also made mention of the medication sapropterin or BH4, a co-factor which directly lowers phenylalanine levels in those intolerant of dietary management. (This medication is very expensive in Australia, and is subsidised by the PBS only for specific deficiency states and not for PKU.) He recommended maintaining phenylalanine levels in PKU below 600 as sufficient, with no evidence supporting the lower levels commonly recommended as necessary.

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Friederike Ehrhart from Maastricht described biological pathway analysis of Rett syndrome transcriptomics, a highly technical exposition which illustrated the extent to which multiple pathways are either up or down regulated in this disorder. She noted the availability of WikiPathways as a repository of biological pathways freely available on the Internet. Further work will help understand the link between the MECP2 deletion and the development of the phenotype of Rett syndrome.

Shruti Garg from Manchester presented a randomised control trial of simvastatin in Neurofibromatosis Type I (NF1) autism. This condition has an incidence of 1:3000 and is a single gene disorder, a mutation at 17q11.2. 50% are the result of dominant inheritance and 50% sporadic. Over 50% have ASD. Previous trials of simvastatin have been negative. This study focused on earlier intervention than previous trials, with 26 patients aged between 5 and 10 years dosed at a rate of 1mg/kg completing the study. The results were negative. Rather than the reduction of GABA expected in frontal white matter there was an increase. There were

no differences in parent behaviour ratings but no adverse effects. NF1 inhibits GABA in animals and NF1 animal models show an excess of GABA. Recent human studies however have found a relative deficiency of GABA in NF1. This disappointing result highlights the problems of extrapolating from animal models to humans in genetic disorders, and the pitfalls in the development of specific drug treatments.

Ernesto Burgio from Palermo gave a fascinating but alarming presentation on the rise of neurodevelopmental disorders, highlighting the shift in emphasis from genetics to epigenetics. He described brain hardware as genetically derived but the connectome as epigenetic. Genes require regulation and this is not done by DNA. The difference between cells is secondary to epigenetics given that all have identical DNA. He described environment as a continuous stream of outside information, and the brain as the most plastic organ of the body throughout life. Many rare genetic variants show substantial overlap between disorders such as schizophrenia, bipolar disorder, ASD and ID. He noted that autism is a disorder of the connectome not of the hardware. The concordance between dizygotic twins is increasing, whereas that between monozygotic twins is decreasing, indicating an increase in the contribution made by epigenetic factors. He noted that early maternal childhood abuse leads to an increased risk of autism in her child. He also noted that pregnant women in Europe have been found to contain between 200 and 300 exogenous chemicals or pollutants. Living near a freeway increases the risk of autistic offspring by 2 or 3 times. There has been a major increase in the prevalence of cancer in the first two years of life over the last 40 years, likely also to be due to epigenetic factors such as pollutants and obesity.

Jim Harris from Johns Hopkins described progress over the last 25 years in understanding Lesch Nyhan syndrome. This is an X-linked recessive disorder of purine metabolism, with an incidence of 1:300 000. There is a spectrum of severity, with only those with classic LNS who have less than 1% of expected enzyme levels exhibiting the classic pattern of severe self-injury. Less severely affected individuals show motor abnormalities varying from clumsiness to severe dystonia, and cognitive impairment ranging from problems of attention to intellectual disability. Impulsivity is common. All individuals have increased urate levels with the standard complications that require active medical management. Lowering serum urate with allopurinol does not improve the behavioural phenotype. A few patients in an open label trial have benefited from S-adenyl methionine. The dopamine D1 agonist ecopisan has shown early promise in reducing SIB. Deep brain stimulation in the globus pallidus can be transformational, with one patient still doing well 15 years later.

