

IFCC Working Group on Standardization of Thyroid Function Tests (WG-STFT)
Meeting at AACC 2009, Chicago, IL, Monday July 20th (2:00 - 5:00 pm)

PARTICIPANTS

The meeting attendance list is attached in annex.

OBJECTIVES OF THE MEETING

After a warm welcome of the meeting attendees, the Chair of the WG-STFT described the 2 agenda points of the meeting as follows:

1. Presentation by Prof. Dr. Paolo Beck-Peccoz, Director of the Institute of Endocrine Sciences, University of Milano, IT
2. Preliminary report on the proof of concept study for FT4 and TSH.

PRESENTATION BY PROF. DR. P. BECK-PECCOZ (see also attached ppt-presentation)

The Chair introduced Prof. Beck-Peccoz as guest speaker to the WG-STFT. She mentioned that he is Professor of Endocrinology, Chief Editor of the European Journal of Endocrinology and a member of different societies/associations (Italian Society of Endocrinology (SIE), European Thyroid Association (ETA), European Neuroendocrine Association (ENEA), Endocrine Society (USA), American Thyroid Association (ATA) and the Pituitary Society (USA)). Subsequently, the Chair invited him to give his view, as endocrinologist/thyroidologist, on the need of standardization of FT4 and TSH measurements.

Having his major research interest is the pathophysiology of the hypothalamic-pituitary-thyroid axis, Prof. Beck-Peccoz gave an overview of the pathophysiology of thyroid disease, however, with the focus on new 'players', such as the A2/B5 heterodimeric glycoprotein hormone, named thyrostimulin on the basis of its thyroid-stimulating activity, thyroid transcription factors regulating the transcriptional activity of thyroid-specific genes, etc. With regard to the utility of diagnostic testing for thyroid dysfunction, he definitely placed TSH and FT4 testing in the forefront, and summarized the results for the testing typically encountered in the different thyroid pathologies. Whereas the added value of FT3 testing for diagnosis of certain pathologies was mentioned, total T3 was deemed by the speaker "*the analyte of the past century*". He also deprecated thyroid hormone testing in patients from intensive care units (ICUs), because the so-called 'low T3 syndrome' observed in this patient category mostly originates from non-thyroidal causes. With regard to the need for standardization of the key assays (FT4 and TSH), the speaker definitely was in favor, because it would allow transferability of results between laboratories, the use of common reference intervals and decision points, ..., however, he unarguably associated standardization of an assay to good quality of performance. A key sentence that regularly came across was "*standardization only helps when one knows what is measured*". In this regard he showed data on the outcome of dilution tests to assess the analytical validity of FT4 immunoassays, and dealt with the prevalence of interferences (samples with antibodies) in TSH and FT4 testing, etc. Striking was his statement that because of the use of free thyroid hormone assays with poor to very poor (FT3) analytical quality, his experience was that 53% of the patients with suspected thyroid disease and referred to him at the University of Milano were normal. In reply to the question which % difference in TSH results he was willing to cope with as thyroidologist, his answer was 40%. This was considered quite a lot and put the discussions (mainly the cons) about lowering the upper limit of normal into perspective. This statement became later on a subject of discussion with the meeting attendees.

REPORT ON THE PHASE II PROOF OF CONCEPT STUDY FOR FT4 AND TSH (see also attached ppt-presentation)

The content of the report comprised:

- investigation of the consistency of results between the phase I- and phase II study

- the status of comparability between FT4 and TSH assays after recalibration of the data by the respective manufacturers on the basis of the method comparison with the panel of 40 sera from apparently healthy donors (samples further referred to as ‘normal’ – eventually ‘clinical’ – samples)
- investigation of the method comparison data on the 65 clinical TSH samples
- Miscellaneous items, i.e. the status of the phase I manuscripts (Action-01), of the transferability of the FT4 candidate reference measurement procedure (RMP) of Ghent University to the laboratory of the Reference Material Institute for Clinical Chemistry Standards (ReCCS, Kawasaki, Japan) (Action-02), the clotting experiments (Action-03), of the 1st draft on the implementation of total thyroxine standardization (Action-09) and of the ‘open letter’ to EQA/PT-providers (Action-10).

