Clinical genetics and other aspects of neuropsychiatric disorders

Gholson Lyon, M.D. Ph.D.
Figure 4. NAT activity of recombinant hNaa10p WT or p.Ser37Pro towards synthetic N-terminal peptides. 

A) and B) Purified MBP-hNaa10p WT or p.Ser37Pro were mixed with the indicated oligopeptide substrates (200 µM for SESSS and 250 µM for DDDIA) and saturated levels of acetyl-CoA (400 µM). Aliquots were collected at indicated time points and the acetylation reactions were quantified using reverse phase HPLC peptide separation. Error bars indicate the standard deviation based on three independent experiments. The five first amino acids in the peptides are indicated, for further details see materials and methods. Time dependent acetylation reactions were performed to determine initial velocity conditions when comparing the WT and Ser37Pro NAT-activities towards different oligopeptides.

C) Purified MBP-hNaa10p WT or p.Ser37Pro were mixed with the indicated oligopeptide substrates (200 µM for SESSS and AVFAD, and 250 µM for DDDIA and EEEIA) and saturated levels of acetyl-CoA (400 µM) and incubated for 15 minutes (DDDIA and EEEIA) or 20 minutes (SESSS and AVFAD), at 37°C in acetylation buffer. The acetylation activity was determined as above. Error bars indicate the standard deviation based on three independent experiments. Black bars indicate the acetylation capacity of the MBP-hNaa10p wild type (WT), while white bars indicate the acetylation capacity of the MBP-hNaa10p mutant p.Ser37Pro. The five first amino acids in the peptides are indicated.

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Vignette #1: The genetic basis of a new syndrome with severe developmental delay and cardiac abnormalities.
Family now in October 2011, with five mutation-positive boys dying from the disease.
These are the Major Features of the Syndrome.

<table>
<thead>
<tr>
<th>Table 1. Features of the syndrome</th>
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<tbody>
<tr>
<td><strong>Growth</strong></td>
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<td><strong>Development</strong></td>
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<tr>
<td><strong>Facial</strong></td>
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<tr>
<td><strong>Skeletal</strong></td>
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<td></td>
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<tr>
<td><strong>Integument</strong></td>
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<td></td>
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<td><strong>Cardiac</strong></td>
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<tr>
<td><strong>Genital</strong></td>
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<td><strong>Neurologic</strong></td>
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Shaded regions include features of the syndrome demonstrating variability. Though variable findings of the cardiac, genital and neurologic systems were observed, all affected individuals manifested some pathologic finding of each.
This is the mutation we found… one nucleotide change out of 6 billion nucleotides in a diploid genome…

- **Pro37**: C C C C
- **Ser37**: C T T T T C C T

**Mutation**

- **WT**: G G
- **Unaffected Brother**
- **Proband**: T C

---
An unrelated second family was also identified, due to having the same mutation, but in a different genetic background.
These two families are UNRELATED, i.e. no common founder.

Courtesy of Chad Huff and Lynn Jorde
Tentative name: Ogden Syndrome, in honor of where the first family lives, in Ogden, Utah
Proving the mutation likely plays some role

- Present in two unrelated families with very similar phenotype of affected boys.

- Blinded Sanger sequencing showed perfect segregation of the mutation with the disease. Mutation present in Proband, Carrier Mother, Carrier Grandmother and other carrier mothers. Absent in unaffected brother and unaffected uncle.

- Also present in DNA from formalin-fixed paraffin-embedded tissue from two other deceased affected boys, found in pathology department, saved in one case for 30 years.

- Mutation NOT present in ~6000 exomes or genomes sequenced at BGI, CHOP and Utah for other projects.
NAT activity of recombinant hNaa10p WT or p.Ser37Pro towards synthetic N-terminal peptides
The mutation is a missense resulting in Serine to Proline change in Naa10p

- Ser 37 is conserved from yeast to human
- Ser37Pro is predicted to affect functionality (SIFT and other prediction programs)
- Structural modelling of hNaa10p wt (cyan) and S37P (pink)
The mutation disrupts the N-terminal acetylation machinery (NatA) in human cells.

Slide courtesy of Thomas Arnesen
Open question:
Function of N-terminal acetylation?

Protein stability? Protein secretion?

Figure courtesy of Kris Gevaert
hNaa10p-S37P is functionally impaired *in vivo* using a yeast model.

Unpublished data, do not further distribute.
Big Question though:

Simulated structure of S37P mutant
Waddington claimed that canals form in the epigenetic landscape during evolution, and that this heuristic is useful for understanding the unique qualities of biological robustness.
E. coli adapting to low glucose conditions, in the context of media containing citrate. – Richard Lemski experiment

"Finally, novel functions often emerge in rudimentary forms that must be refined to exploit the ecological opportunities. This three-step process — in which potentiation makes a trait possible, actualization makes the trait manifest, and refinement makes it effective — is probably typical of many new functions.” - Lemski
The Biology of MENTAL DEFECT

BY

LIONEL S. PENROSE, M.A., M.D.

