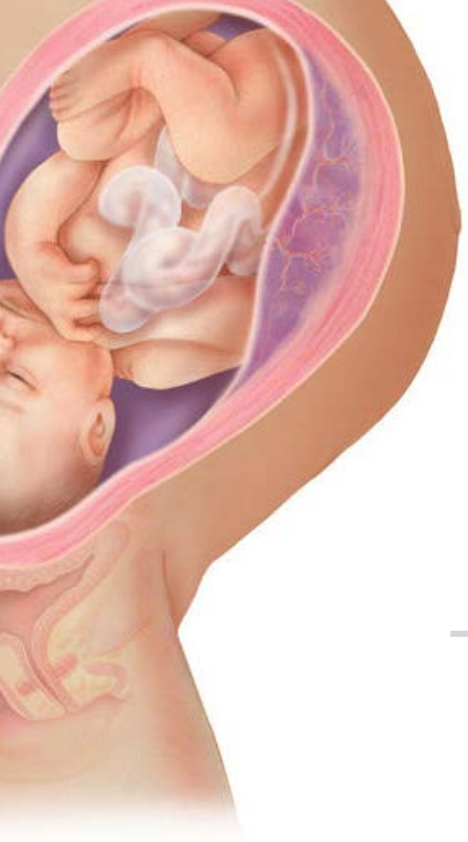


Noninvasive prenatal testing of α-thalassemia and β-thalassemia

Current Practice and Future Trend

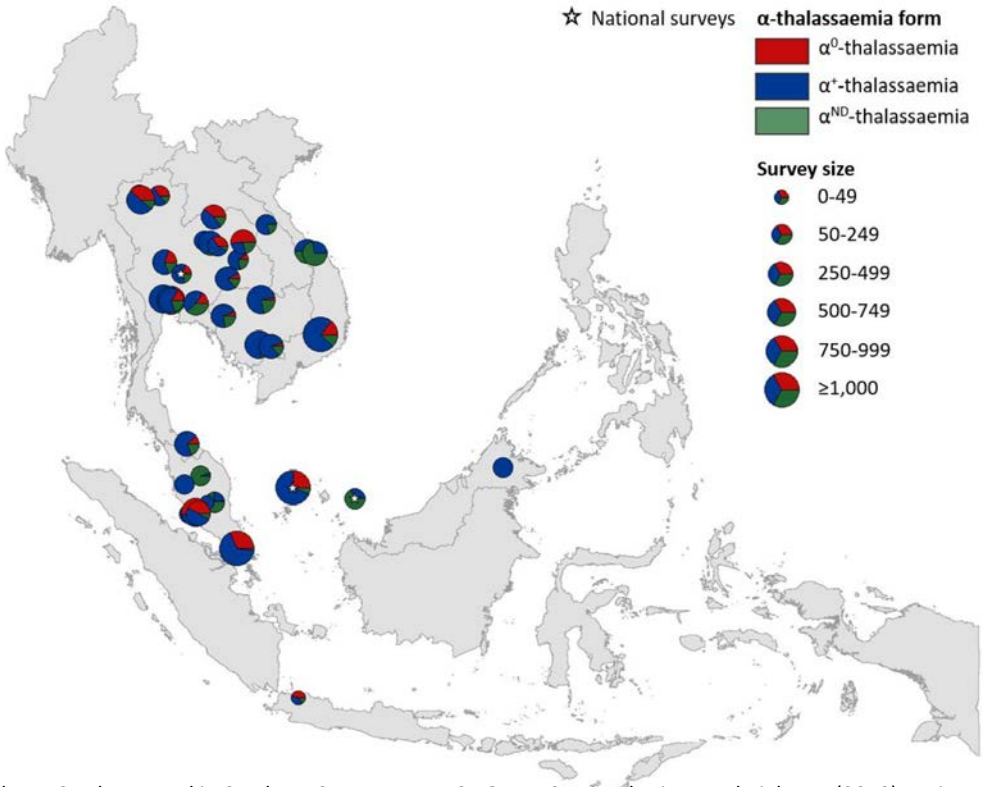
Prof. Vip Viprakasit, MD., D. Phil (Oxon)

Division of Haematology, Department of Paediatrics, Faculty of Medicine, Siriraj Hospital,

Mahidol University, Bangkok

THAILAND

Thalassemia distribution in Thailand



Hockham, C., Ekwattanakit, S., Bhatt, S., Penman, B.S., Gupta S., Viprakasit V. and Piel, F.B. (2019). Estimating the burden of α-thalassaemia in Thailand using a comprehensive prevalence database for Southeast Asia eLife 8:e40580.

40-50% of the Thai population are carriers of at least one of these abnormal genes*

α-thalassemia is at about 20–40%
β-thalassemia is at 3–9%

Variant types

1. Single nucleotide variants (SNVs)
2. Small insertions/deletions (InDels)
3. Copy number variants (CNVs)

Thalassemia **prevention** and control programs were introduced using **post conception screening in couples** and **prenatal diagnosis (PND)** for the prevention of new thalassemic births.

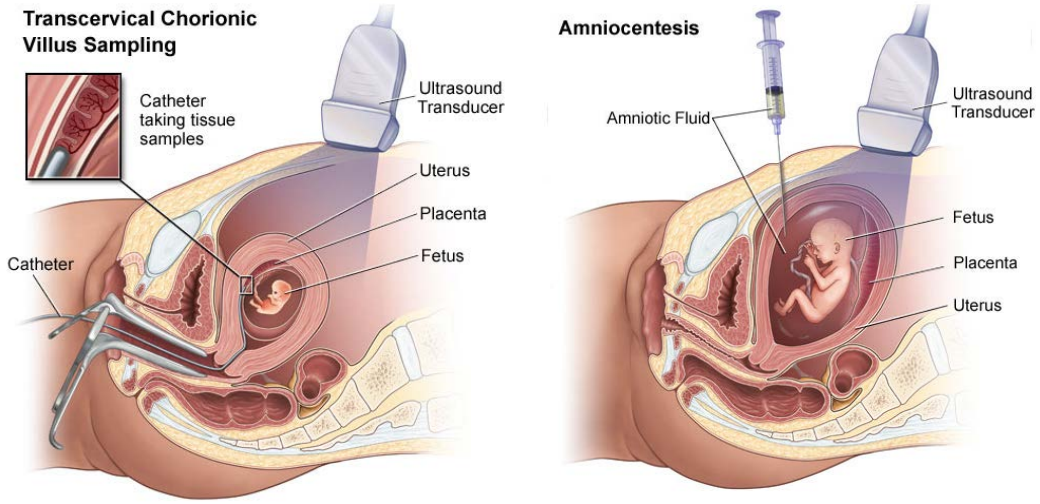
*Xu *et al.* BMC Public Health 2022

https://www.tandfonline.com/doi/full/10.1080/03630269.2022.2025824?scroll=top&needAccess=true&role=tab

Prenatal screening

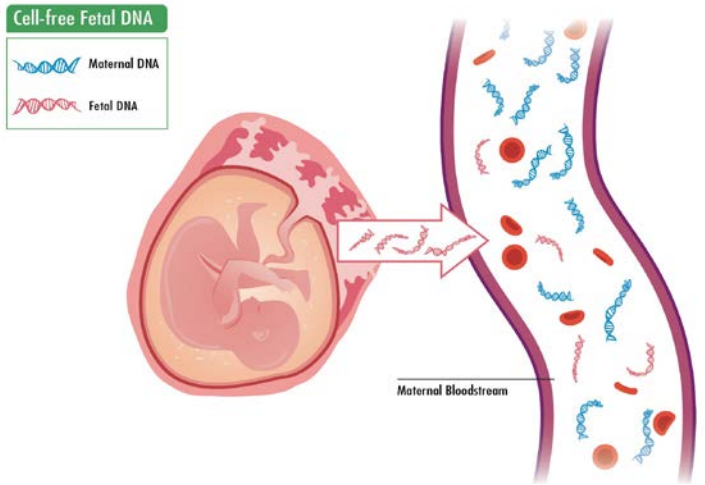
Traditional method (Invasive)

is the most commonly used to **diagnose** chromosomal abnormalities.



Non-Invasive Prenatal Testing (NIPT)

is a **screening** that helps to detect the presence of some genetic diseases and anomalies in the fetus before birth from **cell-free fetal DNA in mother's blood**.



Comparison between traditional method and NIPT

Characteristic	Amniocentesis	NIPT
Objective	For diagnosing To confirm/rule out genetic problems	For screening To evaluate the risk of problems
Type	Invasive procedure	Non-invasive
Miscarriage	1-2% risk	None
Time	> 17^{th} weeks of pregnancy	> 10^{th} weeks
Sample	Amniotic fluid	Mother blood

Non-Invasive Prenatal Testing (NIPT) for screening of genetic disorders in fetal

1. Chromosomal disorders

- Down Syndrome (Trisomy 21)
- Edwards Syndrome (Trisomy 18)
- Patau Syndrome (Trisomy 13)

2. Disorders found in the sex chromosome (X and Y)

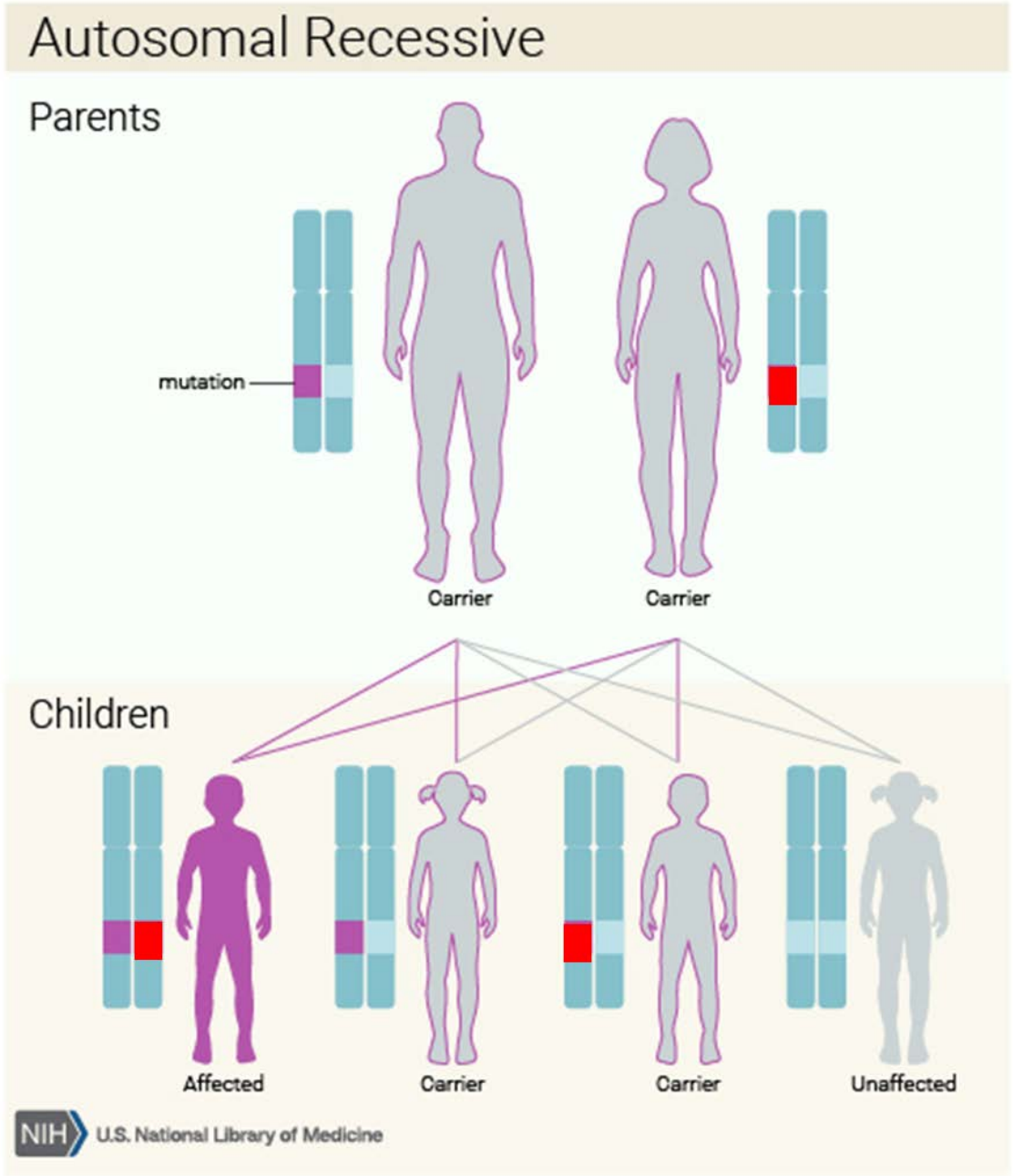
- Jacobs Syndrome (XYY)
- Turner Syndrome (Monosomy X)
- Klinefelter Syndrome (XXY)
- Triple X Syndrome (Trisomy X)
- XXYY Syndrome

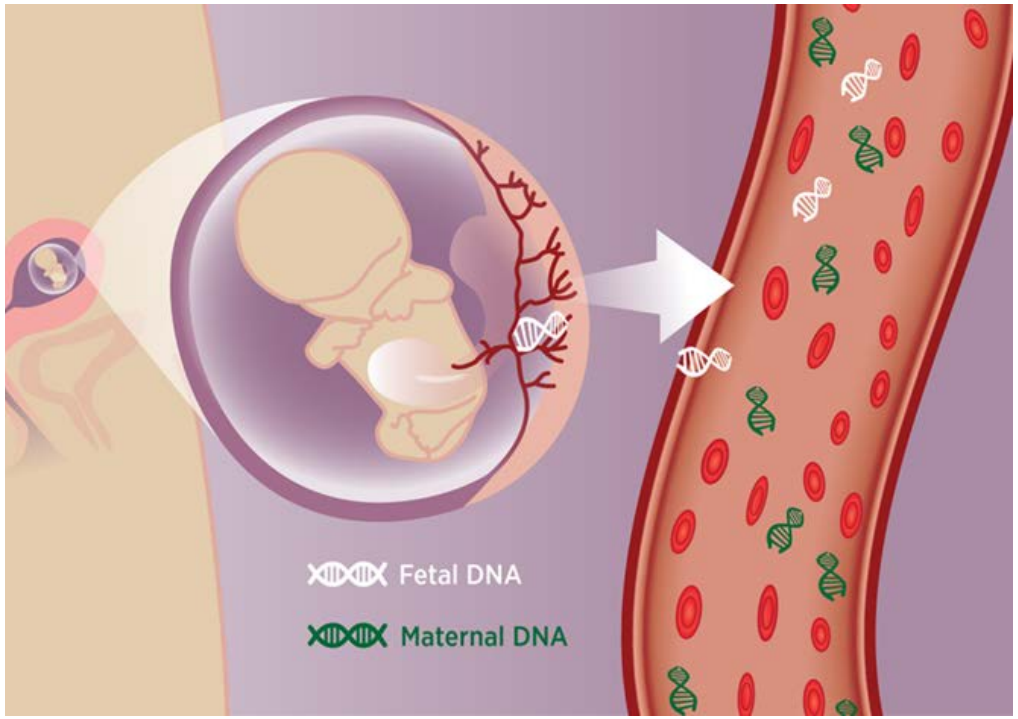
3. Disorders caused by some gene sequences (microdeletions)

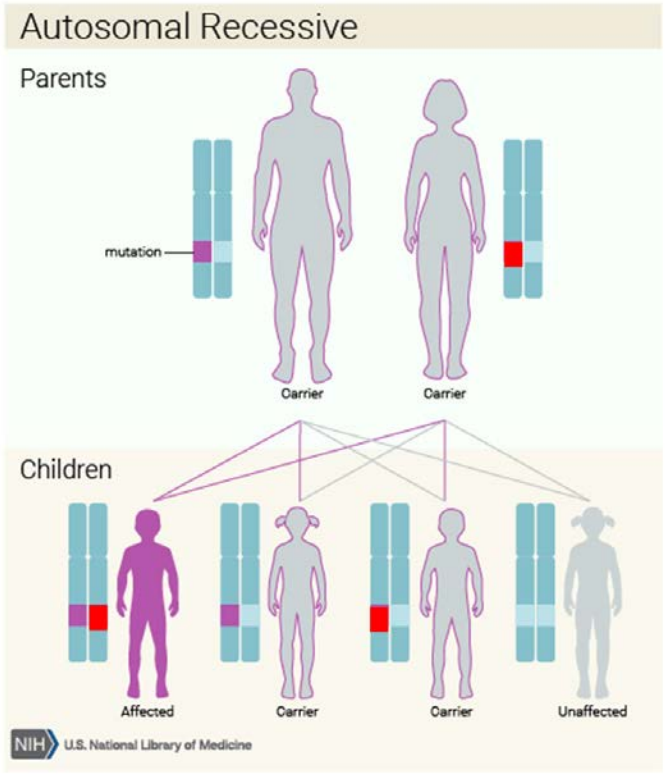
- Smith-Magennis Syndrome
- DiGeorge Syndrome
- 1p36 deletion Syndrome
- Wolf-Hirschhorn Syndrome

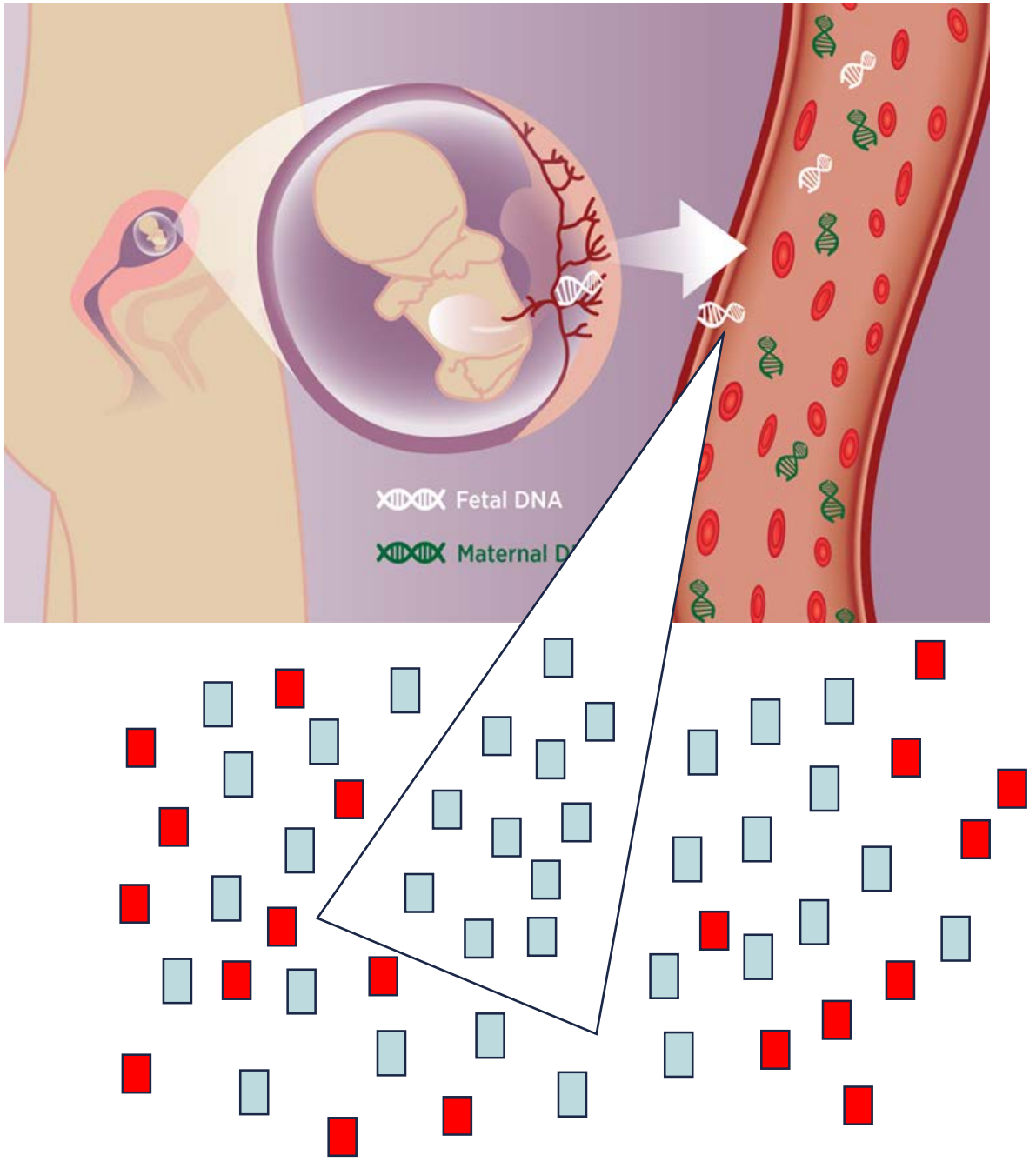
4. Some of the nearly 100 monogenic disorders

- Alpha-thalassemia (α-thalassemia)
- Beta-thalassemia (β-thalassemia)
- Sickle cell anemia
- Cystic fibrosis
- Gaucher's disease
- Phenylketonuria
- etc..


