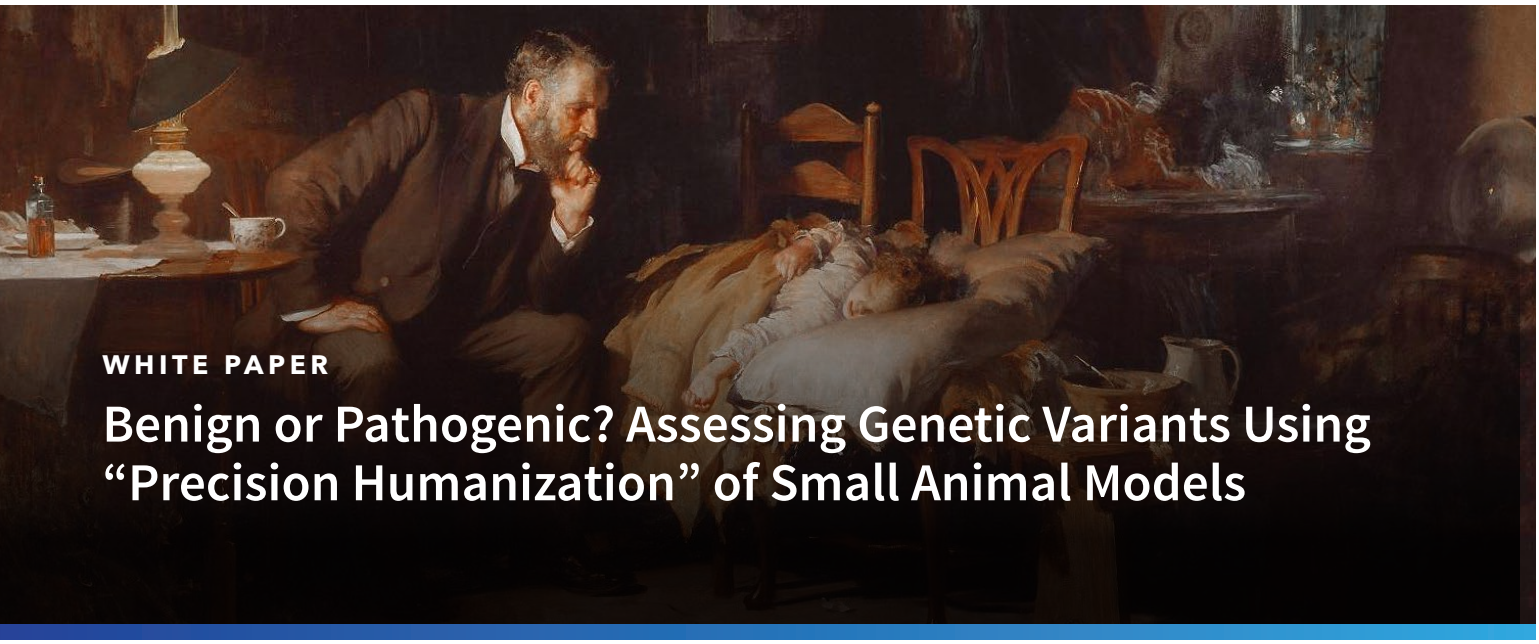
NemaMetrix

Connect genotypes to phenotypes. Decode human health.



WHITE PAPER

Benign or Pathogenic? Assessing Genetic Variants Using "Precision Humanization" of Small Animal Models

The age of genomic and precision medicine is revolutionizing medical practices, but it is also revealing gaps in our knowledge of genetics. Each individual's genome harbors a significant number of unique alleles that may impact the individual's health in unpredictable ways. These are the clinical Variants of Uncertain (or Unknown) Significance (VUS). How do the physician and clinical geneticist make decisions in this noisy background when trying to determine whether the gene mutation seen in the patient is benign or pathogenic?

The Need for Alternative Approaches to Assess Clinical Variants

DOCTORS DILEMMA

Genome data reveals many genes suspected for involvement in a patient's disease contain high loads of VUS alleles. These are the clinical variants in patient DNA that are suspected to alter gene function and cause disease. Yet, many of these variations may actually be benign and harmless. To the physician's chagrin, the presence of VUS alleles in a patient population clouds the ability of the clinical geneticist to make clear diagnostic recommendations.