Petrus de Vries from Cape Town presented the results of a large trial of everolimus adjunctive therapy for the treatment of refractory seizures in patients with Tuberous Sclerosis Complex (TSC). 70% of these individuals have epilepsy, often refractory. Everolimus is an inhibitor of mTOR. The study was strongly supported by the manufacturer Novartis. No studies are done with the related medication sirolimus (rapamycin) which is significantly cheaper and off patent. Everolimus is already licensed for use in shrinking SEGAs and renal AMLs. Subjects in this trial had to have at least 16 seizures in a four-week period, but the median seizure frequency pre-treatment was 35-40. 40% of subjects on high dose everolimus showed a greater than 50% reduction in seizure frequency, while 5% became seizure free. Discontinuations were low despite the high prevalence of side effects, including stomatitis, diarrhoea, mouth ulceration and hyperlipidaemia. Vineland and quality of life questionnaires have been administered and analysis is ongoing. Future trials are planned to look at the impact on psychiatric and behavioural disorders.

Bissell from Birmingham compared difficult behaviours in younger and older children with TSC, finding that young children had high rates of self-injury and aggression, indicating that behavioural problems in TSC begin early. The average age of diagnosis of autism in 2000 individuals with TSE was very late at 7. Inflexibility and resultant tantrums are very problematic.

Shahid Zaman from Cambridge discussed evaluating the earliest manifestations of Alzheimer's disease in Down syndrome. 40% of those with Down syndrome aged between 50 and 59 will have clinical dementia, compared to 5% of those over 65 without Down syndrome. 15% of Down individuals have Alzheimer's histopathology at the age of 15, with 100% by the age of 35. Pathology is evident perhaps 20 years before clinical onset. Clinical onset however is not inevitable and this remains unexplained, but must be due to neuroprotective factors. The cascade of damage appears to result from the existence of three copies of the amyloid precursor protein (APP) gene on chromosome 21.

Ines Pote from Kings College described the developmental trajectory of glutamate in the human brain from the foetus to the early infant. Glutamate is an excitatory neurotransmitter, which is vital for the development of neurons and circuits. It is the immediate precursor of GABA which is inhibitory, and in the healthy adult brain these two neurotransmitters are in balance. In the immature nervous system however GABA is excitatory, and possibly switches functions at birth. In this small sample size of individuals at high risk of autism because of a sibling with autism, glutamate levels were higher. Remarkably proton magnetic resonance spectroscopy (HMRS) can measure glutamate concentrations in foetal brains. Data are preliminary and the sample size is so far small.

Rosalyn Hithersay from UCL described the use of functional near infrared spectroscopy (FNIS) to measure executive functioning in adults with Down syndrome. This technology relies on the fact that near infrared light passes through brain tissues and bone, but is differentially absorbed by haemoglobin depending on the degree of oxygen saturation. Light penetrating the skull traverses an arc before exit. A cap similar to an EEG cap can be used to both transmit and detect light, with the depth of imaging dependent on the distance between source and sensor. Thick hair creates problems with using this technology in older children and adults. The investigation is well tolerated. The machine automatically subtracts the part of signal due to variations in skin blood flow. Healthy adults were scanned while performing four tasks involving different executive functions. This is a preliminary study to determine the likely effectiveness of the method in individuals with Down and other syndromes. Larger cross-sectional and longitudinal studies are planned.

Jessica Penhallow from Birmingham described long term predictors of quality of life for adults with genetic syndromes. Various measures were used in a sample of 69 parents and carers of individuals with Angelman's, Cri du Chat, Cornelia de Lange, FXS and Prader Willi syndromes. The mean age was 29 and 68% were male. Measurements were taken in 2003 and 2015. The main predictor of poor quality of life was low mood. Quality of life was measured across four domains, physical, psychological, social relationships and quality of the environment. The presence of autism and challenging behaviours were significant negative factors. Self-reported quality of life in those able to participate closely paralleled the information obtained from informants. (Pat Howlin described finding surprisingly good quality of life in adult autistics, leading her to question the validity in that population of the tool used.) This study suggests that early intervention to address low mood may have significant later benefits.