Autosomal Recessive
Parents
mutation
Carrier
Carrier
Children
Affected
Carrier
Carrier
Unaffected
NIH
U.S. National Library of Medicine

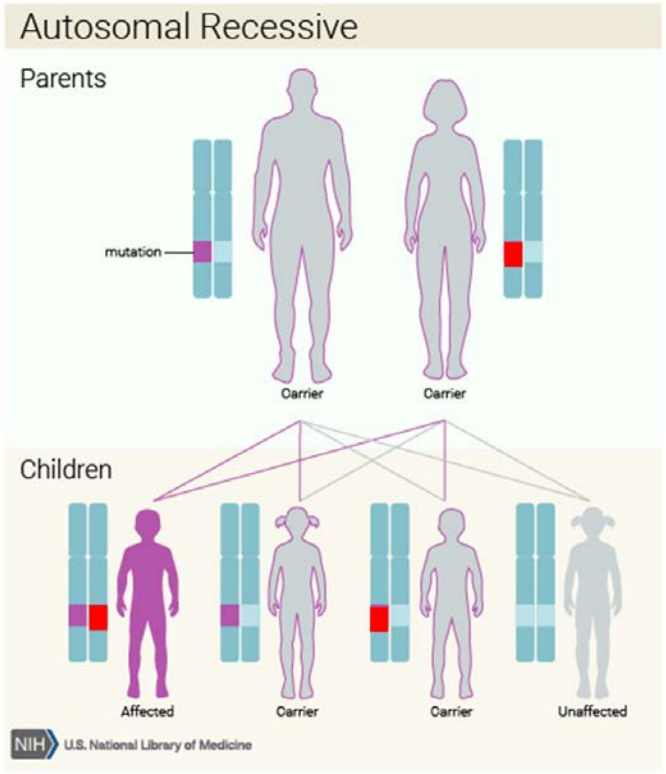

Fetal DNA
Maternal DNA

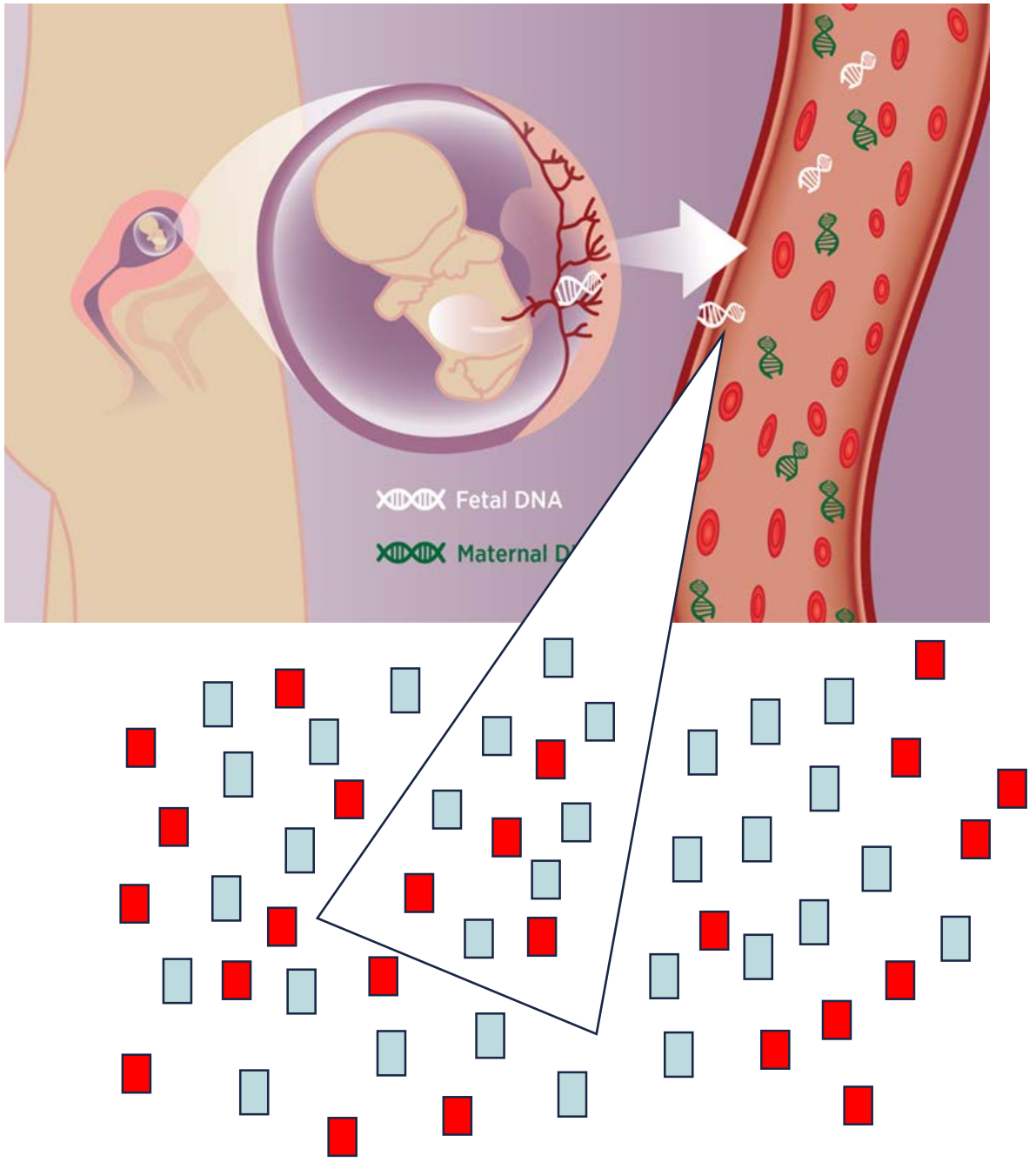




No detection of mutated paternal alleles

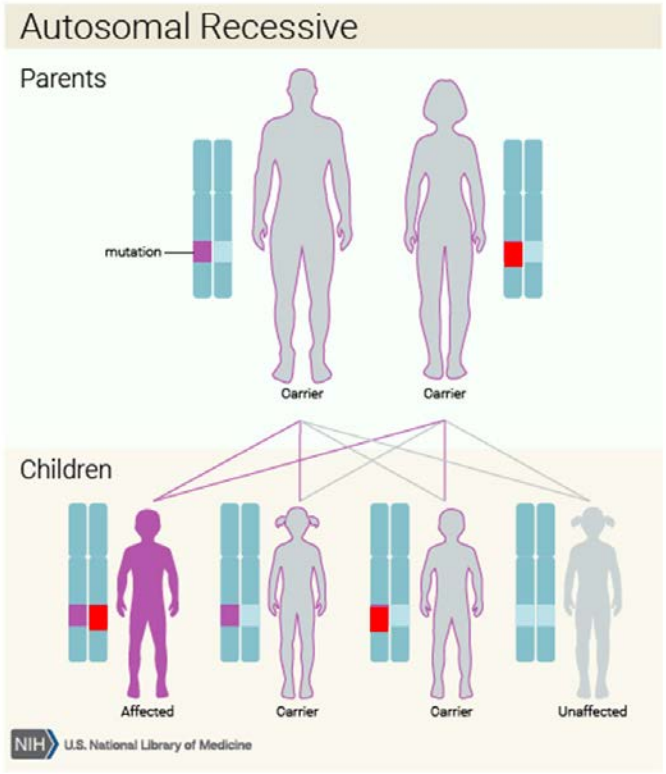
- normal fetus





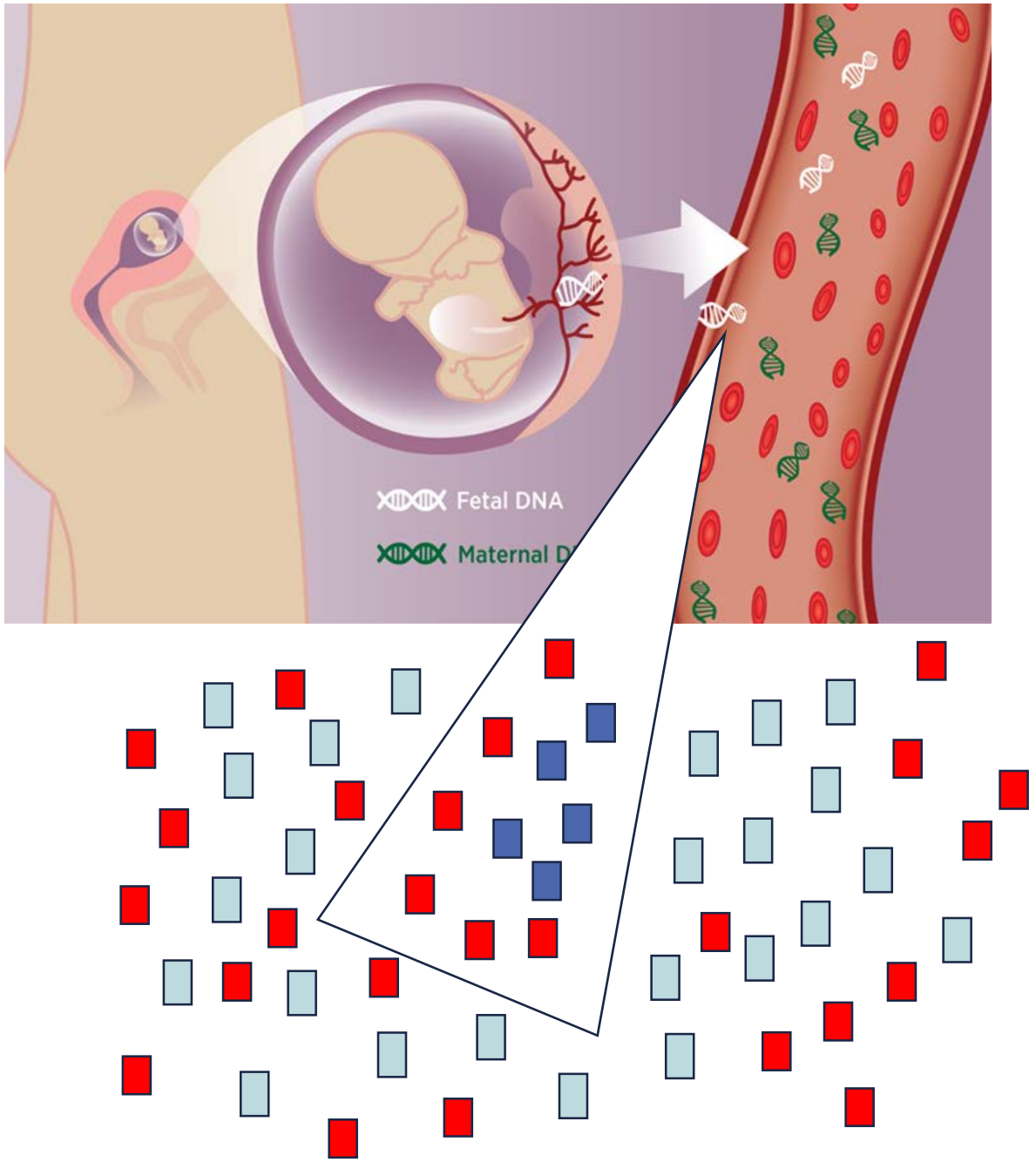
No detection of mutated paternal alleles

- normal fetus
- maternal carrier



Detection of paternal alleles

- Affected fetus

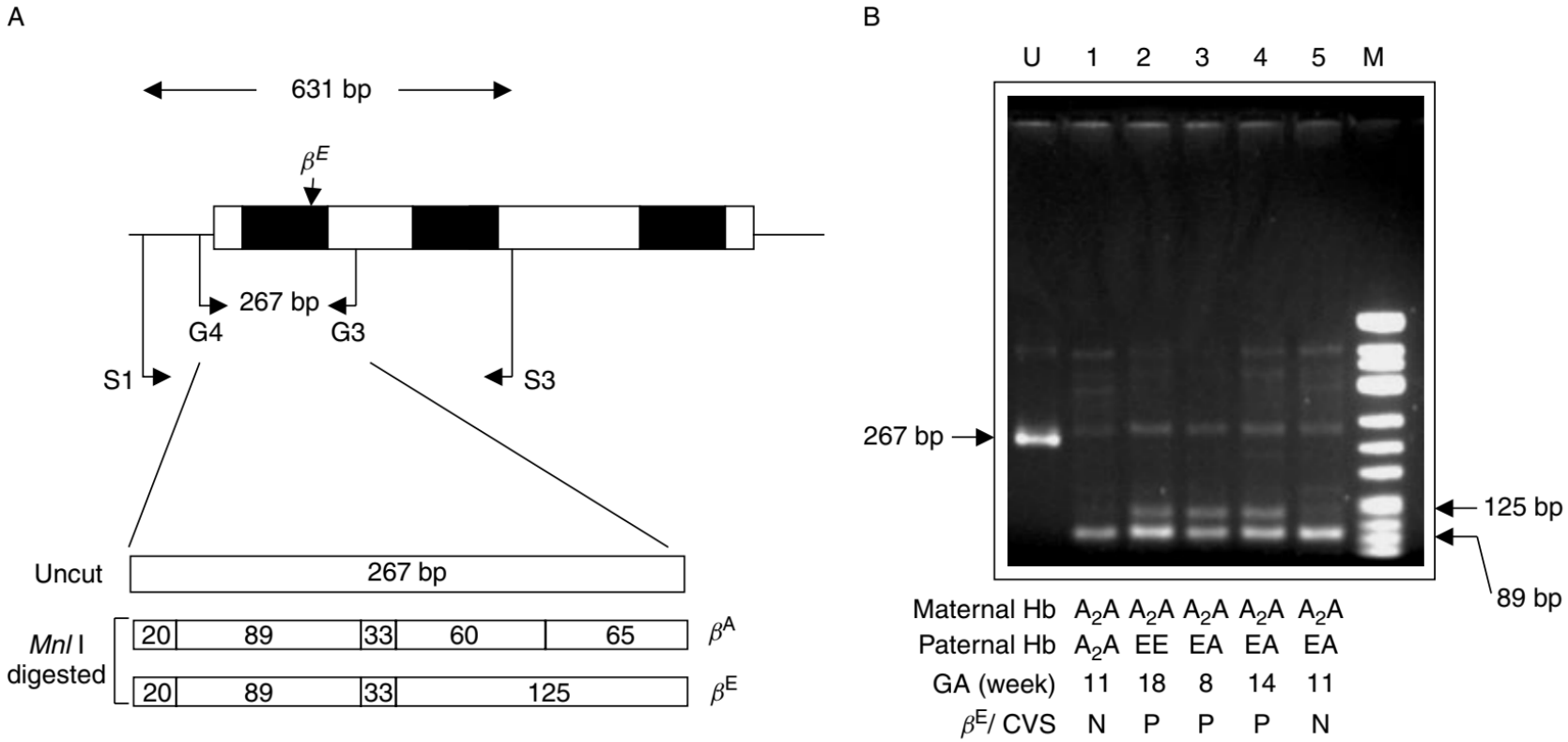


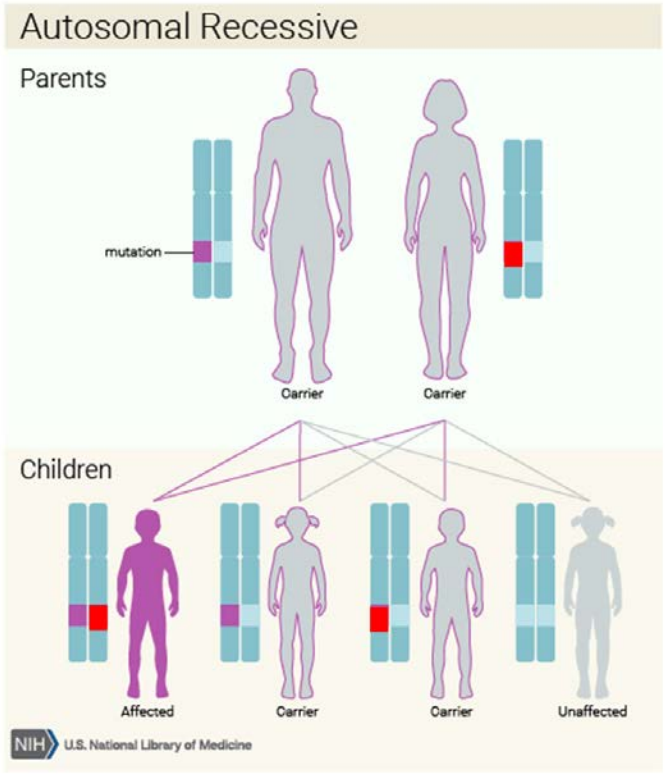
PRENATAL DIAGNOSIS
Prenat Diagn 2003; **23**: 393–396.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/pd.607

