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.
Vignette #1: The genetic basis of a new syndrome with severe developmental delay and cardiac abnormalities.

Using VAAST to Identify an X-Linked Disorder Resulting in Lethality in Male Infants Due to N-Terminal Acetyltransferase Deficiency

Alan F. Rope,1 Kai Wang,2,19 Rune Evjenth,3 Jinchuan Xing,4 Jennifer J. Johnston,5 Jeffrey J. Swensen,6,7 W. Evan Johnson,8 Barry Moore,4 Chad D. Huff,4 Lynne M. Bird,9 John C. Carey,1 John M. Opitz,1,4,6,10,11 Cathy A. Stevens,12 Tao Jiang,13,14 Christa Schank,8 Heidi Deborah Fain,15 Reid Robison,15 Brian Dalley,16 Steven Chin,6 Sarah T. South,1,7 Theodore J. Pysher,6 Lynn B. Jorde,4 Hakon Hakonarson,2 Johan R. Lillehaug,3 Leslie G. Biesecker,5 Mark Yandell,4 Thomas Arnesen,3,17 and Gholson J. Lyon15,18,20,*

The American Journal of Human Genetics 89, 1–16, July 15, 2011
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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</thead>
<tbody>
<tr>
<td>Growth</td>
</tr>
<tr>
<td>Development</td>
</tr>
<tr>
<td>Facial</td>
</tr>
<tr>
<td>Skeletal</td>
</tr>
<tr>
<td>Integument</td>
</tr>
<tr>
<td>Cardiac</td>
</tr>
<tr>
<td>Genital</td>
</tr>
<tr>
<td>Neurologic</td>
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</tbody>
</table>

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…
An unrelated second family was also identified, due to having the same mutation, but in a different genetic background.
Tentative name: Ogden Syndrome, in honor of where the first family lives, in Ogden, Utah
Bring clinical standards to human-genetics research

Study protocols need to be rigorous, because more than science is at stake. Sometimes participants’ lives depend on the results, writes Gholson J. Lyon.


Guest post: Time to bring human genome sequencing into the clinic
02/16/2012
PRIVACY and PROGRESS in Whole Genome Sequencing

Presidential Commission for the Study of Bioethical Issues

October 2012
Recommendation 4.1

Funders of whole genome sequencing research, relevant clinical entities, and the commercial sector should facilitate explicit exchange of information between genomic researchers and clinicians, while maintaining robust data protection safeguards, so that whole genome sequence and health data can be shared to advance genomic medicine.

Performing all whole genome sequencing in CLIA-approved laboratories would remove one of the barriers to data sharing. It would help ensure that whole genome sequencing generates high-quality data that clinicians and researchers can use to draw clinically relevant conclusions. It would also ensure that individuals who obtain their whole genome sequence data could share them more confidently in patient-driven research initiatives, producing more meaningful data. That said, current sequencing technologies and those in development are diverse and evolving, and standardization is a substantial challenge. Ongoing efforts, such as those by the Standardization of Clinical Testing working group are critical to achieving standards for ensuring the reliability of whole genome sequencing results, and facilitating the exchange and use of these data.\textsuperscript{216}
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.” - Lemsik
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
“This laboratory test was developed, and its performance characteristics were determined by the Illumina Clinical Services Laboratory (CLIA-certified, CAP-accredited). Consistent with laboratory-developed tests, it has not been cleared or approved by the U.S. Food and Drug Administration. If you have any questions or concerns about what you might learn through your genome sequence information, you should contact your doctor or a genetic counselor. Please note that Illumina does not accept orders for Individual Genome Sequencing services from Florida and New York.”
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

![Bar graph showing YBOCS score before and after surgery](image)

(View PDF) (Download additional files)
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 Moléculaire, Centre National de la Recherche Scientifique/INSERM, CHU Cochin, Paris

<table>
<thead>
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<th>Gene</th>
<th>Locus</th>
<th>Exon</th>
<th>Protein</th>
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<tbody>
<tr>
<td>ZNF41</td>
<td>X:47307978</td>
<td>5</td>
<td>p.As p.397Glu</td>
</tr>
<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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</table>

**Non-coding**

<table>
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<tr>
<th>Gene</th>
<th>Locus</th>
<th>Exon</th>
<th>Protein</th>
</tr>
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<tbody>
<tr>
<td>UTR3 AR</td>
<td>X:66945414</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>FAM155B</td>
<td>X:68453113</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>(dist=271971)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIR221</td>
<td>X:45569979</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>(dist=35606)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMD-AS2</td>
<td>X:31284835</td>
<td>------</td>
<td>------</td>
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<tr>
<td>intronic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MID1</td>
<td>X:10383096</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>(dist=30252)</td>
<td></td>
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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.
Figure 6 Northern blot hybridization of ZNF41, by use of a probe corresponding to nucleotides 621–1099 of ZNF41 transcript variant 1. A, Adult tissues (left to right): heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. B, Fetal tissues (left to right): brain, lung, liver, and kidney. C, Adult brain structures (left to right): amygdala, caudate nucleus, corpus callosum, hippocampus, whole brain, substantia nigra, and thalamus. Black arrowheads highlight the presence of a novel 6-kb transcript. Actin (A and C) or GAPDH (B) served as controls for RNA loading.

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*1,2 and Kai Wang*2,3

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

Gholson J. Lyon a,b,*, Jeremy P. Segal c,**

a Stanley Institute for Cognitive Genomics, Cold Spring Harbor Laboratory, NY, United States
b Utah Foundation for Biomedical Research, Salt Lake City, UT, United States
c New York Genome Center, New York City, NY, United States
The End
Low concordance of multiple variant-calling pipelines: practical implications for exome and genome sequencing

Jason O’Rawe¹,², Tao Jiang³, Guangqing Sun³, Yiyang Wu¹,², Wei Wang⁴, Jingchu Hu³, Paul Bodily⁵, Lifeng Tian⁶, Hakon Hakonarson⁶, W Evan Johnson⁷, Zhi Wei⁴, Kai Wang⁸,⁹* and Gholson J Lyon¹,²,⁹*
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.
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/Frameshift 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.

• 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
“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.”