Molecular Methods in Urology and Nephrology

DR. MAJID KHEIROLLAHI
ASSOCIATE PROFESSOR OF MEDICAL GENETICS
ISFAHAN UNIVERSITY OF MEDICAL SCIENCES
CLASSIFICATION OF CHROMOSOMES
GENE, NUCLEOTIDE AND NUCLEOSIDE

Adenine (A)  Guanine (G)
Cytosine (C)  Thymine (T)  Uracil (U)

Adenosine  Adenosine 5’-monophosphate (AMP)  2’-Deoxyadenosine 5’-triphosphate (dATP)

Figure 1.4: A 3’-5’ phosphodiester bond.
DNA double helix
DNA replication
CHROMOSOME STRUCTURE
<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Genes</th>
<th>Total base pairs</th>
<th>% of bases</th>
<th>Sequenced base pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2000</td>
<td>247,199,719</td>
<td>8.0</td>
<td>224,999,719</td>
</tr>
<tr>
<td>2</td>
<td>1300</td>
<td>242,751,149</td>
<td>7.9</td>
<td>237,712,849</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>199,446,827</td>
<td>6.5</td>
<td>194,704,827</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>191,263,063</td>
<td>6.2</td>
<td>187,297,063</td>
</tr>
<tr>
<td>5</td>
<td>900</td>
<td>180,837,866</td>
<td>5.9</td>
<td>177,702,766</td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
<td>170,896,993</td>
<td>5.5</td>
<td>167,273,993</td>
</tr>
<tr>
<td>7</td>
<td>900</td>
<td>158,821,424</td>
<td>5.2</td>
<td>154,952,424</td>
</tr>
<tr>
<td>8</td>
<td>700</td>
<td>146,274,826</td>
<td>4.7</td>
<td>142,612,826</td>
</tr>
<tr>
<td>9</td>
<td>800</td>
<td>140,442,295</td>
<td>4.6</td>
<td>139,312,298</td>
</tr>
<tr>
<td>10</td>
<td>700</td>
<td>135,374,737</td>
<td>4.4</td>
<td>131,624,737</td>
</tr>
<tr>
<td>11</td>
<td>1300</td>
<td>134,452,384</td>
<td>4.4</td>
<td>131,130,853</td>
</tr>
<tr>
<td>12</td>
<td>1100</td>
<td>132,289,534</td>
<td>4.3</td>
<td>130,303,534</td>
</tr>
<tr>
<td>13</td>
<td>300</td>
<td>114,127,980</td>
<td>3.7</td>
<td>95,569,980</td>
</tr>
<tr>
<td>14</td>
<td>800</td>
<td>106,360,585</td>
<td>3.5</td>
<td>88,290,585</td>
</tr>
<tr>
<td>15</td>
<td>600</td>
<td>100,338,915</td>
<td>3.3</td>
<td>81,341,915</td>
</tr>
<tr>
<td>16</td>
<td>800</td>
<td>86,822,254</td>
<td>2.9</td>
<td>78,884,754</td>
</tr>
<tr>
<td>17</td>
<td>1200</td>
<td>78,654,742</td>
<td>2.6</td>
<td>77,800,220</td>
</tr>
<tr>
<td>18</td>
<td>200</td>
<td>76,117,153</td>
<td>2.5</td>
<td>74,656,155</td>
</tr>
<tr>
<td>19</td>
<td>1500</td>
<td>63,806,651</td>
<td>2.1</td>
<td>55,785,651</td>
</tr>
<tr>
<td>20</td>
<td>500</td>
<td>62,435,965</td>
<td>2.0</td>
<td>59,505,254</td>
</tr>
<tr>
<td>21</td>
<td>200</td>
<td>46,944,323</td>
<td>1.5</td>
<td>34,171,998</td>
</tr>
<tr>
<td>22</td>
<td>500</td>
<td>49,628,953</td>
<td>1.6</td>
<td>34,893,953</td>
</tr>
<tr>
<td>X (sex chromosome)</td>
<td>800</td>
<td>154,913,754</td>
<td>5.0</td>
<td>151,058,754</td>
</tr>
<tr>
<td>Y (sex chromosome)</td>
<td>50</td>
<td>57,741,652</td>
<td>1.9</td>
<td>25,121,652</td>
</tr>
<tr>
<td>Total</td>
<td>21,000</td>
<td>3,079,043,747</td>
<td>100.0</td>
<td>2,857,698,560</td>
</tr>
</tbody>
</table>
Base pairs of Genes
GENE STRUCTURE
REGULATION OF GENE EXPRESSION
Transcription, post-transcriptional processing, translation, and post-translational processing
ALLELLE AND LOCUS
GENETIC DISORDERS