The Chair explained that due to the late arrival of some of the results she had not been able to send the report to the participants before the meeting. The only information distributed beforehand was the code used for each of the manufacturers (in order to ensure in situ identification of the coded data in the slides). The Chair assured that all results that were received, even those on the evening before the meeting, were included in the presented report. This was for the normal FT4 and TSH samples, 13 sets of results, for the clinical TSH samples, 10 sets, for the recalibrated normal FT4 and TSH 12 and 13 sets, respectively.

With regard to the consistency of the results between phase I and II (slides 3 & 4), it was considered reasonable to expect that the discrepancy would be below 10% for both FT4 and TSH. This was achieved by most of the assays, apart from a few exceptions. It was the Chair's point of view that for the future the aim probably should be a consistency within 5%. With regard to recalibration, it was shown that most of the manufacturers had successfully reassigned values to their master calibrators, so that the agreement of the results for the normal FT4 and TSH samples with the reference (on the x-axis) was of the order of the achievable agreement by mathematical recalibration demonstrated in phase I (slides 5-9; 15-19). To recall: for FT4 the reference was based on the values obtained by the equilibrium dialysis isotope dilution mass spectrometry (ED ID-MS) candidate RMP, for TSH on the all methods' trimmed mean (TM). The procedure used for calculating the all methods' TM was clarified (slides 11-15). Some recalibrated assays were looked at in more detail, because of apparent issues, most probably due to inclusion of outliers. The between-assay CVs before and after recalibration (slide 10 & 20) gave evidence of the achievable improvement in comparability of results through recalibration.

When it came to the discussion of the between-assay comparability of the clinical TSH results, the observations were that the high results (>12 mIU/L) showed a markedly higher between-assay variation than the normal samples (slide 21), that with some assays the high clinical samples had a moderate to strong negative or positive bias versus the normal and the low clinical samples, that the ratio of the latter 2 was typically not constant, but followed the same trend (slide 22 & 23). It was hypothesized that the deviate behavior of the high clinical samples may indicate a different recognition of glycosylation forms by the different assays, unless, it would be due to the matrix of the high samples. To exclude the latter, the Chair proposed to repeat the experiment with 30 high clinical and 30 normal TSH samples obtained from the same source and prepared by the same, exactly defined protocol. She said it would be sufficient to measure the samples with 4 assays (2 compatible and the 2 extremes). The Chair concluded that, apart from the underlying reason of the deviate behavior of the high clinical samples, the data were such that the assays cannot be standardized. Also the results for the low clinical versus the normal samples were discussed in some more detail (slides 27-31), with special emphasis on the fact that a few assays had reported results that were very low, 0 or <0 mIU/L and potentially had

calibration or sensitivity problems. Also the question whether all assays were 3rd generation was shortly discussed.

With regard to the miscellaneous matters:

- Action-01, status of the manuscripts in preparation on the phase I study: the Chair mentioned that the 2nd draft (after thorough revision; circulated on April 23th; deadline for reply: June 26th) had in general been positively received, with comments only editorial of nature. She will wait for the pending replies until mid August. Then it is her intention to submit the manuscripts to the IFCC Scientific Division (SD) for approval and afterwards to Clin. Chem.
- Action-02, transferability of the FT4 candidate RMP: the Chair showed the outcome of a blind intercomparison between Ghent University and the laboratory of ReCCS (slides 32 & 33). She was pleased that the data gives sufficient evidence that the transfer of the FT4 RMP with sufficient agreement is nearly a fact and requires that only some calibration and reproducibility issues are resolved.
- Action-03, clotting experiments (slides 34 & 35): the Chair mentioned that, unfortunately, they were not finalized (at the time of the meeting only 1 set of results from 1 affected TT3 assay was received; the FT3 results still are pending). She showed the preliminary results, and drew the attention on the positive effect on the clotting process from adding thrombin, in that no sample-related effects and outliers were observed.
- Action-09 and Action-10, make a 1st draft on the implementation of total thyroxine standardization and the 'open letter' to EQA/PT-providers: the Chair mentioned that she had decided to postpone these actions until clinicians, laboratory medicine, manufacturers and regulatory bodies reached agreement on standardization, with clear elaboration of timelines and responsibilities for implementation.

