



Khunying Sudsakorn Tuchinda Memorial Lecture:

**“Current status of thalassemia prevention”
Mar 18, 2025**



Current Status of Thalassemia Prevention

Kitti Torcharus, M.D.

Department of Pediatrics,

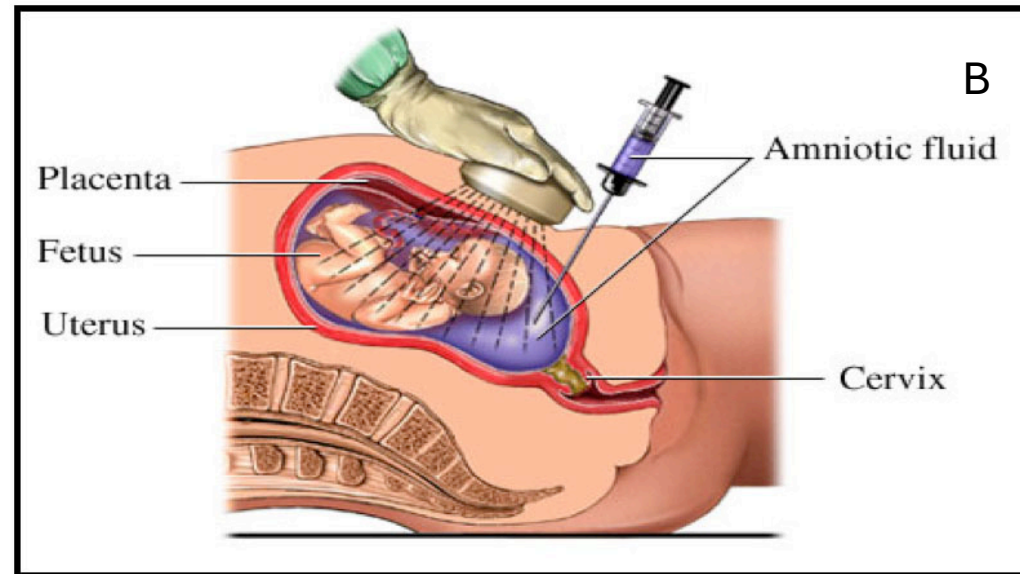
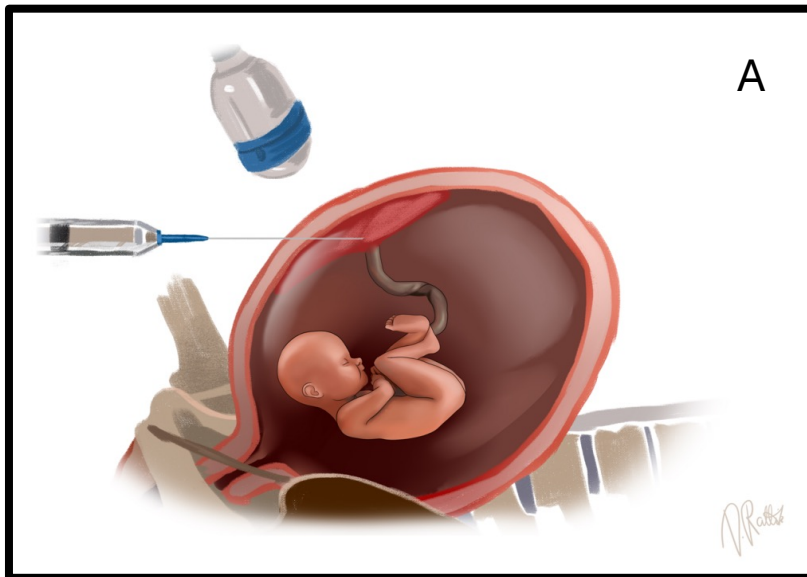
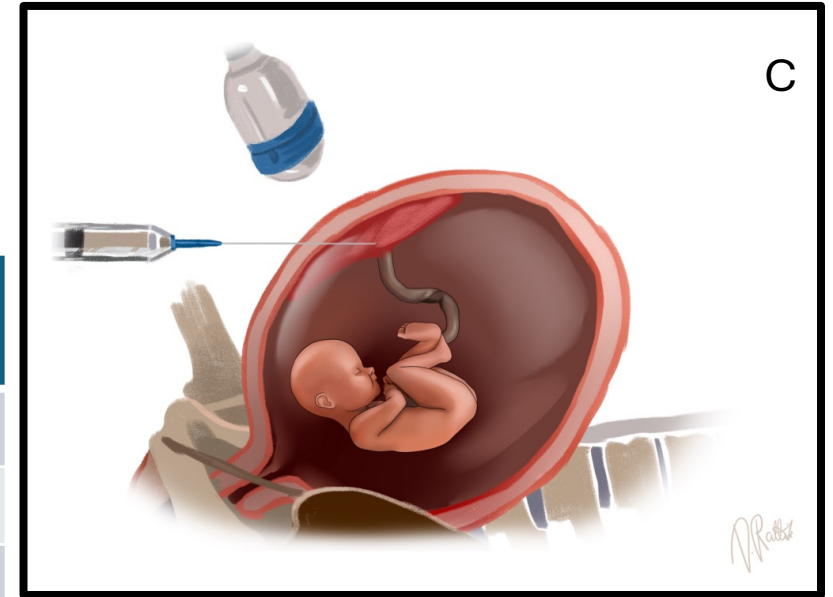
Phramongkutklao Hospital and College of Medicine

Thalassemia Prevention: Prenatal diagnosis (PND)

- Genetic counselling
- Molecular study
 - Father and mother
 - Fetus
- Ultra sound
- Obstetric procedures
 - CVS (chorionic villus sampling): 11th -13rd wk G.A.
 - Amniocentesis: 16th-18th wk G.A.
 - Cordocentesis: 18th-22nd wk G.A.
- Prenatal diagnosis (PND)
 - Termination of affected fetus (options)
- Preimplantation genetic diagnosis (PGD)
- Noninvasive prenatal diagnosis (NIPD)

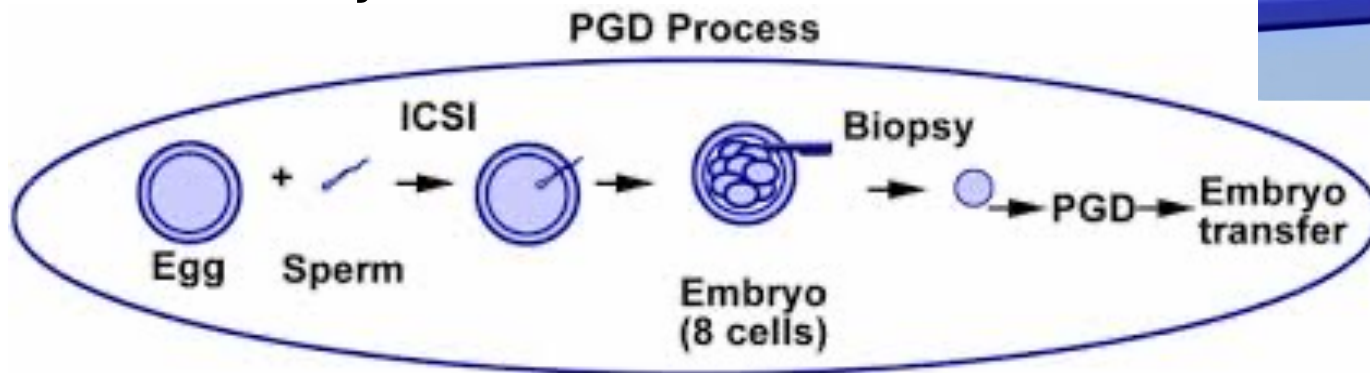
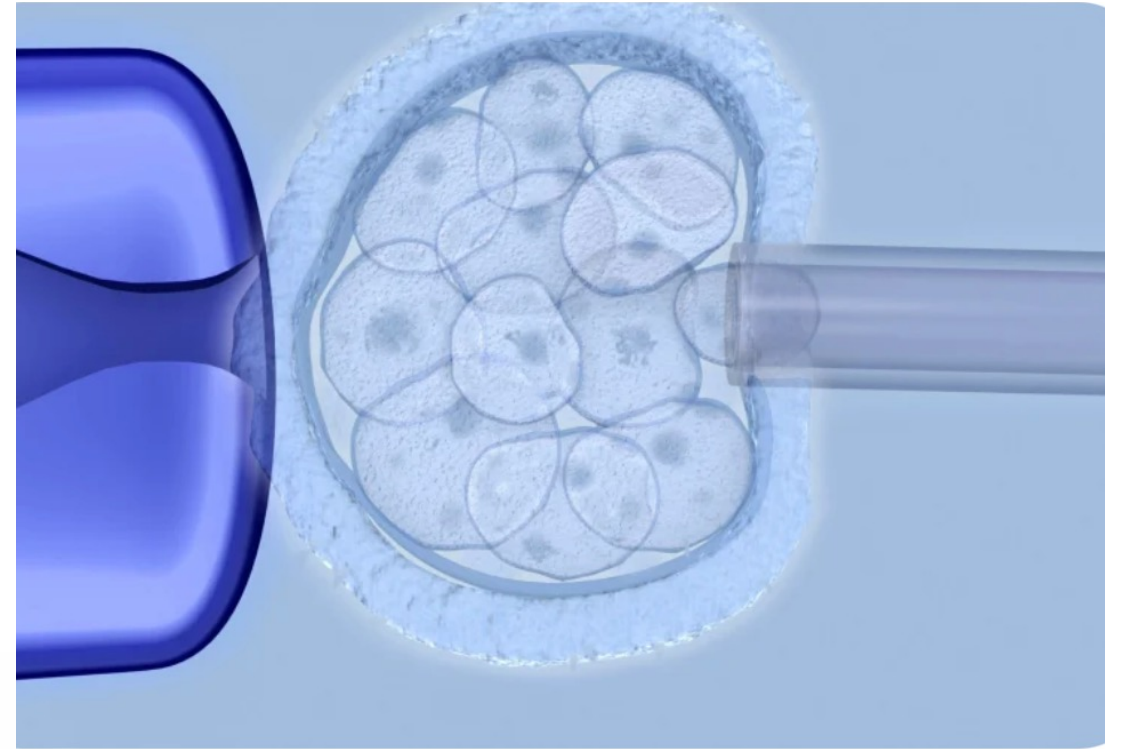
Prenatal diagnosis

