CHROMOSOME IN DIABETES: ROLES AND DETECTION

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Genetic diseases

- Genetic diseases ~ genetic disorder
  - The illness caused by abnormality in gene or chromosome

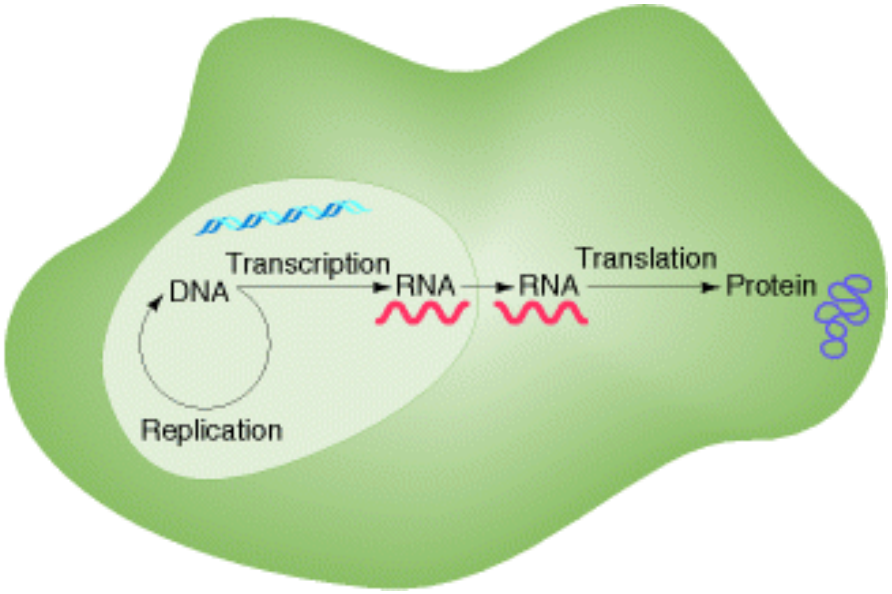
Clinical genetics: **genetic disorders**, **incl.congenital anomalies**

Do: Pre-postnatal dx; presymptomatic dx; genetic counseling; care for patients and their families

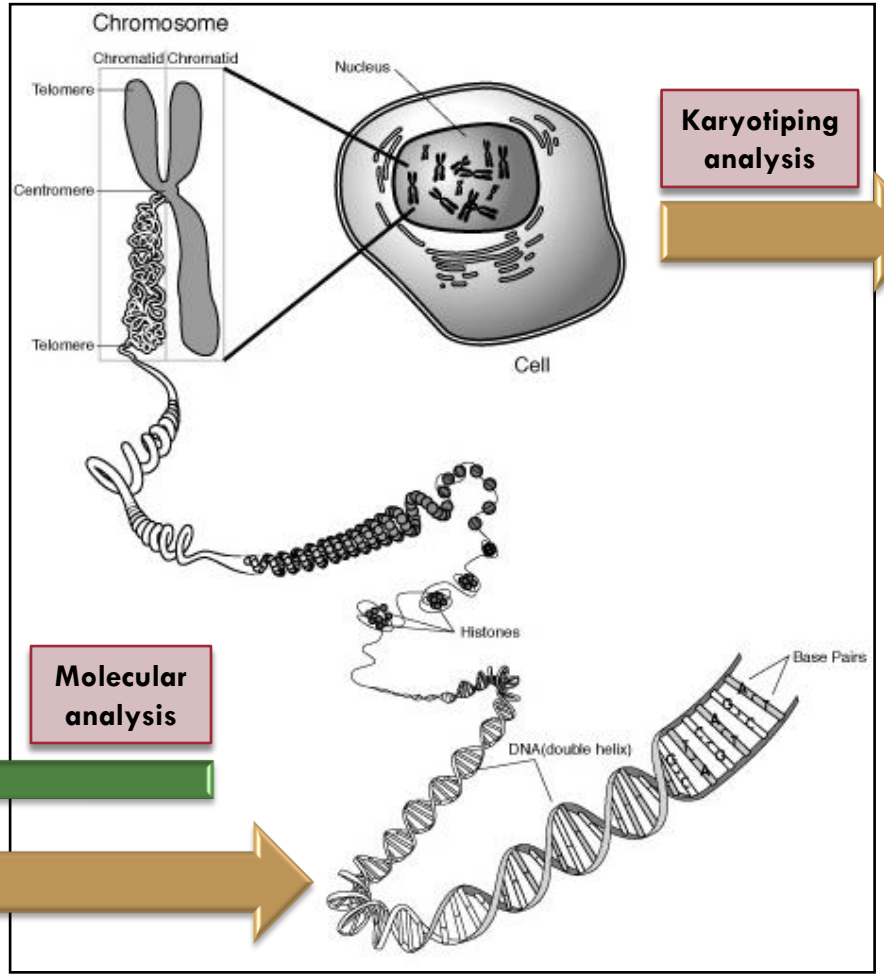
- Causes of genetic disorders
  1. Monogenic/single gene defect
  2. Chromosomal aberation
  3. Multifactorial/polygenic

The central dogma

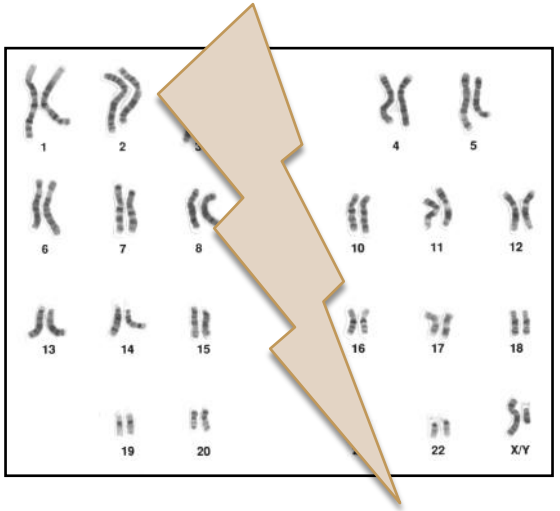
- DNA carries the genetic information of a cell and consists of thousands of genes which serves as a recipe on how to build a protein molecule.
- Proteins perform important tasks for the cell functions.
- The information from the genes determines the protein composition and the functions of the cell.