DISCUSSION

The Chair declared the report open for discussion.

FT4

The manufacturers stated that, although for the proof of concept they had been prepared to recalibrate their assays only on the results for the panel with 40 normal specimens, the recalibration process requires samples covering a much broader FT4 concentration range. This can of course only be achieved by including clinical samples. It was proposed that the recalibration panel should comprise the following specimens (and numbers): from subjects with eu- (n = 30), hypo- (down to 3 pmol/L) (n = 30) and hyperthyroid FT4 concentrations (up to 40 pmol/L) (n = 30) and from pregnant subjects (n = 30). For recalibration, the WG considered it not of relevance to include samples from ICU patients or subjects with Familial dysalbuminemic hyperthyroxenemia (FDH). These are samples that each manufacturer uses to analyze for validation of his assay according to the recommendations of the National Academy of Clinical Biochemistry. The manufacturers expressed their expectance that again Ghent University would assign values to the sera with the ED ID-MS candidate RMP. The WG asked the Chair whether ED ID-MS was able to measure FT4 concentrations as low as 3 pmol/L, which she confirmed. The WG also urged the Chair to optimize the candidate RMP to the point that it was ready for nomination for listing by the Joint Committee for Traceability in Laboratory Medicine (JCTLM). Availability of a free hormone reference measurement system like there is one for total thyroid hormones is a *conditio sine qua non* for IVD industry to proceed to recalibration. The Chair will give priority to this demand on her return in the laboratory.

When SeraCare was brought up as source of the specimens, Prof. P. Beck-Peccoz kindly offered to help the WG with providing the samples. It would only be necessary to send him clear instructions on required volume, tubes etc. With regard to the latter, the Chair asked whether it

was not necessary to decide on the type of tubes to be used for blood collection. This question was put forward in view of the problems encountered with certain FT3 and TT3 assays in phase I. Although Prof. P. Beck-Peccoz said he could use whatever tube the WG prefers, there was consensus to use the tube that is most common all over the world, i.e., with gel separator. The rationale was that this would best represent laboratory medicine practice.

TSH

The observation that the clinical TSH samples behave differently from the normal ones with some of the assays was further discussed, in particular the question whether the matrix of the samples or a difference in glycosilation pattern could be the cause. Some manufacturers argued against a matrix effect because, the measurement range of some assays being not that broad, had necessitated dilution of the samples, without any visible influence on the behavior of the dilutions in comparison to the non-diluted clinical samples. It is namely expected that dilution so-called 'neutralizes' matrix effects. Therefore, it was proposed that, when the study is repeated as suggested by the Chair (to recall: with 30 high clinical and 30 normal samples obtained from the same source and prepared by the same but exactly specified protocol), sample dilutions (1/2, 1/4, 1/8, 1/16) should be included to (dis)prove linearity of results.

With regard to the source to provide the samples, the WG has now an alternative to the previously used source, i.e., Prof. Beck-Peccoz versus SeraCare.

As mentioned before, the statement of Prof. Beck-Peccoz regarding the acceptability of a difference of 40% was brought up again in the discussion. If indeed this difference is acceptable, there would be no need for standardization of the current TSH assays, since phase I and II showed for the normal TSH concentrations a difference <40%. Dr. G. Klee made the statement that, although this difference may be acceptable when the results of only 1 patient are concerned, he considered it huge when the between-assay comparability of results is considered. This is because the aim of standardization in general is to allow the use of common reference intervals and to establish practice guidelines with common decision points. He referred to the impact of a systematic difference on the false positive or negative interpretation of a result against a common reference interval or decision point. According to Dr. Klee, the calibration bias of a TSH assay should be <5%. Also Dr. G. Beastall, mentioned that in the UK patients are hopelessly confused by non-comparability of results among assays, in consequence, that he suspected that a 40% difference between TSH results would run up against a brick wall.

CONCLUSION

After the meeting, the Chair will prioritize writing the minutes and making the report. She will contact some individual manufacturers, for whom issues, e.g., in the recalibration procedure, have been seen.