Anne Bassett from Toronto described the neurocogntiive profile of 22q11.2 deletion syndrome. She emphasised that this is now the universal name for this syndrome, and older terms such as VCFS are now discouraged. This is a multisystem syndrome. The facial features can be subtle. There are high rates of ASD, ADHD, seizures, mood and anxiety symptoms, Parkinsonism and schizophrenia, which the incidence of the latter approaching 30%. Lifespan is reduced. IQ ranges from normal to intellectual disability, but those with an average IQ still have impairment in social and communication skills. Adaptive function is proportional to IQ and to the presence of schizophrenia. The onset of schizophrenia in this population has a mean age of 21, the same as in neurotypicals. The neurocognitive profiles of those with schizophrenia with and without this syndrome are similar. There are no tests that will predict which individuals with this syndrome will develop schizophrenia. This investigation included individuals with schizophrenia who had been stabilised on medication. The impact of that medication on these results is therefore unknown. Most cases of this syndrome occur de novo but 5-10% are inherited.

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Kate Woodcock from Belfast described the development of a pilot battery to measure executive function (EF) (also termed cognitive control) in individuals with genetic neurodevelopmental disorders. Three EFs are typically studied – inhibition, task switching, and updating working memory. EF is typically measured indirectly by task performance, which requires competence in numerous components. EF measures therefore have poor reliability, and rely on a number of assumptions. All of those individuals we study have patchy cognitive skills. She described the development of a battery of 25 tests designed to minimise the influence of cognitive strengths and weaknesses, piloted with 125 neurotypical children aged 6-12, and 12 with genetic syndromes. This tool, the CAN measure assessment battery, has now been administered to almost 700 neurotypical 6-12 year olds and should facilitate more valid assessment of EF in those with neurodevelopmental disorders. At this stage assessment of children aged less than 6 is not possible. Her team plans to make this instrument freely available.

Kate Wolfe from UCL described the phenotypic features of individuals with the rare CNVs 2q13 and 4p16. Microarrays of 202 adults recruited from ID psychiatry services across England showed 11% with CNVs classed as pathogenic. 4 patients were examined with 2q13 deletions and 5 with 4p16.3 duplications. A deletion at the latter site causes Wolf Hirschorn syndrome. No consistent phenotypes emerged. In one individual the non affected parent also had a deletion on two q13, raising questions about pathogenicity, and raising questions about the concept of benign familial variants. It is of course possible that the CNVs found in these individuals were not causally linked to the clinical presentation. The study of CNVs revealed by genome wide microarrays remains in its infancy, and there are many uncertainties about interpretation.

Andre Strydom from UCL presented on cognitive decline and dementia in Down syndrome. 40% of those with Down syndrome in the UK are now aged over 40. The lifetime risk of dementia in this population is now estimated to be 80-90%. The median age at diagnosis is 55 years, with 25% occurring before 50 and 25% after 60. Down syndrome results from an overdose of 300 known, normal genes. There are problems of microcephaly, hypofrontality, small hippocampi and impaired memory, executive function and expressive language before the onset of Alzheimer's disease. In contrast to those with Down syndrome and therefore three copies of the APP gene, the syndrome of duplication of the APP gene results in the universal onset of dementia by the age of 60. The overproduction of amyloid cannot therefore be the whole story in the dementia of Down syndrome, so there must be neuroprotective factors that prevent a similar outcome. Good sleep protects the brain and 40% of individuals with Down syndrome have obstructive sleep apnoea. Intervention in children is likely to be more successful than in adults. Anti-amyloid treatments are often toxic and need to be given early. Most trials are conducted in those already exhibiting clinical dementia, and these trials have typically failed. Trials of antioxidants have been ineffective. Trials of a green tea extract which inhibits the kinase DYRK1A are underway as a result of successful treatment in Down syndrome mice. Andre and his team are developing a cognitive scale for Down Syndrome focusing on memory, EF and language, and will be exploring the use of this in early prediction.

Sophie van Rijn from the TRIXY (trisomy of X and Y) Centre in Leiden presented on social, emotional and behavioural problems in individuals with an extra X chromosome. Most are shy, timid, with poor peer relationships and high social anxiety. There is an increased prevalence of autism. There are global deficits in communication skills and language problems. Affective socialisation depends on intact executive function, including attention, inhibition, flexibility and working memory, which are all diminished in these syndromes. Similarly poor cognitive skills, such as recognition of facial expression and body language, contribute. Individuals with TRIXY but without autism show reduced social attention but heightened social arousal, whereas individuals with ASD show reductions in both domains.

