TOPIC 3 – GENETIC TESTING

Fiona Karet, Shih-Hua Lin, Rosa Vargas-Poussou

Disclosure of Interests

None

Genetic testing – Gitelman syndrome

1. Utility
2. Technique
3. Familial studies

Genetic testing – GS – Utility

What is the validity and clinical utility of genetic testing in the proband?

1. Diagnosis confirmation
   - Sensitivity: 65~80%
   - Specificity: 90~100%
2. Natural history
   - CKD (6 % Tseng MH, JCEM 2012)
   - HTA (44% Berry MR, NDT 2013). Largest cohorts ? Mechanism?
   - Chondrocalcinosis

Genetic testing – GS – Utility

3. Phenotype/genotype correlation
    - Type of mutation
        - Severe phenotype and splicing mutations (Rivera-Muñoz, JASN 2007)
        - HTA and C-terminal domain mutations (Berry MR, NDT 2013)
    - Sex
        - More severe in males (Rivera-Muñoz, JASN 2007; Tseng MH, JCEM 2012)
        - More severe in females (Berry MR, NDT 2013)

Genetic testing – GS – Utility

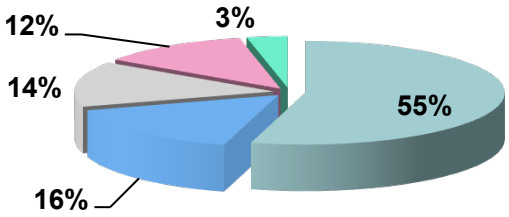
4. Early and appropriate treatment
   - Effect on long-term evolution and complications ?
5. Diagnosis of exclusion
   - Psychiatric patients
   - Sjögren syndrome
   - Long QT syndrome

Genetic testing – GS – Utility

6. Genetic counselling
    - Siblings
        - 25% risk
        - All? Only siblings with biological abnormalities ?
    - Pregnancy
        - Is the father carrier ?
        - Prenatal diagnosis : feasible but not recommended

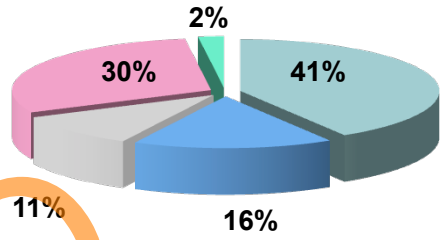
Genetic testing – Gitelman syndrome

662 probands-French cohort (2001-2012)



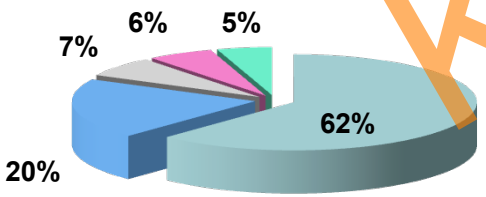
- Compound heterozygous (n = 360)
- Homozygous (n = 105)
- Only one htz mut. (n = 94)
- No mutation (n = 81)
- CLCNKB (n=22)

240 probands-Netherland cohort



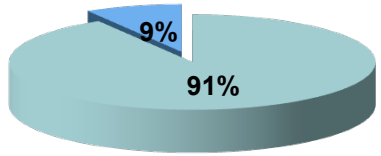
- Compound heterozygous (n = 99)
- Homozygous (n = 39)
- Only one htz mut. (n = 25)
- No mutation (n = 72)
- CLCNKB (n=5)