With regard to the path forward for the WG, the meeting led to the following plans:

- Action-12: extend the FT4 method comparison and recalibration with clinical samples, i.e. 120 in total, comprising hypo-, eu-, hyperthyroid and pregnant subjects (1);
- Action-13: describe repetition of the experiments with clinical TSH samples, ensuring full control of the source and protocol for serum generation (2);
- Related to Action-12 & 13, make an estimate of the costs for plan (1) and (2)
- Action-14: optimize the FT4 candidate RMP towards nomination for listing by the JCTLM
- Action-01: revise draft 2 of the 3 manuscripts on the phase I study, submit for approval to the IFCC-SD and finally to Clin. Chem.

Annex

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Working Group Standardization of Thyroid Function Tests (WG-STFT)

Phase II Proof of concept AACC 2009

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Overview

Consistency phase I/phase II

FT4 recalibration

TSH recalibration

TSH clinical samples

Miscellaneous

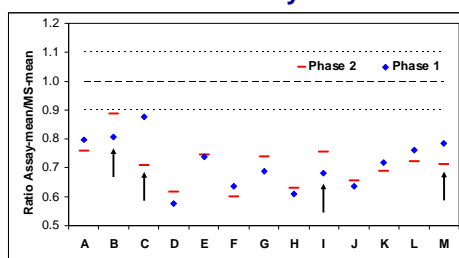
- FT4 reference measurement procedure
- Clotting experiments
- TT4 standardization & letter to PT/EQA-providers
- Status of phase I manuscripts

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Consistency – FT4



Differences $\geq 10\%$

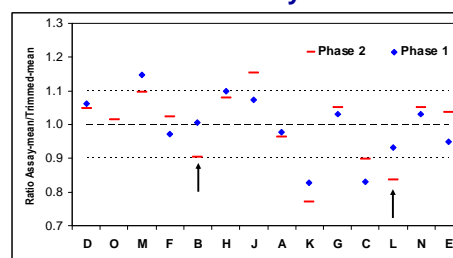
C: -21.5% (intentionally?); I: 10.4%; M: -9.9%; B: 9.5%

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Consistency – TSH



Differences $\geq 10\%$

B: -10.6%; L: -10.9%

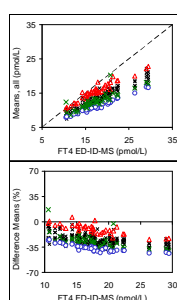
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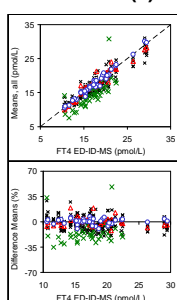
FT4 recalibration (9 of 13)

Before



After

Problems: C(x) & M



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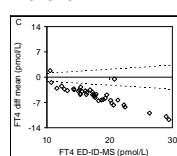


FT4 recalibration

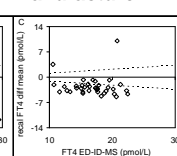
Recalibration problems assay C

Possible reason: inclusion of outliers in the method comparison regression equation

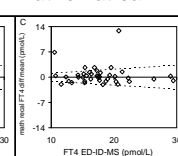
Before



Manufacturer



Mathematical



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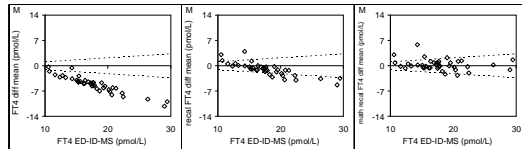


FT4 recalibration

Recalibration problems assay M

Possible reason: inclusion of outliers in the method comparison regression equation

Before Manufacturer Mathematical



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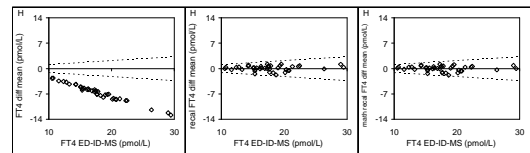
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FT4 recalibration