Obstetric procedures	G.A. (wks)	Miscarriage (%)
A. Chorionic villus sampling	11-13	1.5-2.5
B. Amniocentesis	16-18	0.3-0.5
C. Cordocentesis	18-22	1.0-1.5

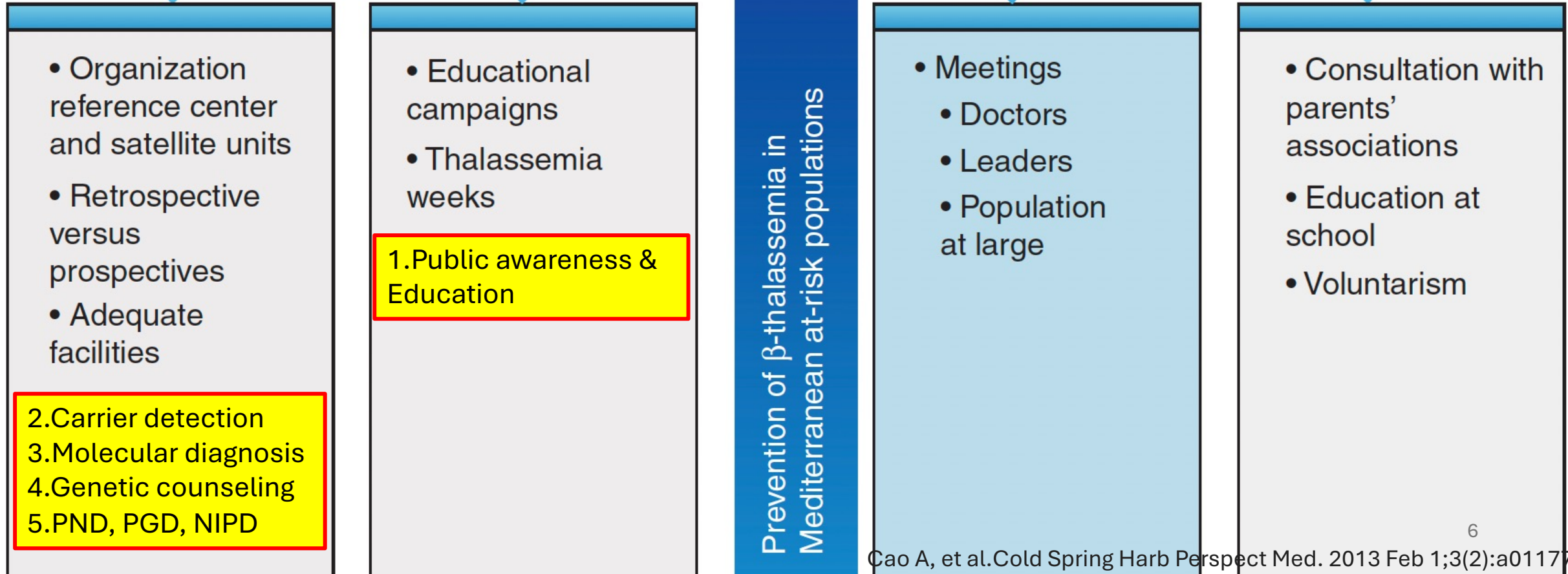


Preimplantation Genetic Diagnosis (PGD)

- Egg
- Sperm, ICSI (intracytoplasmic sperm injection)
- Embryo (8 cells)
- Biopsy
- One cell PGD
- Embryo transfer



Strategy for prevention of β -thalassemias in Mediterranean at-risk populations



β -thalassemia Prevention Model: 40 years of Single Center Experience (1975-2017)

- **Microcitemico Pediatric Hospital**, Cagliari, Sardinia, Italy
 - Population: 1.7 million, carrier 12%, newborn affect 1/250 newborn
- **Carrier screening:**
 - Extensive information & education: mass media, family doctors, obstetricians, pediatricians, midwives
 - Population screening to identify β -thal carrier (hemato & mol. mutation)
- **Genetic counselling:**
 - β -thal, genetic mutation, reproductive options, PGD, efficacy, accuracy & risks of fetal diagnosis
 - neonatal treatment, HSCT
 - 97% of pregnant & 93% of non-pregnant accepted PND

β -thalassemia Prevention Model: 40 years of Single Center Experience (1975-2017)

- Fetal & Embryo Invasive Procedure, outcomes:
 - 8,748 cases:
 - 8,564 prenatal diagnosis
 - Trans-abdominal CVS =77.2% (6,615/8,564); fetal loss 0.6%
 - PND affected fetus 24.96% (2,138/8,564)
 - Misdiagnosis (inability to amp. DNA, Mispaternity, maternal contamination)
 - 184 PGD procedures
 - PGD (start in 2022) 38/184 > clinical pregnancy

Fall in the birth rate of homozygous β -thalassemia

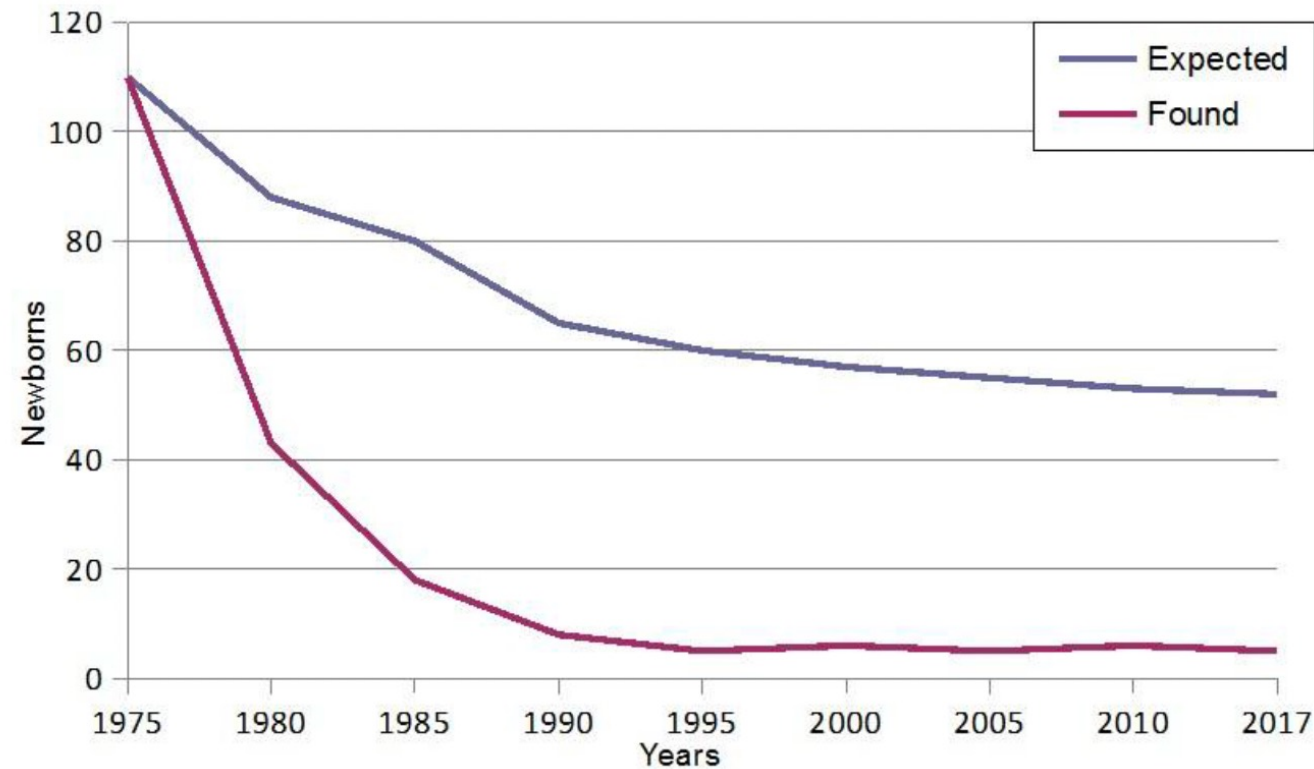


Figure 1. Fall in the birth rate of homozygous β -thalassemia in Sardinia.