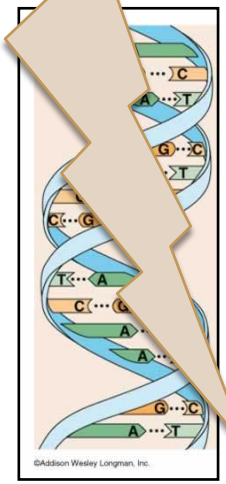
DNA to Chromosome



Karyotiping analysis



Molecular analysis



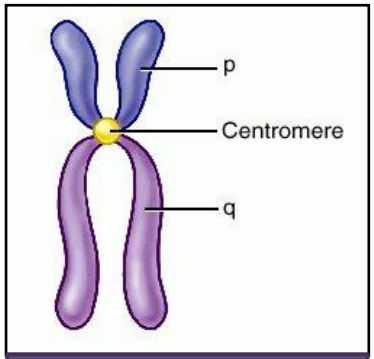
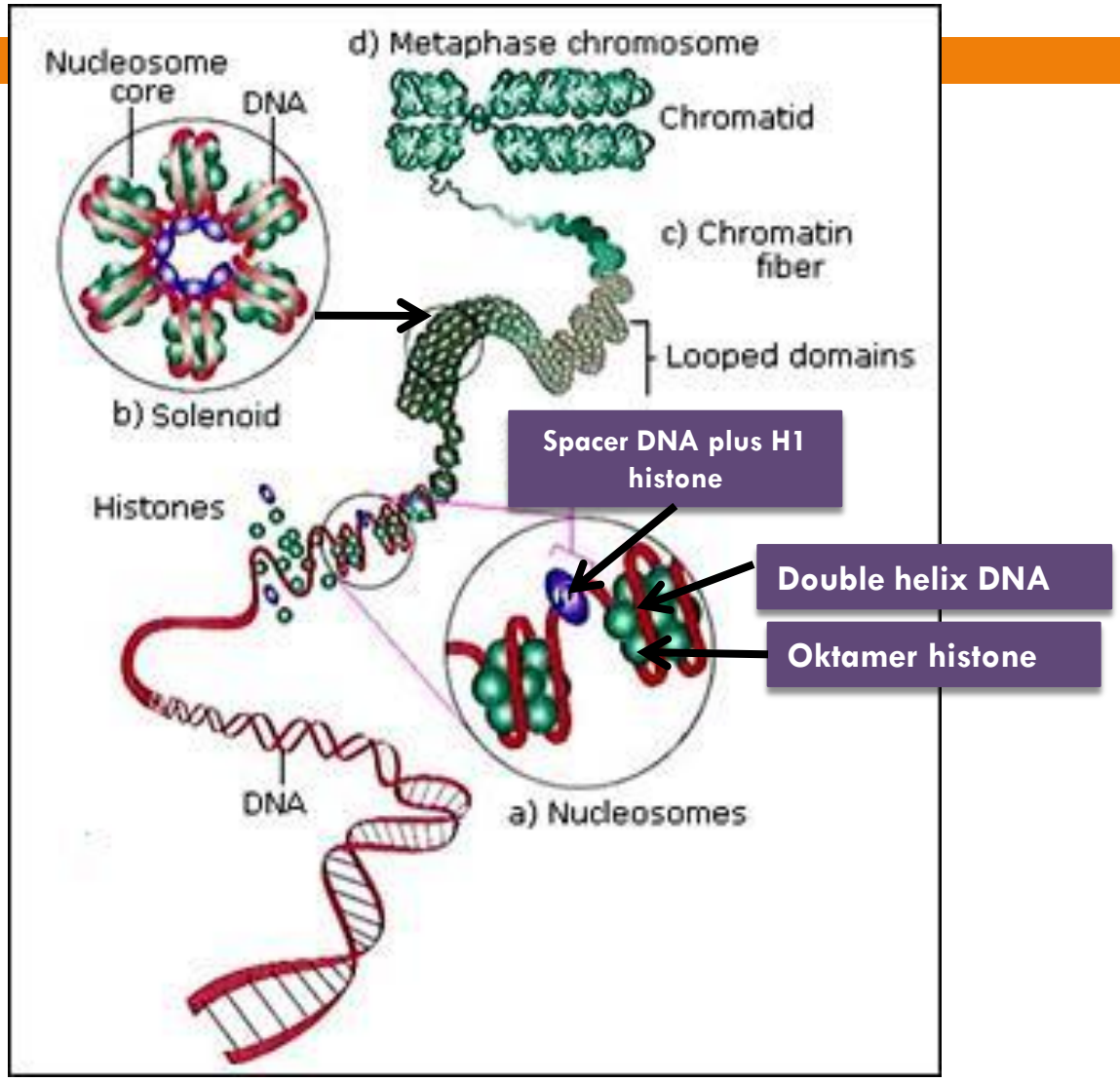


Fig. Morphology of duplicated chromosome

Chromosome packing



Mutation level

- DNA level
  - Single gene disorder
  - Polygenic
  - Multifactorial
- Chromosome level (chromosome aberration)
  - Translocation
  - Inversion
  - Deletion
  - Insertion
  - Duplication
  - Ring chromosome
  - Isochromosome
  - UPD
  - Etc.

Technique of Genetic Analysis

Each types of genetic disorders needs the difference tools for analysis.

