Translating Tech-Knowledge into Patient Care in the Plain Community

--- from a low-tech country clinic to a molecular diagnostic center ---

Baozhong Xin, PhD
CME Conference
8/15/2014
DDC Clinic for Special Needs Children (2005)

1700-square foot facility
Plain Technology in a Cyber World
Austrian Monk grew garden peas in his backyard
Mendelian inheritance

Mendel's Laws of Inheritance

1. Law of Segregation
2. Law of Independent Assortment
3. Law of Dominance

Gregor Mendel (1822-1884)
Father of Genetics
List of Genetic and Metabolic Diseases in DDC Clinic (2005)

Disease

- Adenylosuccinase (adenylosuccinase lyase, ADSL) deficiency
- Alagille syndrome
- Amish albinism
- Amish brittle hair syndrome
- Autism spectrum disorders
- Beal’s syndrome
- Byler disease
- Cartilage-hair hypoplasia dwarfism
- Celiac disease
- Cerebral palsy with late onset IgA nephropathy (?)
- Chicken breast disease (Amish nemaline myopathy)
- Chromosome 1p duplication
- Cockayne syndrome
- Cohen syndrome
- Cortical dysplasia and focal epilepsy syndrome
- Crigler-Najjar syndrome Type I
- Down syndrome
- Duchenne muscular dystrophy
- Familial cleft lip with or without cleft palate (?)
- Familial craniosynostosis (?)
- Familial deafness (?)
- Familial seizure with mental retardation (?)
- Gangliosidosis GM2 synthase (alpha 2,3-sialyltransferase) deficiency
- Glucose/galactose malabsorption
- Glutaric acidemia I (glutaryl-CoA dehydrogenase deficiency)
- Hemophilia B – Factor IX deficiency
- Hypertrophic cardiomyopathy (?)
- Hypotonia, ataxia and developmental delay (?)
- Hypotonia, excessive height, pectus excavatum & mental retardation (?)
- Hyperglycemia – lipoprotein lipase deficiency or apolipoprotein C-II deficiency
- Infantile lethal cardiomyopathy (?)
- Juvenile glaucoma, failure to thrive and leukodystrophy (?)
- Leigh syndrome
- Lesch-Nyhan syndrome
- Maple syrup urine disease
- Metachromatic leukodystrophy
- Microcephalic osteodysplastic primordial dwarfism, Type I
- Mitochondrial respiratory chain complex IV deficiency
- Phenylketonuria (PKU) – phenylalanine hydroxylase deficiency
- Prolidase deficiency
- Propionic acidemia – propionyl-CoA carboxylase deficiency
- Pyruvate kinase deficiency of red cell
- Rett syndrome
- Tarsal coalition
- Troyer syndrome
- Usher syndrome, Type II
Amish Disease?

- Infantile lethal cardiomyopathy (heart baby)
- Hypotonia, excessive height, pectus excavatum & mental retardation
- Short stature, skin frostbite, and development delay
- Cortical dysplasia and focal epilepsy syndrome
- Cerebral palsy with late onset nephropathy
- Chicken breast disease
- Amish brittle hair syndrome
- Familial cleft lip with or without cleft palate
- Familial seizure with mental retardation
- Hypotonia, ataxia and developmental delay
- Juvenile glaucoma, failure to thrive and leukodystrophy
- Microcephalic osteodysplastic primordial dwarfism
Our Vision

The genetic disorders seen are not exclusive to the Amish. These disorders also appear in the general population. We believe that any research obtained from these efforts has the potential to benefit special needs children throughout the world.
Breakthrough Research

- Cerebral vasculopathy is a common hallmark in individuals with SAMHD1 mutations. *Proc Natl Acad Sci USA* 2011 108:E233.
- Mutations in U4atac snRNA, a component of the minor spliceosome, in the developmental disorder MOPD I. *Science* 2011 332:238-240.
- Homozygous frameshift mutation in TMCO1 causes a syndrome with craniofacial dysmorphism, skeletal anomalies, and mental retardation. *Proc Natl Acad Sci USA* 2010 107:258-263.
A Brief History of the Lab