103 probands-Chinese cohort



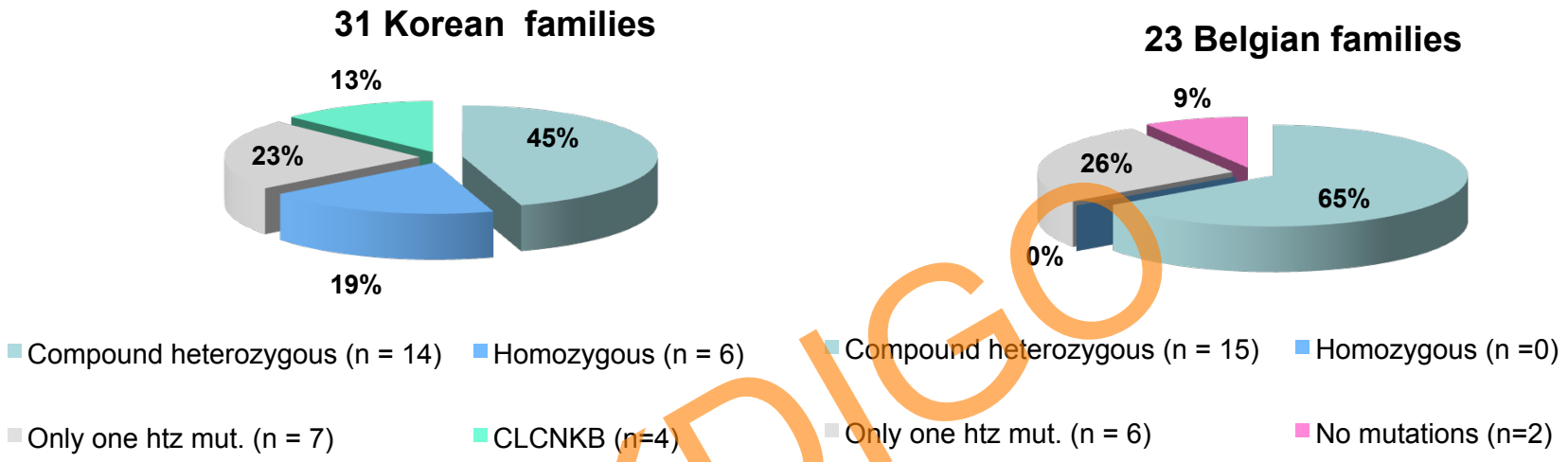
- Compoun heterozygous (n=64)
- Homozygous (n=21)
- Only one htz mut. (n = 7)
- No mutation (n=6)
- CLCNKB (n=5)

35 probands-UK cohort



- Coumpound heterozygous (n=32)
- Homozygous (n=3)
- Only one htz mut.

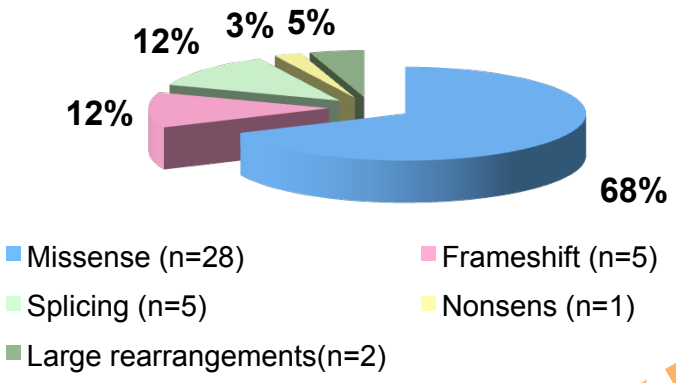
Genetic testing – Gitelman syndrome



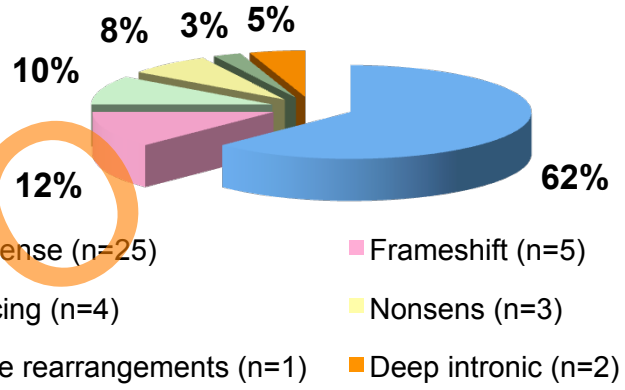
Mutation detection rate: 65-80%
Only one heterozygous mutation: 11-26%
CLCNKB mutations: 2-13%

Genetic testing – Gitelman syndrome
Type of mutation

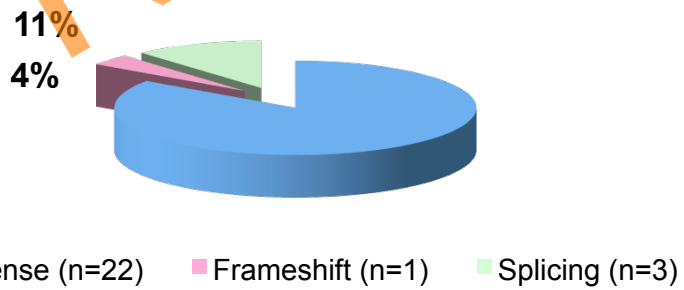
UK (n=41)



China (n=40)