- SINGLE-GENE DISORDER
- CHROMOSOME ABNORMALITY
- MULTIFACTORIAL DISORDER
<table>
<thead>
<tr>
<th>MIM</th>
<th>Name of Disease</th>
<th>Inheritance</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>263100</td>
<td>POLYCYSTIC KIDNEY, CATARACT, AND CONGENITAL BLINDNESS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>600273</td>
<td>POLYCYSTIC KIDNEY DISEASE, INFANTILE SEVERE, WITH TUBEROUS SCLEROSIS; PKDS</td>
<td>AD</td>
<td>-</td>
</tr>
<tr>
<td>600666</td>
<td>POLYCYSTIC KIDNEY DISEASE 3 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD3</td>
<td>AD</td>
<td>GANAB</td>
</tr>
<tr>
<td>618061</td>
<td>POLYCYSTIC KIDNEY DISEASE 6 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD6</td>
<td>AD</td>
<td>DNAJ811</td>
</tr>
<tr>
<td>617874</td>
<td>POLYCYSTIC LIVER DISEASE 3 WITH OR WITHOUT KIDNEY CYSTS; PCLD3</td>
<td>AD</td>
<td>ALG8</td>
</tr>
<tr>
<td>617610</td>
<td>POLYCYSTIC KIDNEY DISEASE 5; PKD5</td>
<td>AR</td>
<td>DZIP1L</td>
</tr>
<tr>
<td>617875</td>
<td>POLYCYSTIC LIVER DISEASE 4 WITH OR WITHOUT KIDNEY CYSTS; PCLD4</td>
<td>AD</td>
<td>LRP5</td>
</tr>
<tr>
<td>617004</td>
<td>POLYCYSTIC LIVER DISEASE 2 WITH OR WITHOUT KIDNEY CYSTS; PCLD2</td>
<td>AD</td>
<td>SEC63</td>
</tr>
<tr>
<td>263210</td>
<td>GILLESSEN-KAESBACH-NISHIMURA SYNDROME; GIKANIS</td>
<td>AR</td>
<td>ALG9</td>
</tr>
<tr>
<td>173900</td>
<td>POLYCYSTIC KIDNEY DISEASE 1 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD1</td>
<td>AD</td>
<td>PKD1</td>
</tr>
<tr>
<td>613095</td>
<td>POLYCYSTIC KIDNEY DISEASE 2 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD2</td>
<td>AD</td>
<td>PKD2</td>
</tr>
<tr>
<td>606702</td>
<td>PKHD1 GENE; PKHD1 FIBROCYSTIN; FCYT POLYDUCTIN POLYCYSTIC KIDNEY AND HEPATIC DISEASE 1 GENE</td>
<td>AR</td>
<td>-</td>
</tr>
<tr>
<td>263200</td>
<td>POLYCYSTIC KIDNEY DISEASE 4 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD4</td>
<td>AR</td>
<td>FCYT</td>
</tr>
<tr>
<td>174000</td>
<td>MEDULLARY CYSTIC KIDNEY DISEASE 1; MCKD1</td>
<td>AD</td>
<td>MUC1</td>
</tr>
<tr>
<td>102500</td>
<td>HAJDU-CHENEY SYNDROME; HJCYS SERPENTINE FIBULA-POLYCYSTIC KIDNEY SYNDROME; SFPKS</td>
<td>AD</td>
<td>NOTCH2</td>
</tr>
<tr>
<td>609886</td>
<td>GLOMERULOCYSTIC KIDNEY DISEASE WITH HYPERURICEMIA AND ISOSTHENURIA</td>
<td>-</td>
<td>UMOD</td>
</tr>
</tbody>
</table>
Polycystic kidney disease