WITH A PREFACE BY

PROFESSOR J. B. S. HALDANE, F.R.S.

GRUNE & STRATTON
New York
1949
Beyond our Kuhnian inheritance
A recent lecture by Prof Greg Radick questions our scientific inheritance, through textbook histories of genetics and Thomas Kuhn's legacy
http://www.guardian.co.uk/science/the-h-word/2012/aug/28/thomas-kuhn

Plate I.

Expression Issues

• We do not really know the expression of pretty much ALL mutations in humans, as we have not systematically sequenced or karyotyped any genetic alteration in Thousands to Millions of randomly selected people, nor categorized into ethnic classes, i.e. clans.

• There is a MAJOR clash of world-views, i.e. do single mutations drive outcome predominately, or are the results modified substantially by genetic background and/or environment? i.e. is there really such a thing as genetic determinism for MANY mutations?
Vignette #2: One person with very severe obsessive compulsive disorder, depression and intermittent psychoses
One person with very severe obsessive compulsive disorder, depression and intermittent psychoses

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Genomic coordinates</th>
<th>Amino acid change</th>
<th>Zygosity</th>
<th>Mutation type</th>
<th>Population Frequency</th>
<th>Clinical significance</th>
</tr>
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<tbody>
<tr>
<td>MTHFR</td>
<td>chr1: 11854476</td>
<td>Glu&gt;Ala</td>
<td>heterozygous</td>
<td>non-synon</td>
<td>T:77% G:23%</td>
<td>Susceptibility to psychoses, schizophrenia, occlusive vascular disease, neural tube defects, colon cancer, acute leukemia, and methylenetetra-hydrofolate reductase deficiency</td>
</tr>
<tr>
<td>BDNF</td>
<td>chr11: 27679916</td>
<td>Val&gt;Met</td>
<td>heterozygous</td>
<td>non-synon</td>
<td>C:77% T:23%</td>
<td>Susceptibility to OCD, psychosis, and diminished response to exposure therapy</td>
</tr>
<tr>
<td>CHAT</td>
<td>chr10: 50824117</td>
<td>Asp&gt;Asn</td>
<td>heterozygous</td>
<td>non-synon</td>
<td>G:85% A:15%</td>
<td>Susceptibility to schizophrenia and other psychopathological disorders.</td>
</tr>
</tbody>
</table>
**DBS lead**
Thin, insulated, coiled wires, each ending in a 1.5 mm electrode, that deliver stimulation to the targeted areas.

**Extension**
An insulated wire that connects the lead to the neurostimulator.

**Neurostimulator**
A pacemaker-like device that contains a battery and circuitry to generate electrical signals that are delivered by the leads to the targeted structures deep within the brain.

The clinician can program and adjust the settings of the neurostimulator externally via a hand-held device.
Nucleus accumbens
Approximate projections of the medial forebrain bundle to striatum, basal forebrain and prefrontal cortex (blue). Credit: Geoff B Hall, Via Wikimedia Commons (modified for current use)
Fig. 1. Coronal section of the brain near the nucleus accumbens with the track of the electrodes on the left and right side.
Two year follow-up

![Graph showing YBOCS scores before and after surgery](image-url)
Vignette #3: New Syndrome with Mental Retardation, “Autism”, “ADHD”

Likely X-linked or Autosomal Recessive, with X-linked being supported by extreme X-skewing in the mother.
Dysmorphic
Mental Retardation
“autism”
“ADHD”
Hearing difficulties
Workup Ongoing for past 10 years

- Numerous genetic tests negative, including negative for Fragile X and MANY candidate genes.
- Found one missense mutation in a known mental retardation gene, but the mutation is a very conservative nonsynonymous Asp to Glu. Is it relevant or not? What about the whole rest of the genome?
2 mutations present in mother and two boys, on X-chromosome, not in father, not in dbSNP135, not in 1000Genomes April 2012 release, and not in NHLBI 6500 Exomes

- Nonsyn SNV  ZNF41  c.1191C>A  p.Asp397Glu

- Nonsyn SNV  TAF1  c.4010T>C  p.Ile1337Thr

TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa
Mutations in the ZNF41 Gene Are Associated with Cognitive Deficits: Identification of a New Candidate for X-Linked Mental Retardation