Monni G, et al. J Clin Med. 2018 Feb 20;7(2):35.

Prenatal Diagnosis of Thalassemia and Hemoglobinopathies: 20 Years Experience at Siriraj Hospital (1985-2005)

- PND started in 1985 (2528 BE):
 - Placentocentesis or Fetoscope & globin chain analysis
 - Cordocentesis (Ultra Sound guided) & DNA analysis (2534 BE)
 - Chorionic villus sampling (CVS): transvaginal vs transabdominal
 - Amniocentesis
- Laboratory¹:
 - β/γ ratio, DNA mapping (RFLP)
 - Radioactive ASO probe, RDB

• Methods of Fetal sampling²

Fetal sampling	1985 - 1990	1991 - 2005
Fetal blood sampling	50	398
Placentocentesis	5	-
Fetoscope	13	-
Cordocentesis	32	398
CVS	12	368
Amniocentesis	5	2
U/S	12	-
Combined	2	55
Total	75	829

1. Fucharoen S, et al. Southeast Asian J Trop Med Public Health. 1991; 22(1):16-29

2. Kanokpongsakdi S, et al. Siriraj Med J 2007; 59: 274-277

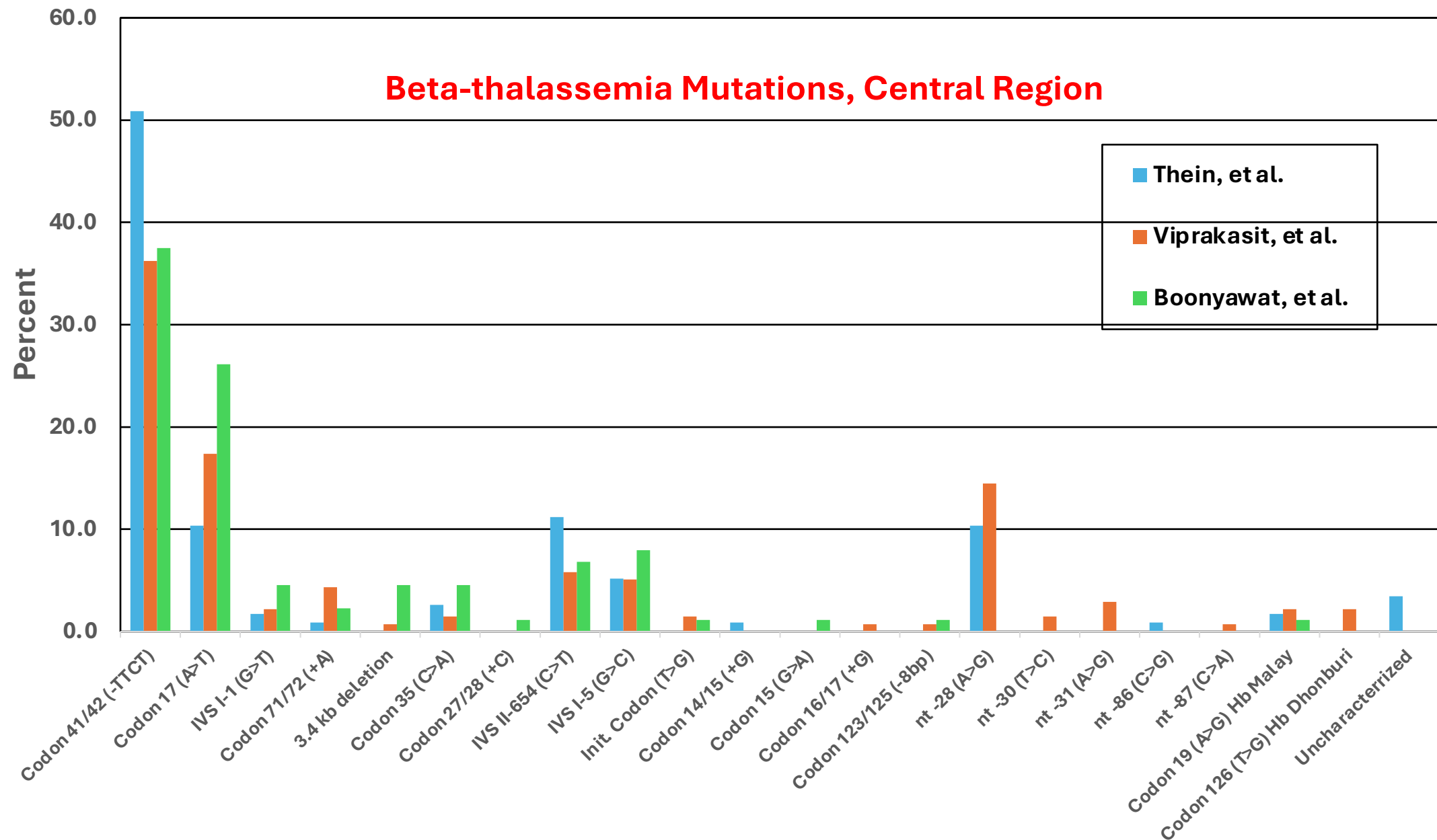
Summary of PND at Siriraj Hospital (1985-2005)

High-risk	Hb Bart's Hydrops Fetalist	Homozygous Beta - Thalassemia	Beta - Thalassemia/ HbE disease	Total
No. of pregnancy	228 (26%)	126 (14%)	550 (60%)	904 (100%)
Method of sampling				
CVS	103	48	229	380
Amniocentesis	3	1	3	7
Fetal blood sampling	89	69	290	448
U/S	12	-	-	12
Combined method	21	7	29	57
No. of Dx achieved	227	125	539	891 (98.5%)
No. of Dx not achieved	1	1	11	13 (1.5%)
Result				
Unaffected	143	107	409	659
Affected	85 (37%)	18 (15%)	129 (24%)	232 (26%)
Fetal Loss	2	1	7	10 (1%)
Misdiagnosis of the disease	3	2	2	7

Kanokpongsakdi S, et al. Siriraj Med J 2007; 59: 274-277

Results: 20 Years Experience at Siriraj Hospital (1985-2005)

- Total 940 PND cases, achieved diagnosis 891 cases (98.5%)
- Fetal loss 10 cases (1%):
 - 7 cases: dead fetus inutero (2nd trimester FBS)
 - 3 cases: developed complications:
 - sampling failure amniitis (N=1)
 - dry amniotic fluid (N=1)
 - spontaneous abortions (D₁₀ after trnsvaginal chorion biopsy) (N=1)
- Misdiagnosis 7 cases:
 - risk for Hb Bart's hydrops (N=3)
 - risk for homo. β -thalassemia (N=2)
 - risk for β -thal/Hb E (N=2)



Prenatal control of severe thalassemia: Chiang Mai Strategy

Chiang Mai Strategy

- (1) Genetic counselling
- (2) Identification of pregnancy at risk
 - 2.1 Retrospective screening (history review for known risk)
 - 2.2 Prospective screening (Figure 2)