1. Single-gene disorders analyzed by:
   - Polymerase Chain Reaction (PCR)
   - RFLP (restriction fragment length polymorphism)
   - ARMS (amplification refractory mutation system)
   - MLPA (multiplex ligation dependent probe amplification)
   - Microarray
2. Chromosome disorders analyzed by:
   - Karyotype (karyotyping)
   - Microarray
3. Multifactorial disorders analyzed by combine the previous analysis

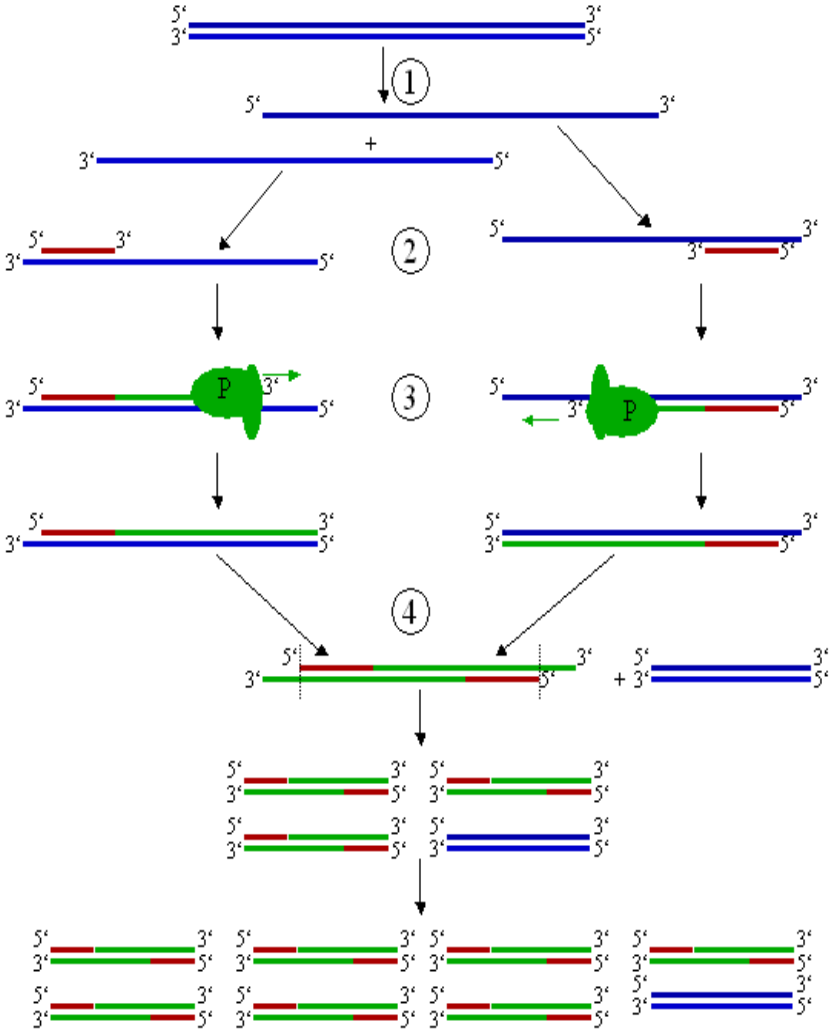
Molecular testing

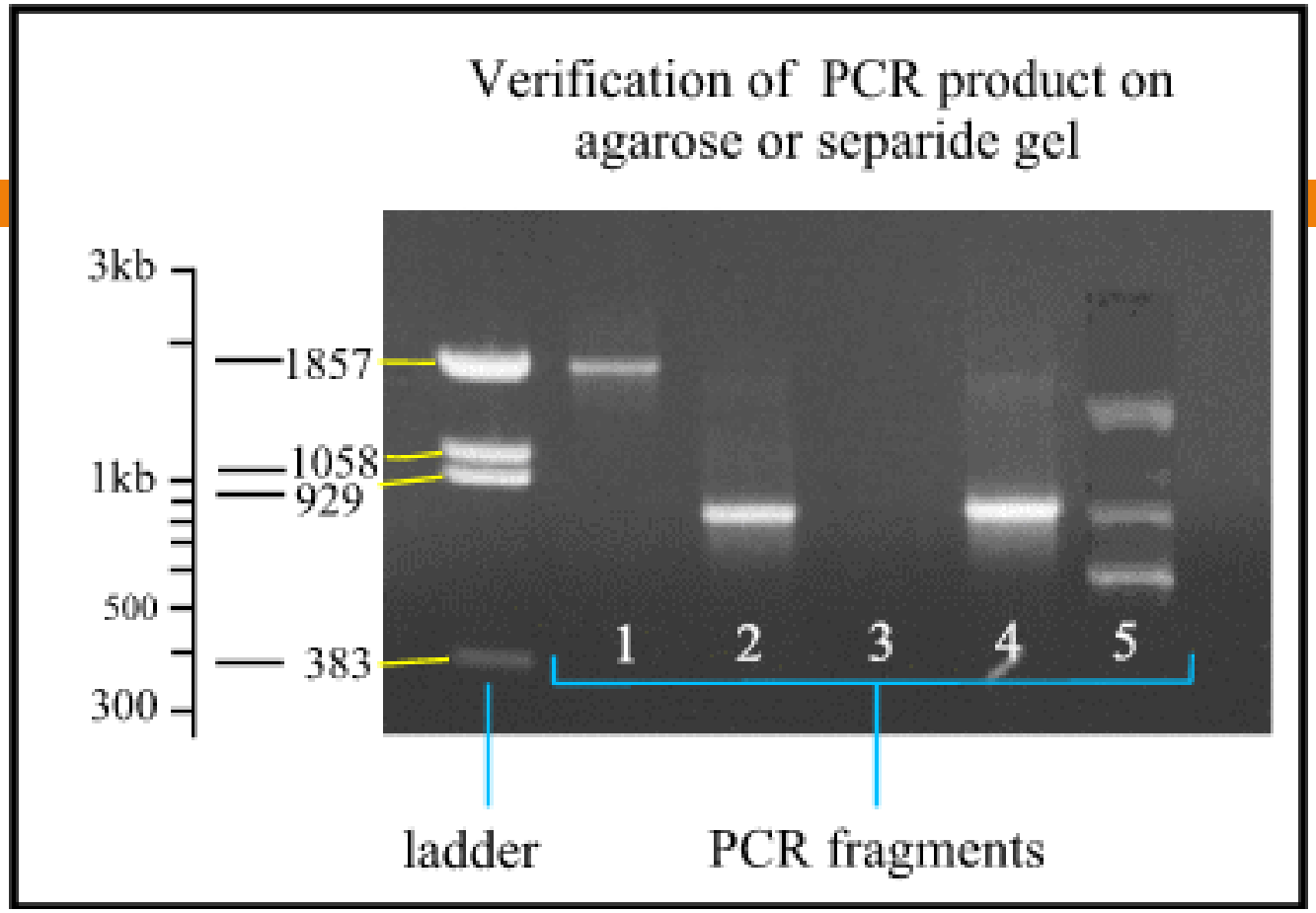
Principle of PCR

- A technique in which a short DNA or RNA sequence (~500 kb) can be amplified $>10^6$ times. Based on the ***enzymatic amplification*** of a fragment of DNA that is flanked by two "***primers***" (a short oligonucleotides that hybridize to the opposite strands of the target sequence and prime synthesis of the cDNA sequence by enzyme DNA polymerase).

Steps of the PCR

1. **Denaturation** at 94°C :
   - the double strand melts open to single stranded DNA
2. **Annealing** at 54°C :
   - Ionic bonds are formed (specify) between the single stranded primer and the single stranded template.
3. **Extension/elongation** (temperature depend on the DNA polymerase used)
4. **Cycle is repeated**





The ladder is a mixture of fragments with known size to compare with the PCR fragments**. Lane 1** : PCR fragment is approximately 1850 bases long. **Lane 2 and 4** : the fragments are approximately 800 bases long. **Lane 3** : no product is formed, so the PCR failed. **Lane 5** : multiple bands are formed because one of the primers fits on different places.

PCR-RFLP

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TNFa sequence:

  1 GAATTCCGGGTGATTTCACTCCCGGCTGTCCAGGCTTGTCCTGCTACCCCACCCAGCCTT

 61 TCCTGAGGCCTCAAGCCTGCCACCAAGCCCCCAGCTCCTTCTCCCCGCAGGACCCAAACA

121 CAGGCCTCAGGACTCAACACAGCTTTTCCCTCCAACCCGTTTTCTCTCCCTCAACGGACT

181 CAGCTTTCTGAAGCCCCTCCCAGTTCTAGTTCTATCTTTTTCCTGCATCCTGTCTGGAAG

241 TTAGAAGGAAACAGACCACAGACCTGGTCCCCAAAAGAAATGGAGGCAATAGGTTTTGAG

301 GGG↓CATGGGGACGGGGTTCAGCCTCCAGGGTCCTACACACAAATCAGTCAGTGGCCCAGA
Recognition site of NcoI (C CATGG→A -308 promoter region)