Sarah A. Shoichet, Kirsten Hoffmann, Corinna Menzel, Udo Trautmann, Bettina Moser, Maria Hoeltzenbein, Bernard Echenne, Michael Partington, Hans van Bokhoven, Claude Moraine, Jean-Pierre Fryns, Jamel Chelly, Hans-Dieter Rott, Hans-Hilger Ropers, and Vera M. Kalscheuer

1Max-Planck-Institute for Molecular Genetics, Berlin; 2Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen-Nuremberg; 3Centre Hospitalier Universitaire de Montpellier, Hôpital Saint-Eloi, Montpellier, France; 4Hunter Genetics and University of Newcastle, Waratah, Australia; 5Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; 6Services de Génétique–INSERM U316, CHU Bretonneau, Tours, France; 7Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium; and 8Institut Cochin de Génétique Moleculaire, Centre National de la Recherche Scientifique/INSERM, CHU Cochin, Paris

### X-linked

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<th>Gene</th>
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<tr>
<td>ZNF41</td>
<td>X:47307978</td>
<td>5</td>
<td>p.Asp397Glu</td>
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<tr>
<td>ASB12</td>
<td>X:63444792</td>
<td>2</td>
<td>p.Gly247Cys</td>
</tr>
<tr>
<td>TAF1</td>
<td>X:70621541</td>
<td>25</td>
<td>p.Ile1337Thr</td>
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### Non-coding

<table>
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<tr>
<td>UTR3 AR</td>
<td>X:66945414</td>
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<td>---</td>
</tr>
<tr>
<td>FAM155B</td>
<td>(dist=271971)</td>
<td>X:68453113</td>
<td>---</td>
</tr>
<tr>
<td>MIR221</td>
<td>(dist=35606)</td>
<td>X:45569979</td>
<td>---</td>
</tr>
<tr>
<td>DMD-AS2</td>
<td>intronic</td>
<td>X:31284835</td>
<td>---</td>
</tr>
<tr>
<td>MID1</td>
<td>(dist=30252)</td>
<td>X:10383096</td>
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</table>
The two brothers with the P111L mutations reported in the prior paper do have mental deficiency, hyperkinesia, no motor or neurologic sign except for the delay, and slight dysmorphic facial anomalies: large low-set ears, thin upper lip, slight downward palpebral slants, but no upturned nose, and a short philtrum. The mother was normal in appearance.
• Previously reported P111L change in the ZNF41 protein has now also been found in two "male controls" (EVS server, ESP6500), and furthermore, there are two rare, likely heterozygous ZNF41 frameshift mutations and one heterozygous stop-gained mutation reported in control individuals (ESP6500) (personal communication from Dr. Vera Kalscheuer).
• KRAB (Kruppel-associated box) domain -A box.
• The KRAB domain is a transcription repression module, found in a subgroup of the zinc finger proteins (ZFPs) of the C2H2 family, KRAB-ZFPs. KRAB-ZFPs comprise the largest group of transcriptional regulators in mammals, and are only found in tetrapods.
• The KRAB domain is a protein-protein interaction module which represses transcription through recruiting corepressors. The KAP1/ KRAB-AFP complex in turn recruits the heterochromatin protein 1 (HP1) family, and other chromatin modulating proteins, leading to transcriptional repression through heterochromatin formation.
Proving Causality

• Will need to find a second, unrelated family with same exact mutation and similar phenotype.
• Can also perform in vitro/in vivo studies and structural modeling, and make knock-in mice and/or test in zebrafish, etc... for biological function.
Genotype First, Phenotype Second AND Longitudinally

Phenotypic variability and genetic susceptibility to genomic disorders

Santhosh Girirajan and Evan E. Eichler

Department of Genome Sciences, Howard Hughes Medical Institute, University of Washington School of Medicine, PO Box 355065, Foege S413C, 3720 15th Avenue NE, Seattle, WA 98195, USA

Genome-Wide Association Study of Multiplex Schizophrenia Pedigrees

Am J Psychiatry Levinson et al.; AiA:1–11

“Rare CNVs were observed in regions with strong previously documented association with schizophrenia, but with variable patterns of segregation. This should serve as a reminder that we still know relatively little about the distribution of these CNVs in the entire population (e.g., in individuals with no or only mild cognitive problems) or about the reasons for the emergence of schizophrenia in only a minority of carriers, so great caution is required in genetic counseling and prediagnosis.”
Clinical Validity?