Screening test

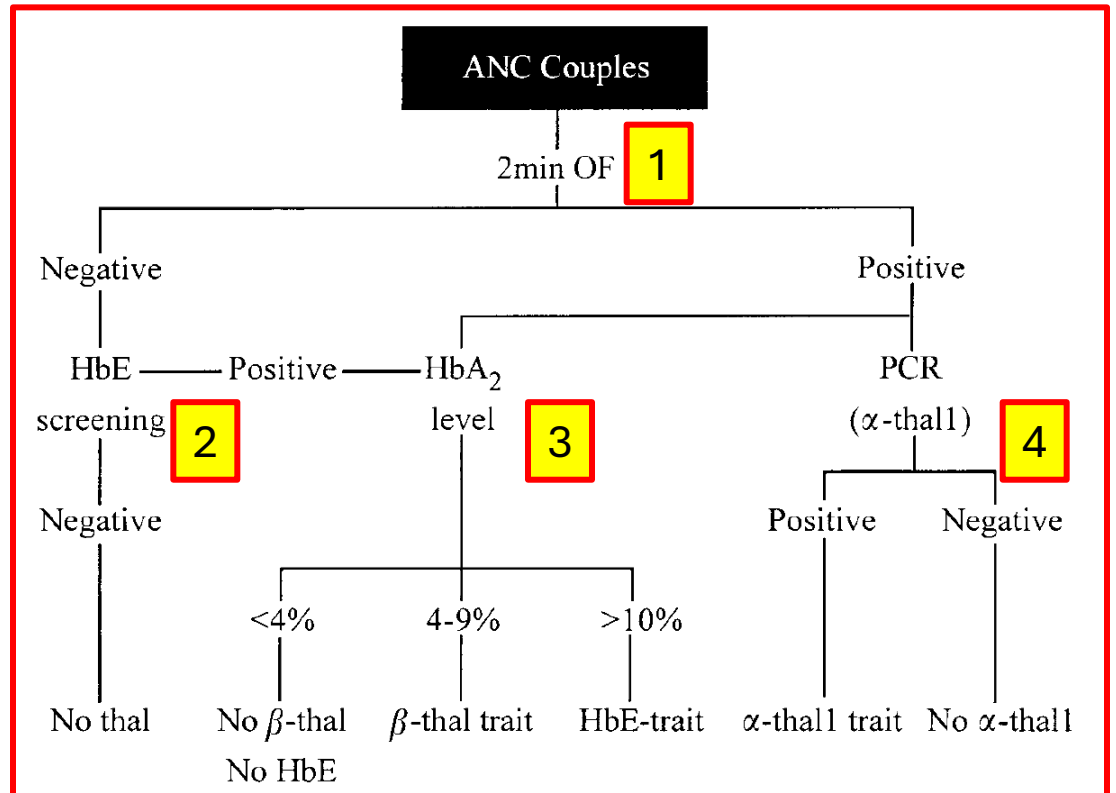
2 min OF (2 min osmotic fragility test)

HbE screening test (only if 2 min OF is negative)

Diagnostic test (if both of the couple are positive in the screening test)

HbA₂ level and PCR for α -thal1 if 2 min OF is positive

HbA₂ level (negative 2 min OF but positive HbE)
- (3) Prenatal diagnosis for pregnancy at risk
 - 3.1 Prenatal counselling
 - 3.2 Cordocentesis (16–22 weeks of gestation)
 - 3.3 Fetal blood analysis (checking maternal blood with acid elution test and haemoglobin typing with HPLC)
- (4) Counselling and termination of affected pregnancy



Tongsong T, et al. Prenat Diagn. 2000;20:229-34

Results: Chiang Mai Strategy

Retrospective & Prospective screening

Risks by screening	Cordocentesis*	$\alpha 1/\alpha 1$	β/β	β/E	Total
Retrospective	108	12	9	8	29
Prospective	134	8	7	18	33
Total	242	20	16	26	62

***Fetal loss 1.4%**

$\alpha 1/\alpha 1$: Hb Bart's hydrops; β/β : Homo. β -thal; β/E : β -thal/Hb E

Prospective screening

Table 1—Prospective screening in identifying pregnancies at risk

Total women attending antenatal clinic	11 124
Total screened women	7954
Women with positive screening test	2282 (28.7%)
Screened husbands	1586
Couples with positive screening test	312
Couples at risk (positive diagnostic test)	181
No prenatal diagnosis	27
Serial ultrasound (<i>Risk for alpha only</i>)	20
Early hydrops fetalis	5/20 (25.0%)
Cordocentesis	134

*Couple at-risk; 181, Did not perform PND = 27/181 (15%)

Tongsong T, et al. Prenat Diagn. 2000;20:229-34

PND in Northern Thailand (mol. era: 2012-2017)

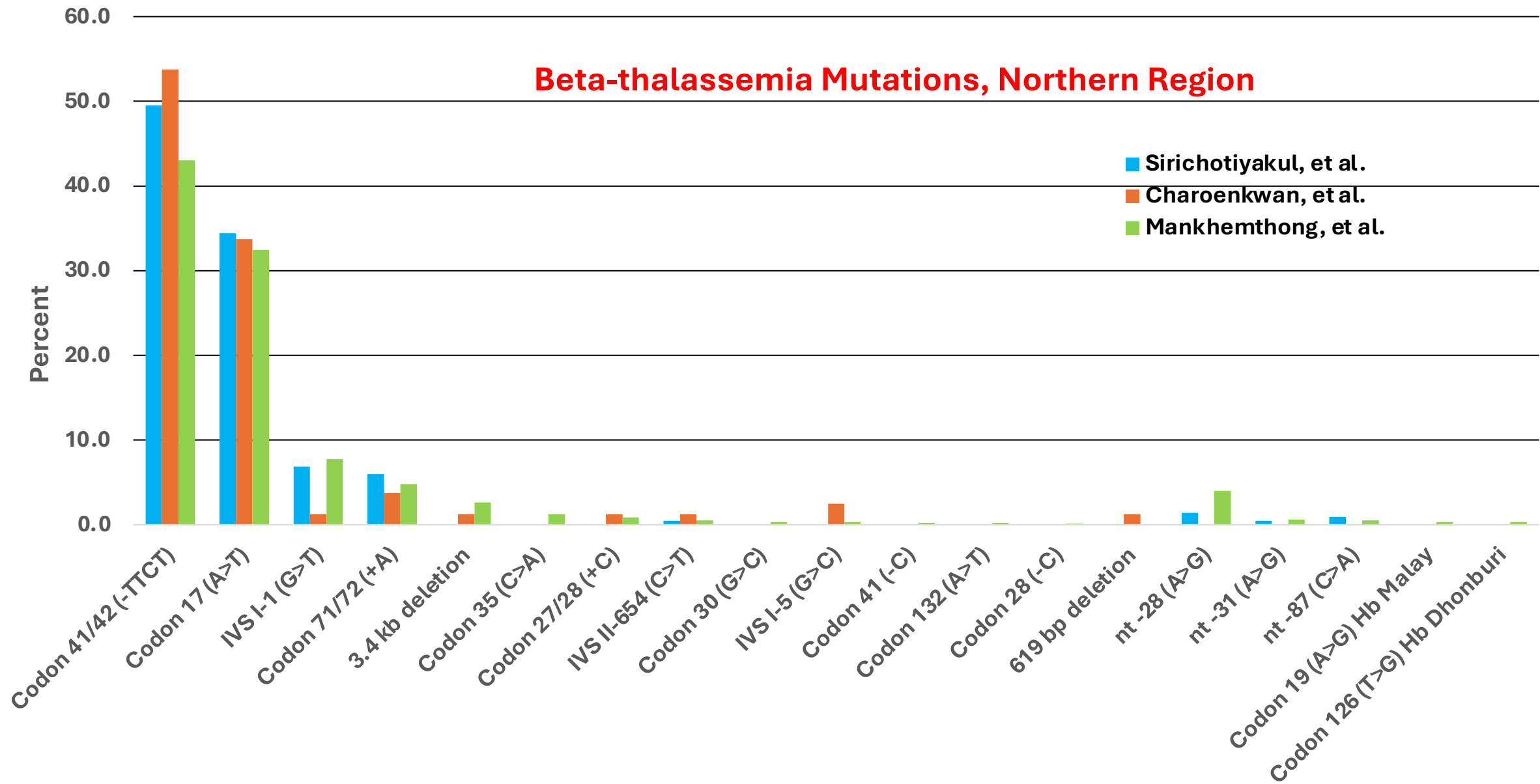
- 1,290 couples at risk for severe thalassemia, at CMU
- Screening: MCV, Hb E screen, if positive: spouse screening
- Confirm: HB analysis, PCR for α^0 -thalassemia
- Molecular/alternative diagnosis:
 - α -thal: multiplex PCR with high-resolution melting (HRM) analysis
 - β -thal: PCR with HRM analysis, DNA sequencing (unknown mutations)
 - Hb Bart's Hydrops: serial U/S for sign of fetal anemia, confirm by invasive fetal diagnosis
- Genotype:
 - α^0 -thal: SEA deletion (99.7%) and Thai deletion (0.3%)
 - β -thal: Codon 41/42 (-TTCT), codon 17 (A>T), IVS-I-1 (G>T) and codon 71/72 (+ A) (90%)

Results: 1,290 couples at risk, 1,301 fetus (11 pairs of twins)

Risk of thalassemia	Number of couples at risk (%)	Number of fetuses (%)	Number of affected fetuses (%)
Alpha-thalassemia			
Hb Bart's hydrops fetalis	444 (34.4)	446 (34.3)	118 (33.0)
Beta-thalassemia			
BTM	196 (15.2)	198 (15.2)	56 (15.6)
BE	642 (49.8)	649 (49.9)	182 (50.8)
BTM and BE	4 (0.3)	4 (0.3)	1 BE (0.3)
Both alpha and beta-thalassemia			
Hb Bart's hydrops fetalis and BTM	1 (0.1)	1 (0.1)	0
Hb Bart's hydrops fetalis and BE	3 (0.2)	3 (0.2)	1 BE (0.3)
Total	1290 (100.0)	1301 (100.0)	358 (100.0)

*BTM= β -thal major, BE= β -thal/Hb E

Mankhemthong K, et al. Int J Hematol. 2019;110(4):474-81



PND of α - and β -thalassemia in Southern Thailand (2003-2017)

- Songklanagarin Hospital: screening 26,021 pregnant
- Couples at risk=1,906 (7.3%)
 - risk for severe thal. = 1,704 (89.4%): β/β , β/E , α^0/α^0
- Amniocentesis at 15-20 wks
- Mutations:
 - β -mutations: 21 point + 7 del.
 - (14 common mutations=96.5%)
 - α^0 -thal: 98% SEA, 2% Thai del.