Paul Hagerman from UC Davis spoke about pathogenic mechanisms of FXTAS, a condition caused by a premutation expansion of the FMR1 gene. This is a multisystem disorder with problems likely to be a result of the development of inclusions in the cell nucleus. These are prominent in neuronal nuclei and are considered to be the result of the accumulation of problems associated with chronic DNA damage repair. The FX gene is too active in the premutation, whereas it is inactivated in the full mutation. Levels of FMRP are normal but there is a substantial increase in mRNA which is toxic to DNA, in part through the formation of transcriptional loops. Individuals manifest an intention rather than a resting tremor, gait ataxia, peripheral neuropathy, cognitive decline, Parkinsonism and problems with bladder and bowel control.

Carol Samango-Sprouse from Washington DC presented results from a large prospective study of the neurodevelopmental outcome in prenatally diagnosed males with 47 XXY. She stated that it is naïve to think that low testosterone levels in these individuals are not significant, yet many have not been offered testosterone replacements. Individuals with this syndrome treated with testosterone show improvement in fine and gross motor skills, reasoning, self-esteem, muscle tone and comprehension, with reduced anxiety levels and a lower risk of osteoporosis. This syndrome is not associated with intellectual disability, and if this is present there should be a further search for additional causes. Motor milestones are delayed even with testosterone treatment and physical therapies are still required. This was a placebo controlled trial of 158 individuals. Treated subjects were given three shots of testosterone between the ages of 4 and 12 months. Booster shots between the age of 5 and 8 are recommended, with regular supplementation from the age of 11. No adverse effects were reported.

Randi Hagerman from UC Davis reported on advances in research in Fragile X. She noted that advances include both new treatments and the elucidation of new mechanisms of dysfunction when FMRP is missing or deficient. FMRP has been found to control the translation of multiple measures important in other disorders such as schizophrenia and ASD. A number of these new mechanisms have potential for identifying new targets for treatment. Intervention in early childhood is the best way to demonstrate treatment efficacy. She notes that there are early deficits in tryptophan pathways whatever the aetiology of ASD. SSRIs increase BDNF, but sertraline is the only one to increase dopamine in the striatum and nucleus accumbens, which can result in improvements in attention. Individuals with FXS have a GABA deficiency, which results in a reduction in habituation to stimuli and therefore an increase in sympathetic tone. As a result, they disproportionately attend to threatening information in their environment. Sertraline 2.5-5mg a day in children aged 2-6 with FXS can improve language especially in those with comorbid ASD.

The Novartis trial of AFq056 failed to demonstrate a statistically significant benefit, but some individuals experienced benefit from this mGluR5 antagonist. It is possible that administration in the trial did not occur at an early enough age. There is further work ongoing. There is a controlled trial underway of lovastatin in 10-18 year olds. This medication lowers RAS-ERK 1/2 and reduces excess protein synthesis. Benefit has been shown in FXS in an open label study. Parent Implemented Language Intervention (PILI) has been included in all participants in this lovastatin trial. This is conducted by therapists via Skype twice a week, and assists parents develop skill in stimulating language in the FXS child.

Studies of the GABA agonists ganaxolone and alphaxolone are underway, with one study producing only very modest results. Two companies are considering undertaking trials of cannabidiol, a non-psychotropic component of cannabis. Perhaps 10% of individuals with FXS show a Prader Wili phenotype and metformin can help with the insulin dysregulation seen in this population. There may be additional benefits on mood stimulation. Acamprosate has benefited some individuals. The challenge in all of this research is choosing robust outcome measures. High placebo responses can obscure subsets of individuals who respond positively to these agents. Eye tracking data can be a useful outcome measure.

The next meeting will be held in Leiden, Netherlands from 14th to 16th September 2017.