- PKD is a genetic disorder in which the renal tubules become structurally abnormal, resulting in the development and growth of multiple cysts within the kidney.

- These cysts may begin to develop in utero, in infancy, in childhood, or in adulthood.

- PKD is caused by abnormal genes which produce a specific abnormal protein which has an adverse effect on tubule development.

- PKD is a general term for two types, each having their own pathology and genetic cause:
  - **Autosomal dominant polycystic kidney disease** (ADPKD)
  - and **autosomal recessive polycystic kidney disease** (ARPKD).
Autosomal dominant polycystic kidney disease (ADPKD) is the most common of all the inherited cystic kidney diseases with an incidence of 1:500 live births.

Studies show that 10% of end-stage kidney disease (ESKD) patients being treated with dialysis in Europe and the U.S. were initially diagnosed and treated for ADPKD.

There are three genetic mutations in the PKD-1, PKD-2, and PKD3 gene with similar phenotypical presentations.
Gene PKD1 is located on chromosome 16 and codes for a protein involved in regulation of cell cycle and intracellular calcium transport in epithelial cells, and is responsible for 85% of the cases of ADPKD.

A group of voltage-linked calcium channels are coded for by PKD2 on chromosome 4.

PKD3 recently appeared in research papers as a postulated third gene. Fewer than 10% of cases of ADPKD appear in non-ADPKD families.
Cyst formation begins in utero from any point along the nephron, although fewer than 5% of nephrons are thought to be involved.

As the cysts accumulate fluid, they enlarge, separate entirely from the nephron, compress the neighboring kidney parenchyma, and progressively compromise kidney function.
Autosomal recessive polycystic kidney disease (ARPKD) (OMIM #263200) is the lesser common of the two types of PKD, with an incidence of 1:20,000 live births.

And is typically identified in the first few weeks after birth.

Unfortunately, the kidneys are often underdeveloped resulting in a 30% death rate in newborns with ARPKD.
Hyperoxaluria is an excessive urinary excretion of oxalate.

Individuals with hyperoxaluria often have calcium oxalate kidney stones.

It is sometimes called Bird's disease, after Golding Bird, who first described the condition.

Types
- Primary hyperoxaluria
- Enteric hyperoxaluria
- Idiopathic hyperoxaluria
- Oxalate poisoning
<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Inheritance</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>259900</td>
<td>HYPEROXALURIA, PRIMARY, TYPE I; HP1</td>
<td>AR</td>
<td>AGXT</td>
</tr>
<tr>
<td></td>
<td>OXALOSIS I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLYCOLIC ACIDURIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALANINE-GLYOXYLATE AMINOTRANSFERASE DEFICIENCY</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEROxisomal ALANINE:GLYOXYLATE AMINOTRANSFERASE DEFICIENCY</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEPATIC AGT DEFICIENCY</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SERINE:PYRUVATE AMINOTRANSFERASE DEFICIENCY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>260000</td>
<td>HYPEROXALURIA, PRIMARY, TYPE II; HP2</td>
<td>AR</td>
<td>GRHPR</td>
</tr>
<tr>
<td></td>
<td>OXALOSIS II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLYCERIC ACIDURIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLYOXYLATE REDUCTASE/HYROXYPYRUVATE REDUCTASE DEFICIENCY</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-GLYCERATE DEHYDROGENASE DEFICIENCY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>613616</td>
<td>HYPEROXALURIA, PRIMARY, TYPE III; HP3</td>
<td>-</td>
<td>HOGA1</td>
</tr>
</tbody>
</table>
Type I (PH1) is associated with AGXT protein, a key enzyme involved in breakdown of oxalate.