Table 5 Outcome of 1906 pregnancies for which a prenatal diagnosis was achieved

Fetuses at risk for	Fetal diagnosis			Total (%)
	Normal	Carrier	Affects	
β -thal/ β -thal	43	90	53	186 (9.76)
β -thal/HbE	281	673	365	1319 (69.20)
β -thal/ $\delta\beta$ -thal	7	20	10	37 (1.94)
$\delta\beta$ -thal/ $\delta\beta$ -thal	1	1	2	4 (0.21)
$\delta\beta$ -thal/HbE	36	83	38	157 (8.24)
α^0 -thal/ α^0 -thal	52	106	41	199 (10.44)
α^0 -thal/ α^{CS} -thal	0	2	2	4 (0.21)
Total (%)	420 (22.04)	975 (51.15)	511 (26.81)	1906 (100)

$\delta\beta$ -thal mutations 12.5 kb deletion or Asian Ind-inv-del or HPFH6

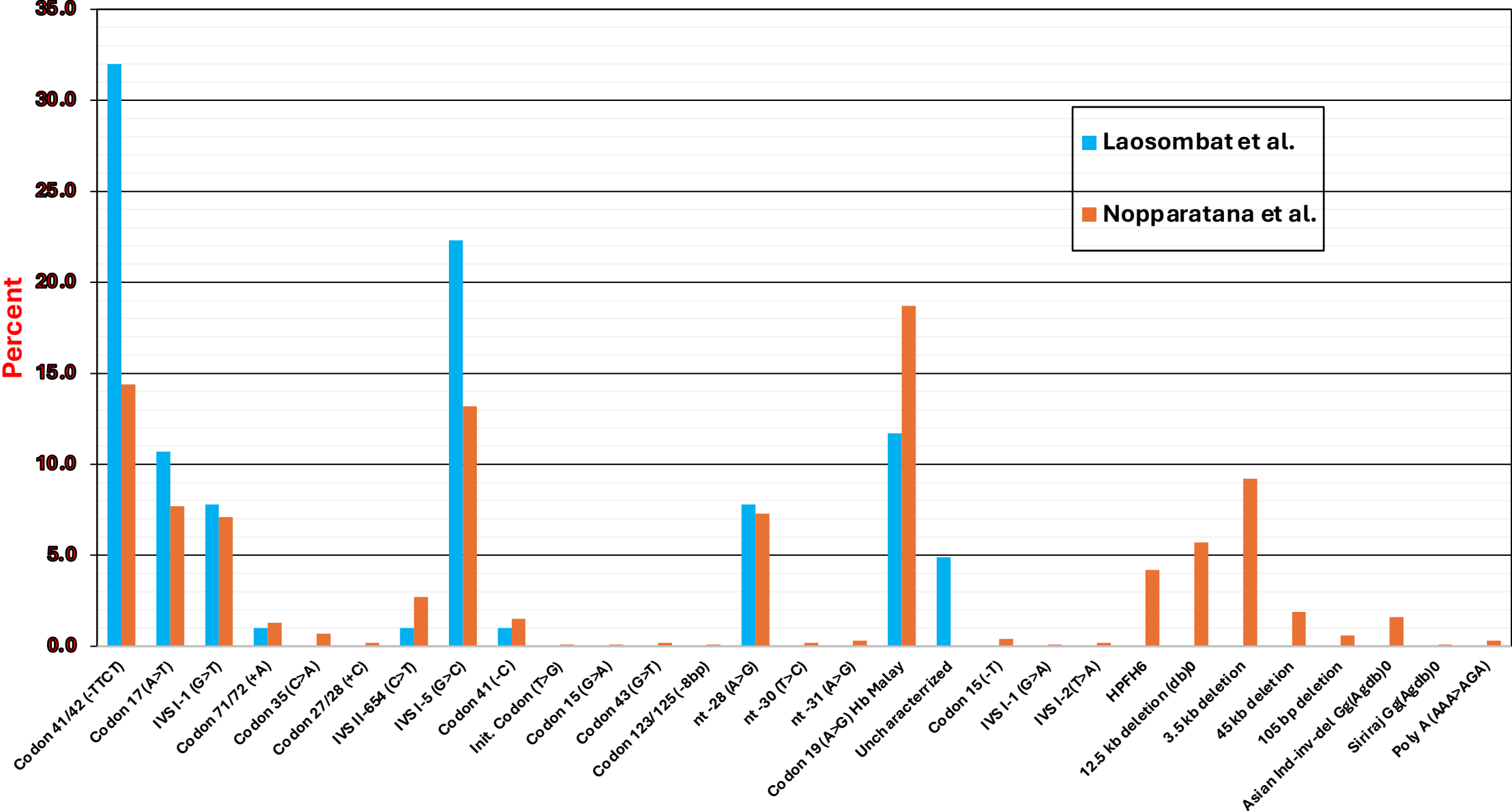
Outcomes: PND at Songklanagarind Hospital

- 1,004 pregnant women who sought treatment at Song. Hos
- 183 affected pregnancies
- 153 (83.6%) terminated pregnancies (TOP)
- 30 (16.4%) did not TOP

Table 6 Outcome of prenatal diagnosis of pregnant women with severe thalassemia diseases who sought treatment at Songklanagarind Hospital

Type of thalassemia	Number of affected pregnancies	Number of terminated pregnancies
β^0 -thal/ β^0 -thal or β^0 -thal/ β^+ -thal	24	20
β^0 -thal/HbE	136	110
α^0 -thal/ α^0 -thal	23	23
Total (%)	183	153 (83.6%)

Beta-thalassemia Mutations, Southern Region



Prevention of severe thalassemia in northeast

Thailand: 16 years of experience at KKU
(Jan 1993-Dec 2008)

- Positive screening (MCV,DCIP): 1,422
- True positive couples (Hb analysis): 1,254
- Risk couples for severe thal (DNA analysis): 986
- Did not perform PND: 212
- Performed PND: 756
 - Fetal diagnosis: 846
- Misdiagnosis: N=2
 - α^0 -thal trait for homo α^0 (maternal contaminate): 1
 - β -thal trait for β -thal/Hb E (technical PCR): 1
- Fetal loss: 1/75 Cordocentesis: 1.3%