361 AGACCCCCCTCGGAATCGGAGCAGGGAGGATGGGGAGTGTGAGGGGTATCCTTGATGCTT

421 GTGTGTCCCCAACTTTCCAAATCCCCGCCCCCGCGATGGAGAAGAAACCGAGACAGAAGG

481 TGCAGGGCCCACTACCGCTTCCTCCAGATGAGCTCATGGGTTTCTCCACCAAGGAAGTTT

541 TCCGCTGGTTGAATGATTCTTTCCCCGCCCTCCTCTCGCCCCAGGGACATATAAAGGCAG

601 TTGTTGGCACACCCAGCCAGCAGACGCTCCCTCAGCAAGGACAGCAGAGGACCAGCTAAG

661 AGGGAGAGAAGCAACTACAGACCCCCCCTGAAAACAACCCTCAGACGCCACATCCCCTGA

721 CAAGCTGCCAGGCAGGTTCTCTTCCTCTCACATACTGACCCACGGCTTCACCCTCTCTCC

781 CCTGGAAAGGACACC ATG AGC ACT GAA AGC ATG ATC CGG GAC GTG GAG…
                met ser thr glu ser met ile arg asp val glu
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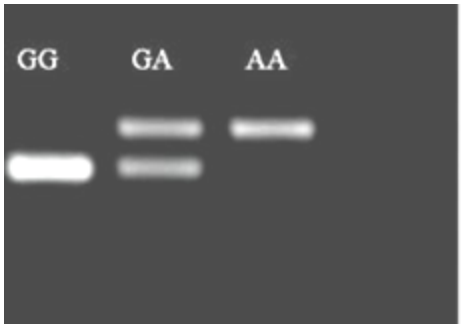
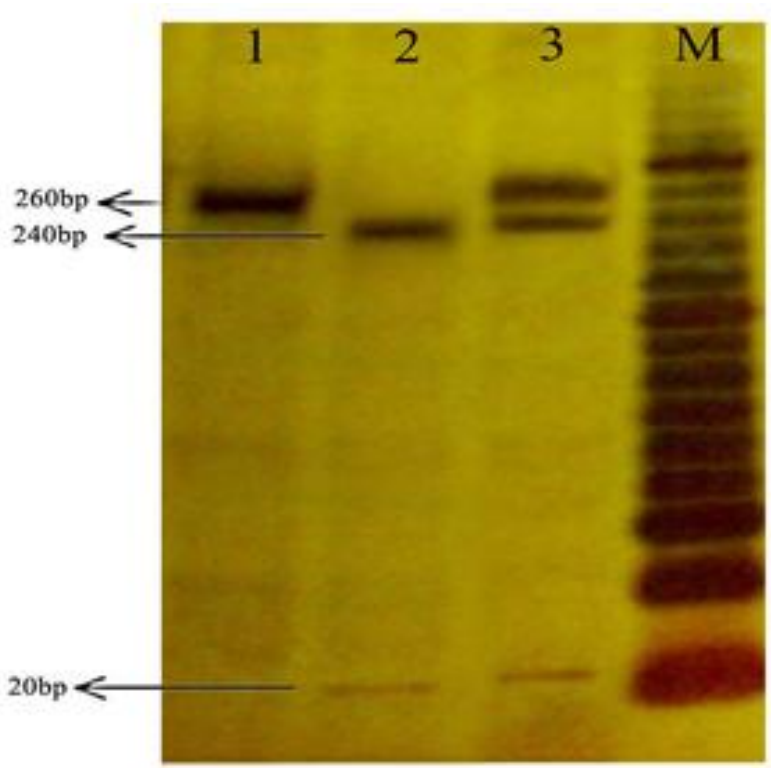
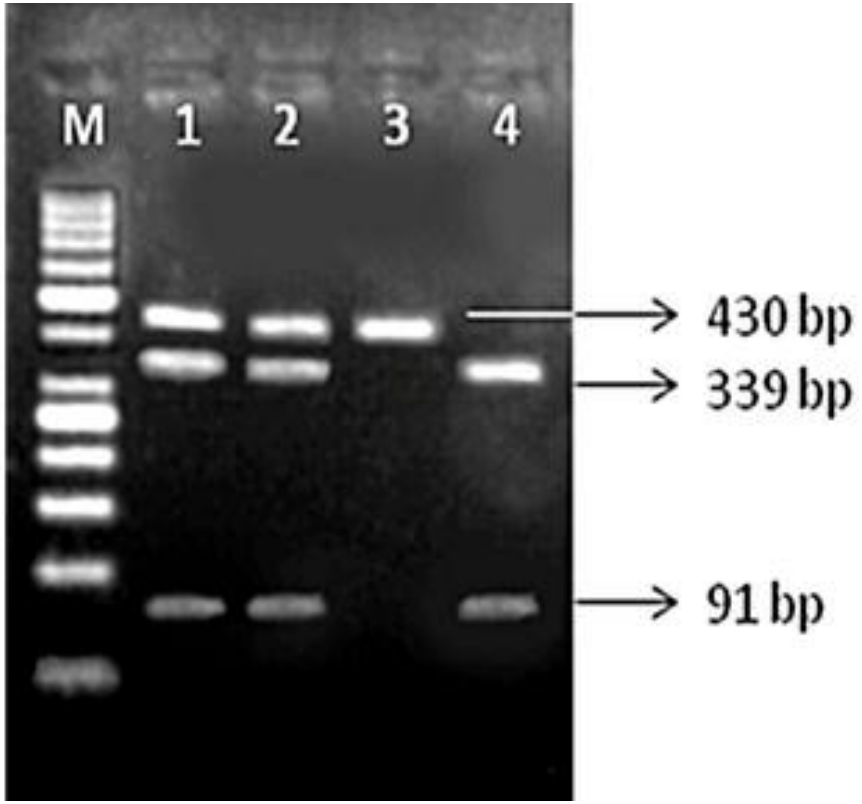