THE PREVALENCE OF VUS ALLELES

As genomic data accumulates, sequence analysis is revealing that each person has about 500 sequence variants, including missense or indel mutations, in the coding regions of their genome. It is estimated that as many as 25% of the genes in the human genome may be involved in disease biology, so any one individual may harbor over 100 codon-changing alleles in their important "disease" genes.

Surprisingly, frameshifting indels account for nearly 7% of these variants and are quite likely to be pathogenic. As a result, a significant number of questionable alleles are part of the background of anyone's personal genome.

DISCOVERY OF SIGNIFICANCE

A variety of bioinformatics approaches can elucidate the effect of suspect alleles. Tracking a suspect allele in a large family pedigree can be used to show linkage of a suspect allele to disease outcome. Another approach is to explore gene similarity across multiple species. At the sequence level, duplicated genes, and genes from other species, are aligned and scored for their levels of conservation of the amino acid sequence.

ClinVar, a database supported by the US Federal Government, archives and makes accessible known relationships between human variations and phenotypic outcomes of those variations (www.ncbi.nlm.nih.gov/clinvar/).

There are five ClinVar categories of clinical variant activity:

(1) Pathogenic

(2) Likely Pathogenic

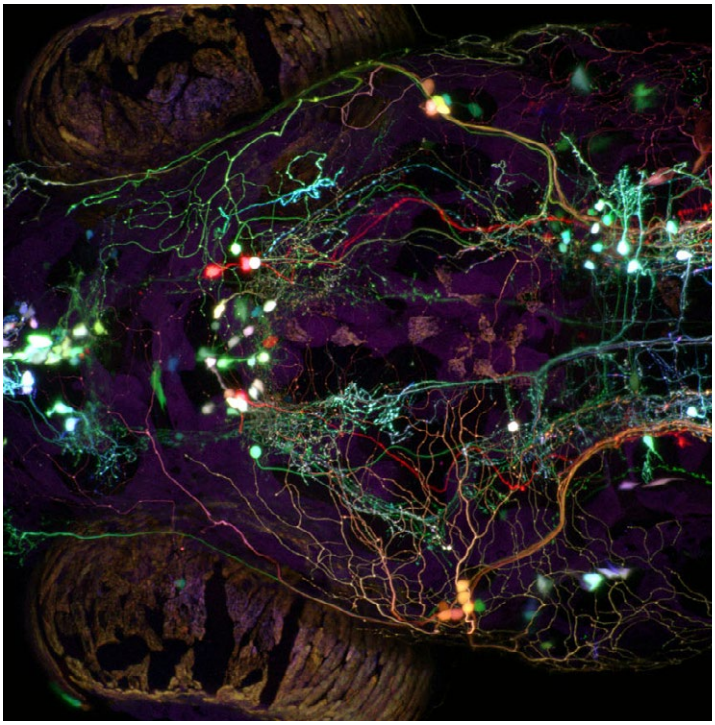
(3) Uncertain Significance

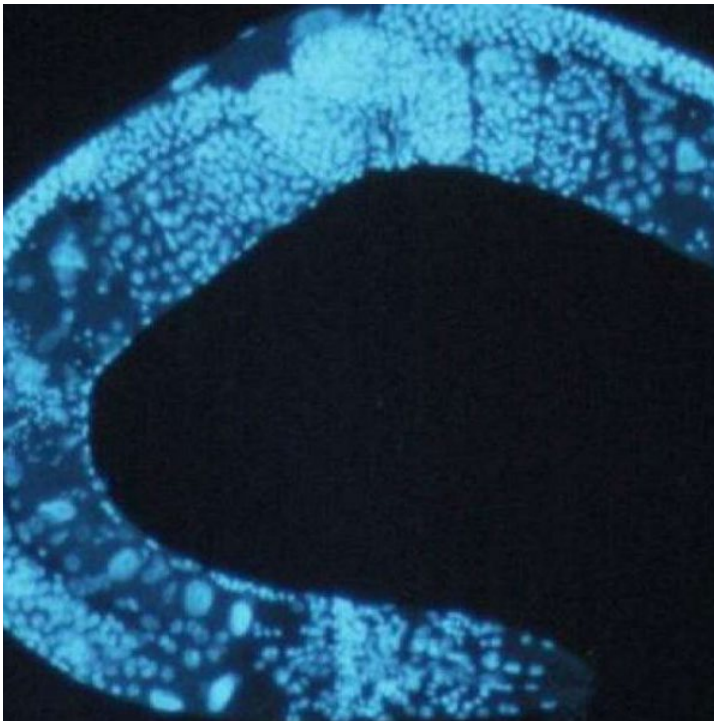
(4) Likely Benign

(5) Benign

Clinical variants in conserved regions of a gene become suspect for possible pathogenicity and are typically categorized in either the first or second ClinVar category. However, many clinical variants occur in non-conserved sequences, which leaves about ¼ of a patient's clinical variants categorized as Variant with Uncertain Significance. These Variants of Uncertain Significance, or VUS alleles, leave health care providers at a loss for how to treat these patients and in need of the tools to shift the assignment towards either pathogenic or benign status.

The Future of Screening Clinical Variants Using Small Animal Models





The ability to quickly install variant alleles in small animals and quantitatively measure the variant phenotypes provides clinicians and researchers a path to rapidly address the consequence of gene variation in their specific patient populations. Animal models with human clinical variants installed are a precision medicine approach for answering questions of disease biology. When applied in the clinic, this type of animal model screening platform is able to:

- Detect the presence of pathogenicity in clinical variants
- Create a high-throughput platform for drug discovery
- Uncover the treatment options that lead to better patient outcomes

While there are many animal models to choose from, we have chosen to work in zebrafish and *C. elegans* because:

1. Both are well established models of human disease
2. Both zebrafish and *C. elegans* are easy to genetically manipulate using CRISPR
3. Cross-species validation of the pathogenicity of genetic variants can be easily accomplished by exploiting the strengths of each organism