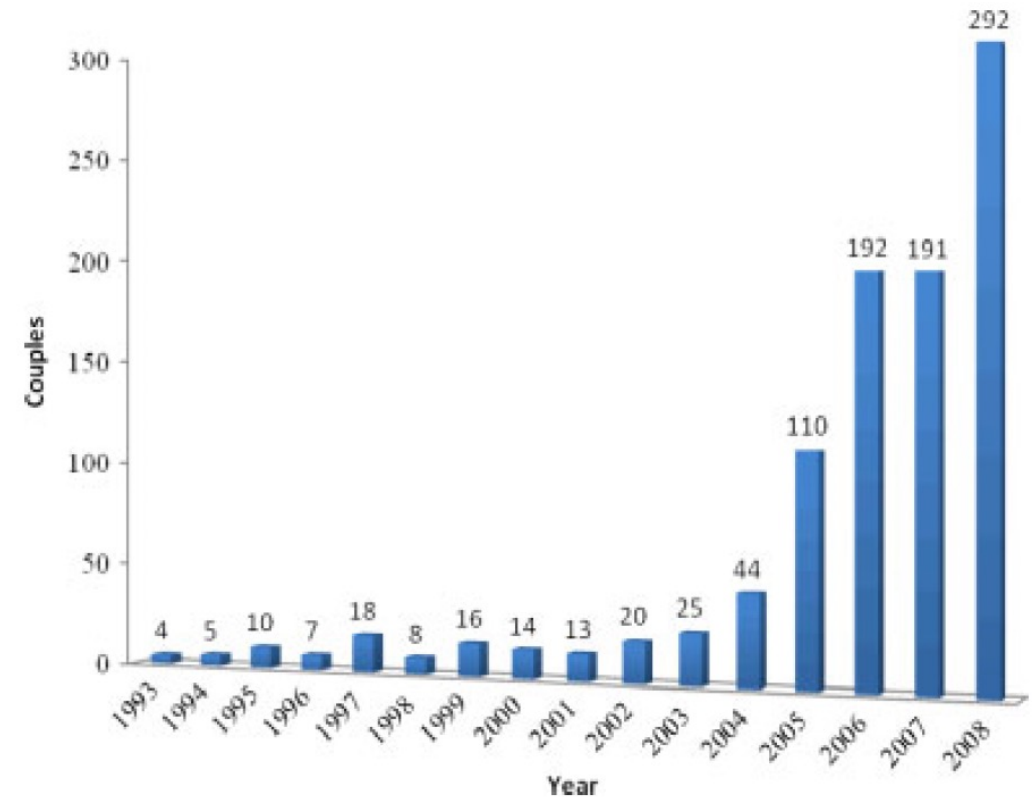


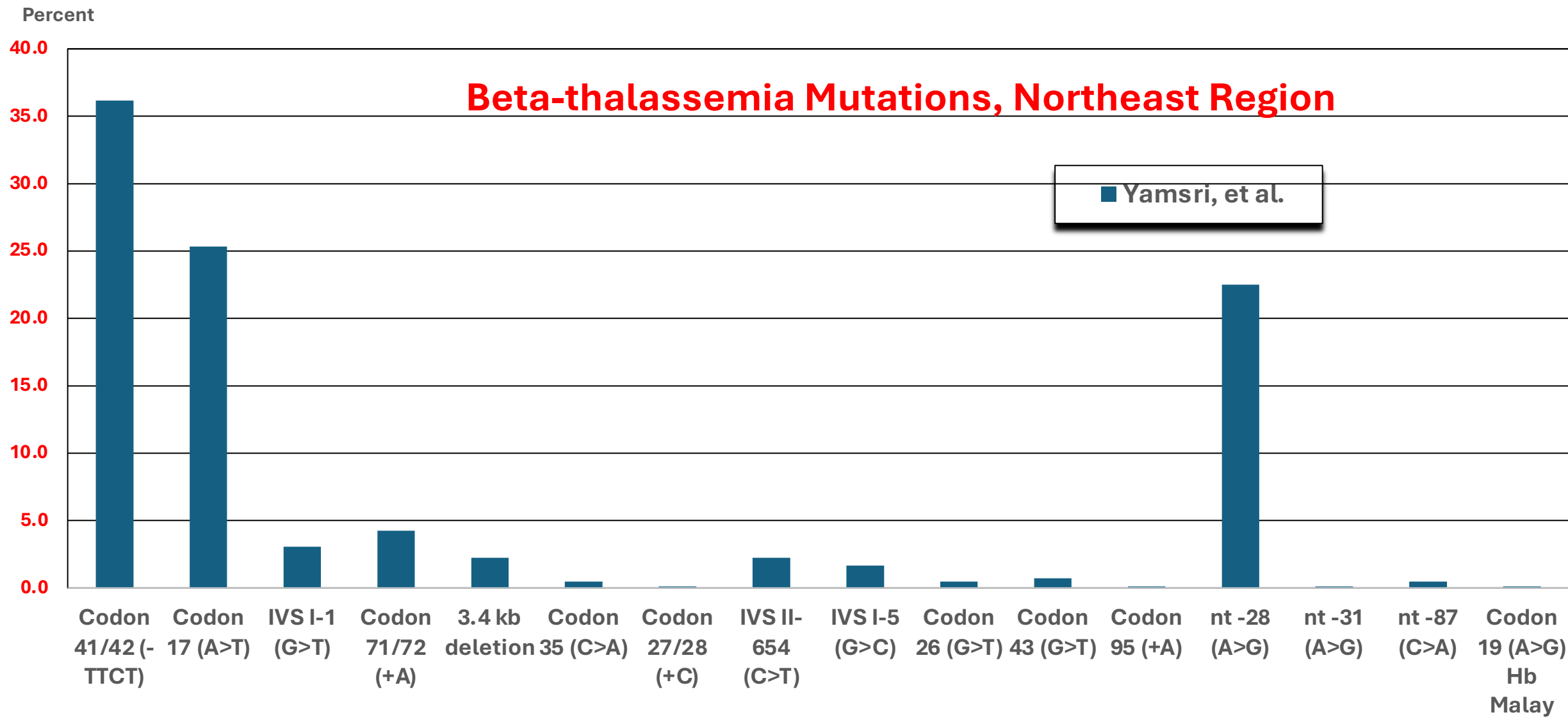
Figure 1—Number of couples attending at Khon Kaen University in northeast Thailand for thalassemia screening and prenatal diagnosis during 1993–2008

Prevention of severe thal in northeast Thailand: 16 years of experience at KKU (Jan 1993-Dec 2008)

- **1422 at Risk Couples**
- **1254 True positive couples**
- **968 Risk for Severe Thalassemia**
- **846 Fetal diagnosis**
- **2 misdiagnosis (α^0 -thal trait > Hb Bart's Hydrops; Hb E trait for β -thal/Hb E)**
- **1 Fetal loss (Cordocentesis)**

Table 4—Proportions of the fetuses diagnosed as affected, thalassemia carrier and unaffected for the true-risk couples

Diseases being at-risk (no.)	Number of fetuses (%)		
	Affected	Carrier	Unaffected
β -thalassemia/Hb E disease (487)	127 (26.1)	253 (52.0)	90 (18.5)
Homozygous α^0 -thalassemia (341)	92 (27.0)	162 (47.5)	87 (25.5)
Homozygous β -thalassemia (9)	4 (44.4)	4 (44.4)	1 (11.1)
Homozygous β -thalassemia and β -thalassemia/Hb E disease (4)	3 (75.0)	1 (25.0)	0
Homozygous α^0 -thalassemia and β -thalassemia/Hb E disease (5)	2 (40)	3 (60)	0
Total (846)	228 (26.9)	423 (50.0)	195 (23.0)



Effectiveness of the **Model for the prenatal control of severe thalassemia**

- Multi-center, 6 hospitals, during Aug 2009 -Dec 2011
- 12,874 pregnancies; 7,008 analysis, 281 couples at risk, 58 affected fetuses
- All neonates were evaluated for thalassemia

Table 1 Number of couples and neonates participating through the programs

Institute	No. of woman	No. of positive screened women	No. fetal diagnosis
Bhumibol Hospital	999	286	232
Chiang Mai University	3280	986	1766
Khonkaen University	810	339	778
Pramongkutklao Hospital	3554	955	1455
Prince of Songkla University	722	266	239
Rajavithi Hospital	3509	1151	2538
Total	12 874	3983	7008

Tongsong T, et al. Prenat Diagn 2013;33(5):477-83

Results

Table 2 Prenatal diagnosis of severe thalassemia syndromes among pregnancies at risk

Diseases being at risk (no.)	PND	Unaffected	Affected
β -thalassemia/HbE disease	151	124	27
HbBart's disease	87	67	20
β -thalassemia major	43	35	8
HbBart's disease + β major	3	2	1
HbBart's disease + β -thalassemia/HbE disease	5	3	2
Total ^a	281	231	58

PND, prenatal diagnosis.

^aSome pregnancies had risk for two severe diseases.

Effectiveness of the model in prenatal prediction of severe thalassemia

- Prevalence 0.83%
- Sen. 100%, Spec 96.8%
- Pos. predictive. 20.6%, Neg. predictive. 100%

Table 3 Effectiveness of the model in prenatal detection of severe thalassemia syndromes

Model prediction	Severe thalassemia	No severe thalassemia	Total
Couples at risk	58 a	223 b	281
Couples at no risk	0 c	6727 d	6727
Total	58	7008	7008
Prevalence of severe thalassemia	0.83% (58/7008)		
Sensitivity (58/58)	100.0 % (95% CI: 100;100)		
Specificity (6727/6950)	96.8% (95% CI: 96.4;97.2)		
Positive predictive value (58/281)	20.6 % (95% CI: 15.9;25.4)		
Negative predictive value (6727/6727)	100.0 % (95% CI: 100;100)		

Tongsong T, et al. Prenat Diagn 2013;33(5):477-83

Model for the prenatal control of severe thalassemia

- 1) Genetic counseling
- 2) Screening: MCV, CMU-E screen
- 3) Screening husbands if wife +ve screen
- 4) Diagnosis of carrier
- 5) Identify the matched couples at risk
- 6) PND (β -thal/ β -thal, β -thal/Hb E, α -thal1/ α -thal1)
- 7) Counseling and option for Termination of affected pregnancy