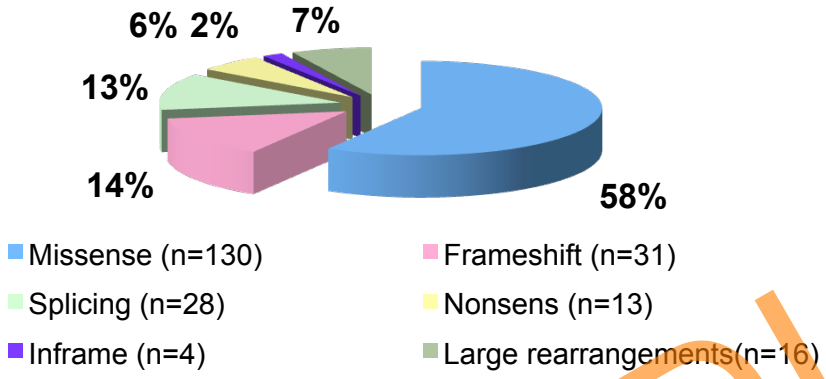
Belgium (n=26)



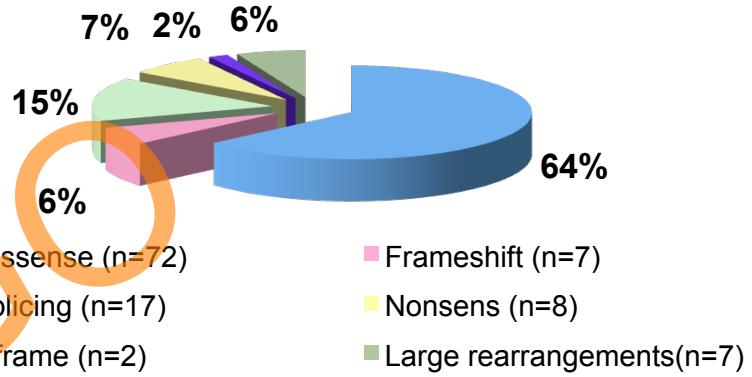
85%

Genetic testing – Gitelman syndrome
Type of mutation

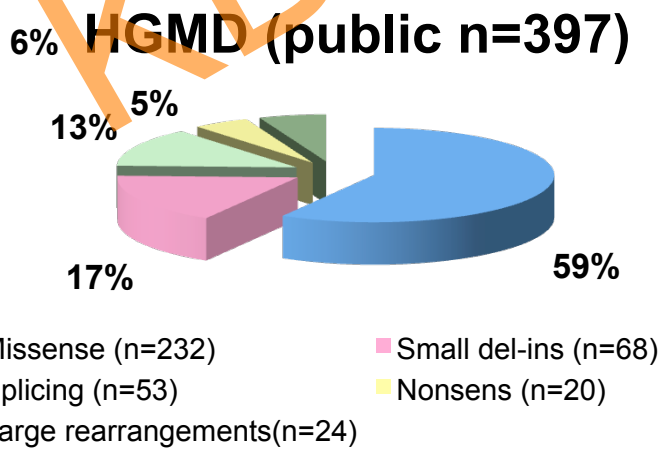
France (n=222)



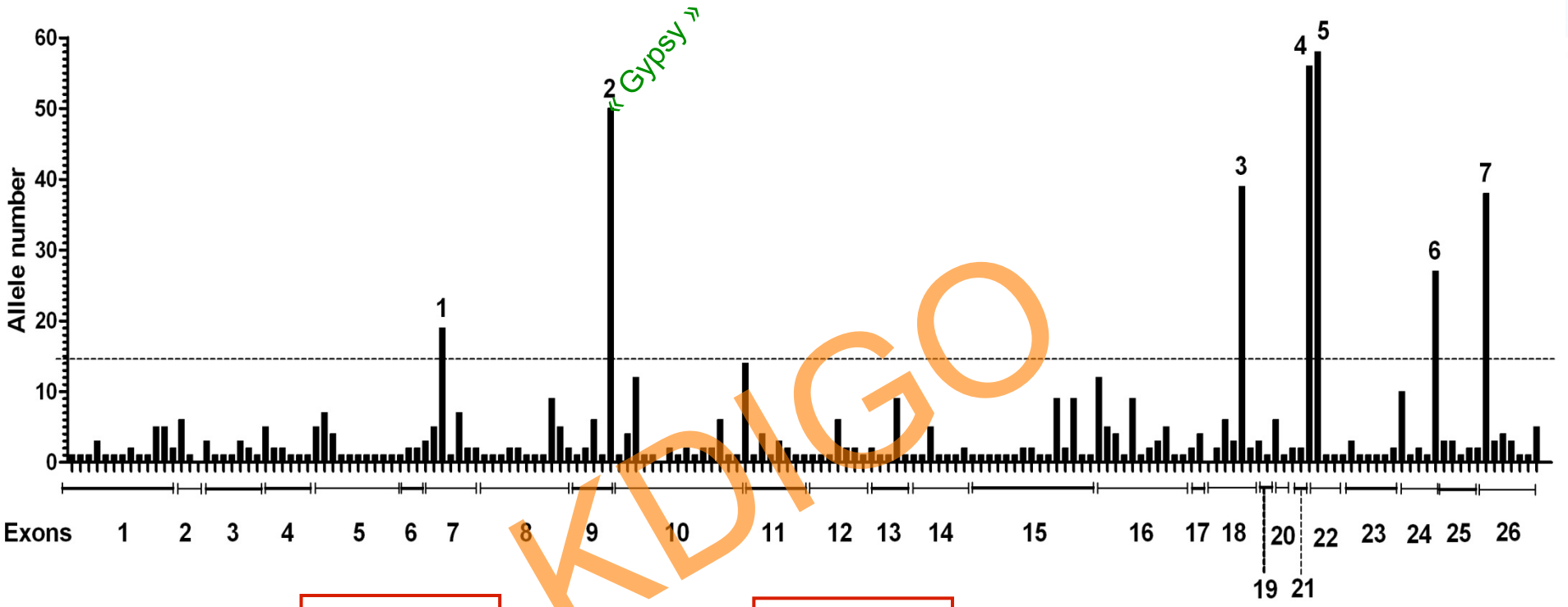
Netherlands (n=114)