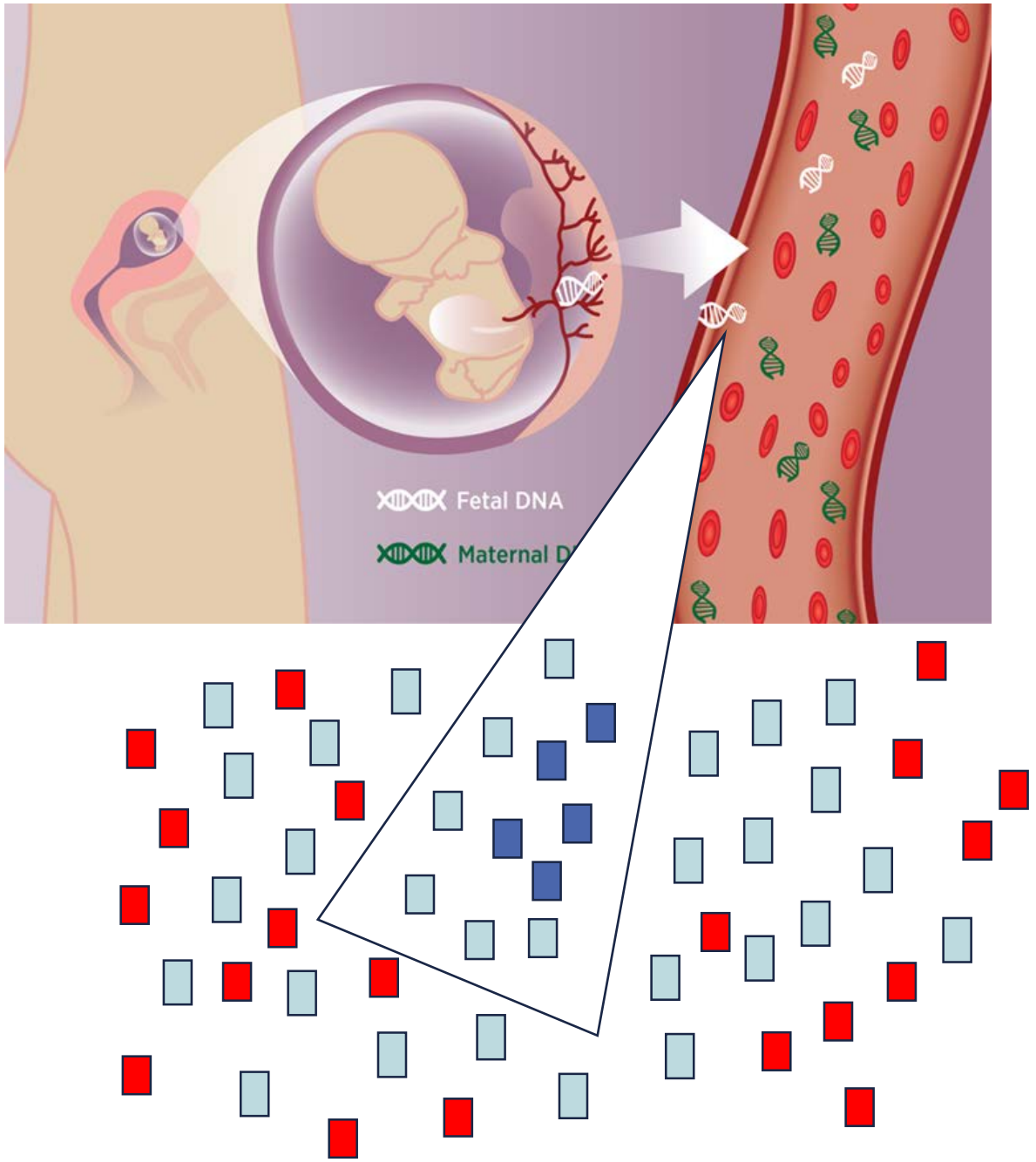
Prenatal detection of fetal hemoglobin E gene from maternal plasma

Goonnapa Fucharoen[1,4], Warunee Tungwiwat[1], Thawalwong Ratanasiri[2], Kanokwan Sanchaisuriya[1,4] and Supan Fucharoen[3,4]*

[1]*Department of Clinical Microscopy, Khon Kaen University, Khon Kaen, Thailand*
[2]*Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
[3]*Department of Clinical Chemistry, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand*
[4]*Centre for Research and Development in Medical Diagnostic Laboratories, Khon Kaen University, Khon Kaen, Thailand*



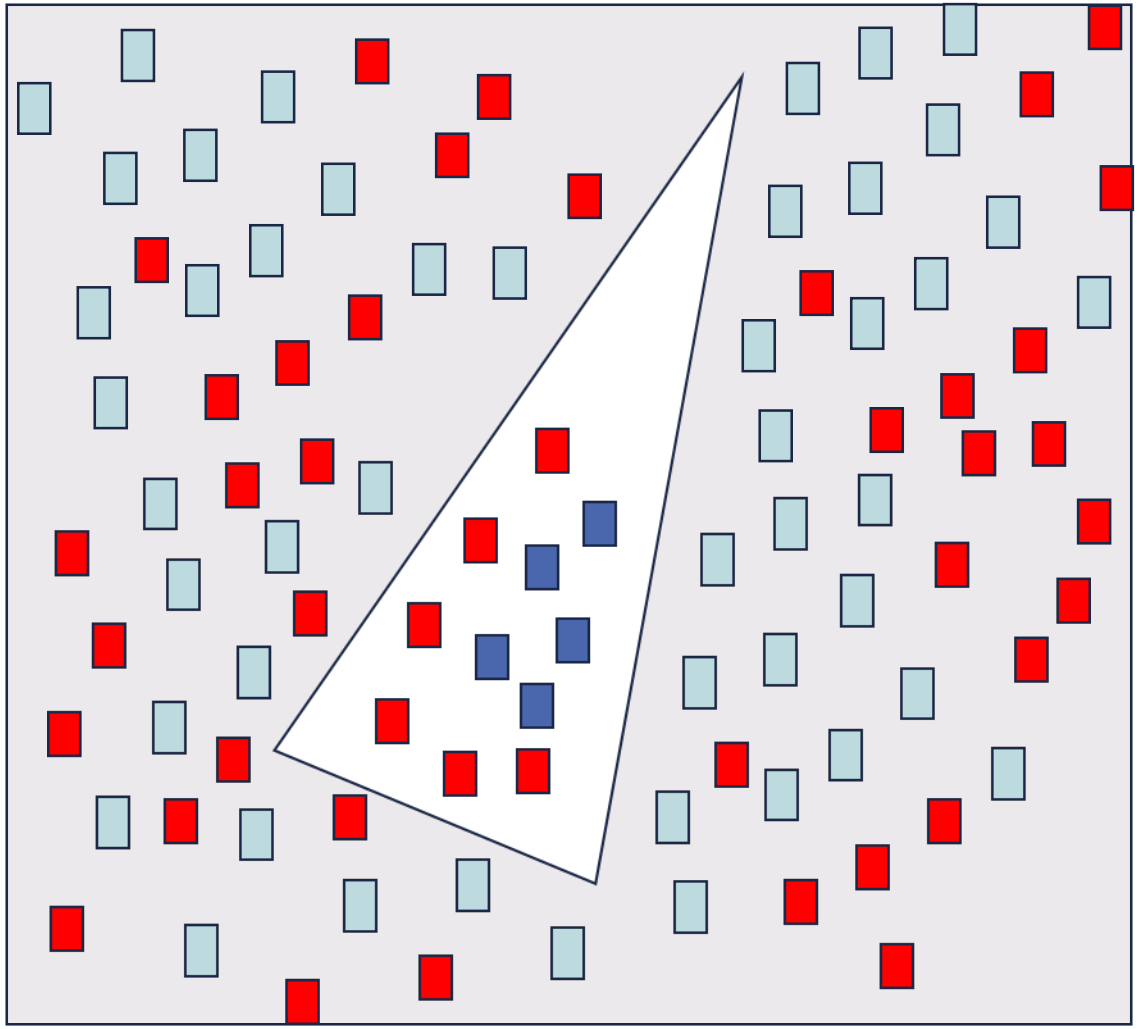


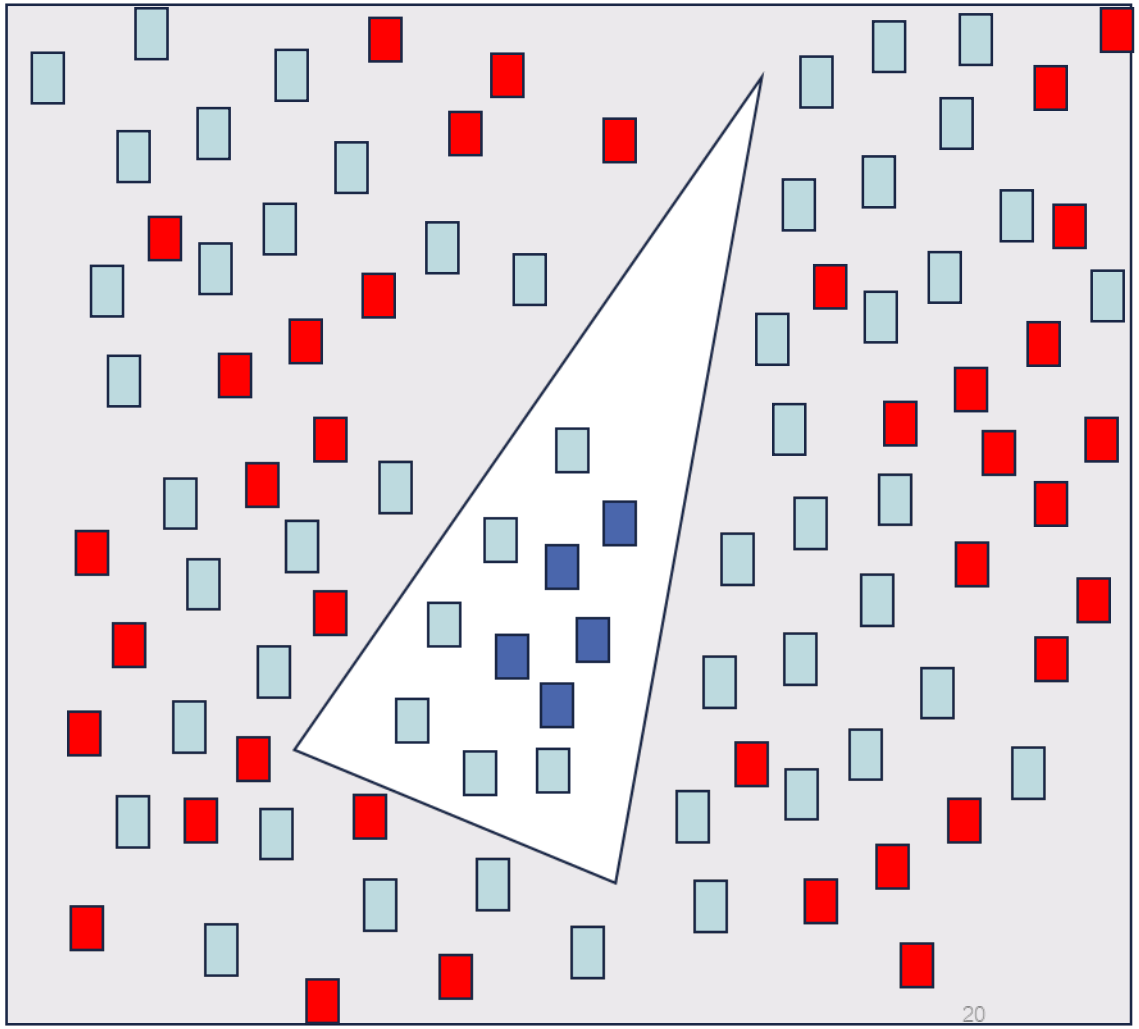


Detection of paternal alleles

- Affected fetus
- Paternal carrier

Principle on how to discriminate between affected fetus and paternal carrier

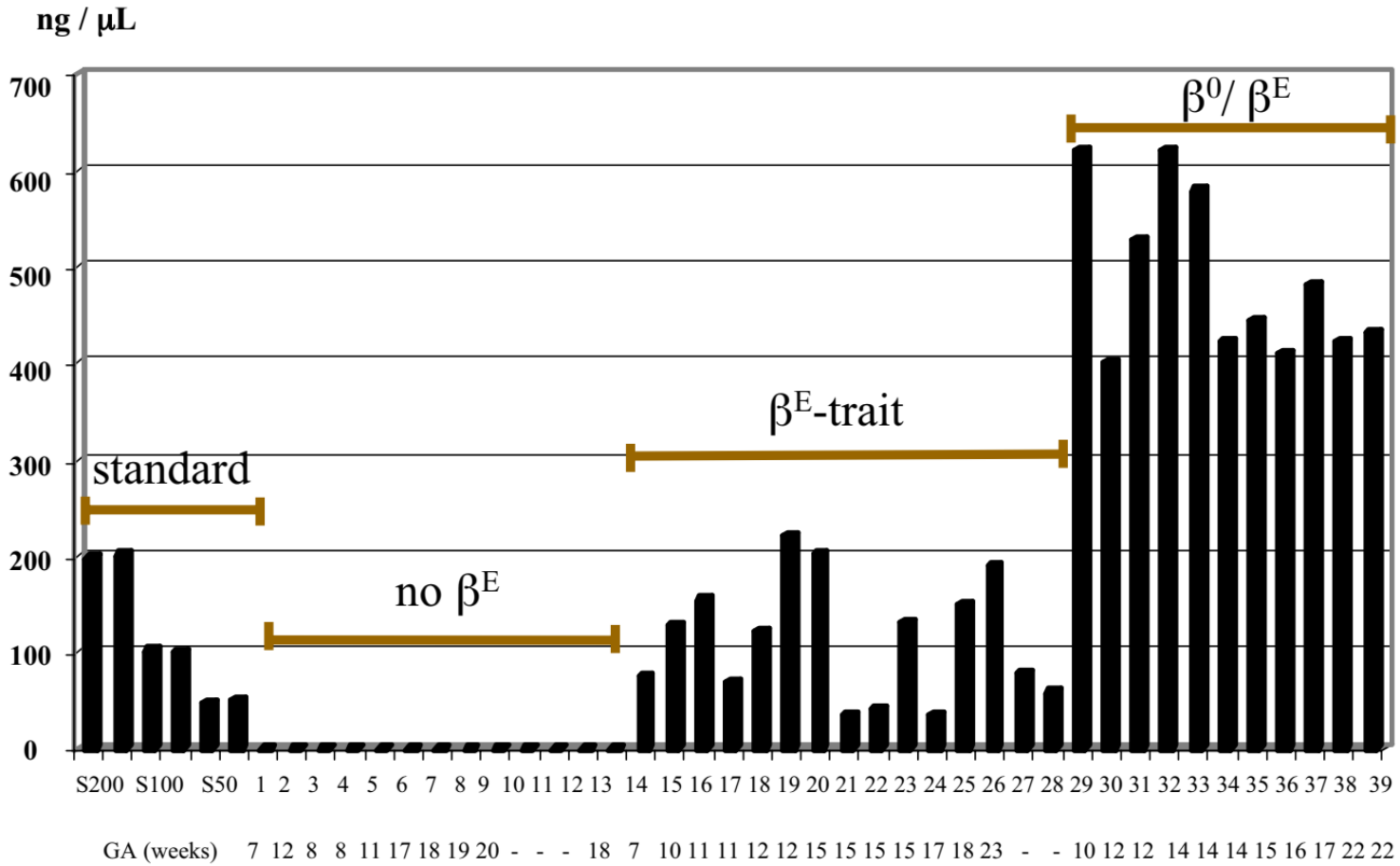




Application of maternal plasma DNA analysis for noninvasive prenatal diagnosis of Hb E-β-thalassemia

WARUNEE TUNGWIWAT, GOONNAPA FUCHAROEN, SUPAN FUCHAROEN, THAWALWONG RATANASIRI, KANOKWAN SANCHAISURIYA, and NATTAYA SAE-UNG

KOHN KAEN, THAILAND

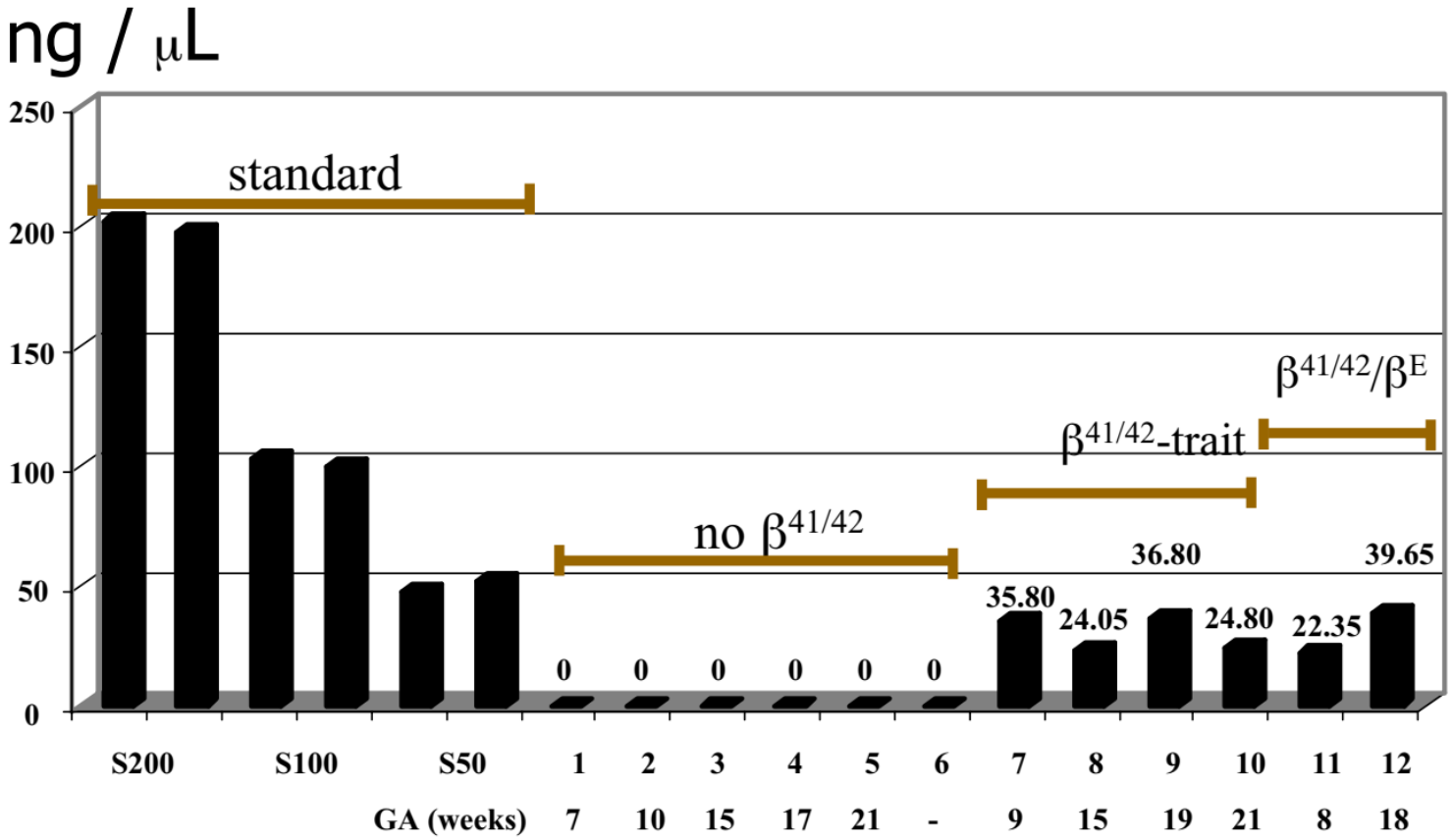


Translational Research 2007

Application of maternal plasma DNA analysis for noninvasive prenatal diagnosis of Hb E-β-thalassemia