Tongsong T, et al. Prenat Diagn 2013;33(5):477-83

Box 1 : The Model for Prenatal Control of Severe Thalassemia

- 1) Genetic counseling
- 2) Screening pregnant women for a carrier using the following screening tests
 - MCV screening for thalassemia trait (α -thal-1, or β -thal)
 - CMU-E screen for HbE trait
- 3) Screening the husbands whose wife had a positive screen (MCV and CMU-E)
- 4) Diagnostic test for a carrier if both had positive screens;
 - Positive MCV (risk for α -thal-1 or β -thal trait):
 - HbA2/HbE microcolumn for β -thalassemia trait
 - IC strip/PCR test for diagnosis for α -thal-1
 - Positive CMU-E only: (risk for HbE trait but not α -thal-1 or β -thal trait)
 - HbA2/HbE microcolumn for diagnosis of HbE trait

*if the test was positive in one of the couple then the other was also tested
if the test was negative in one of the couple no further test was needed*
- 5) Identify the matched couples at risk for a certain disease
 - Homozygous β thalassemia:
 - β thal trait and β thal trait
 - β thal trait and β thal / HbE
 - β thalassemia / HbE :
 - β thal trait and HbE trait
 - β thal trait and homozygous HbE
 - β thal trait and β thal/HbE disease
 - Hb Bart's disease
 - α thal 1 trait and α thal 1 trait
- 6) Prenatal diagnosis for pregnancies at risk
 - Risk for β thalassemia major, β -thalassemia / HbE disease
 - CVS or Amniocentesis or Cordocentesis for fetal DNA or blood analysis
 - Risk for Hb Bart's disease
 - Invasive diagnosis as above OR
 - Serial ultrasound
 - No ultrasound marker: repeat ultrasound every 2 weeks
 - +ve for ultrasound marker: invasive fetal diagnosis
- 7) Counseling and options for termination of affected pregnancy

Factors affecting Thai pregnant women's decisions concerning PND and termination of pregnancy (TOP) for β -thalassemia

- **Study:** Div. Maternal Fetal Medicine, Dep. Obstetrics and Gynecology, Siriraj Hosp (May 2018 and June 2019)
- **Participants:** 142 Thai Buddhist pregnant women waiting for PND
- **Question:** “reasons for PND and their decisions and reasoning concerning pregnancy if the fetus was found to be affected”
- **Accepted PND for 3 reasons:**
 - 1).to know whether their pregnancies were affected
 - 2).to confirm that their pregnancies were unaffected
 - 3).to terminate if their pregnancies were affected
- **Results:** acceptance of PND was not always associated with TOP. Multiple factors influenced the decision to terminate, but not their religious affiliation

Preimplantation Genetic Diagnosis (PGD)

- Superovulation (recombinant FSH)
- Oocytes fertilization
 - Intra Cytoplasmic Sperm Injection (ICSI)
- Cleavage stage day 3 embryo biopsy
- Molecular genetic diagnosis (single cell)
- Transfer of normal embryo

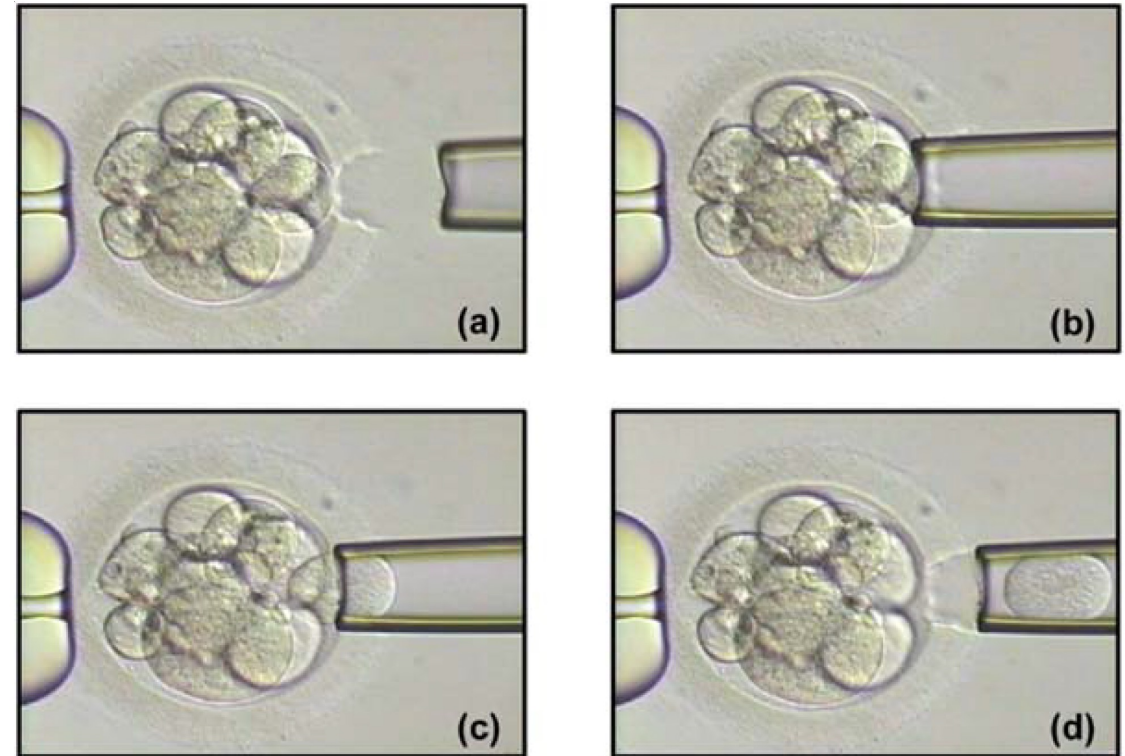


Fig. 1 Cleavage stage embryo biopsy in the present study: (a) the embryo is stabilized by a holding pipette (left) with negative pressure, a hole is created in the zona pellucida (zona drilling) using laser, (b), (c) and (d) a blastomere is gently pulled away from the embryo using a biopsy pipette (right) with negative pressure through the hole in the zona pellucida. Procedure performed by TV

PGD for severe thalassaemia

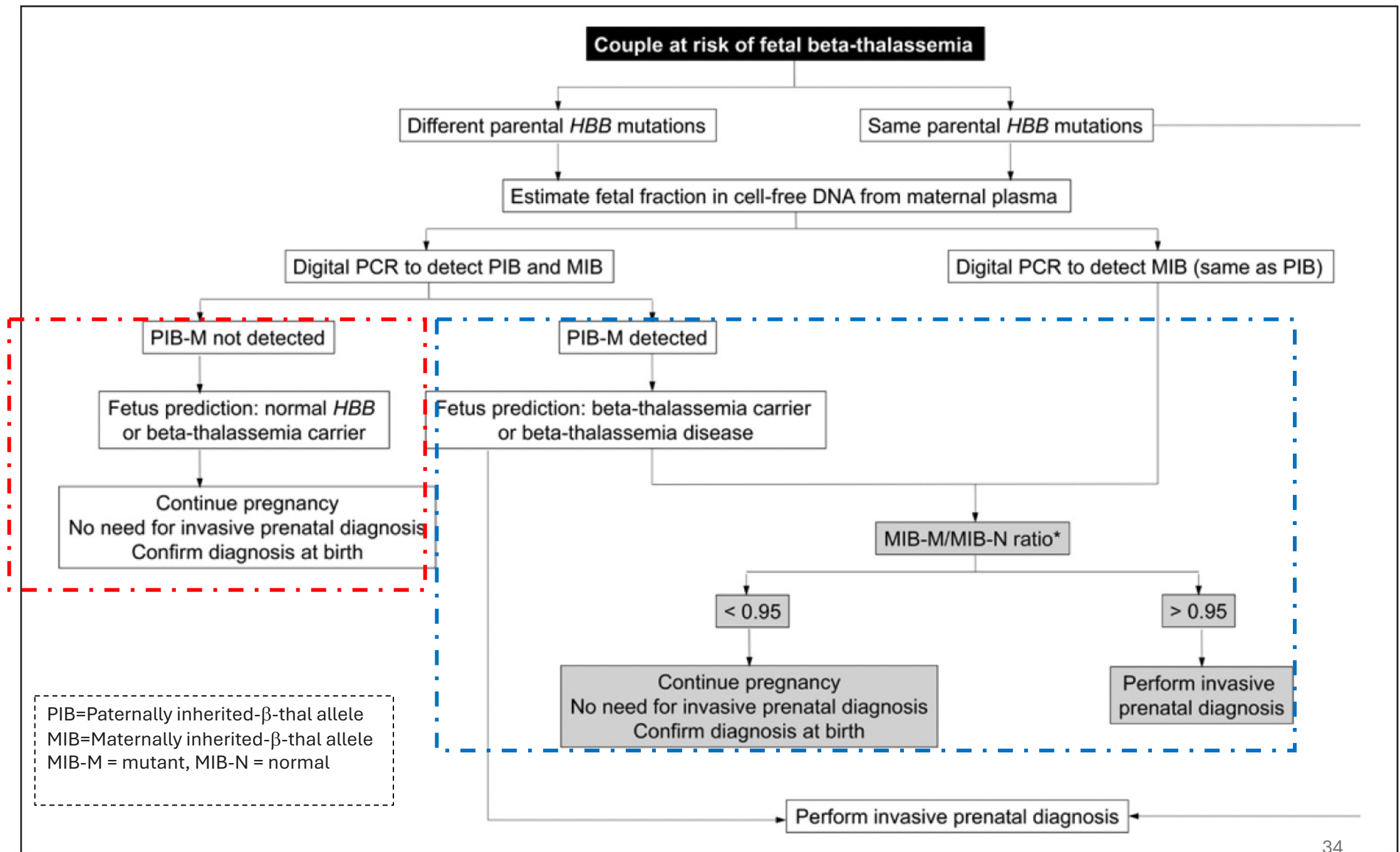
- Alternative to the traditional PND, without Termination of pregnancy (TOP)
- 1st reported (2547 BE) couples at risk of homo. β -thal ($\beta^{CD41/42}/\beta^{CD41/42}$)
- Dep. Of OBGYN, Faculty of Medicine, CMU
- During 2547-2561 BE
 - 24 pregnancies at risk, 64 PGD cycles (37 homo α^0 -thal, 5 β/β and 22 β -thal/Hb E)
 - 24 healthy pregnancies delivered 27 babies