HGMD (public n=397)



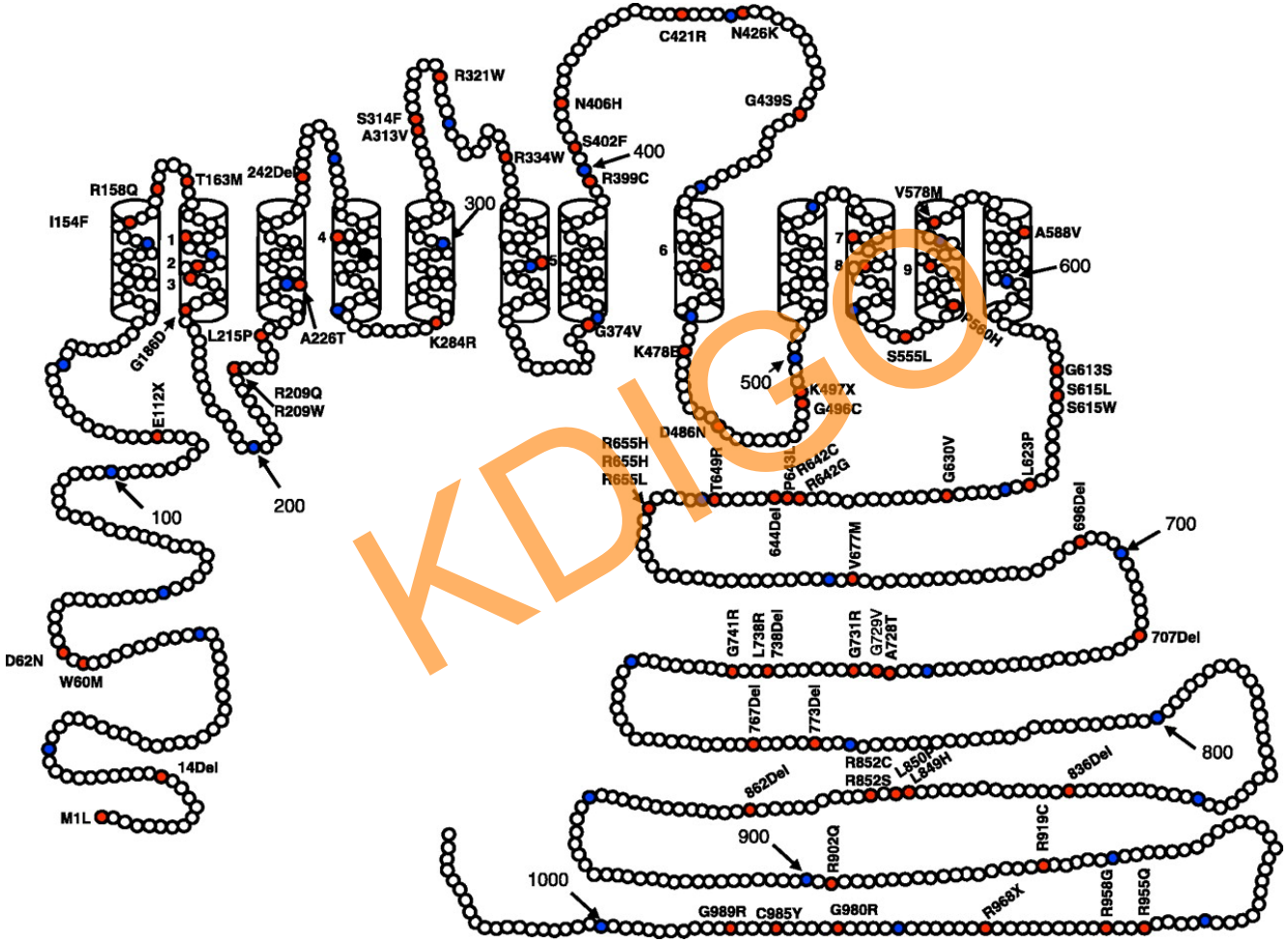
SLC12A3 – Mutations



1. p.Ala313Val
2. c.1180+1G>T
3. p.Gly741Arg
4. p.Leu859Pro
5. p.Arg861Cys
6. c.2883+1G>T
7. p.Cys994Tyr

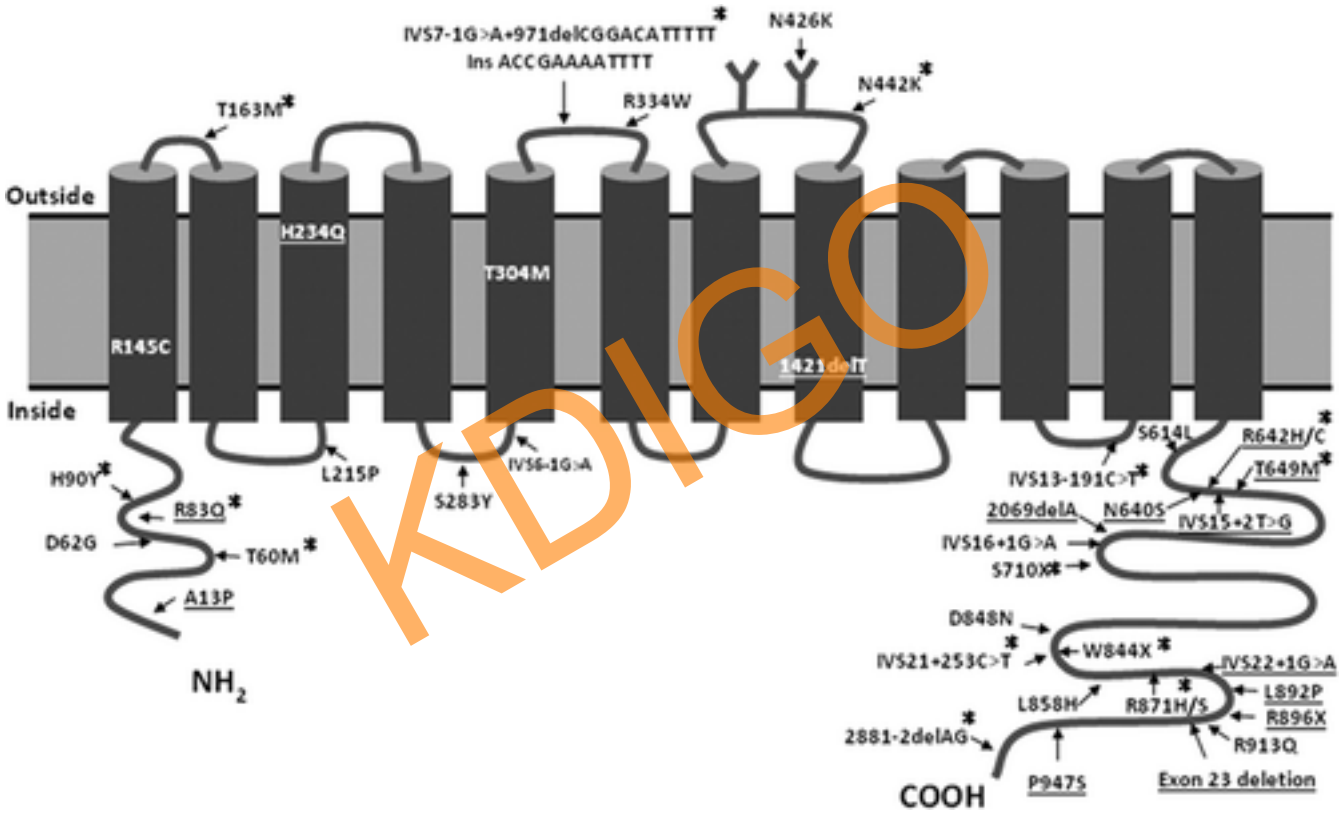
Kunchaparty, S, et al. *Am J Physiol*, 1999.
De Jong, JC, et al. *J Am Soc Nephrol*, 2002

Mutations in human thiazide-sensitive Na+-Cl− cotransporter TSC reported in patients with Gitelman's disease.