- **2006**: Sanger Sequencing
- **2010**: Next-generation Sequencing (NGS)
- **2012**: Chromosomal Microarray Analysis (CMA)
- **2013**: CLIA Certification for high-complexity genetic testing
Molecular Diagnostics Laboratory

New “Toy-land”

“Toy-land” players
Targeted mutation testing by Sanger sequencing

*Sanger sequencing*: the gold standard of DNA sequencing methods

Ideal sequencing technology for rapid and cost-effective mutational analysis in plain populations
## Targeted mutation testing at DDC Clinic

<table>
<thead>
<tr>
<th>Diseases (OMIM #)</th>
<th>Gene symbol</th>
<th>Sequence variant</th>
<th>Genomic coordinate (GRCh37/hg19)</th>
<th>Strand</th>
<th>Ref/Alt</th>
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<tbody>
<tr>
<td>Amish brittle hair syndrome (234050)</td>
<td>MPLKIP (TTDN1)</td>
<td>c.430A&gt;G</td>
<td>chr7:40,172,768-40,172,768</td>
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<td>T/C</td>
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<td>Amish nemaline myopathy (Chicken breast disease) (605355)</td>
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<td>chr35,657,945-35,657,945</td>
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<td>GDAPI</td>
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<td>Cockayne syndrome (216400)</td>
<td>ERCC6</td>
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<td>Cohen syndrome (216550)</td>
<td>VPS13B (COH1)</td>
<td>c.8459T&gt;C + c.9260dupT</td>
<td>chr8:100,830,701-100,830,701</td>
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<td>Cortical dysplasia and focal epilepsy syndrome (610042)</td>
<td>CNTNAP2</td>
<td>c.3709delG</td>
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<td>Crigler-Najjar syndrome Type I (218800)</td>
<td>UGT1A1</td>
<td>c.222C&gt;A</td>
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<td>Ellis-Van Creveld syndrome (225500)</td>
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<td>IVS13+5G&gt;T</td>
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<td>TJP2</td>
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<td>BAAT</td>
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<td>Ganglioside GM3 synthase deficiency (609056)</td>
<td>ST3GAL5 (SIAT9)</td>
<td>c.694C&gt;T</td>
<td>chr2:286,071,665-286,071,665</td>
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<td>Gaucher disease (231000)</td>
<td>GBA</td>
<td>c.1226A&gt;G</td>
<td>chr1:155,205,634-155,205,634</td>
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<td>Glucose/galactose malabsorption (606824)</td>
<td>SLC5A1(SGLT1)</td>
<td>c.1673G&gt;A</td>
<td>chr22:32,500,780-32,500,780</td>
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<td>Glutaric acidemia type I (231670)</td>
<td>GCDH</td>
<td>c.1262C&gt;T</td>
<td>chr12:19,010,300-12,10,300</td>
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<td>Hallervorden-Spatz syndrome</td>
<td>PANK2</td>
<td>c.927_933del</td>
<td>chr20:3,888,871-3,888,877</td>
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<td>Hemophilia B – Factor IX deficiency (306900)</td>
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<td>c.1025C&gt;T</td>
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<td>HERC2</td>
<td>c.1781C&gt;T</td>
<td>chr1:15,28,510,853-28,510,853</td>
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<td>Homocystinuria due to MTHFR deficiency</td>
<td>MTHFR</td>
<td>c.1129C&gt;T</td>
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<td>Hypertrophic cardiomyopathy (115197)</td>
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<td>IVS3+2T&gt;G</td>
<td>chr11:47,354,743-47,354,743</td>