Noninvasive PND of β -Thalassemia Disease by Using Digital PCR Analysis of Cell-Free Fetal DNA in Maternal Plasma

- dPCR detect (in maternal plasma)
 - Paternally inherited- β -thal allele (PIB)
 - Maternally inherited- β -thal allele (MIB)
- Study : pregnancies couples at risk of β -thal/Hb E, β -thal/ β -thal (N 35)
 - GA: 12-22 weeks (18.4 ± 2), mutation: CD 17(A>T), IVS I-1(G>T), CD 41/42 (-TTCT), CD 26(G>A)
- Result & Interpretation (different parental mutation, N=29):
 - Absent PIB-M = normal or β -thal trait (from maternal) = **Exclude thal. Disease**
 - Present PIB-M (mutant) = β -thal trait or thal. Disease
 - MIB-M/MIB-N ratio = 0.87 ± 0.07 = **Fetus not inherited maternal mutation**
 - MIB-M/MIB-N ratio = 1.01 ± 0.05 = **Fetus inherited maternal mutation**
 - **Sensitivity 100%, Specificity 92.3%, false-positive rate 7.7%, AUC: 0.976 (95%CI 0.925-1.0)**

Results & Interpretation (continue)

- 4 couples with same parental mutation:
 - MIB-M/MIB-N ratio:
 - 0.90 = normal *HBB* (v=1)
 - 1.01 and 1.11 = heterozygous mutation (N=2)
 - 1.45 = homo. β -thal (N=1)
- 2 couples with paternal β -thal/Hb E, maternal β -thal trait
 - PIB-M present 25 and 35 positive partition
 - MIB-M/MIB-N ratio: 0.96 and 1.10
 - All two cases diag β -thal/Hb E



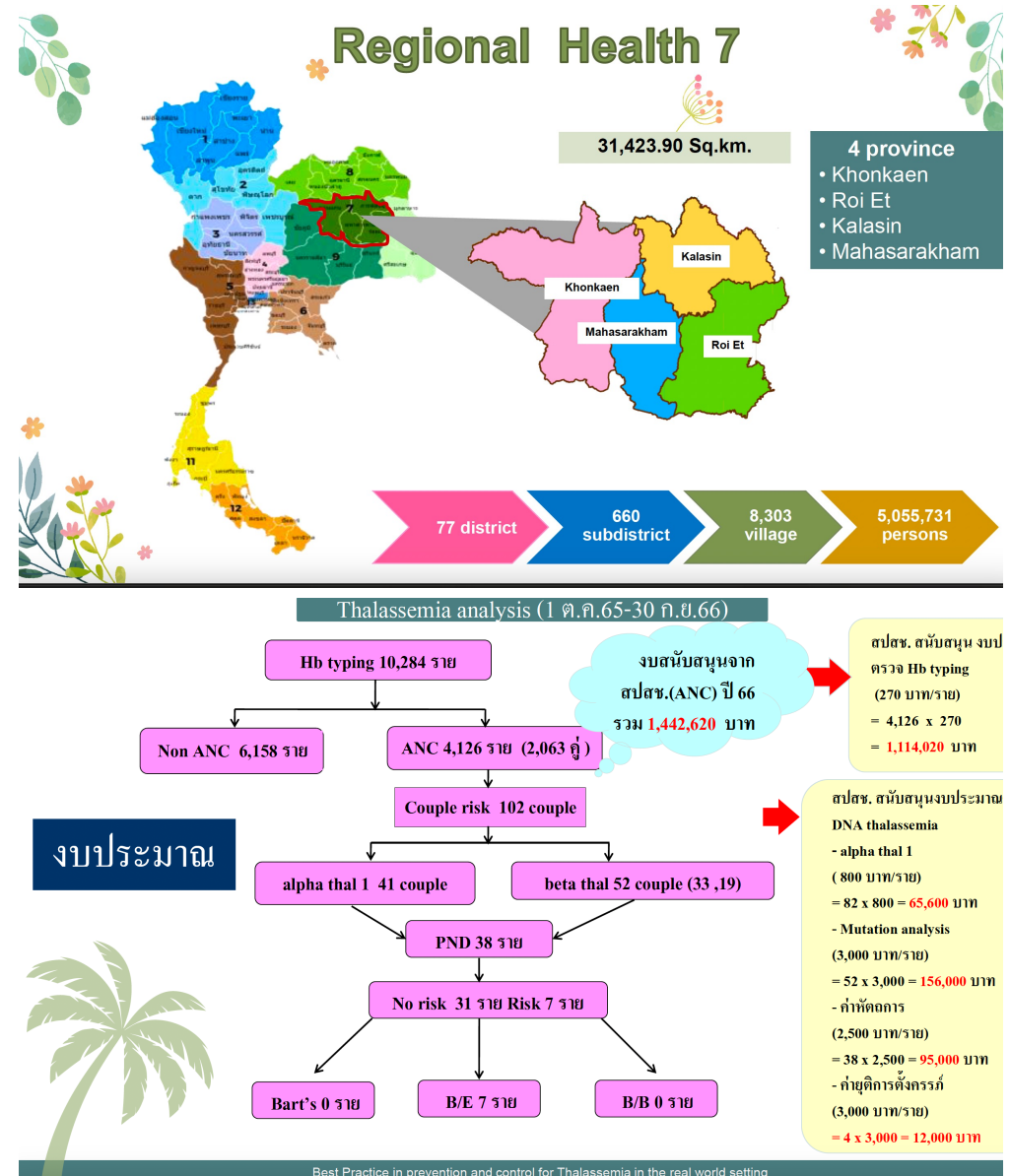
The evaluate the Thalassemia Prevention and Control in Pregnancy Program: HITAP (2561 BE)

- The Ministry of Public Health (MPH) introduced the National Thalassemia Action Plan 2550-2554 BE aim:
 - to reduce the number of newborns with severe thalassemia
 - to improve patients' quality of life
- The National Health Security Office (NHSO) implemented a strategic plan for prevention and control of thalassemia 2557-2559 BE: **“Thalassemia Prevention and Control in Pregnancy Program”**
- In 2561 BE, Health Intervention and Technology Assessment Program (HITAP) evaluate the Thalassemia Prevention and Control in Pregnancy Program

Evaluated Items:	Results:
All new pregnant women received thal. Screening	83.7-99.8%
Husban screening	60.7-87.4%
Couples at risk received PND	28.8-87.4%
TOP (termination of preg nancy)	24.6-100%
New pregnancy thal. Screening at GA. (wk.)	9.7-17 wk.
Incidence of new thal. (0-4 yrs.) decline, 2559 BE	20 to 19/100,000 patiens

Prevention & control of severe thalassemia: Khon Kaen Hospital (2022-2023)

- Screening: 10,284
- Couple at risk : 102
- PND: 38
- β -thal/Hb E: 7
- TOP: 3



Summary: Current Status of Thalassemia Prevention

- **Public awareness & Education**

- Carrier detection &
- Molecular diagnosis
- Genetic counseling
- Options for Prevention:
 - Prenatal diagnosis
 - Preimplantation genetics diagnosis
 - Noninvasive prenatal diagnosis

- Organizations:

- The Ministry of Public Health
- The National Health Security Office
- **Thalassemia Foundation of Thailand**
- **Thai Society of Hematology**
- The Royal College of Physicians of Thailand
- The Royal College of Peditricians of Thailand
- The Royal Thai College of Obstetricians and Gynaecologists

Thank you for your attention

Nang Paya Sua Krong @ Doi Luang Chiang Dao: Jan 20, 2025