WARUNEE TUNGWIWAT, GOONNAPA FUCHAROEN, SUPAN FUCHAROEN, THAWALWONG RATANASIRI, KANOKWAN SANCHAISURIYA, and NATTAYA SAE-UNG

KOHN KAEN, THAILAND



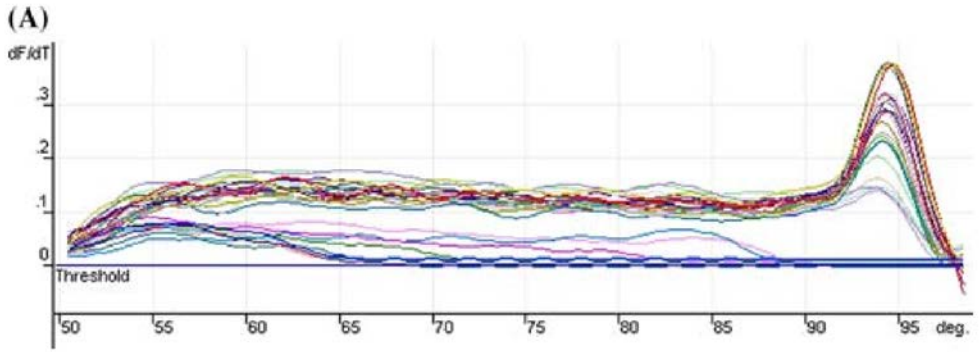
"…The noninvasive prenatal diagnostic methods developed should potentially prove useful for detection of paternally inherited mutation and for providing the exclusion of pregnancies at risk for this common genetic disorder in the region…."

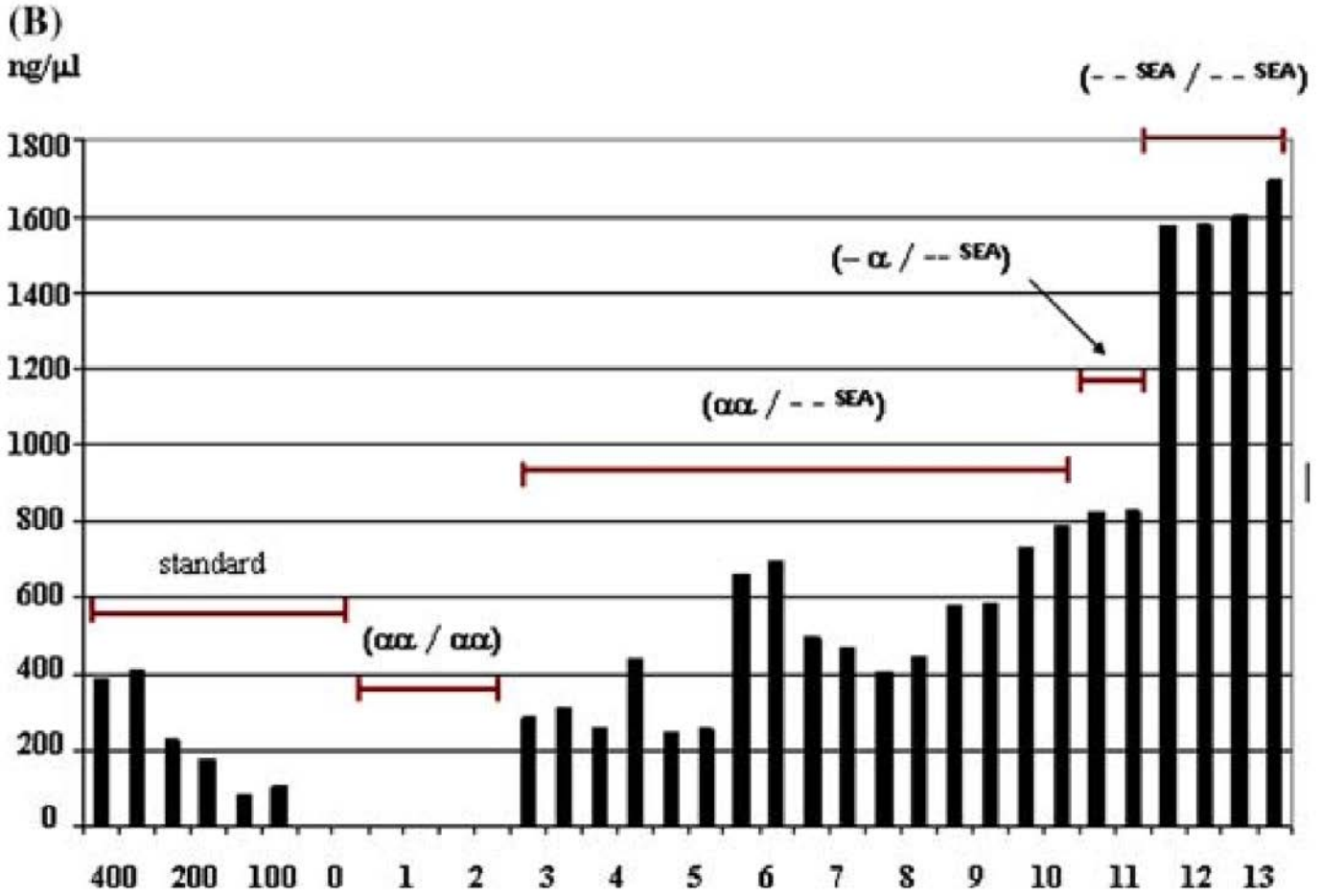
Translational Research 2007

Development and Application of a Real-Time Quantitative PCR for Prenatal Detection of Fetal α^0-Thalassemia from Maternal Plasma

WARUNEE TUNGWIWAT,[a,b] SUPAN FUCHAROEN,[b] GOONNAPA FUCHAROEN,[b] THAWALWONG RATANASIRI,[c] AND KANOKWAN SANCHAISURIYA[b]

"…Differences in the CT (threshold cycle) values and calculated concentrations of amplified DNA among different genotypes were clearly observed, which could help in prenatal prediction of the fetal genotype…"





Ann NY Acad Sci 2006

Non-invasive prenatal diagnosis of beta-thalassemia and sickle-cell disease using pyrophosphorolysis-activated polymerization and melting curve analysis

Marion Phylipsen[1]*, Supawadee Yamsri[2], Emmely E. Treffers[1], Diahann T. S. L. Jansen[1], Warsha A. Kanhai[1], Elles M. J. Boon[1], Piero C. Giordano[1], Supan Fucharoen[2], Egbert Bakker[1] and Cornelis L. Harteveld[1]

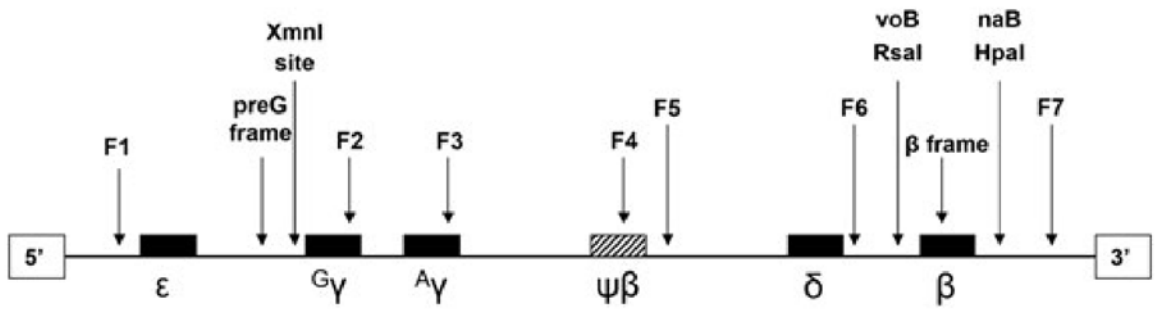
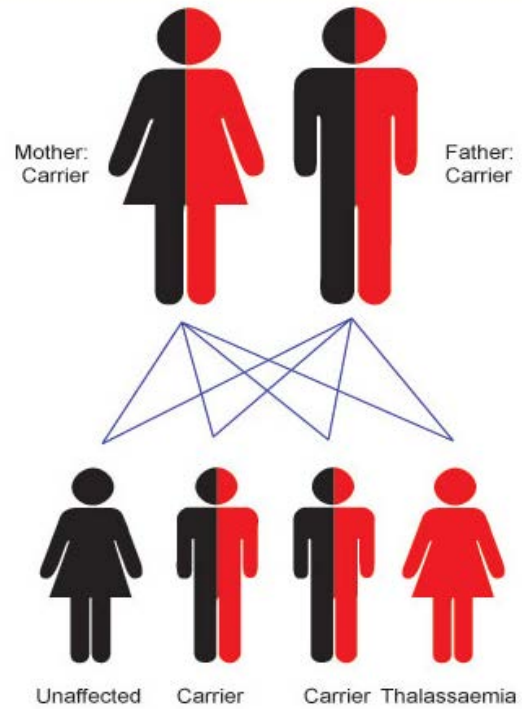


Table 3 **Overview of the frequencies of the informative SNPs in each of the control populations**

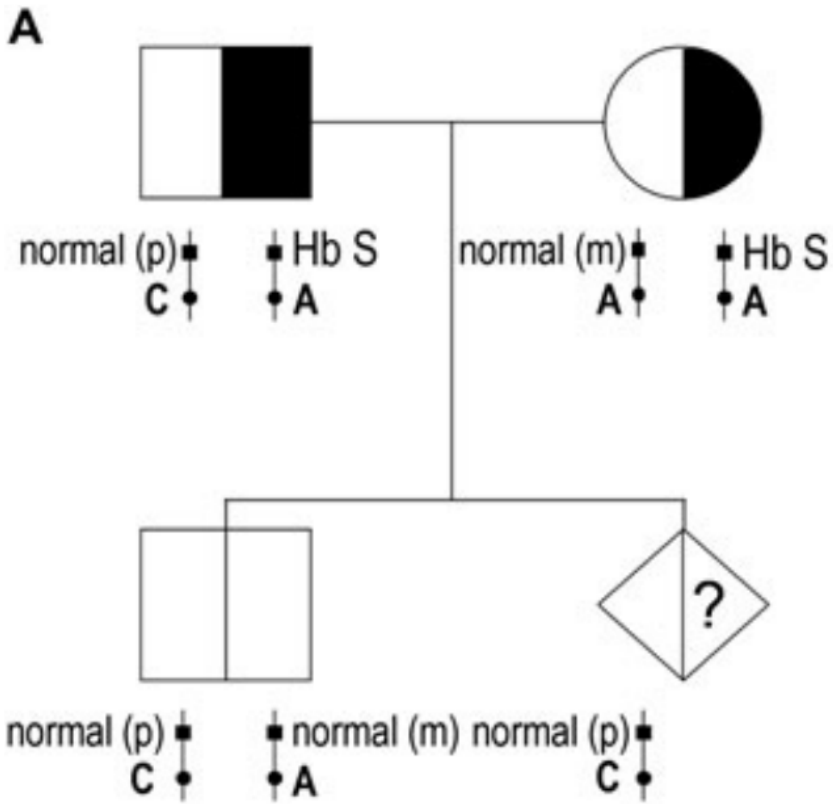
	F1 rs113040651	PreG frame SNP1 rs2855121	PreG frame SNP2 rs2855122	XmnI site rs7482144	F2 SNP2 rs2070972	F2 SNP4 rs60097179	F3 SNP1 rs28379094
Turks ($n=40$)		70.0	42.1		42.5	70.0	70.0
Moroccans ($n=40$)	50.0	80.0	58.3	77.5	50.0	0.0	71.1
Czechs ($n=100$)	54.2	68.0	44.7	68.0	44.0	66.0	67.0
Surinamese ($n=60$)	33.3	86.7	73.3	86.7	63.8	83.3	73.3
Cypriots ($n=40$)	40.0	77.5	ND	78.9	62.5	ND	77.8
Greek ($n=40$)	47.5	69.4	ND	70.0	52.5	ND	71.1
Dutch ($n=100$)	59.2	57.1	43.6	58.2	37.0	59.0	45.9
Total ($n=420$)	50.2	70.5	50.9	71.2	48.1	59.6	64.9

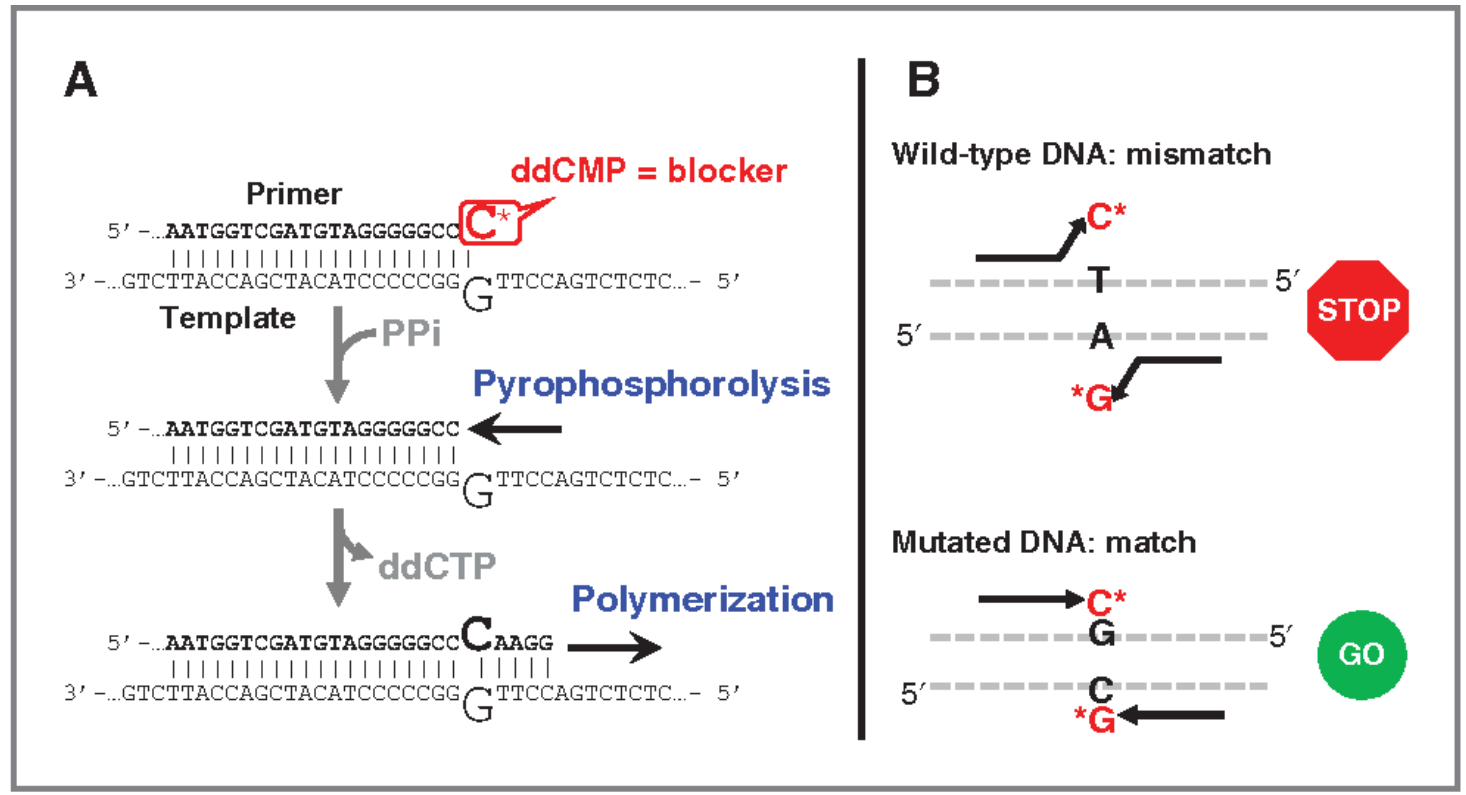
Out of the 24 selected SNPs, 17 appeared informative (frequency >5% and <95%) and were used to design the pyrophosphorolysis-activated polymerization (PAP) assay. The numbers indicate the percentage of alleles in the population containing the SNP. ND, not determined; n = number of alleles tested.