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<tr>
<td>ITCH deficiency</td>
<td>ITCH</td>
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<td>chr2:23,301,001-23,301,001</td>
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<td>A/AA</td>
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<td>Limb-girdle muscular dystrophy type 2A (LGMD2A)</td>
<td>CAPN3</td>
<td>c.2306G&gt;A</td>
<td>chr15:42,703,124-42,703,124</td>
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<td>G/A</td>
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<td>Maple syrup urine disease (248600)</td>
<td>BCKDHA</td>
<td>c.1312T&gt;A</td>
<td>chr19:41,930,471-41,930,471</td>
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<td>Mast syndrome (248900)</td>
<td>SPG21</td>
<td>c.601dupA</td>
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<td>T/TT</td>
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<td>McKusick-Kaufman syndrome (236700)</td>
<td>MKKS</td>
<td>c.250C&gt;T + c.724G&gt;T</td>
<td>chr20:10,393,913-10,393,913</td>
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<td>Microcephalic osteodysplastic primordial dwarfism type 1 (MOPD RNU4ATAC)</td>
<td>GCATG</td>
<td>g.51G&gt;A</td>
<td>chr2:122,288,506-122,288,506</td>
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<td>G/A</td>
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<td>Osteopetrosis</td>
<td>TCIRG1</td>
<td>c.1228G&gt;A</td>
<td>chr11:67,814,962-67,814,962</td>
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<td>Phenylketonuria (PKU) (261600)</td>
<td>PAH</td>
<td>IVS10-11G&gt;A</td>
<td>chr12:103,237,568-103,237,568</td>
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<td>Primary ciliary dyskinesia</td>
<td>DNAH5</td>
<td>c.4348C&gt;T</td>
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<td>Prolidase deficiency (170100)</td>
<td>PEPD</td>
<td>c.793C&gt;T</td>
<td>chr19:33,902,603-33,902,603</td>
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<td>Propionic acidemia (606054)</td>
<td>PCCB</td>
<td>c.1606A&gt;G</td>
<td>chr3:136,048,854-136,048,854</td>
<td>plus</td>
<td>A/G</td>
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<td>Pyruvate kinase deficiency of red cell (266200)</td>
<td>PKLR</td>
<td>c.1436 G&gt;A</td>
<td>chr1:155,262,968-155,262,968</td>
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<td>C/T</td>
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<td>SAMS Association</td>
<td>SAMHD1</td>
<td>c.1411-2A&gt;G</td>
<td>chr20:3,532,654-35,532,654</td>
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<td>T/C</td>
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<td>Spastic ataxia (613672)</td>
<td>MTPAP</td>
<td>c.1432A&gt;G</td>
<td>chr10:30,602,855-30,602,855</td>
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<td>T/C</td>
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<td>chr1:169,519,049-169,519,049</td>
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<td>C/T</td>
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<td>TMCO1 defect syndrome</td>
<td>TMCO1</td>
<td>c.139_140delAG</td>
<td>chr1:165,737,437-165,737,438</td>
<td>minus</td>
<td>CT/--</td>
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<td>Troyer syndrome (275900)</td>
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<td>c.1110delA</td>
<td>chr13:36,903,553-36,903,553</td>
<td>minus</td>
<td>T/-</td>
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<td>Yoder dystonia</td>
<td>WDR73</td>
<td>c.888delT</td>
<td>chr15:85,186,950-85,186,950</td>
<td>minus</td>
<td>A/-</td>
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</tbody>
</table>
On-site genetic analyzer