PH1 is also an example of a protein mistargeting disease, wherein AGXT shows a trafficking defect: instead of being trafficked to peroxisomes, it is targeted to mitochondria, where it is metabolically deficient despite being catalytically active.

Type II is associated with GRHPR.

It is also a complication of jejunoileal bypass, or in any patient who has lost much of the ileum with an intact colon.

This is due to excessive absorption of oxalate from the colon.
Cystinuria

- **Cystinuria** is an inherited **autosomal recessive disease** that is characterized by high concentrations of the amino acid **cysteine** in the urine, leading to the formation of cystine stones in the kidneys, ureter, and bladder. It is a type of **aminoaciduria**.

- Cystinuria is caused by mutations in the **SLC3A1** and **SLC7A9** genes.

- These defects prevent proper reabsorption of basic, or positively charged, amino acids: **Cysteine**, **lysine**, **ornithine**, **arginine**.

- Under normal circumstances, this protein allows certain amino acids, including cysteine, to be reabsorbed into the blood from the filtered fluid that will become urine.
CYSTINURIA

Alternative titles; symbols

CSNU
CYSTINURIA, TYPE I, FORMERLY; CSNU1, FORMERLY
CYSTINURIA, TYPE II, FORMERLY
CYSTINURIA, TYPE III, FORMERLY; CSNU3, FORMERLY
CYSTINURIA, TYPE NON-I, FORMERLY

Other entities represented in this entry:

CYSTINURIA, TYPE A, INCLUDED
CYSTINURIA, TYPE B, INCLUDED
CYSTINURIA, TYPE A/B, INCLUDED

**Phenotype-Gene Relationships**

<table>
<thead>
<tr>
<th>Location</th>
<th>Phenotype</th>
<th>Phenotype MIM number</th>
<th>Inheritance</th>
<th>Phenotype mapping key</th>
<th>Gene/Locus</th>
<th>Gene/Locus MIM number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2p21</td>
<td>Cystinuria</td>
<td>220100</td>
<td>AR, AD</td>
<td>3</td>
<td>SLC3A1</td>
<td>104614</td>
</tr>
<tr>
<td>19q13.11</td>
<td>Cystinuria</td>
<td>220100</td>
<td>AR, AD</td>
<td>3</td>
<td>SLC7A9</td>
<td>604144</td>
</tr>
</tbody>
</table>
Mutations in either of these genes disrupt the ability of this transporter protein to reabsorb these amino acids, allowing them to become concentrated in the urine.

As the levels of cysteine in the urine increase, it forms cystine crystals, resulting in kidney stones. Cystine crystals form hexagonal-shaped crystals that can be viewed upon microscopic analysis of the urine.

The other amino acids that are not reabsorbed do not create crystals in urine.

The overall prevalence of cystinuria is approximately 1 in 7,000 neonates (from 1 in 2,500 neonates in Libyan Jews to 1 in 100,000 among Swedes).
Male infertility

- Genetic factors cause account for 10–15% of male infertility, including chromosomal aberrations and single gene mutations.
- Klinefelter Syndrome
- Y chromosome deletions
Klinefelter Syndrome

One of the most commonly known causes of infertility is Klinefelter Syndrome, affecting 1 out of 500-1000 newborn males.

Klinefelter Syndrome is a chromosomal defect that occurs during gamete formation due to a non-disjunction error during cell division.

Resulting in males having smaller testes, reducing the amount of testosterone and sperm production.
Y chromosome deletions

- Y chromosomal infertility is a direct cause of male infertility due to its affects on sperm production, occurring in 1 out of every 2000 males.

- Usually affected men show no sign of symptoms other than at times can exhibit smaller testes size.