Fig above. PCR products (107 bp) were digested by NcoI

*) Coding sequence for TNFa G-308A in the promoter region was referred from Enseml database ENSG00000204490

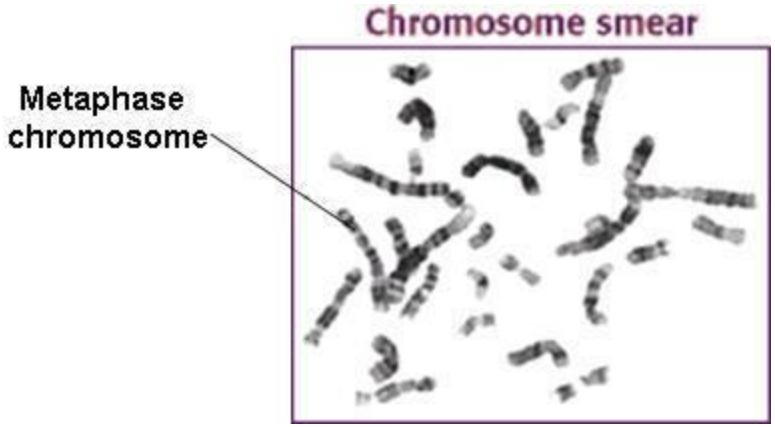

1
2
3
M
260bp
240bp
20bp

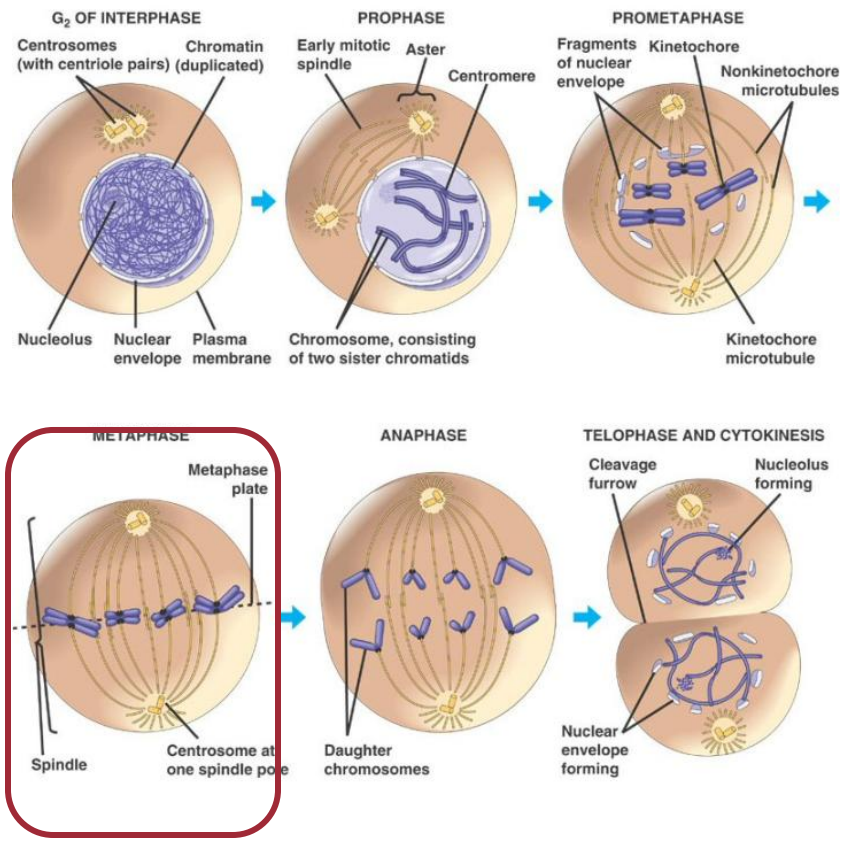

M
1
2
3
4
430 bp
339 bp
91 bp

Cytogenetics

Structure of human chromosomes

- Cytogenetics: the study of chromosome and cell division
  - Chromosome can be seen clearly during metaphase, maximally condensed





Chromosome analysis (karyotyping)

- Chromosome staining for identification and detection if any abnormality **>4Mbp**
  - The light bands on chromosome regions rich in GC and genes.
  - Dark bands rich in AT and few on genes. Ex: Chromosome 19, dense with genes, has few dark bands.