Successful recalibration

Before Manufacturer Mathematical



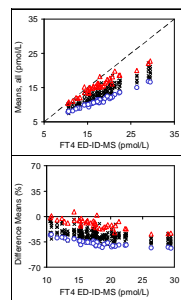
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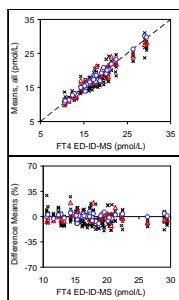


FT4 recalibration (8 of 13)

Before



After
Without assay C



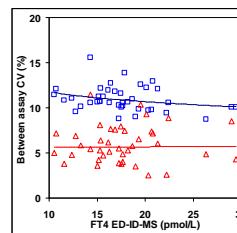
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FT4 recalibration (8 of 13)

Between assay CV before (□) and after (Δ) recalibration (without assay C)



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TSH recalibration

Trimmed mean (except 3 low samples)

Strategies for outlier omission

(note: 13 of 14 assays, 1 came in late)

1. Within assay outliers

Grubbs test (95% probability)

Within assay outliers
(Grubbs test, 95%)

B	1	F	5
J	6	A	0
M	1	H	2
D	4	C	2
O	1	L	0
G	1	N	1
K	0	E	4

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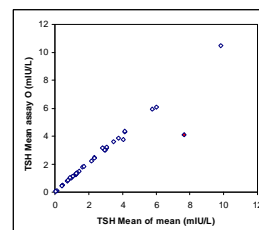


TSH recalibration

Trimmed mean 1

2. Identification of gross outliers

Scatter or bias plot versus overall mean



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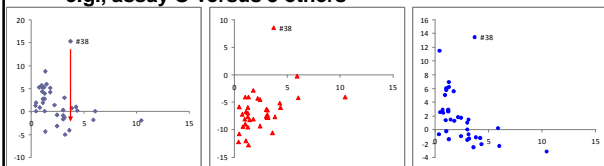


TSH recalibration

Trimmed mean 2

3. Identification of test-specific outliers

- Pairwise bias plots (all combinations)
e.g., assay O versus 3 others



- Removal of outliers after calibration of the assay to the mean, or by assigning a value on the regression line

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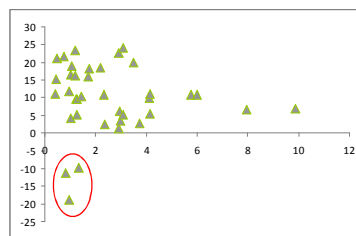
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TSH recalibration

Trimmed mean 3

4. Bias plot versus trimmed mean 2 e.g., assay M



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TSH recalibration

Manufacturers' calculations

- Do regression **without** the identified assay-specific outliers
– was not always followed
- Recalibrate **all** samples, **except** the 3 low ones
– was not always followed: some recalibrated the low; some did not recalibrate the outliers

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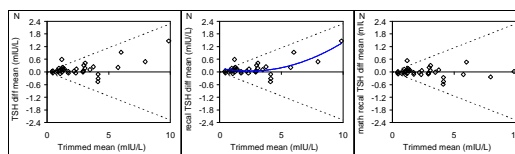


TSH recalibration (8 of 14)

Problem case

Did not recalibrate

Before Manufacturer Mathematical



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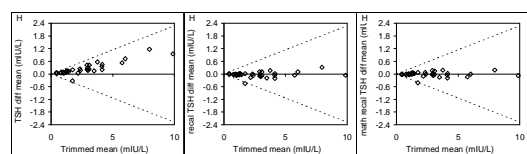
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TSH recalibration (8 of 14)

Successful recalibration

Before Manufacturer Mathematical



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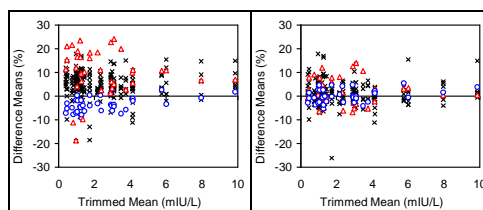
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TSH recalibration (8 of 14)

