

ARTICLES

nature
genetics

Exome sequencing identifies the cause of a mendelian disorder

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We demonstrate the first successful application of exome sequencing to discover the gene for a rare mendelian disorder of unknown cause, Miller syndrome (MIM%263750). For four affected individuals in three independent kindreds, we captured and sequenced coding regions to a mean coverage of 40× and sufficient depth to call variants at ~97% of each targeted exome. Filtering against public SNP databases and eight HapMap exomes for genes with two previously unknown variants in each of the four individuals identified a single candidate gene, *DHODH*, which encodes a key enzyme in the pyrimidine *de novo* biosynthesis pathway. Sanger sequencing confirmed the presence of *DHODH* mutations in three additional families with Miller syndrome. Exome sequencing of a small number of unrelated affected individuals is a powerful, efficient strategy for identifying the genes underlying rare mendelian disorders and will likely transform the genetic analysis of monogenic traits.

Andres Veidenberg 9.03.2010

THE PROBLEM

- Rare variants may have a greater effect than common ones
- Full genome sequencing too expensive
- coding mutations vs. non-coding mutations

IN A NUTSHELL

- Capture and sequence the exomes of controls & cases
- Align exomes with reference genome to find all variants
- Filter out common variation
- Pick the gene(s) which have mutations in all cases

PROOF OF CONCEPT

nature

Vol 461 | 10 September 2009 | doi:10.1038/nature08250

LETTERS

Targeted capture and massively parallel sequencing of 12 human exomes

Sarah B. Ng¹, Emily H. Turner¹, Peggy D. Robertson¹, Steven D. Flygare¹, Abigail W. Bigham², Choli Lee¹, Tristan Shaffer¹, Michelle Wong¹, Arindam Bhattacharjee⁴, Evan E. Eichler^{1,3}, Michael Bamshad², Deborah A. Nickerson¹ & Jay Shendure¹

- Authors pinned down the (pre-known) causative gene of a dominantly inherited disorder (Freeman–Sheldon syndrome)

METHODS

- 10 mg of DNA from blood lymphocytes
- construction of shotgun sequencing library for Illumina Genome Analyzer II
- hybridization to 2x244K custom Agilent arrays
- massively parallel exome sequencing with Illumina GAI

SUBJECTS

- 4 individuals with Miller syndrome (2 related, 2 unrelated)



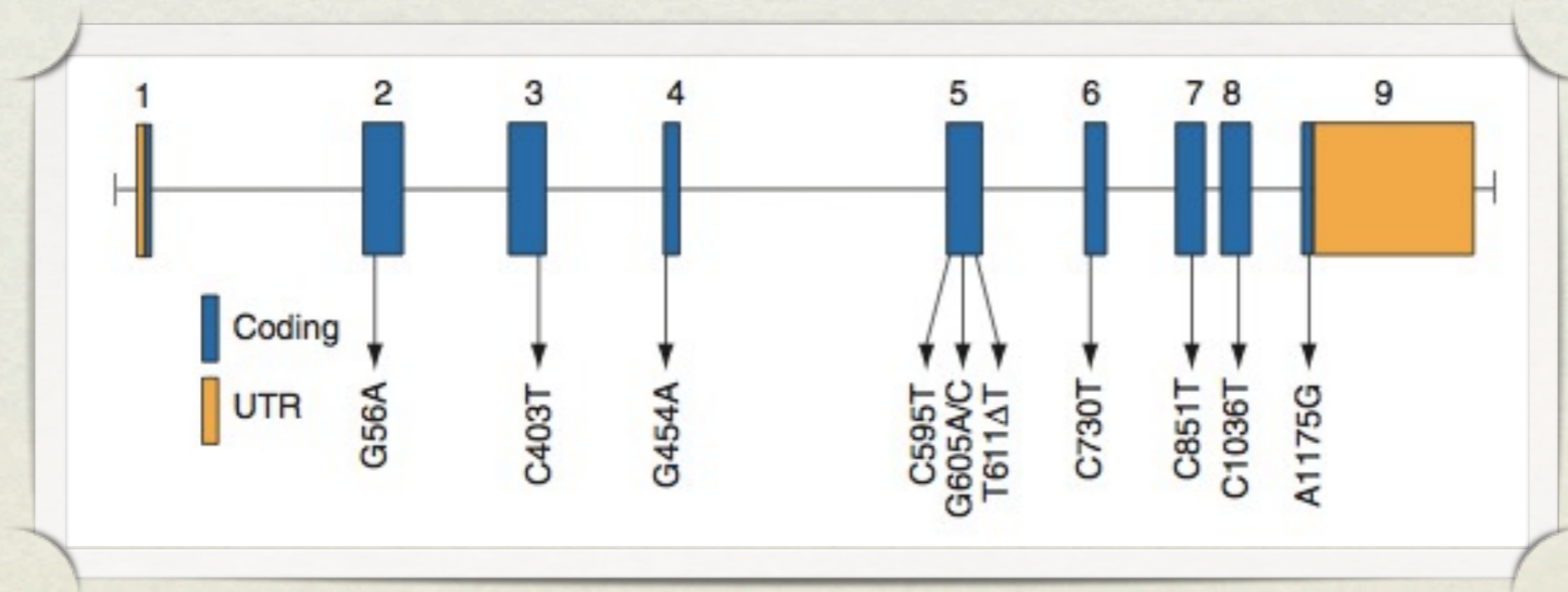
- 8 HapMap individuals from 4 populations

FILTERING OUT GENES

- Include only genes with potentially pathogenic mutations
 - non-synonymous variants, splice acceptor/donor site mutations, short coding insertions/deletions
- dominant model - 228 candidate genes
- recessive model - 9 candidate genes
- exclusion of common variants - 8 candidates / 1 candidate
 - filter with dbSNP database
 - filter with 8 HapMap individual exome sequences
 - comparison with 2 unrelated affected individuals

DHODH

- enzyme dihydroorotate dehydrogenase
- 9 exons (11 mutations in affected families)
- causative mechanism unclear



DISCUSSION

- Sequencing the exomes of few affected individuals with appropriate filtering is sufficient to identify a single candidate gene for rare monogenic disorder
- Several factors are important for a success
 - rare disorder -> mutation not found in dbSNP
 - it's easier to find genes for recessive disease
 - genetic heterogeneity will reduce power

THANKS!