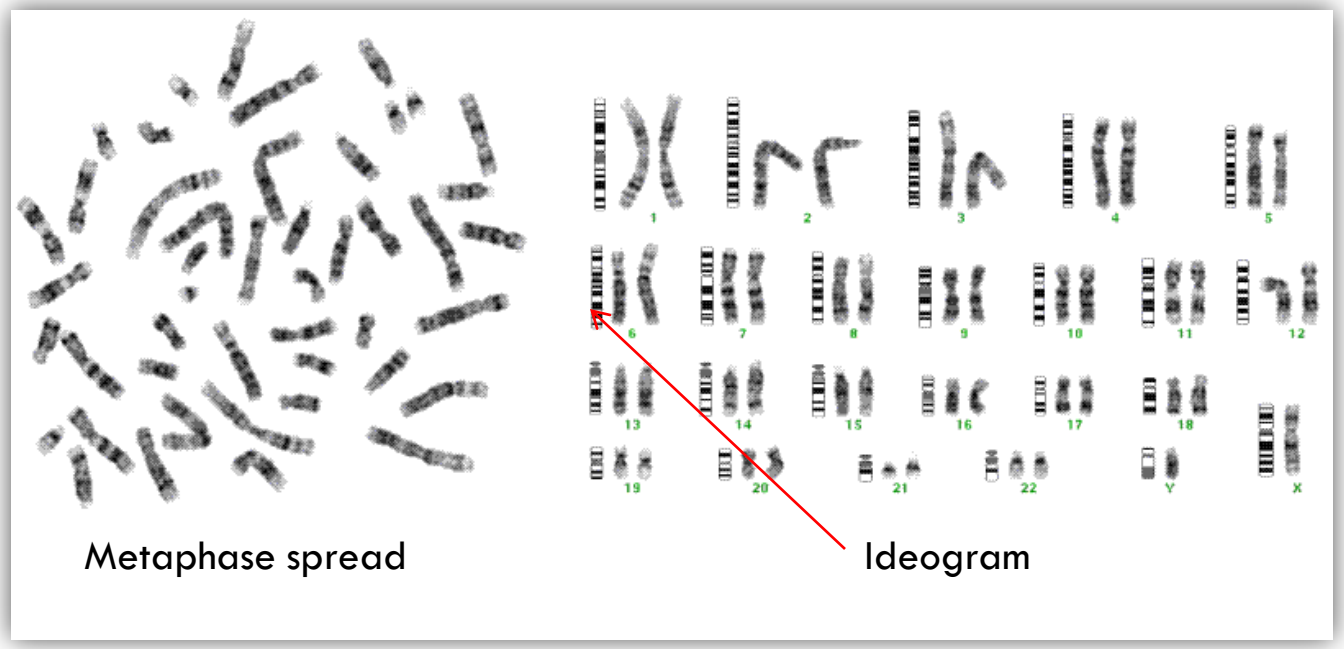
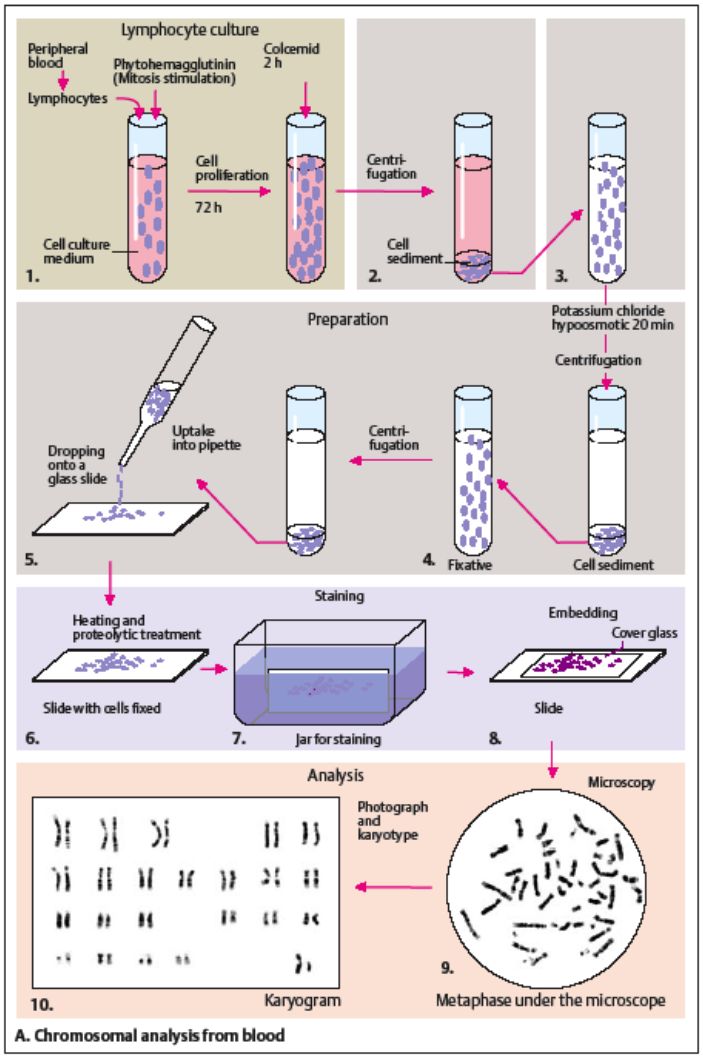