Before

After



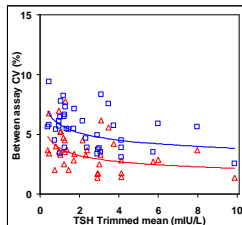
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TSH recalibration (8 of 14)

Between assay CV before (□) and after (Δ) recalibration



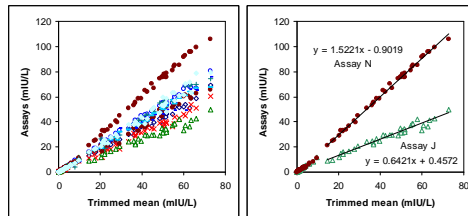
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TSH clinical samples

High clinical samples (Results of 10 assays)
The high clinical samples (>12 mIU/L) showed a markedly higher between-assay variation than the normal samples. Most deviating were assays N & J.



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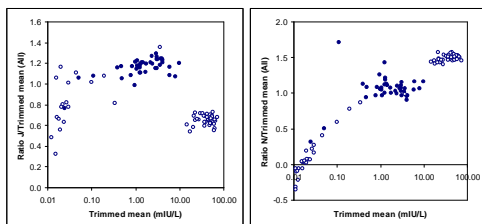
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TSH clinical samples

High clinical samples

The high clinical samples have a moderate to strong negative (J, B, M) or positive bias (N, K, L) versus the normal samples and the low clinical samples. Note, the ratio for the normal and the low samples is typically not constant, however, low clinical and normal samples follow the same trend.



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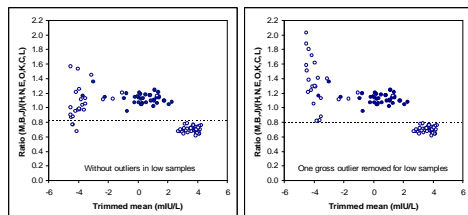
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TSH clinical samples

High clinical samples

A ratio plot between the mean of the assays with negative bias for the high clinical samples (M, B, J) and the mean of the other assays shows the deviate behaviour of the high clinical samples even more clearly



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TSH clinical samples

Clinical samples

The deviate behaviour of the high clinical samples may be due to a different recognition of glycosylation forms by the different assays. However, it cannot be excluded that it is caused by the matrix of the high samples.

It is proposed to repeat the experiment with 30 high clinical and 30 normal samples prepared by the same, exactly defined protocol. It would be sufficient to measure the samples with 4 assays (2 compatible and the 2 extremes).

Apart from the underlying reason of the deviate behaviour of the high clinical samples, the assays CANNOT be standardized on the basis of these results.

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TSH clinical samples

Low clinical samples ("healthy panel" included)

J reported 7 samples <0.005 mIU/L

H reported 6 samples <0.01 mIU/L

N reported 6 values <0 mIU/L

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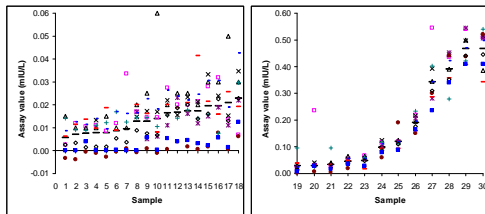


TSH clinical samples

Low clinical samples ("healthy panel" included)

Assays N, L, & C report <0, zero, or "very low" values for samples with overall means (—) of <0.02 mIU/L.

Some assays show gross outliers.



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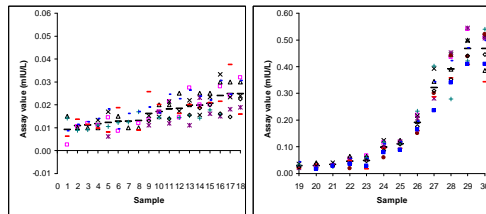


TSH clinical samples

Low clinical samples ("healthy panel" included)

Removal of <0, zero, or "very low" values for assays N, L, C, and others and removal of gross outliers.

Note: samples are resorted!