Gamba G. *Physiol Rev* 2005;85:423-493

SLC12A3 – Mutations



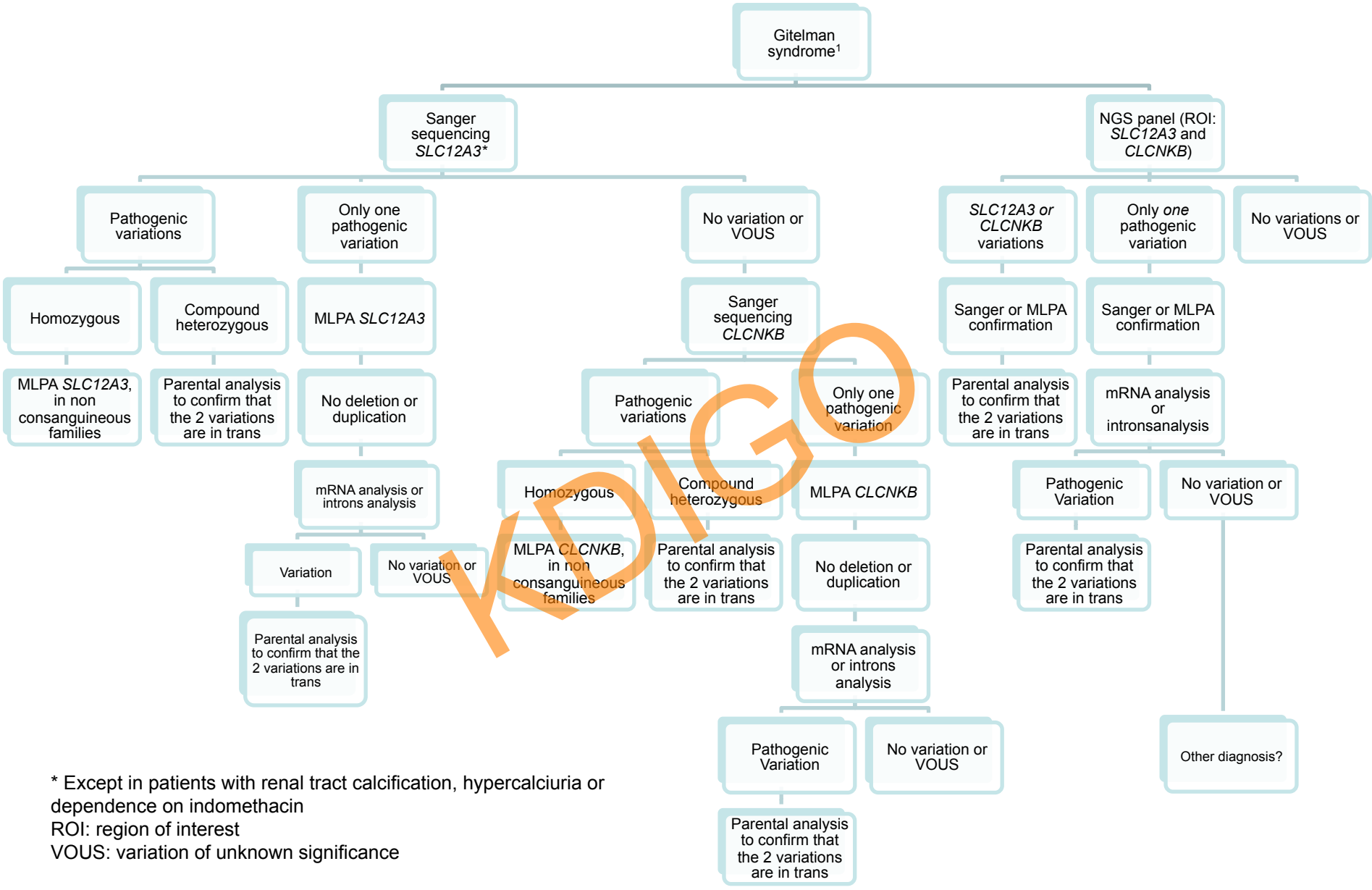
Tseng MH, Yang SS, Hsu YJ, et al. ***J Clin Endocrinol Metab.*** 2012

Genetic testing – GS – Technique

1. Sequencing
    - Sanger
    - NGS
2. Large rearrangements research
    - MLPA
    - QMPSF, qPCR
3. Transcript analysis

Genetic testing – GS – Technique

1. First *SLC12A3*
    1. Targeted exons in some populations (i.e. “Gypsies”)
2. Second: *CLCNKB*
3. Exceptions (*CLCNKB* first): patients with
    1. renal tract calcification;
    2. Hypercalciuria
    3. dependence on indomethacin as part of their therapeutic regimen



