Human Genetics and Genomic Medicine

The amino-terminal acetylation of proteins, with Max Doerfel, Yiyang Wu, Jonathan Crain, and collaborators, including Scott Lyons.

More than 85 % of human proteins are acetylated at their N-terminal amino group, hence, N-terminal acetylation (NTA) is one of the most abundant modifications of eukaryotic proteins. Despite its discovery more than 30 years ago, very little is known about the cellular effects/functions of this modification. In humans, 6 distinct N-terminal amino-acetyltransferases (NATs) catalyze the transfer of an acetyl group from acetyl-CoA to the Na-terminal amino group of their specific target proteins. The major human acetyltransferase, NatA, consists of an auxiliary subunit, Naa15, and a catalytic active subunit, Naa10. We have previously described two families with a lethal X-linked disorder of infancy called Ogden syndrome. This disorder comprises a distinct combination of an aged appearance, craniofacial anomalies, hypotonia, global developmental delays, cryptorchidism and cardiac arrhythmias. Using X chromosome exon sequencing, we identified a c.109T>C (p.Ser37Pro) variant in Naa10 as contributing to this disease. Biochemical analysis and immunoprecipitation assays in combination with LCMS demonstrated a reduced catalytic capacity and revealed an impaired binding of the S37P mutant towards specific interaction partners, including Naa15 and Naa50. Analysis of the N-terminal acetylome of patient cells revealed a decreased acetylation of a subset of NatA substrates, indicating that a reduced binding capability and an affected enzymatic activity of the Naa10 S37P mutation is a prominent feature in Ogden Syndrome. We are studying NTA of proteins in yeast, mice, and patient-derived human cell lines, as it is very likely that the complexity of NTA increased substantially during evolution of higher organisms. As such, the long-term goal is to gain an understanding of this understudied protein modification in yeast (S. cerevisiae) and in mice and humans. We have already conducted extensive analyses of the pathway in yeast, primarily as an easier system in which to understand some of the known phenotypes, including the mating defect in yeast. Characterization of NAA10/NAA15 knockout yeast strains revealed various phenotypes, including growth defects at elevated temperatures and altered sensitivity towards cytotoxic stresses. These effects could be rescued by overexpressing human wild type Naa15/Naa10 from plasmids; however, overexpressing mutant Naa15/Naa10 S37P only partially rescue these effects. Interestingly, introduction of both human Naa15/Naa10 wt and S37P mutant into the endogenous locus of the corresponding yeast genes failed to reverse the effects. We also continued our efforts with establishing induced pluripotent stem cells (iPSCs) from skin fibroblasts from one of the boys with Ogden Syndrome.

The role of NTA, as carried out by any of the NATs, is poorly understood on a whole organism level, particularly in mammals. Therefore, we have been working with several mouse strains, either lacking Naa10 completely or having severe knockdown of Naa10 expression, which display increased early postnatal lethality and various abnormalities, including congenital heart defects, peripheral nervous system (PNS) defects, a C7 cervical vertebrosternal rib, piebaldism, and genital and renal abnormalities. These mice also develop hydrocephaly during embryogenesis, which in some cases gets worse in the first few weeks of life and becomes associated with abnormal gait and neurologic abnormalities, ultimately leading to death. In addition, the majority of the homozygous female mice are infertile, along with some infertility even in the heterozygous female mice. These data demonstrate a relatively specific set of defects that emerge in late embryogenesis and/or early neonatal life, thus arguing that Naa10 is important for normal development, but also hinting that there must be some unknown biological redundancy in this pathway, at least in mice, given that these mice do not all die during embryogenesis. We have been using much of the new equipment at Woodbury, including the CT scanner and ultrasound machine, in active collaboration with Scott Lyons.

Outlier gene expression reveals recurrent dysregulation in rare disease pedigrees, with Sara Ballouz, Max Dörfel, Jonathan Crain, Megan Crow, and Jesse Gillis

We have been generating and analyzing RNA sequencing from blood-derived RNA from one quad and five trios from the rare TAF1 syndrome cohort. We have re-submitted a manuscript in which we describe a new "outlier-based analysis" that reveals otherwise difficult to observe signals, including an outlier gene involving the calcium channel subunit CACNA1I, which recurs in five of the six pedigrees. The sole family in which no signal was present was a CNV carrier, whilst the other probands had different single nucleotide variants, implying a potentially different underlying molecular mechanism. Notably, this gene is recurrently implicated in other neurological diseases such as schizophrenia and autism, making it a very plausible candidate for further functional studies.

Whole genome sequencing of one complex pedigree illustrates challenges with genomic medicine, with Han Fang, Yiyang Wu, Hui Yang, Margaret Yoon, Kai Wang, and others.