Fig. normal male karyotyping

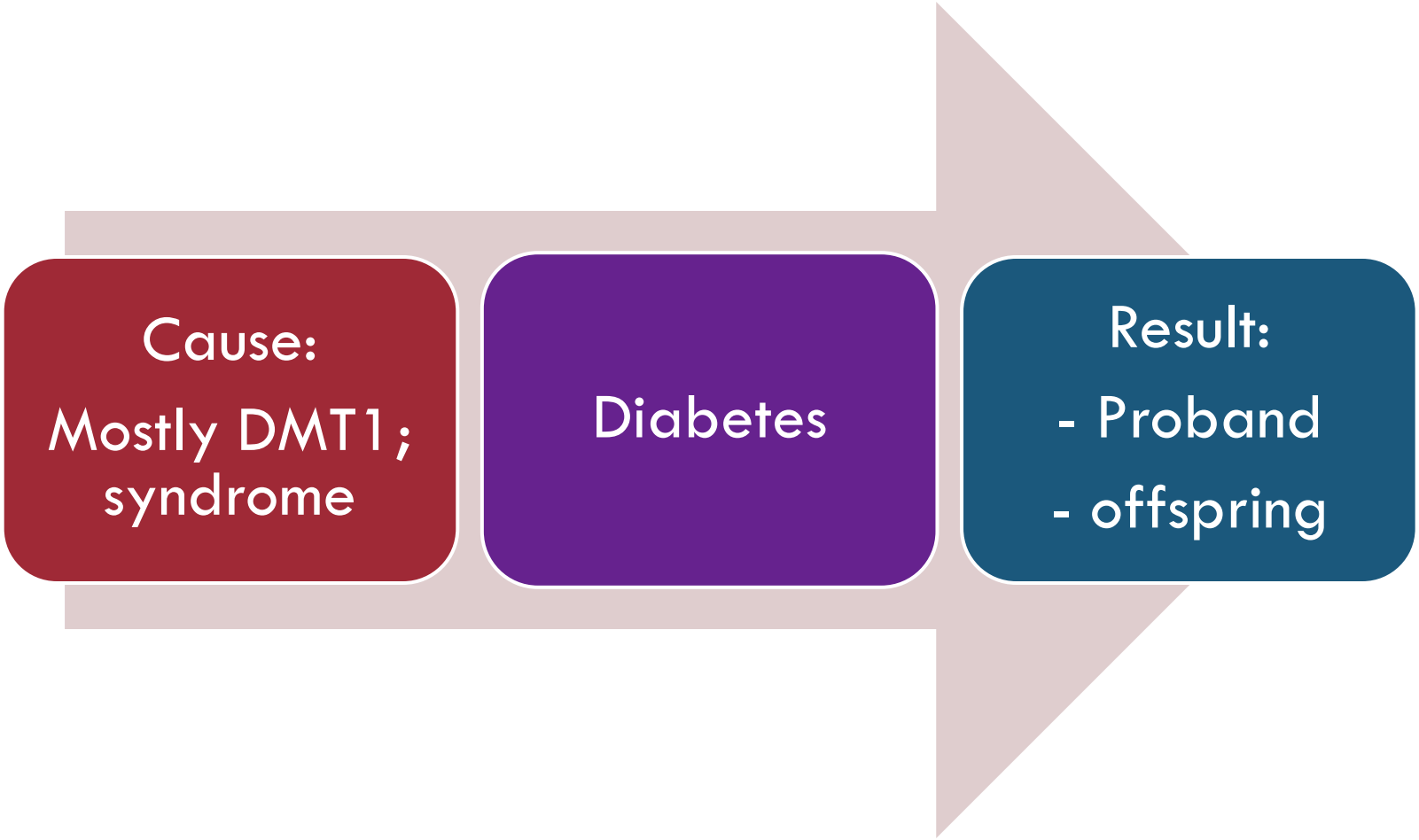
Chromosome analysis



A. Chromosomal analysis from blood

- Staining
  - Non-banding techniques
    - Grouping: based on size and position of centromere
  - Banding techniques
    - Q bands (quinacrine)
    - G (Giemsa) bands
    - R (reverse G) bands
    - C bands for chromatin
    - T bands for telomere

Chromosome damage in diabetes



Uniparental Isodisomy

ORIGINAL ARTICLE

Mosaic Paternal Uniparental Isodisomy and an *ABCC8* Gene Mutation in a Patient With Permanent Neonatal Diabetes and Hemihypertrophy

Julian P.H. Shield,[1] Sarah E. Flanagan,[2] Deborah J. Mackay,[3,4] Lorna W. Harries,[2] Peter Proks,[5] Christophe Girard,[5] Frances M. Ashcroft,[5] I. Karen Temple,[3,4] and Sian Ellard[2]

Ring chromosome 18: IDDM and autoimmune thyroiditis

J Med Genet 1999;**36**:156–158

Insulin dependent diabetes mellitus (IDDM) and autoimmune thyroiditis in a boy with a ring chromosome 18: additional evidence of autoimmunity or IDDM gene(s) on chromosome 18

C Dacou-Voutetakis, A Sertedaki, M Maniatis-Christidis, C Sarri, G Karadima, M B Petersen, A Xaidara, M Kanariou, P Nicolaidou

Abstract

A 4 year 3 month old boy with insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, slight mental retardation, facial dysmorphism, and a de novo ring chromosome 18 (deletion 18q22.3-18qter) is described. This unique association of defects could represent a chance association. Alternatively, the clinical features could be the result of the chromosomal aberration. If so, one could speculate that a gene or genes on chromosome 18 might act as a suppressor or activator of the autoimmune process by itself or in concert with other IDDM loci.

(*J Med Genet* 1999;**36**:156–158)

Keywords: ring chromosome 18; chromosome 18 deletion; IDDM; hypothyroidism

was managed appropriately with fluids, electrolytes, and insulin and he was discharged home on diet and insulin injections with the diagnosis of IDDM.