Non-invasive prenatal diagnosis of beta-thalassemia and sickle-cell disease using pyrophosphorolysis-activated polymerization and melting curve analysis

Marion Phylipsen[1]*, Supawadee Yamsri[2], Emmely E. Treffers[1], Diahann T. S. L. Jansen[1], Warsha A. Kanhai[1], Elles M. J. Boon[1], Piero C. Giordano[1], Supan Fucharoen[2], Egbert Bakker[1] and Cornelis L. Harteveld[1]





Fetal Diagnosis and Therapy

Prenatal Diagnosis

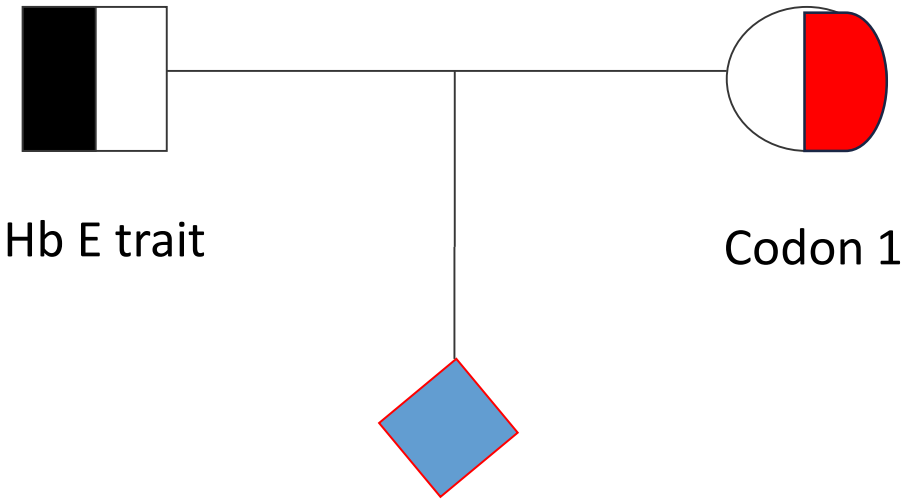
Fetal Diagn Ther 2022;49:468–478
DOI: 10.1159/000528033

Received: June 13, 2022
Accepted: November 5, 2022
Published online: December 27, 2022

Noninvasive Prenatal Diagnosis of Beta-Thalassemia Disease by Using Digital PCR Analysis of Cell-Free Fetal DNA in Maternal Plasma

Pimlak Charoenkwan[a, b] Kuntharee Traisrisilp[b, c] Supatra Sirichotiyakul[b, c]
Arunee Phusua[a, b] Torpong Sanguansermsri[a, b] Theera Tongsong[b, c]

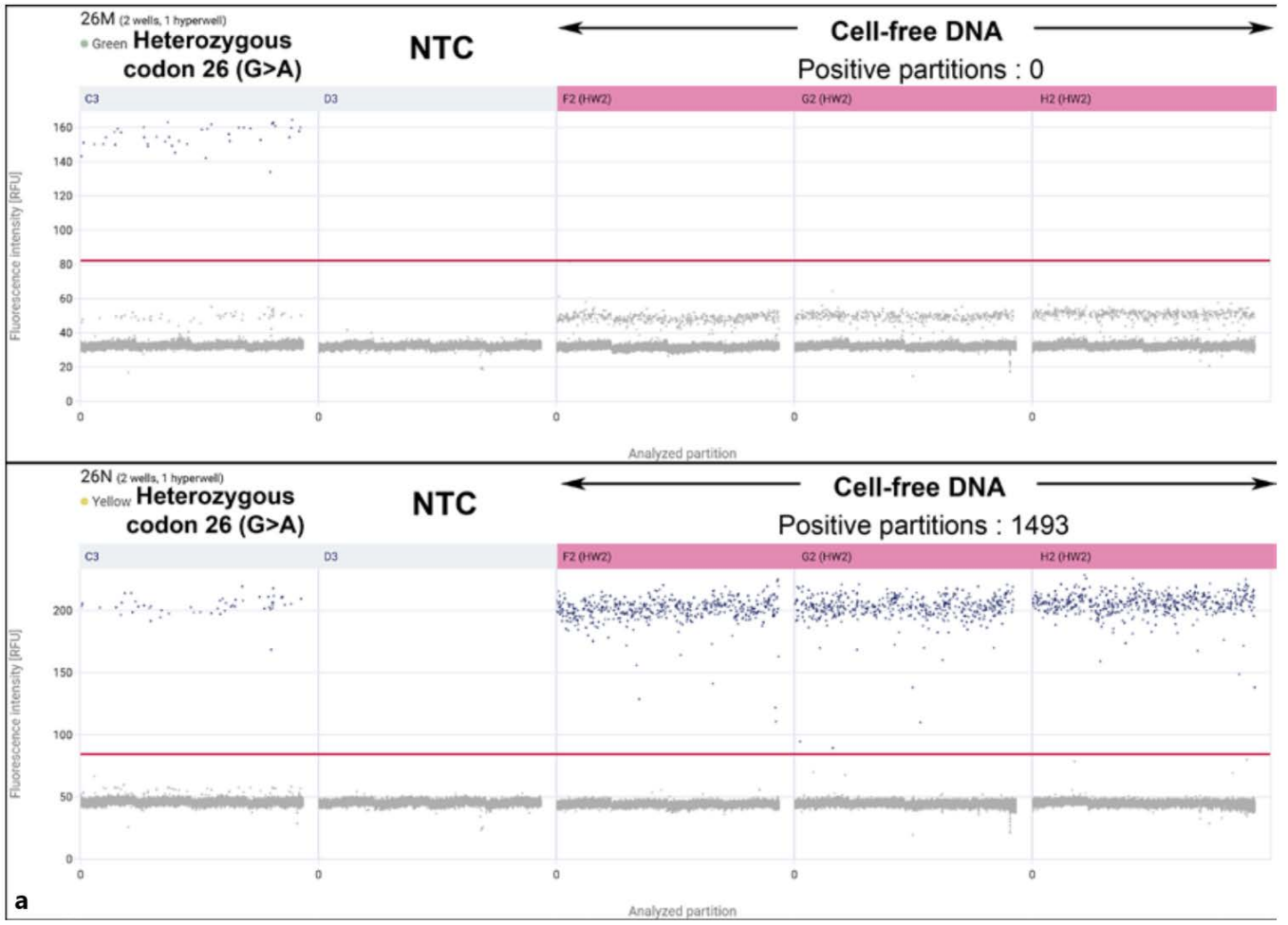
[a]Department of Pediatrics, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand; [b]Thalassemia and Hematology Center, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand; [c]Department of Obstetrics and Gynecology, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand



Hb E trait

Codon 17

Paternal alleles



Color version available c

Charoenkwan P *et al.*, Fetal Diagn Ther 2022

Fetal Diagnosis and Therapy

Prenatal Diagnosis

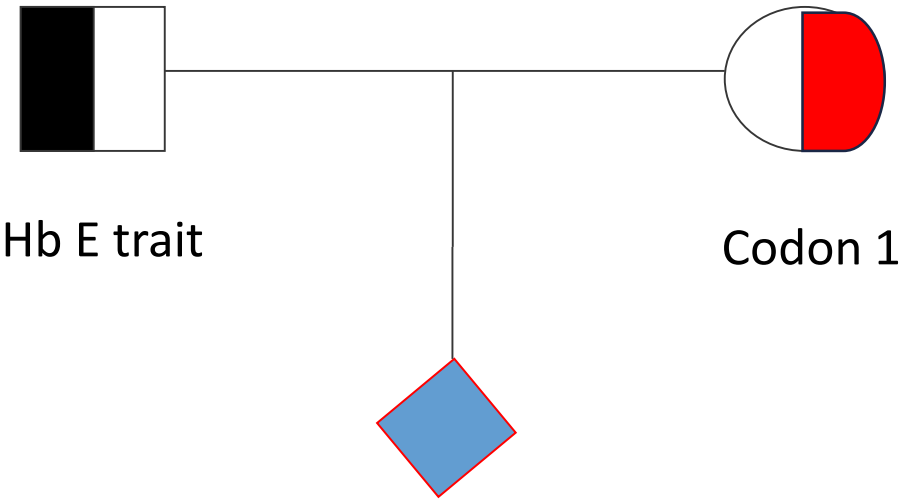
Fetal Diagn Ther 2022;49:468–478
DOI: 10.1159/000528033

Received: June 13, 2022
Accepted: November 5, 2022
Published online: December 27, 2022

Noninvasive Prenatal Diagnosis of Beta-Thalassemia Disease by Using Digital PCR Analysis of Cell-Free Fetal DNA in Maternal Plasma

Pimlak Charoenkwan[a, b] Kuntharee Traisrisilp[b, c] Supatra Sirichotiyakul[b, c]
Arunee Phusua[a, b] Torpong Sanguansermsri[a, b] Theera Tongsong[b, c]

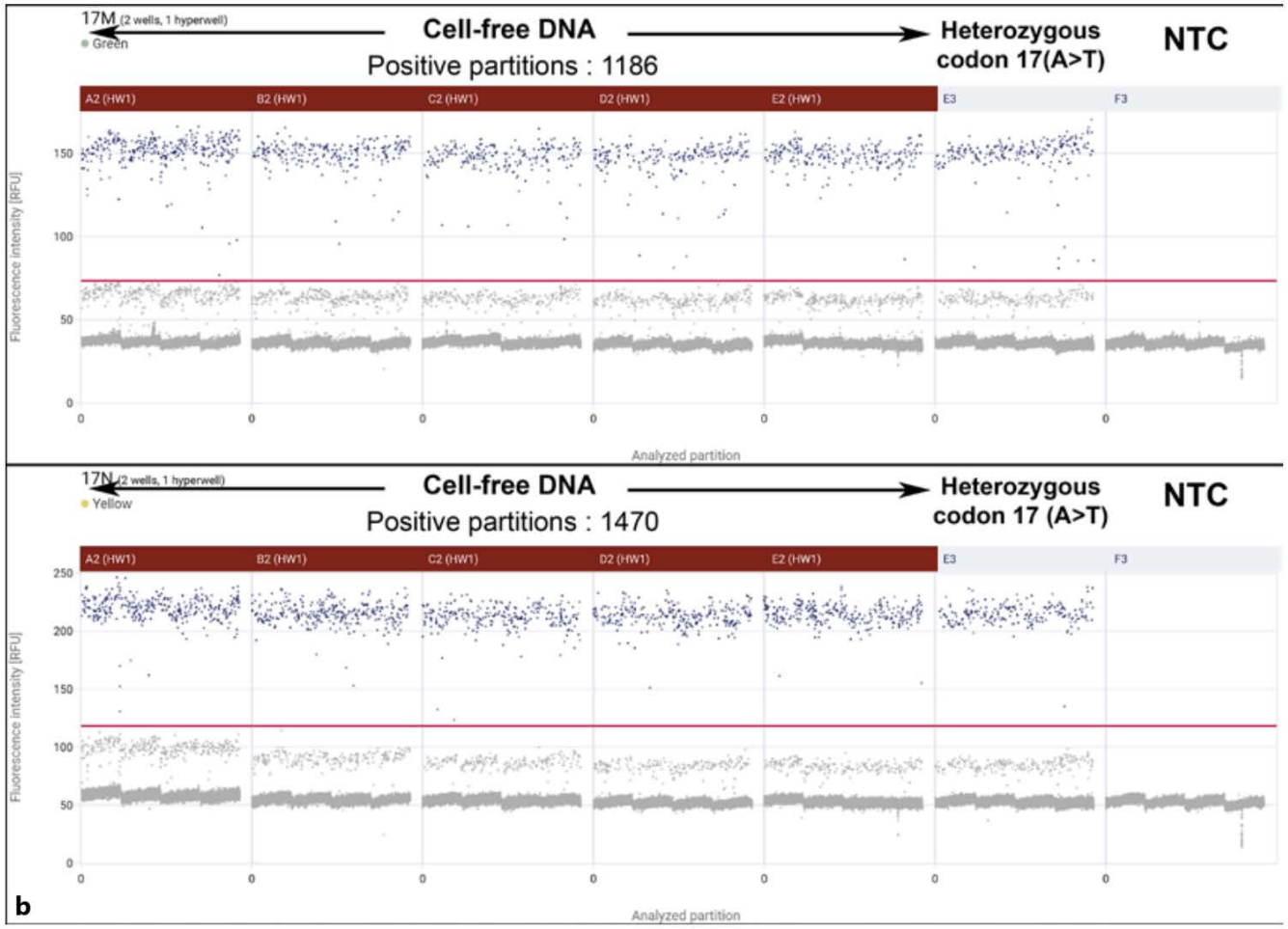
[a]Department of Pediatrics, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand; [b]Thalassemia and Hematology Center, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand; [c]Department of Obstetrics and Gynecology, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand



Hb E trait

Codon 17

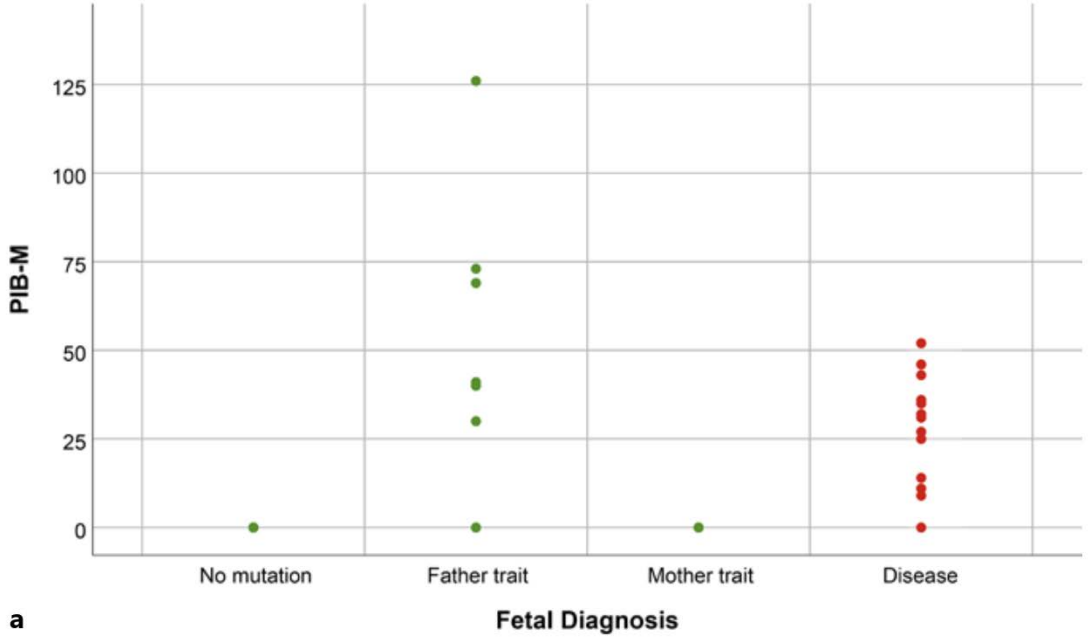
Maternal alleles



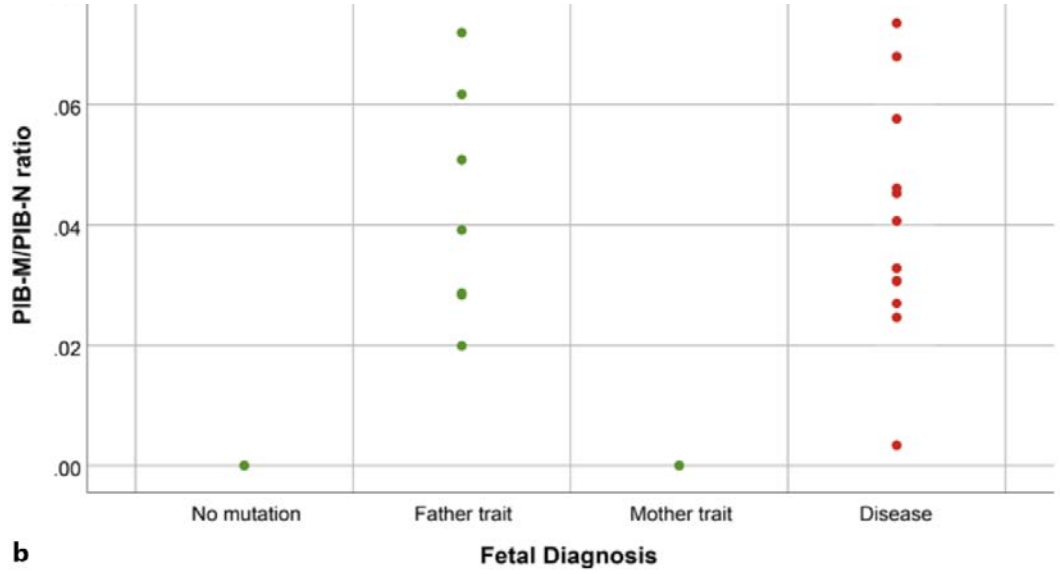
MIB-M/MIB-N = 0.81

Charoenkwan P *et al.*, Fetal Diagn Ther 2022

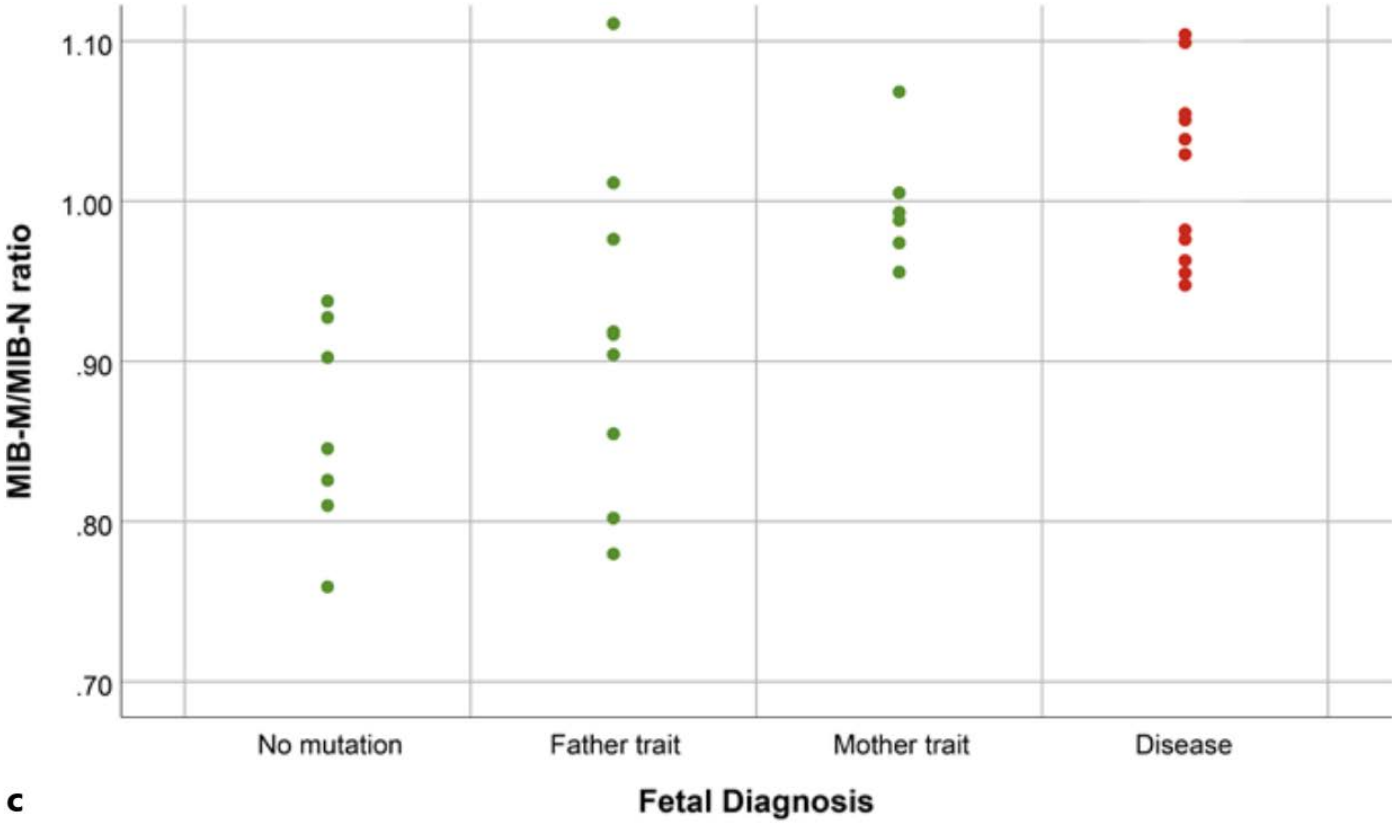
Scatterplots show distribution of PIB-M (a), PIB-M/PIB-N ratio (b), and MIB-M/MIB-N ratio (c) in maternal blood of 29 couples of discordant mutations.