Human Phenotype Ontology (HPO) has risen as a useful tool for precision medicine by providing a standardized vocabulary of phenotypic abnormalities to describe presentations of human pathologies; however, there have been relatively few reports combining whole genome sequencing (WGS) and HPO, especially in the context of structural variants. We illustrate an integrative analysis of WGS and HPO using an extended pedigree, which involves Prader–Willi Syndrome (PWS), hereditary hemochromatosis (HH), and dysautonomia-like symptoms. A comprehensive WGS pipeline was used to ensure reliable detection of genomic variants. Beyond variant filtering, we pursued phenotypic prioritization of candidate genes using Phenolyzer. Regarding PWS, WGS confirmed a 5.5Mb de novo deletion of the parental allele at 15q11.2 to 15q13.1. Phenolyzer successfully returned the diagnosis of PWS, and pinpointed clinically relevant genes in the deletion. Further, Phenolyzer revealed how each of the genes is linked with the phenotypes represented by HPO terms. For HH, WGS identified a known disease variant (p.C282Y) in HFE of an affected female. Analysis of HPO terms alone fails to provide a correct diagnosis, but Phenolyzer successfully revealed the phenotype-genotype relationship using a disease-centric approach. Finally, Phenolyzer also revealed the complexity behind dysautonomia-like symptoms, and seven variants that might be associated with the phenotypes were identified by manual filtering based on a dominant inheritance model. The integration of WGS and HPO can inform comprehensive molecular diagnosis for patients, eliminate false positives and reveal novel insights into undiagnosed diseases. Due to extreme heterogeneity and insufficient knowledge of human diseases, it is also important that phenotypic and genomic data are standardized and shared simultaneously.

KBG syndrome involving a single base insertion in *ANKRD11*, with Janet Malcolmson, Robert Kleyner, David Tegay, Kenneth Ward (Utah), Justine Coppinger (Utah), Annette Maughan (Utah), Glenn Maughan (Utah), Lesa Nelson (Utah), Kai Wang (California), Reid Robison (Utah).

KBG syndrome is a rare autosomal dominant genetic condition characterized by neurological involvement, macrodontia and distinct facial, hand and skeletal features. Over 100 cases have been reported; however, it is likely that KBG syndrome is underdiagnosed due to lack of comprehensive characterization of the heterogeneous phenotypic features. We described the clinical manifestations in a male referred at 11 years of age, who exhibited symptoms including epilepsy, developmental delay, distinct facial features and hand anomalies, without positive genetic diagnosis. Subsequent exome sequencing identified a novel *de novo* heterozygous single base pair insertion (c.6015dupA) in *ANKRD11* which was validated by Sanger sequencing. We predicted that this insertion leads to a premature stop codon and loss of function in *ANKRD11,* thereby implicating it as contributing to the proband's symptoms and yielding a molecular diagnosis of KBG syndrome for the case.

SCN8A Mutation in Child Presenting with Seizures and Developmental Delays, with Janet Malcolmson, Robert Kleyner, David Tegay, Whit Adams (Utah), Kenneth Ward (Utah), Justine Coppinger (Utah), Lesa Nelson (Utah), Kai Wang (California), Reid Robison (Utah).

The *SCN8A* gene encodes the Nav1.6 neuronal voltage-gated sodium channel alpha subunit. Mutations in this gene have been associated with early infantile epileptic encephalopathy type 13. With the use of whole exome sequencing, a missense mutation was identified in a 4-year-old female who initially exhibited symptoms at the age of 5-months, after she received routine vaccinations. Determining the molecular etiology of this proband's epileptic encephalopathy improved her management.

Expanding collection and sequencing of other rare genetic syndromes, with Yiyang Wu, Han Fang, Reid Robison (Utah), Kai Wang (California) Alan Rope (Oregon), and others.

We continue to meet and collect many families in Utah and elsewhere with very rare, idiopathic genetic syndromes. The total number of DNA samples collected to date is approaching 2000, and this includes detailed phenotyping information. We have been making extensive use of Human Phenotype Ontology terms, and the PI was an author on a review concerning the current progress with the development and integration of HPO in various research settings. We also participated in writing a review concerning the indel-calling algorithm, Scalpel. The PI also participated in the long-read sequencing of a Chinese genome, along with reporting a novel mutation in a case of Ehlers-Danlos.

Collaborating on genetics of Tourette Syndrome, with the Tourette Syndrome Association International Consortium for Genetics.

The PI continues to collaborate on this international effort to understand the genetics of Tourette Syndrome. Psychiatric comorbidity is common in Tourette syndrome (TS); when present, these conditions typically cause more distress and impairment than do tics. High rates of attention-deficit/hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD) are well documented and thought to be core components of the TS phenotype; however, few studies have fully characterized other comorbidities. We therefore continue to characterize the prevalence and impact of psychiatric comorbidity in a large sample of individuals with TS and their family members.

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