* Except in patients with renal tract calcification, hypercalciuria or dependence on indomethacin
ROI: region of interest
VOUS: variation of unknown significance

Genetic testing – GS – Family studies

1. Family segregation
    - In compound heterozygous: different alleles
    - Three mutations
        - French cohort 3.5%
        - Taiwan cohort 12 %
    - Pseudo-dominant inheritance
    - Interpretation of VOUS

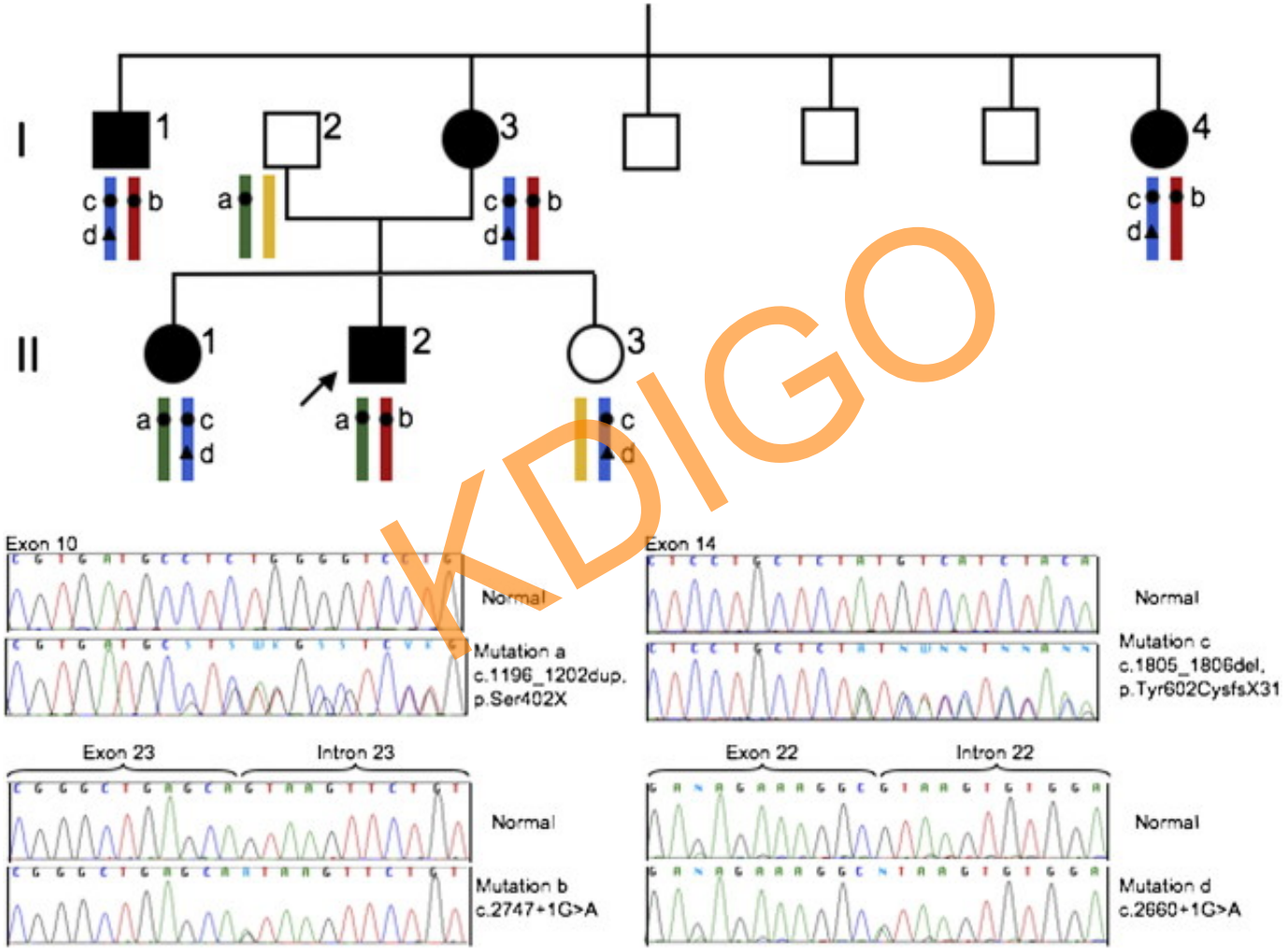
Genetic testing – GS – Family studies

2. Parents:
    1. Both ?
    2. Mother ?
    3. Father ? (paternity issues)

Genetic testing – GS – Family studies

3. Siblings :
   - First : clinical and biochemical evaluation
   - SG suggestive: genetic test to confirm.
4. Carrier testing of the proband's partner
   - Not indicated, frequency of heterozygous 1%
   - Exception if the partner is a relative: couple of heterozygous carrier and GS patient: risk 50%