a



b



c

Charoenkwan P *et al.*, Fetal Diagn Ther 2022

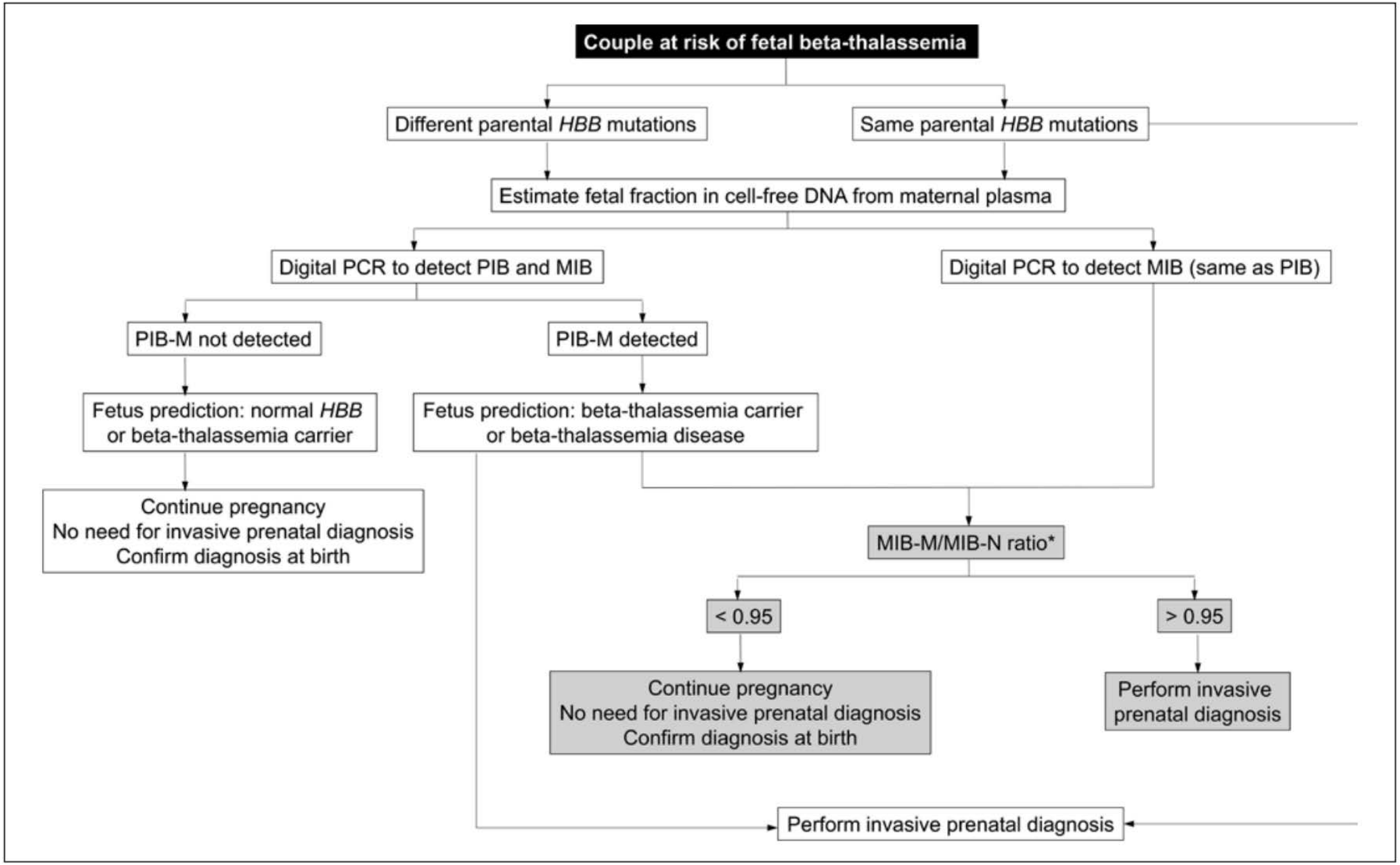
Table 1. PIB-M-positive partition number, PIB-M/PIB-N ratio, MIB-M/MIB-N ratio in fetuses with normal *HBB*, heterozygous and compound heterozygous *HBB* mutations

Fetal diagnosis	Number	PIB-M-positive partition number	PIB-M/PIB-N ratio	MIB-M/MIB-N ratio
Normal *HBB*	6	0	0	0.85±0.07
Beta-thal or Hb E carrier (maternally inherited)	6	0	0	1.00±0.04
Beta-thal or Hb E carrier (paternally inherited)	7	59.9±33.3	0.043±0.019	0.88±0.07
Beta-thalassemia disease	10	30.1±15.0	0.039±0.017	1.02±0.05

Table 2. Comparison of prenatal noninvasive diagnosis of beta-thalassemia by dPCR results with prenatal invasive diagnosis in 29 couples with different paternal and maternal *HBB* mutations

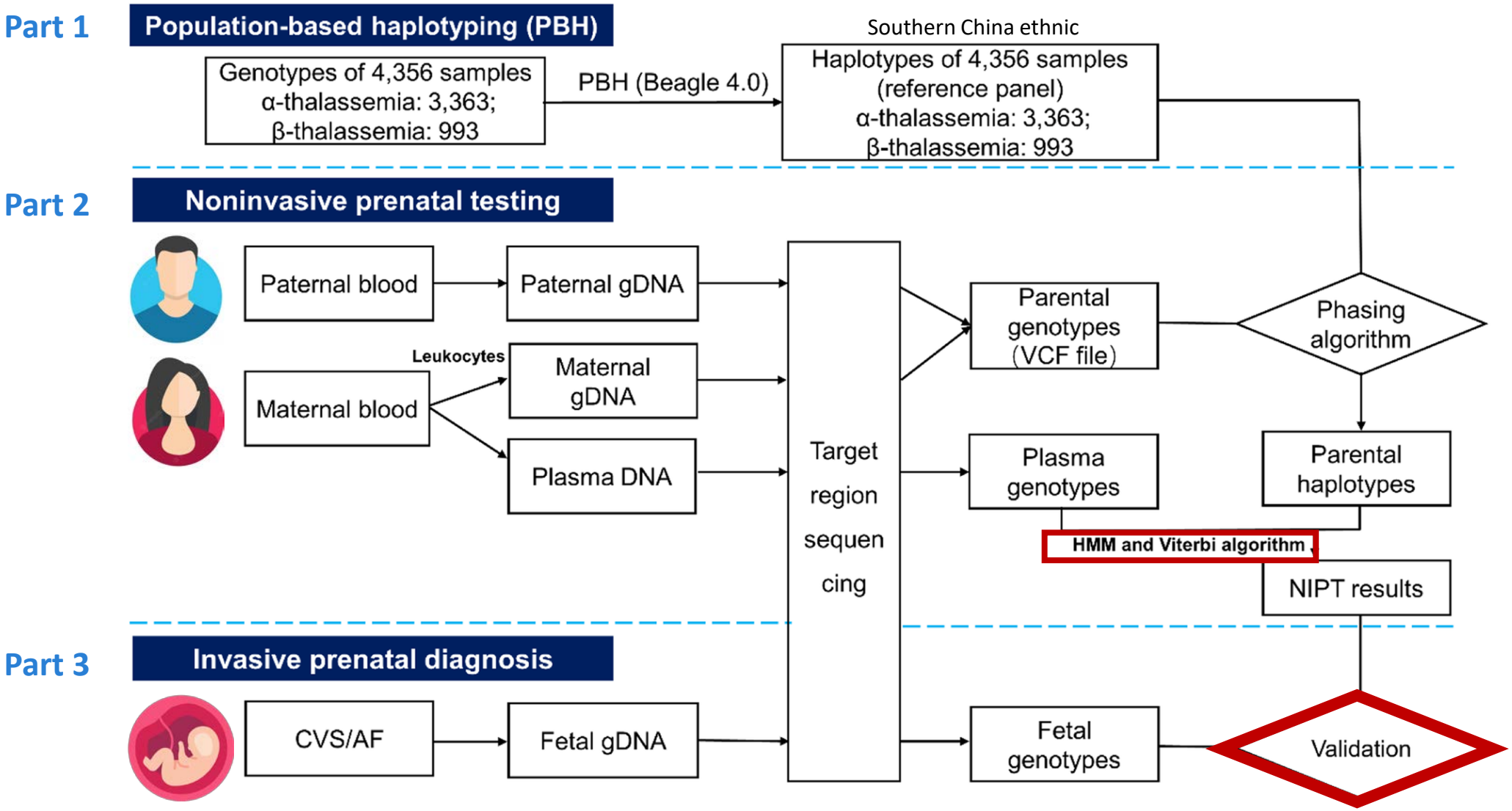
	Fetal diagnosis (*N*)			
	Normal *HBB*	Beta-thal or Hb E carrier (maternally inherited)	Beta-thal or Hb E carrier (paternally inherited)	Beta-thalassemia disease
PIB-M not detected	6	6	0	0
PIB-M detected	0	0	7	10
MIB-M/MIB-N <0.95	6	0	6	0
MIB-M/MIB-N >0.95	0	6	1	10
Total	6	6	7	10

Detection of PIB: sensitivity 100%, specificity 100%. Detection of MIB: sensitivity 100%, specificity 92.3%.



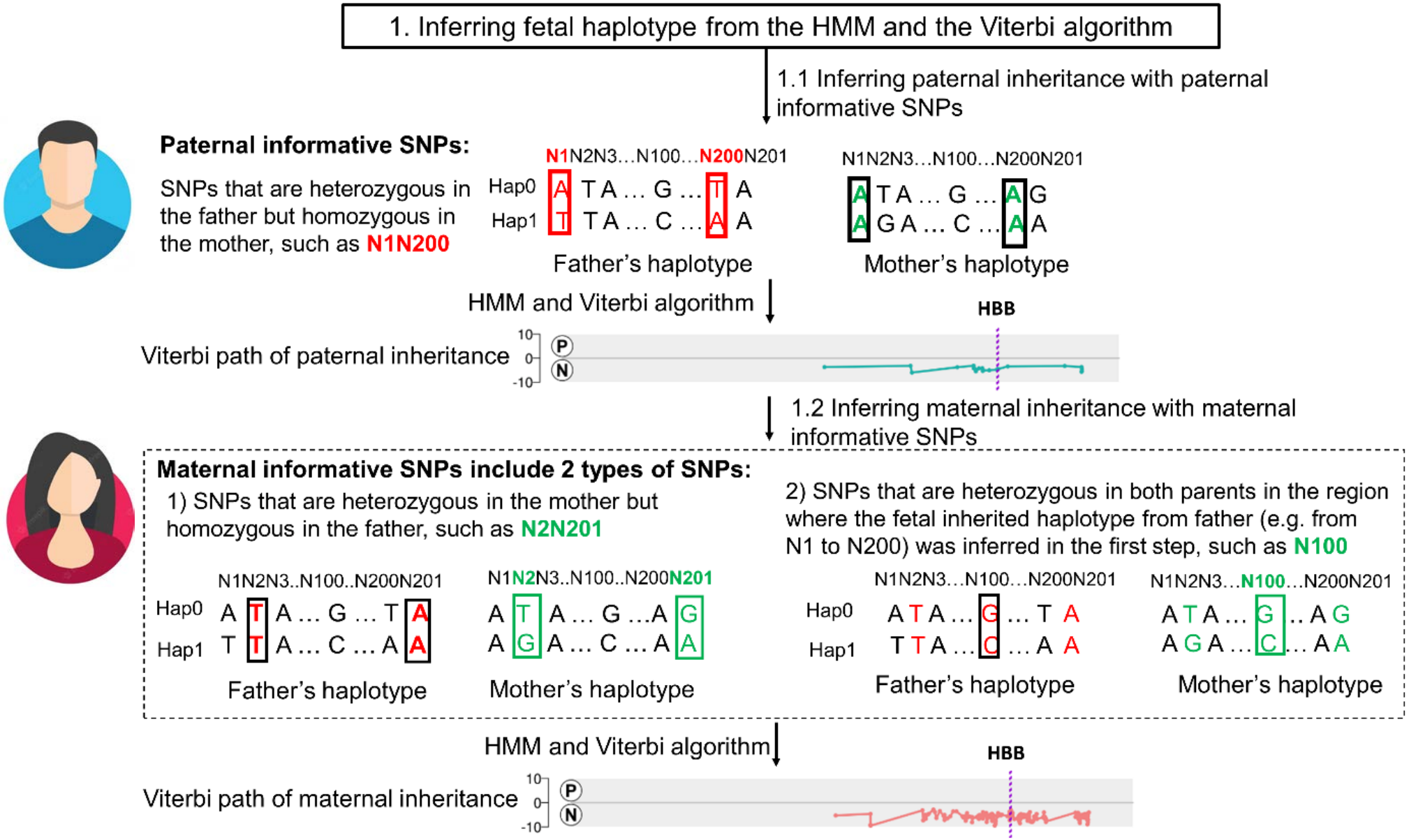
Charoenkwan P *et al.*, Fetal Diagn Ther 2022

Novel population-based haplotyping-NIPT (PBH) for α-thalassemia and β-thalassemia workflow



Chen, C., Li, R., Sun, J. et al. (2021). Noninvasive prenatal testing of α-thalassemia and β-thalassemia through population-based parental haplotyping. Genome Med 13, 18.

The application of the HMM and Viterbi algorithm for NIPT (1)



The application of the HMM and Viterbi algorithm for NIPT (2)

2. Inferring fetal genotype of the pathogenic site from the Viterbi path

The path contains only one haplotype block

The path contains two haplotype blocks

The haplotype block spans the target gene

Normal

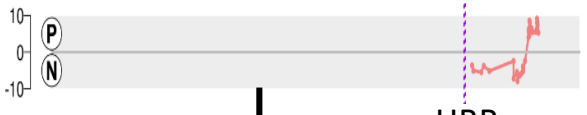
The haplotype block does not span the target gene

Normal

One haplotype block spans the target gene

Pathogenic

Neither haplotype block span the target gene



No-call

The fetal genotype of the pathogenic site is **the state of the haplotype block**

The fetal genotype of the pathogenic site is **the state of the haplotype block**

The fetal genotype of the pathogenic site is **the state of the haplotype block that span the target gene**

The fetal genotype of the pathogenic site is **no-call**

Calculated confidence score (CS)