- Men with this condition can exhibit **azoospermia** (no sperm production), **oligospermia** (small number of sperm production), or they will produce abnormally shaped sperm.

- These individuals are thereby “Y-linked”, although daughters are not affected due to the lack of the Y chromosome.
Microdeletions in the Y chromosome

- Microdeletions in the Y chromosome have been found at a much higher rate in infertile men than in fertile controls and the correlation found may still go up as improved genetic testing techniques for the Y chromosome are developed.

- Much study has been focused upon the "azoospermia factor locus" (AZF), at Yq11.

- A specific partial deletion of AZFc called gr/gr deletion is significantly associated with male infertility among Caucasians in Europe and the Western Pacific region.

- Additional genes associated with spermatogenesis in men and reduced fertility upon Y chromosome deletions include RBM, DAZ, SPGY, and TSPY.
Lab Methods

- Karyotyping
- PCR/Sanger Sequencing
- Deletion/Duplication Analysis
- MLPA
- Next-Gen Sequencing
Karyotyping
PCR and Sanger sequencing
AGXT gene
MLPA

Denaturation and Hybridization

Ligation and Digestion
with methylation-sensitive endonuclease

PCR with universal primers X and Y
exponential amplification of ligated, undigested probes only

Fragment Analysis & Sample Comparison
Whole exome sequencing (NGS)
آزمایش های NGS و پانل موارد زیر مثال هایی از اختلالاتی هستند که به روش NGS و با بررسی تعدادی زن و یا کل زنوم مورد بررسی قرار می‌گیرند.

• سرطان‌ها
• ناتوانی ذهنی و تأخیر تکاملی
• ناشنوایی‌های ارثی
• نابینایی‌های ارثی
• بیماری‌های متابولیک ارثی
• بیماری‌های سیستم اسکلتی و استخوانی
• معلولیت‌های حرکتی
• بیماری‌های سیستم اعصاب مرکزی
• بیماری‌های زننیک‌پوست (زنودرماتوزها)
• بیماری‌های های‌زننیک قلب (آریتمی‌ها و...)
Diagnosis

- Prenatal testing and prenatal expectations
  - Prenatal testing
  - In vitro fertilisation, via preimplantation genetic diagnosis
  - Chorionic villus sampling
  - Amniocentesis
آمنیوسنتز، NIPT و CVS
مشارکت زننیک

مشاوره پیش از ازدواج، پیش از بارداری و حین بارداری
مشاوره موارد مربوط به سطح، تاباری و مرده زایی
انجام تمامی آزمایشات تخصصی زننیک پزشکی
سینوسنثیک و کاربوتیپ
زننیک مولکولی تشخیصی
PND تشخیص پیش از تولد و
NIPT آمیبوستنژی و CVS
آزمایش های NGS و پانل
زننیک سرطان

اسفهان - خیابان شیرینی - بین چهارراه پلیس و حکیم نظامی - پلاک ۲۰۸
۳۵۲۷۰۵۸۷ - ۳۵۲۷۰۵۸۶
۹۱۳۷۲۱۱۱۲
آزمایشگاه زنیتیک پزشکی
شان آزما

مشاوره زنیتیک
مشاوره پیش از زایمان و پزشکی پیش از بارداری
مشاوره موارد مربوط به سفید، نا ایزمار و مرده زایی
آزمایش همه آزمایشات تخصصی زنیتیک پزشکی
آزمایشات تشخیص زنیتیک بر روی بدن ناژادان

فوتبال با علت ناشخص
سندرمژنیتیک کارپوپنیوپ
ژناتیک مولکولی تشخیصی
PND
تشخیص پیش از تولد و NIPPT و CVS
آمینوئستاز
آزمایش های NGS
بنیان
زنیتیک سرطان

موسسه و مستندات
دکتر مهدی پورنژاد

36269587 09137216113 36269586
خبیار شریعتی، بیماری‌های پایین و حکایت نمازی، پلاک ۲۰۸
THANKS