A pseudo-dominant form of Gitelman's syndrome



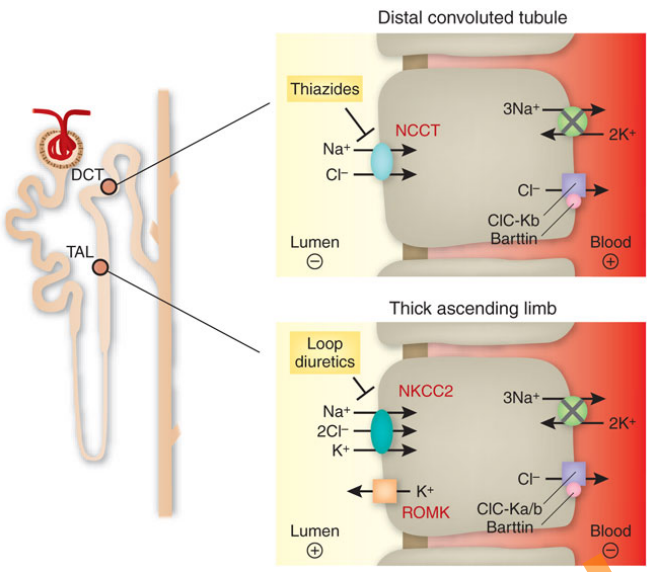
de La Faille R et al. *NDT plus* 2011

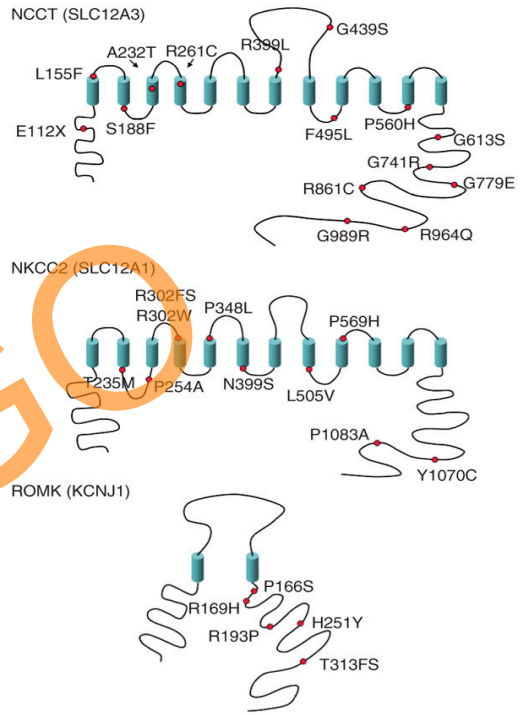
Heterozygous carrier and hypertension

1. Frequency
    1. 1% ; 3% ?
2. Phenotype
    - Increase in dietary salt intake
    - Lower blood pressure (adjusted for age and gender)

Cruz DN 2001, Fava C. 2008

Heterozygous carrier and hypertension





Gitelman syndrome
~prevalence 1/40,000

Bartter syndrome
~prevalence1/1.000.000

In the Framingham cohort
SLC12A3 (NCCT) : freq 1/200 (0,5%)
SLC12A1 (NKCC2) : freq 1/360
KCNJ1 (ROMK1) : freq 1/670
80% of heterozygous carriers had long-term systolic BP below the mean of the cohort

Devuyst O *Nat Genet* 2008; **40**, 495 - 496

Ji et al. *Nat Genet* 2008; **40**, 592-599

Heterozygous carrier and hypertension

3. But GS patients can develop hypertension
   - 44% : Berry MR, 2013
   - Prevalence in largest cohort

Heterozygous carrier and hypertension

Clinical Research Protocol HEPHYGI - Clinical significance of *SLC12A3* heterozygous mutations
(2013-2016, funded by PHRC)

Population :
- GS Patients -/- n= 80
- Relatives +/- n= 80
- Controls +/+ n= 80

Multicentric : 6 French centres

The inclusion process started in November 2013.

January 2016: 83% of inclusions (200 individuals)

Blood Pressure :
- Clinic, BP Self-Measurement, ECG

Na K homeostasis :
- Blood, 24h Urine, renin, aldosterone

Mg, Ca homeostasis :
- Blood, 24h Urine, bone turnover markers

Vessels :
- Pulse wave analysis, Central BP

Metabolism :
- Height, weight, W/H ratio
- Glucose (OGTT) and lipid metabolism, proteinuria

Other researchs:
- Biobanking : Blood, Plasma, Urine,