CS>=0.99

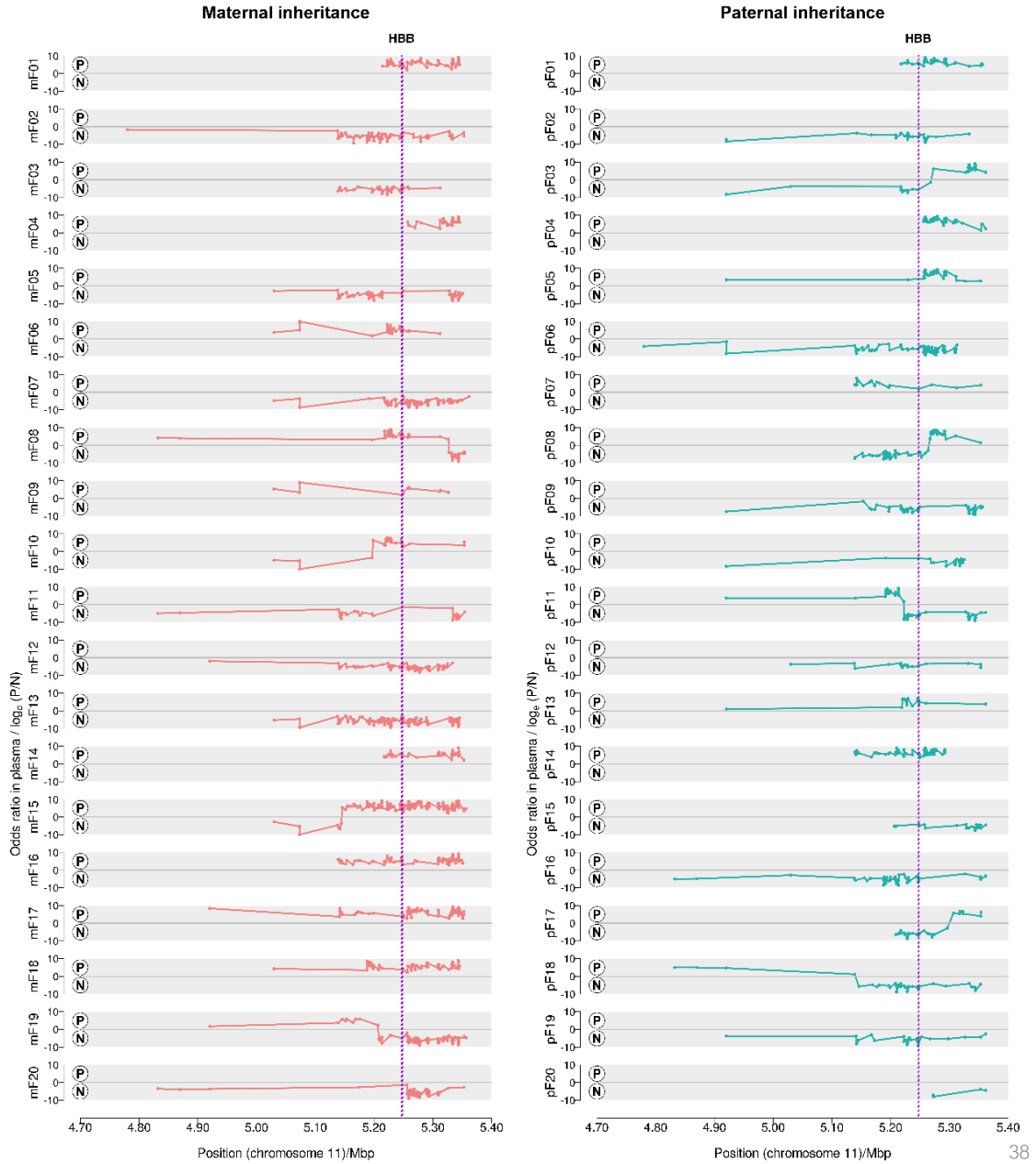
NO → No-call

YES

Final result

Concordance of parental haplotypes deduced by PBH and FBH

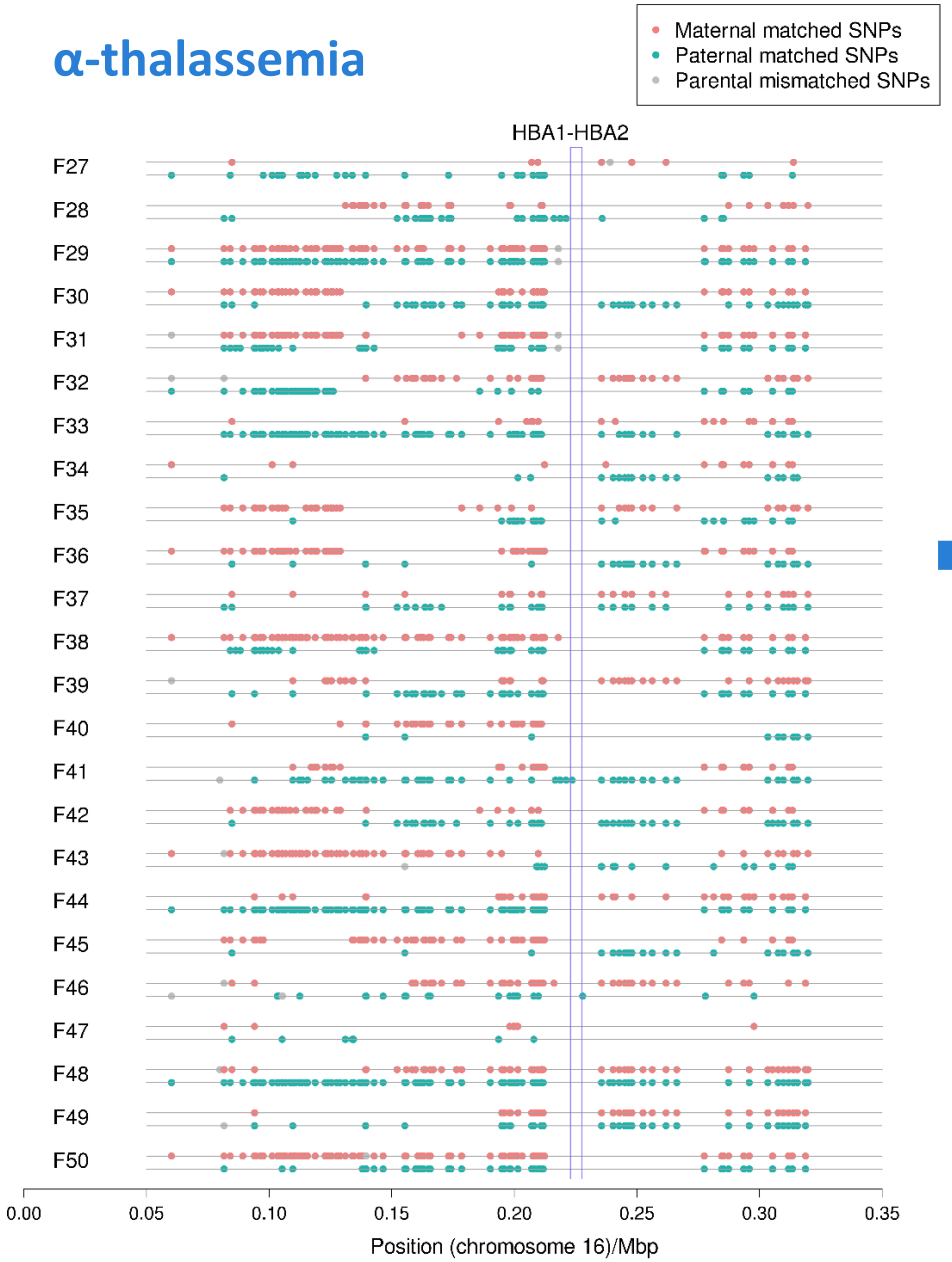
- β-thalassemia



Note: Data selectively presented only the results of the first 20 families.

Concordance of parental haplotypes deduced by PBH and FBH

- α-thalassemia



Note: Data selectively presented only the results of 24 families.

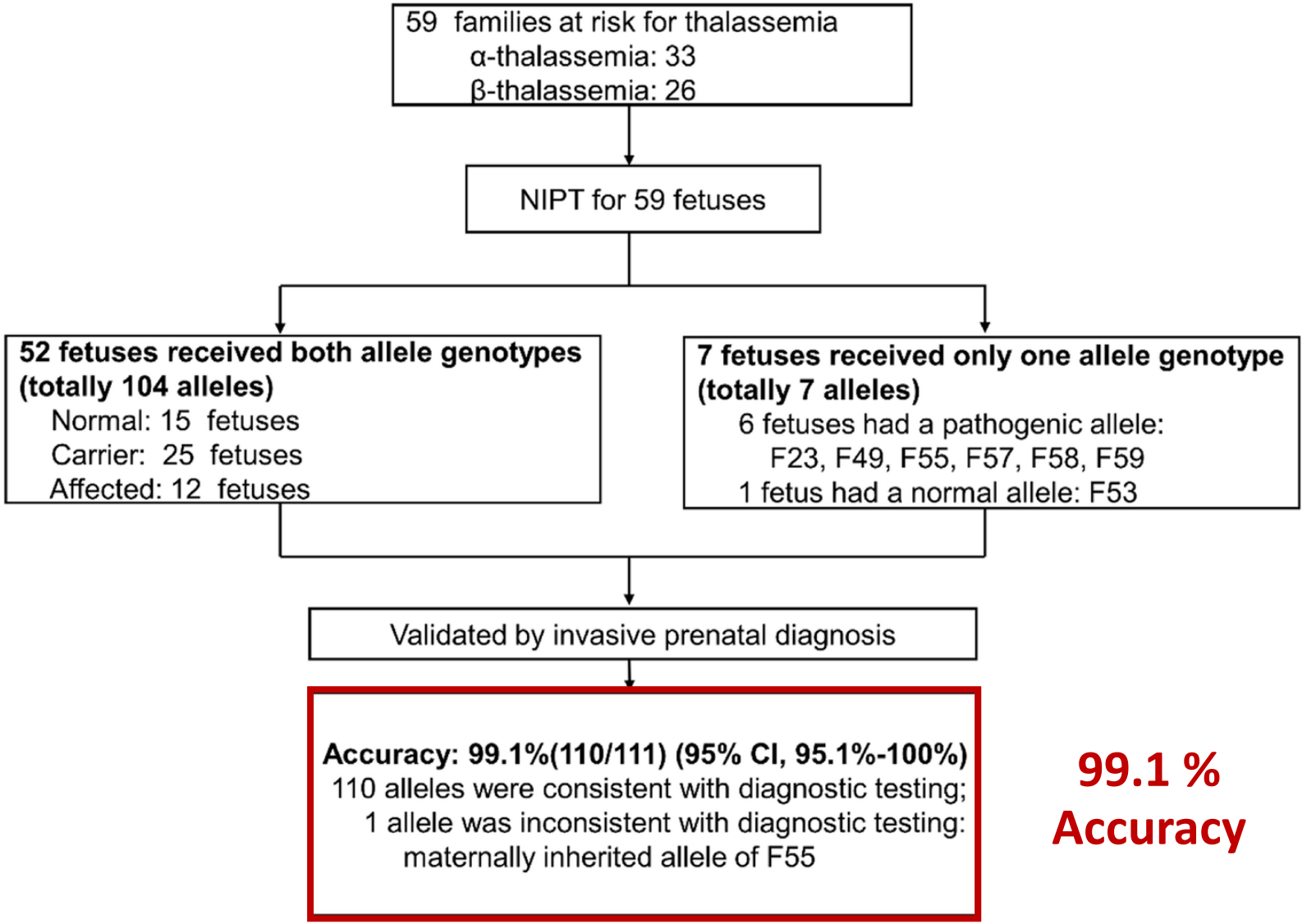
Concordance of parental haplotypes deduced by PBH and FBH

Family	No. of SNPs in the Mother				No. of SNPs in the Father			
	No. of SNPs Phased by PBH [a]	No. of SNPs Phased by PBH and FBH [b]	No. of Consistent SNPs [c]	Concordance Rate [d]	No. of SNPs Phased by PBH [a]	No. of SNPs Phased by PBH and FBH [b]	No. of Consistent SNPs [c]	Concordance Rate [d]
F01	81	66	66	100%	90	75	75	100%
F02	127	105	105	100%	124	102	101	99.0%
F03	56	56	56	100%	71	71	42	59.2%
F04	104	74	72	97.3%	161	123	123	100%
F05	87	55	53	96.4%	144	112	111	99.1%
F06	45	14	14	100%	126	95	95	100%
F07	116	114	114	100%	27	25	25	100%
F08	81	60	58	96.7%	106	85	51	60.0%
F09	13	7	7	100%	81	75	75	100%
F10	36	15	15	100%	48	27	27	100%
F11	51	33	33	100%	109	91	54	59.3%
F12	115	104	104	100%	35	24	24	100%
F13	178	178	178	100%	29	29	27	93.1%
F14	66	60	60	100%	97	91	91	100%
F15	189	164	162	98.8%	55	30	30	100%
F16	94	67	67	100%	83	56	37	66.1%
F17	123	112	112	100%	99	88	75	85.2%
F18	102	81	81	100%	75	54	51	94.4%
F19	119	112	110	98.2%	49	42	41	97.6%
F20	107	107	107	100%	48	48	48	100%
...	...	...	...	....	...	...	...	...

[a] The number of phased SNPs in parents inferred by PBH. [b] The number of phased SNPs in parents inferred by two methods (PBH and FBH).
[c] The number of phased SNPs that were consistent between the two methods. d Concordance rate = c/b.

Note: Data selectively presented only the results of the first 20 families.

Outcomes of PBH-NIPT



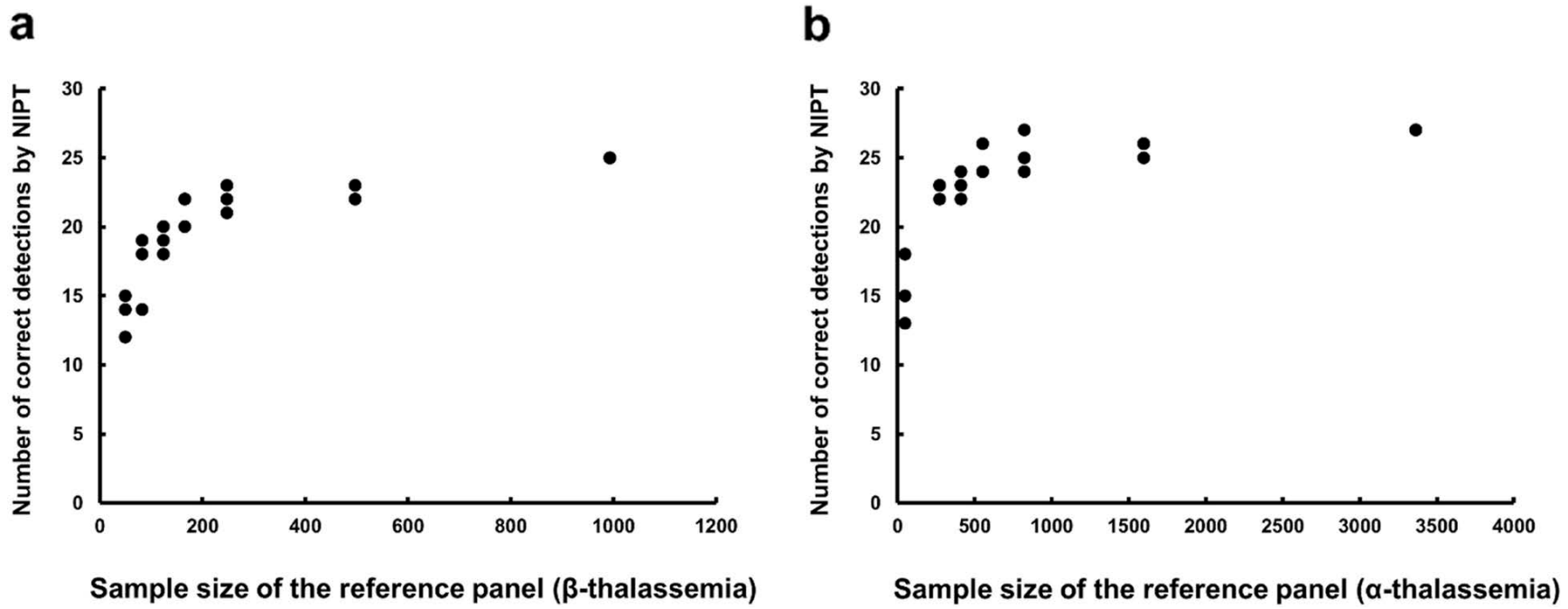
NIPT successfully inferred 111/118 (94.1%) alleles

99.1 % Accuracy

Chen, C., Li, R., Sun, J. et al. (2021). Noninvasive prenatal testing of α-thalassemia and β-thalassemia through population-based parental haplotyping. Genome Med 13, 18.

To evaluate the relationship between the accuracy of NIPT and the reference panel sample size

They randomly selected 1/2, 1/4, 1/6, 1/8, 1/12, and 50 of the samples from the total reference panel and performed 3 independent tests.



The NIPT outcome improved as the reference panel sample size increased

Chen, C., Li, R., Sun, J. et al. (2021). Noninvasive prenatal testing of α-thalassemia and β-thalassemia through population-based parental haplotyping. Genome Med 13, 18.

Genetic testing of thalassemia – carrier screening and NIPT

Medicover Genetics Editorial Team | January 4, 2023

Derived from the Greek words for sea (θάλασσα) and blood (αίμα), thalassemias are a group of inherited, genetic blood disorders. Thalassemias occur when the production of hemoglobin, a protein that carries oxygen within the red blood cells (RBCs) is disrupted. A life-threatening disease, thalassemia is an autosomal recessive condition with over 100,000 affected babies being born every year [1].

Related articles

Pancreatic cancer—The silent killer

Familial Mediterranean fever: a

OVERVIEW

WHAT IS VERAGENE

VERAgene is the only non-invasive prenatal test that can simultaneously screen for aneuploidies, microdeletions and single gene diseases. The diseases screened by VERAgene are associated with moderate to severe phenotype with significant impact on the quality of life. By combining detection of aneuploidies and microdeletions with the screening of monogenic diseases, VERAgene provides a comprehensive solution to prospective parents.

HOW IT WORKS

VERAgene needs a maternal blood sample, and a buccal swab sample from the biological father. The maternal blood contains cell-free DNA from both the mother and the fetus. This cell-free DNA is isolated and analyzed along with the father's DNA sample for any potential genetic mutations using next generation sequencing. Sophisticated bioinformatics algorithms are then used to compute the risk of the fetus having a monogenic disease.

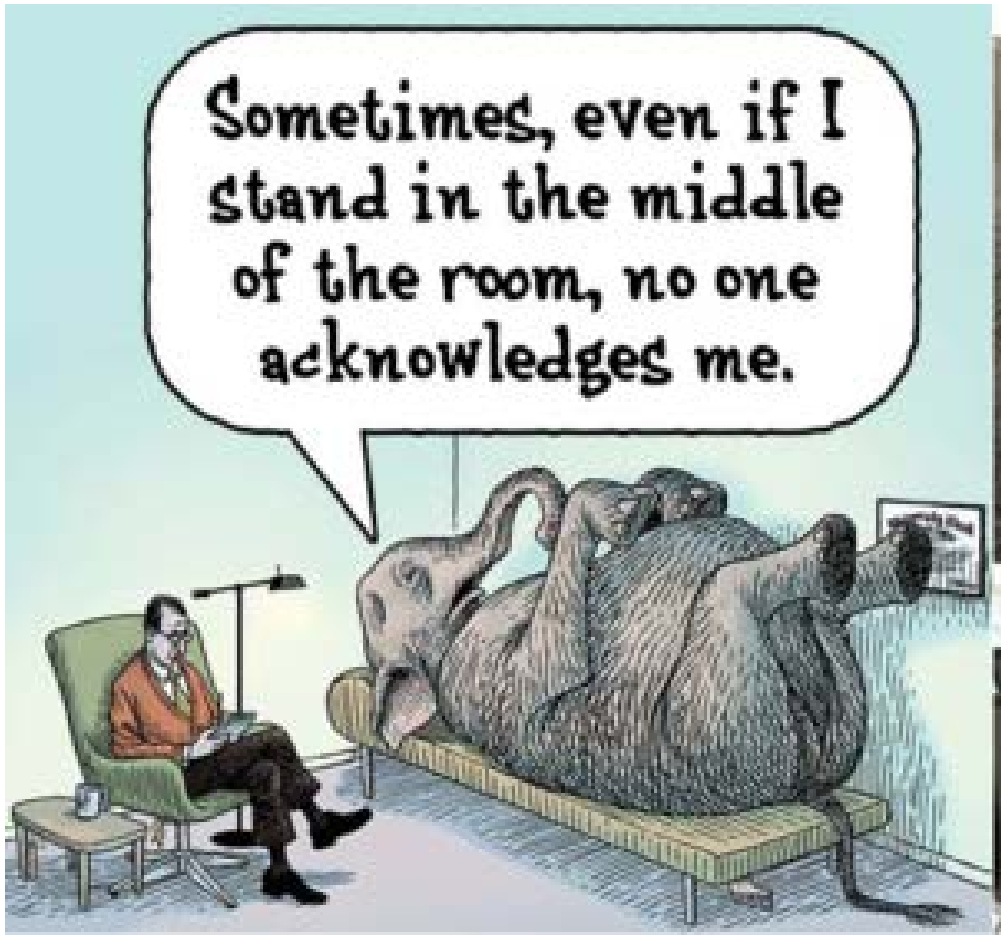
The results are sent to the clinician who communicates them to the parents and provides the necessary counseling.

Cost and turnaround time of PBH-NIPT

Task	Estimated cost per sample	Turnaround time
Cell-free DNA extraction and QC	~$5 (173 THB)	~0.5 day
Library preparation and QC	~$15 (517.70 THB)	~0.5 day
Hybridization capture and QC (8 indexed libraries pooled into one sequencing library)	~$30 (1035.39 THB)	~2 days
Sequencing	~$25 (862.83 THB)	~2 days
Data analysis	~$5 (173 THB)	~1-2 days
Total	**~$80 (2761.04 THB)**	**~7 days**

Chen, C., Li, R., Sun, J. et al. (2021). Noninvasive prenatal testing of α-thalassemia and β-thalassemia through population-based parental haplotyping. Genome Med 13, 18.