ABI Prism 310

ABI Prism 3100
Next-Gen Sequencing (NGS)

- Massively Parallel Sequencing Technology:
  - gDNA Frag. Target Enrichment Sequencing

- High throughput: gene panel, exome, and genome

- Rapid: 2 days~1 week

- Low cost: $10,000/genome
New challenges in sequence variants interpretation

- Mutation
- Polymorphism (SNP)

- Pathogenic
- Likely pathogenic
- Variant of unknown significance (VOUS)
- Likely Benign
- Benign

The $1,000 Genome, the $1 Million Interpretation?
MiSeq Personal Sequencer

First and Only FDA-Cleared IVD NGS System

An ideal NGS platform for rapid, cost-effective genetic analysis
MiSeq Personal Sequencer

- Single gene sequencing analysis

- Gene panels
  - Cardiomyopathy panel (46 genes)
  - Autism Spectrum disorder panel (101 genes)
  - Pediatric recessive disease panel (552 genes)

- Clinically relevant genes panel
TruSight One Sequencing Panel

- Targets 4813 genes associated to a clinical phenotype
- Covers the most commonly ordered molecular assays
- Genomic targets were identified based on information:
  1. Human Gene Mutation Database (HGMD)
  2. Online Mendelian Inheritance in Man (OMIM)
- From DNA sample to report within a week
Two genetic disorders identified on MiSeq

GGM: SLC5A1 c.1673G>A

CH: VPS13B c.[8459T>C; 9260dupT]
### Two genetic disorders identified on MiSeq

- **Cockayne syndrome**: ERCC6 c.2709+1G>T
- **Propionic acidemia**: PCCB c.1606A>G

![Gene View](image)
Cohen syndrome gene testing for a non-Amish patient

Cohen syndrome: VPS13B c.[7751T>C]; [8515C>T]

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Chr</th>
<th>Coordinate</th>
<th>Exon</th>
<th>Intron</th>
<th>HGVSc</th>
<th>HGVSp</th>
<th>Consequence</th>
<th>Genotype</th>
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<td>VPS13B</td>
<td>T&gt;C</td>
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<td>8/61</td>
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<td>NM_017890.4:c.1206+33T&gt;G</td>
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</table>
Chromosomal microarray analysis (CMA)

“CMA technology is now recommended as a first-tier test for detection of gains and losses of genomic DNA associated with intellectual disability, autism, and multiple congenital anomalies.”

— ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis

*Genetics in Medicine* 2013; 15:901-909
GeneChip Microarray Platform

First and Only FDA-cleared IVD Microarray System
The CytoScan HD Array

- Contains more than 2.6 million markers for copy number analysis and approximately 750,000 SNP markers for fully genotyping

- High-resolution genome-wide analysis to detect:
  - DNA copy number gains and losses
  - Loss of heterozygosity (LOH)
  - Uniparental disomy (UPD)
  - Regions identical-by-descent (IBD)
Multiple regions of long stretches of homozygosity
arr(21)x3  single copy gain (trisomy) of chromosome 21
arr 22q13.31(44,091,212-45,173,040)x1 (chr22 1.1Mb deletion)
arr Xp21.1(31,889,056-31,987,608)x0  99kb hemizygous loss on chrX
Molecular diagnostic testing at DDC Clinic

- **Sanger Sequencing**
  - Population-specific targeted mutation analysis

- **Next-generation sequencing**
  - Gene-specific mutational analysis
  - Disease spectrum gene panels
  - Medically relevant genes screening

- **Chromosomal microarray analysis**
  - Genome-wide DNA copy number changes
  - Genotyping
“For 28 years, we didn’t know what was wrong with our daughter. Imagine living all those years with unanswered questions; imagine not knowing what was wrong with your child. Having a diagnosis, knowing what’s wrong, is half the battle. Knowing helps you live with it. Now maybe there is something that can be done in the future to help children with this disorder.”

- The mother of a daughter who passed away from this disease described her excitement about the clinic’s findings
The Journey of Hope

From the Buggy to the Byte
“Special children are not just interesting medical problems, subjects of grants and research. Nor should they be called burdens to their families and communities. They are children who need our help, and if we allow them to, they will teach us compassion. They are children who need our help, and if we allow them to, they will teach us love. If we come to know these children as we should, they will make us better scientists, better physicians, and thoughtful people.”

Dr. Holmes Morton
Co-founder
Clinic for Special Children
The Journey of Hope

“We love these children.”
“我们舍不得这些孩子”

Dr. Heng Wang
Medical Director
DDC Clinic
Acknowledgements

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  - Cleveland State University
  - University Hospitals of Cleveland
  - Clinic for Special Children
  - National Institutes of Health (NIH)
  - University of California, Los Angeles (UCLA)
  - University Hospitals of Cleveland
  - Wake Forest University School of Medicine
  - University of London, UK
  - Beijing Tiantan Hospital, China