This is SO complex that the only solid way forward is with a “networking of science” model, i.e. online database with genotype and phenotype longitudinally tracked for thousands of volunteer families.
Identifying disease mutations in genomic medicine settings: current challenges and how to accelerate progress

Gholson J Lyon and Kai Wang

Applied & Translational Genomics

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journal homepage: www.elsevier.com/locate/atg

Practical, ethical and regulatory considerations for the evolving medical and research genomics landscape

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The End
Low concordance of multiple variant-calling pipelines: practical implications for exome and genome sequencing

Jason O’Rawe\textsuperscript{1,2}, Tao Jiang\textsuperscript{3}, Guangqing Sun\textsuperscript{3}, Yiyang Wu\textsuperscript{1,2}, Wei Wang\textsuperscript{4}, Jingchu Hu\textsuperscript{3}, Paul Bodily\textsuperscript{5}, Lifeng Tian\textsuperscript{6}, Hakon Hakonarson\textsuperscript{6}, W Evan Johnson\textsuperscript{7}, Zhi Wei\textsuperscript{4}, Kai Wang\textsuperscript{8,9*} and Gholson J Lyon\textsuperscript{1,2,9*}
Major Conclusion: Clinical Validity?

This is SO complex that the only solid way forward is with a “networking of science” model, i.e. online database with genotype and phenotype longitudinally tracked for thousands of volunteer families.

ancestry.com

PatientsLikeMe

23andMe
Sequenced whole genomes of Mother, Father and Two Boys, using Complete Genomics

• Sequenced “whole” genomes to obtain noncoding and other non-exonic regions.
• No obvious pathogenic CNVs – microarrays normal.
• ~6 million variants total in the 4 people different from Hg19 reference genome.
• No homozygous autosomal recessive mutations found.
• No Nonsense/Framedshift mutations in both boys.
• 2 mutations present in mother and two boys, on X-chromosome, not in father, not in dbSNP135, not in 1000Genomes April 2012 release, and not in NHLBI 6500 Exomes
DBS-probes shown in X-ray of the skull (white areas around maxilla and mandible represent metal dentures and are unrelated to DBS devices)
“Biological Indeterminacy”

- Bateson became famous as the outspoken Mendelian antagonist of Walter Raphael Weldon, his former teacher, and Karl Pearson who led the biometric school of thinking. This concerned the debate over saltationism versus gradualism (Darwin had been a gradualist, but Bateson was a saltationist). Later, Ronald Fisher and J.B.S. Haldane showed that discrete mutations were compatible with gradual evolution: see the modern evolutionary synthesis.

[Biological Indeterminacy](https://doi.org/10.1093/scienceethics/2012Jul-3) by Greenspan RJ. Sci Eng Ethics. 2012 Jul 3
• Seguin E. 1866, - “our incomplete studies do not permit actual classification; but it is better to leave things by themselves rather than to force them into classes which have their foundation only on paper”- from *Idiocy and its treatment by the physiological method*.

• Walter Frank Raphael Weldon 1902 – “the accumulation of records, in which results are massed together in ill-defined categories of variable and uncertain extent, can only result in harm”.
Diagnostic Criteria for 299.00 Autistic Disorder

Diagnostic and Statistical Manual of Mental Disorders: DSM IV

(I) A total of six (or more) items from (A), (B), and (C), with at least two from (A), and one each from (B) and (C)

(A) qualitative impairment in social interaction, as manifested by at least two of the following:
1. marked impairments in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body posture, and gestures to regulate social interaction
2. failure to develop peer relationships appropriate to developmental level
3. a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people, (e.g., by a lack of showing, bringing, or pointing out objects of interest to other people)
4. lack of social or emotional reciprocity ( note: in the description, it gives the following as examples: not actively participating in simple social play or games, preferring solitary activities, or involving others in activities only as tools or "mechanical" aids )

(B) qualitative impairments in communication as manifested by at least one of the following:
1. delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
2. in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
3. stereotyped and repetitive use of language or idiosyncratic language
4. lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level

(C) restricted repetitive and stereotyped patterns of behavior, interests and activities, as manifested by at least two of the following:
1. encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
2. apparently inflexible adherence to specific, nonfunctional routines or rituals
3. stereotyped and repetitive motor mannerisms (e.g hand or finger flapping or twisting, or complex whole-body movements)
4. persistent preoccupation with parts of objects

(II) Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:
(A) social interaction
(B) language as used in social communication
(C) symbolic or imaginative play

(III) The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder
OBSERVATIONS ON AN ETHNIC CLASSIFICATION OF IDIOTS *

J. LANGDON H. DOWN M.D., London

London Hospital Clinical Lecture Report, 3, 259-262, 1866.

“Those who have given any attention to congenital mental lesions, must have been frequently puzzled how to arrange, in any satisfactory way, the different classes of this defect which may have come under their observation. Nor will the difficulty be lessened by an appeal to what has been written on the subject. The systems of classification are generally so vague and artificial, that, not only do they assist but feebly, in any mental arrangement of the phenomena represented, but they completely fail in exerting any practical influence on the subject.”