   a. **Zebrafish** – as a vertebrate model, zebrafish has organ systems that are identical to humans, has gene counterparts for more than 80% of human diseases, and is known to recapitulate phenotypes of human diseases.
   b. ***C. elegans*** – as a simple organism with strong genetic homology to humans, *C. elegans* can be quickly and inexpensively genetically altered to investigate the impacts of large numbers of patient specific variant alleles.

Cross-species validation of the same human mutation installed in the zebrafish and *C. elegans* genome provides greater clarity of the phenotypic consequence of an installed clinical variant.

New advances in genome engineering and phenotypic assessment are making it possible to go confidently from identification of a novel patient allele to a ClinVar assignment of variant activity with speed and ease using *in vitro* functional data.

Steps for using *in vitro* models for Functional Analysis of Variant Activity

1. Confirm gene has impact on the disease pathway in zebrafish and *C. elegans*

- Loss-of-function (LOF): Knock-outs of genes of interest are made in both zebrafish and *C. elegans* to confirm genes whose activity is necessary for health (loss-of-function pathogenic alleles).
- Gain-of-function (GOF): Knock-ins of a known pathogenic GOF allele into *C. elegans* genome are made to confirm genes whose overactivity is sufficient to cause pathogenicity.

2. Create Humanized Model: Confirm Canonical Sequence is Functionally Benign

- Knock-ins of the canonical human gene, which can be easily created in *C. elegans*, are made to demonstrate restoration of function. If the human gene restores normal function in the *C. elegans* knock-out model, we have now created a humanized system for installing and assessing unique clinical variants of interest.

3. Assess pathogenicity of VUS alleles in native *C. elegans* genome and Precision Humanization System

Individual alleles can be most easily manipulated in *C. elegans* to determine their specific effects. There are two different paths to understanding each variant.

- If a gene homolog exists, introduce the unique human point mutation directly in the homologous *C. elegans* sequence.
- Using a "precision humanization" system of the *C. elegans* worm, install the variant of interest in the installed human gene.

"Precision Humanization" of animal models and the resulting quantitative phenotypic data will become a powerful tool to drive translational science to the next level and further advance personalized medicine.

About NemaMetrix

Our mission is to enable scientists and researchers around the world to better understand human health and explore potential treatments for high-impact disorders such as Alzheimer's disease, epilepsy and cancer by offering a more affordable and accessible platform.

We develop solutions for the genetic modification of simple microscopic living animals to act as human proxies for disease research and drug discovery and help our customers reveal new insights into the true nature of genetic mutations.

Contact us at:
www.nemametrix.com/product-inquiries/

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