The Elephant in the Room for NIPT for Thalassemia



Risks of Down Syndrome babies based on Maternal Ages

Maternal Age	Incidence of Down syndrome
20	1 in 2,000
21	1 in 1,700
22	1 in 1,500
23	1 in 1,400
24	1 in 1,300
25	1 in 1,200
26	1 in 1,100
27	1 in 1,050
28	1 in 1,000
29	1 in 950

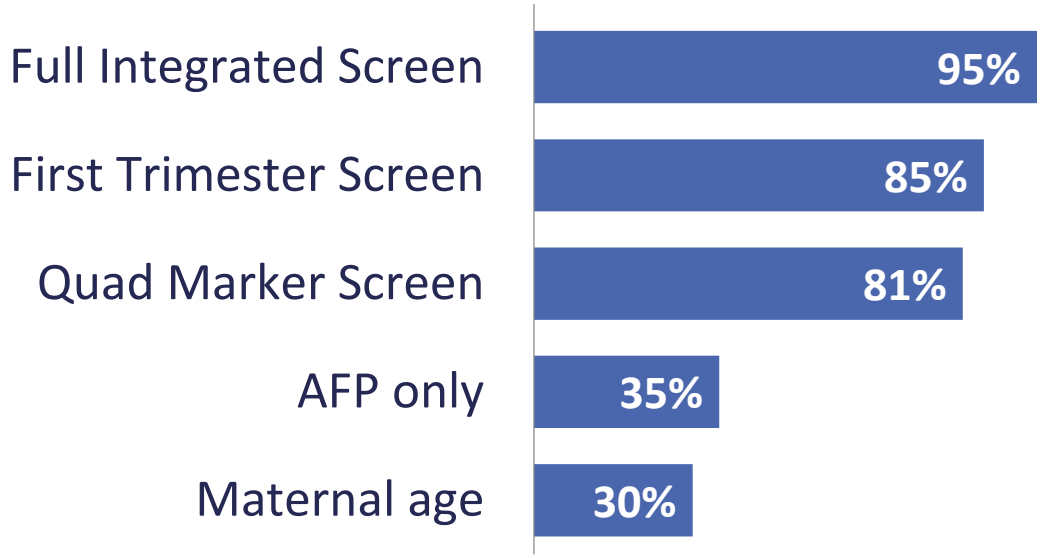
Maternal Age	Incidence of Down syndrome
30	1 in 900
31	1 in 800
32	1 in 720
33	1 in 600
34	1 in 450
35	1 in 350
36	1 in 300
37	1 in 250
38	1 in 200
39	1 in 150

Maternal Age	Incidence of Down syndrome
40	1 in 100
41	1 in 80
42	1 in 70
43	1 in 50
44	1 in 40
45	1 in 30
46	1 in 25
47	1 in 20
48	1 in 15
49	1 in 10

We can not PND 800 cases to get one case

Detection rate for Down syndrome Using Current Recommendation

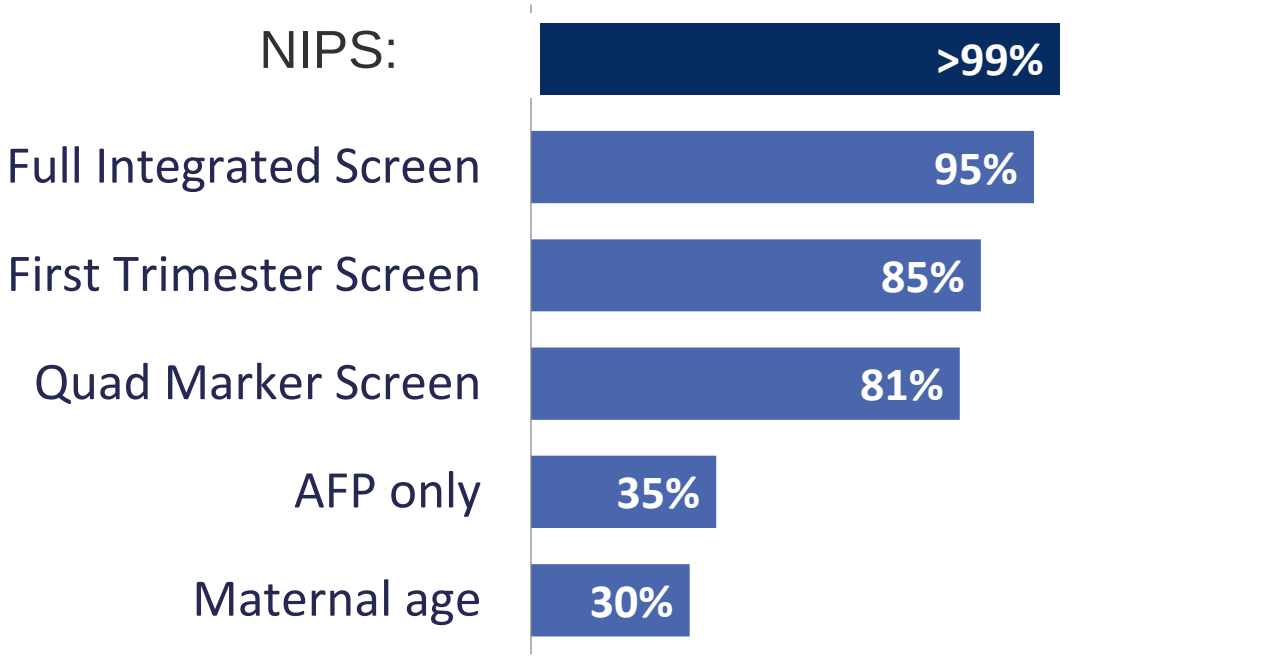
Detection rate for Down syndrome



False positive rate for conventional screening: 5%

ACOG Practice Bulletin No. 77. Obstet Gynecol 2007;109:217-27.

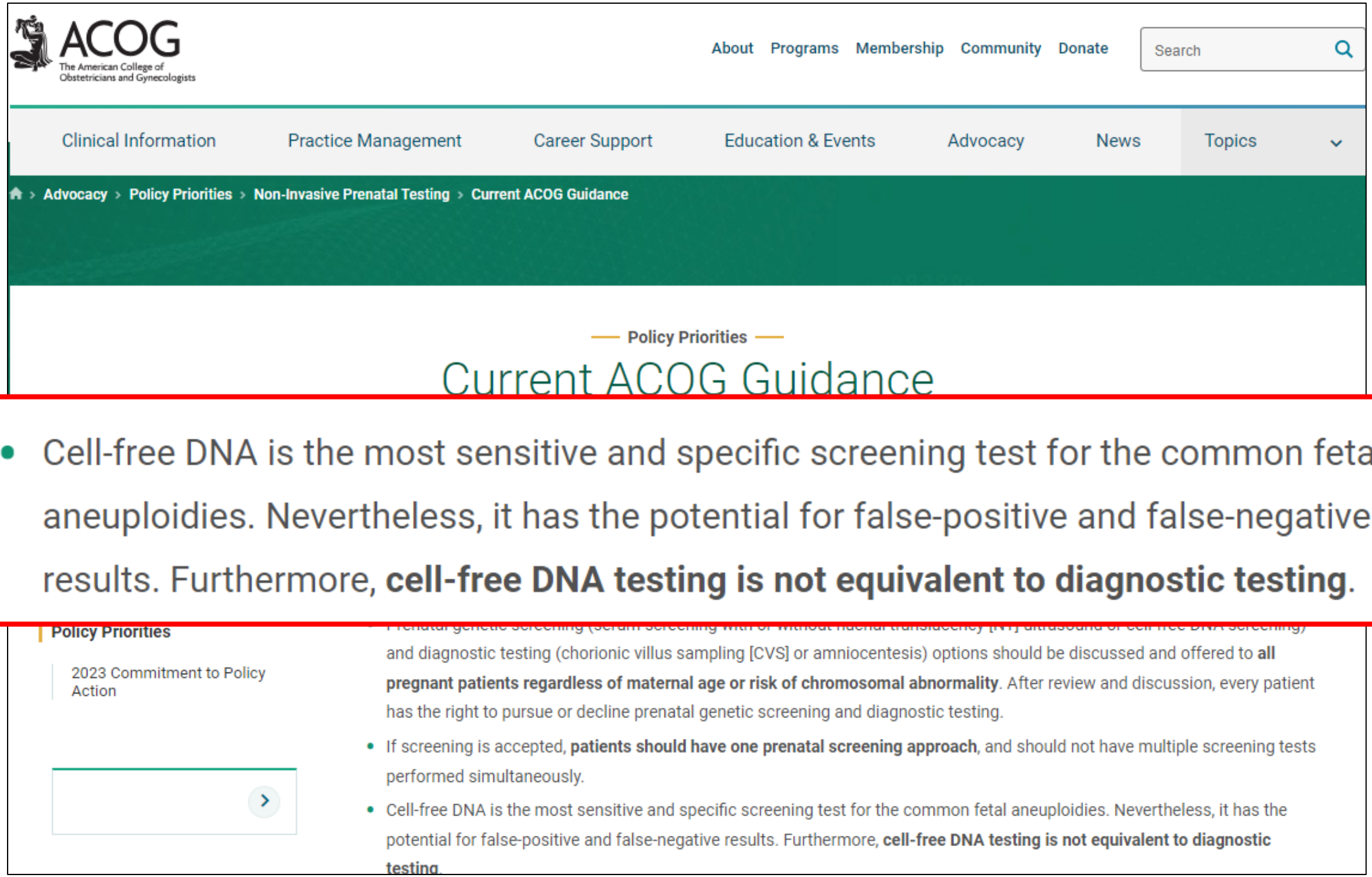
Detection rate for Down syndrome Using NIPS



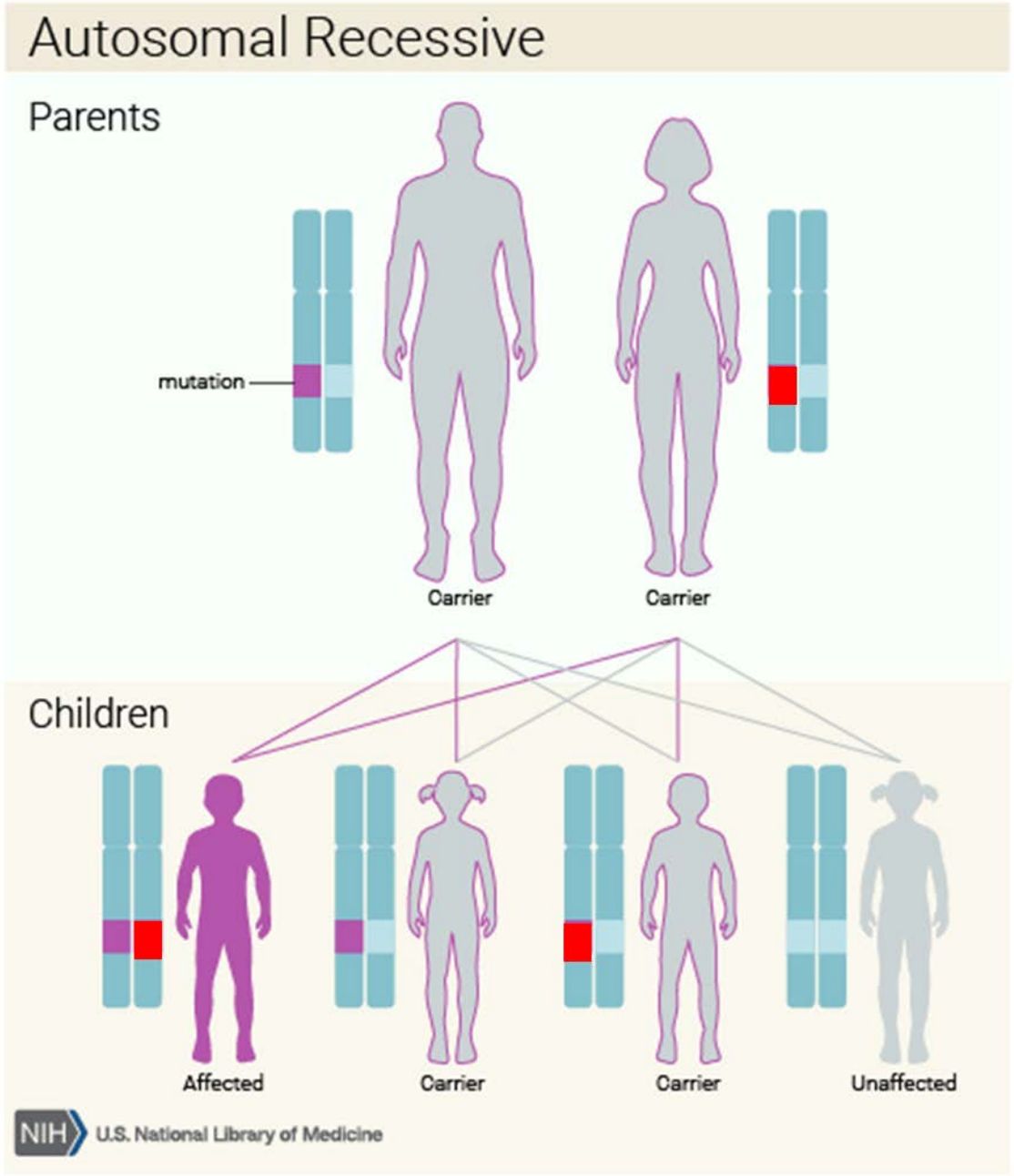
False positive rate for NIPT : < 0.1%

ACOG Practice Bulletin No. 77. Obstet Gynecol 2007;109:217-27.

NIPS: Non-Invasive Prenatal Screening



- Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, **cell-free DNA testing is not equivalent to diagnostic testing**.



Why there is no room for NIPT for thalassemia ?

- Risk for thalassemia syndrome ranges from 25% to 50% (not 1 in 800 or 1 in 2000)
- PPV and NPV: unknown ?
- False positive: unknown ?
- False negative: unknown ?

Principles of Screening:

1. The disease should be an important public health problem in terms of its frequency and/or severity

2. The natural history of the disease presents a window of opportunity for early detection

3. An effective treatment should be available that favorably alters the natural history of the disease

4. A suitable screening test should be available, that is, one that is accurate, acceptable to the population, fairly easy to administer, safe, and relatively inexpensive

WHO Guideline 2020

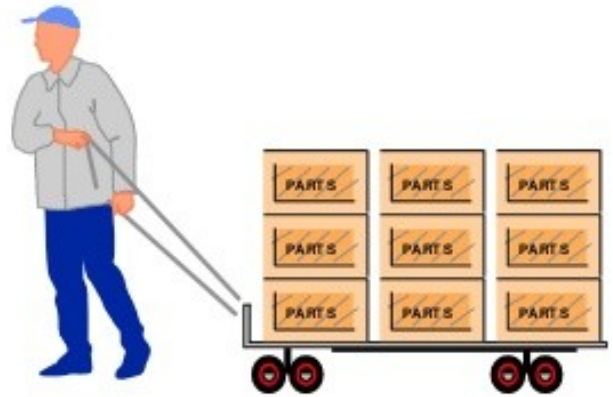
Technology Push vs. Demand Pull

Push vs. Pull

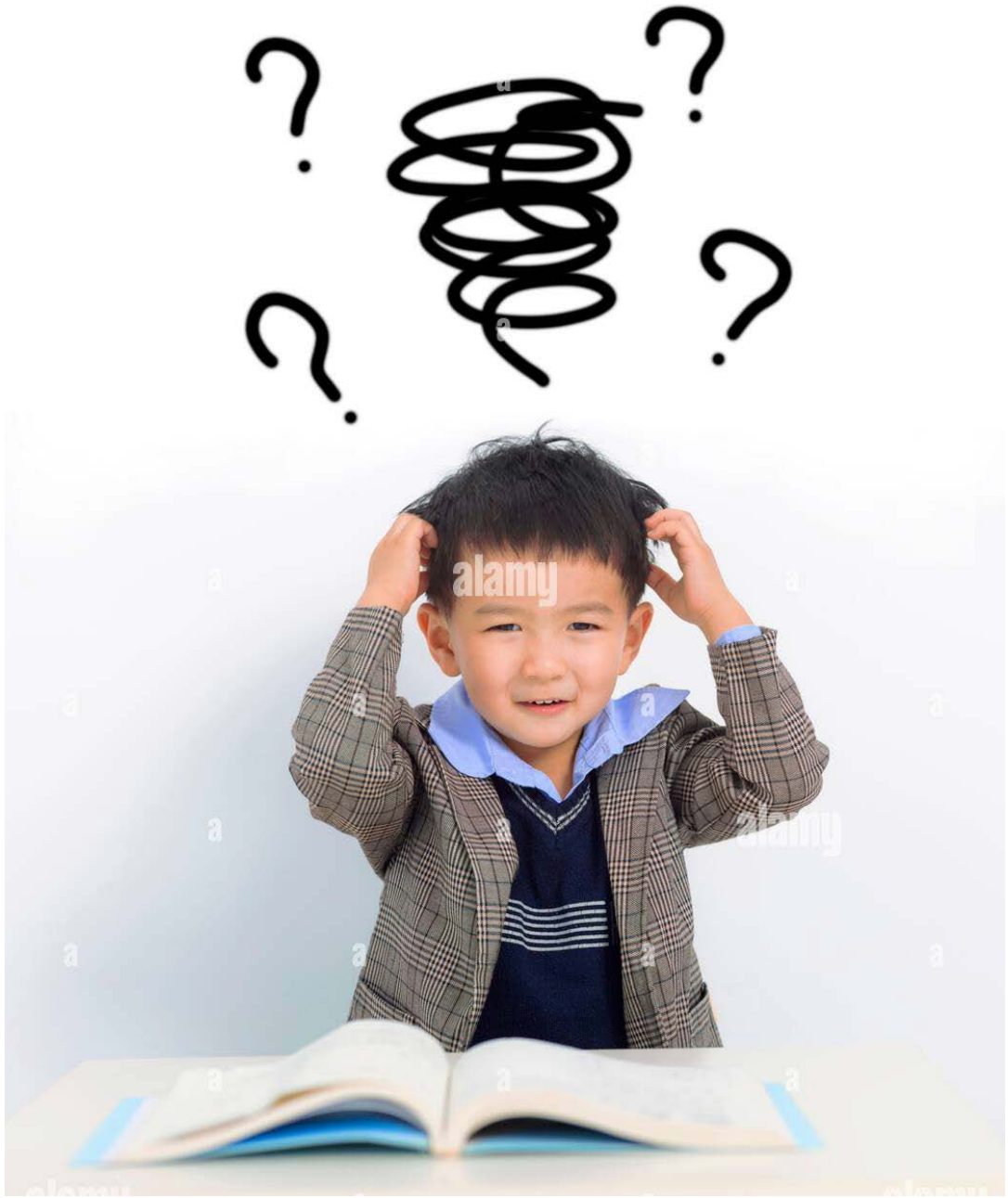
Make all we can just in case.

- Production Approximation
- Anticipated Usage's
- Large Lots
- High Inventories
- Waste
- Management by Firefighting
- Poor Communication

Make what's needed when we need it



- Production Precision
- Actual Consumption
- Small Lots
- Low Inventories
- Waste Reduction
- Management by Sight
- Better Communication



Room for Discussion