PAST AND FAMILY HISTORY

He was born after an uneventful term pregnancy to a 24 year old mother, para 1. His father was 37 years old. The delivery was normal with cephalic presentation and the birth weight was 3150 g. He cried immediately and had no neonatal problems. He was late to achieve developmental milestones. At 17 months he had not walked and was admitted to our clinic for evaluation. Abdominal sonography showed that the urinary tract and other abdominal organs were normal, but lithiasis of the gall bladder was present. The blood amino acids were normal and antibodies against rubella, herpes simplex virus, and CMV, as well

Klinefelter syndrome with DMT1

Case report

A case of Klinefelter's syndrome with type 1 diabetes mellitus

CAI Xiao-pin, ZHAO Li, MAO Min, YANG Zhao-jun, XING Xiao-yan and LI Guang-wei

Keywords: *Klinefelter's syndrome; type 1 diabetes mellitus; autoimmune*

Klinefelter's syndrome (KS) is the most common sex chromosome disease in men. Classical features of the syndrome include a eunuchoidal body habitus, small testes and hypergonadotrophic hypogonadism. There has been an increased risk of diabetes mellitus and autoimmune disease for KS patients. This paper reports a case of KS in association with type 1 diabetes mellitus. The patient was a 21-year-old man, who has been confirmed by absolute insulin deficiency and positive IA-2 autoantibody. The hyperinsulinemic euglycemic clamp test indicated his insulin sensitivity in normal range, and his blood glucose was controlled well by the insulin therapy.

Chin Med J 2012;125(5):937-940

Gestational diabetes

J Clin Res Pediatr Endocrinol 2012;4(4):223-225
DOI: 10.4274/Jcrpe.764

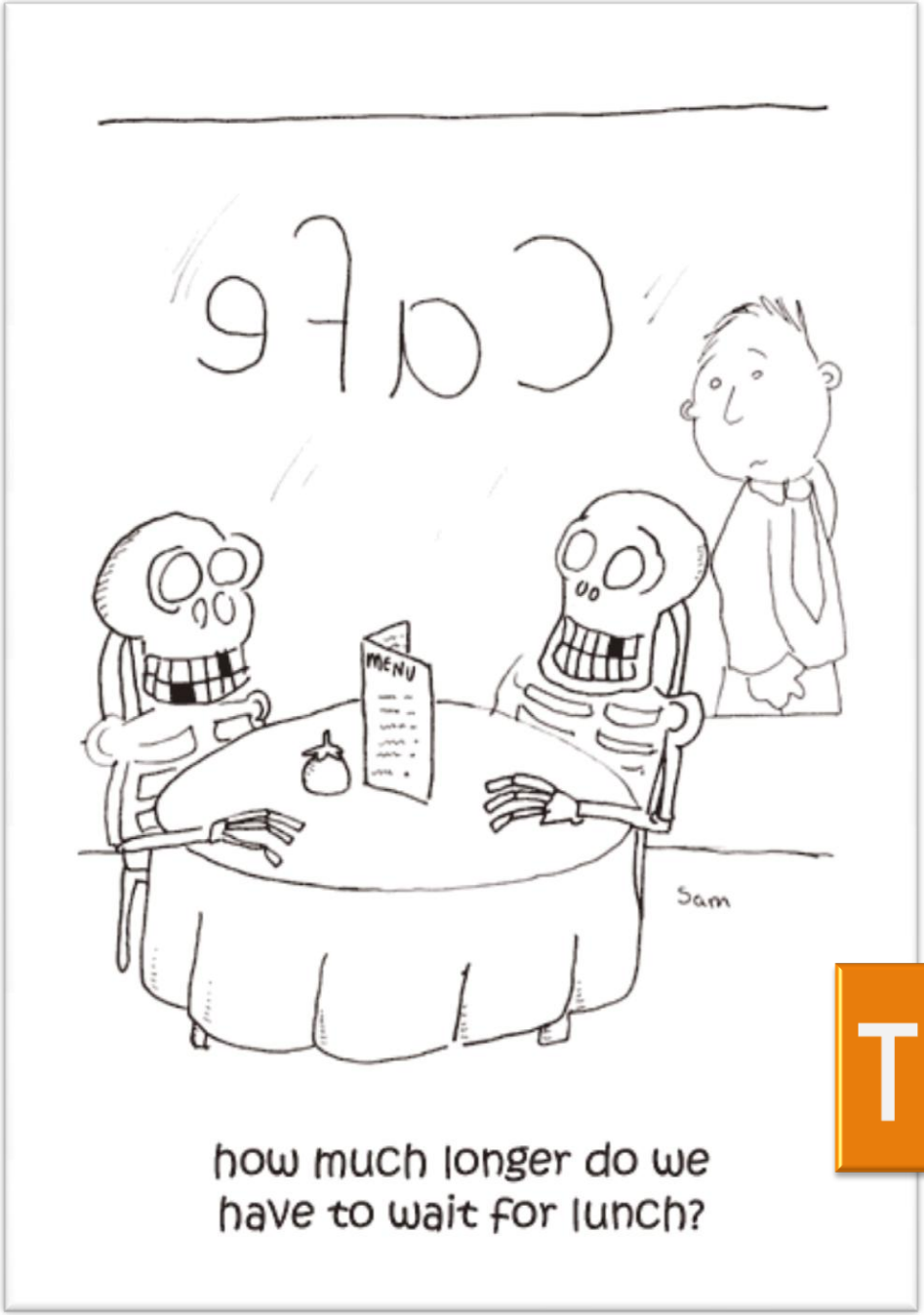
Case Report

An Infant Born to a Mother with Gestational Diabetes Presenting with 49,XXXXY Syndrome and Renal Agenesis-A Case Report

Dulika Sumathipala[1], Thilini Gamage[1], Bandula Wijesiriwardena[2], Rohan W. Jayasekara[1], Vajira H.W. Dissanayake[1]

[1]Human Genetics Unit, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka

[2]National Hospital of Sri Lanka, Colombo, Sri Lanka



Thank You