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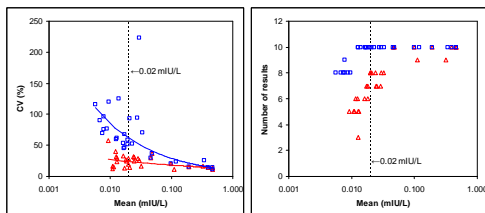
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TSH clinical samples

Low clinical samples ("healthy panel" included)

Between assay CV and n before (□) and after (Δ) removal of outliers.



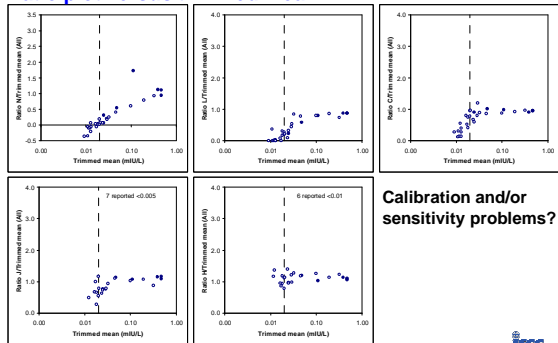
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TSH low "healthy" & clinical samples

Ratio plot versus trimmed mean



Calibration and/or sensitivity problems?

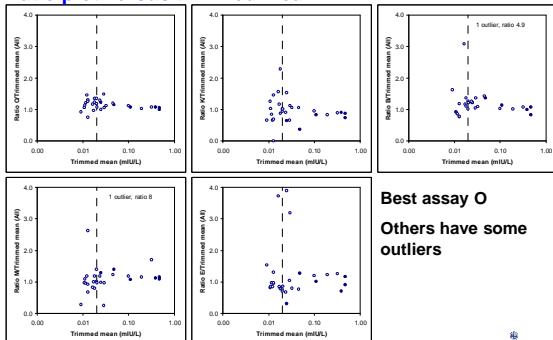
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TSH low "healthy" & clinical samples

Ratio plot versus trimmed mean



Best assay O
Others have some outliers

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TSH low "healthy" & clinical samples

3rd generation assays?

Phase I (LoQ and precision data)

N, L, J, K

Phase II (no precision data)

N, L, J?, C?

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FT4 reference measurement procedure

Action-02: Transferability study of the ED ID-MS international conventional RMP; intercomparison study UGent-ReCCS¹

Experiments

Sera

10 samples from phase II panel, spread over the concentration range

Measurements

- UGent: mean of 3 singlicates
- ReCCS: mean of 3 duplicates

¹Reference Material Institute for Clinical Chemistry Standards (ReCCS, Kawasaki, Japan)

31

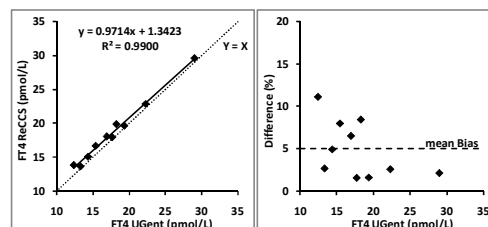
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FT4 reference measurement procedure

Results

- Bias ~5%
- Somewhat high scatter
- >We are "nearly there"; some calibration and reproducibility issues need to be resolved



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Clotting experiments

Action-03: Investigate outlier problematic observed with some TT3/FT3 assays on CLSI C37-A serum; do clotting experiments, measure and compare with results on sera according to the regular C37-A protocol.

Experiments

Sera

- 20 sera with thrombin, clotted at room temperature
- Phase II panel

Measurements

- TT3 and FT3: selected unaffected and affected assays

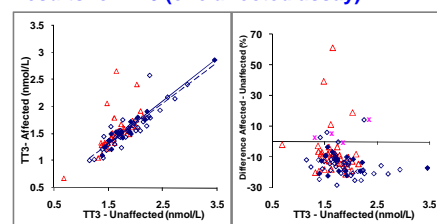
33

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Clotting experiments

Results for TT3 (one affected assay)



Legend: ◊: phase II; ♦: samples with thrombin; △: phase I; --: phase II; -: thrombin samples

- Positive effect of thrombin for TT3: no sample related effect, no outliers
- Phase II panel better than phase I panel, but still slight problems (x)

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TT4 standardization & PT/EQA-providers

Action-09: Provide 1st draft on the implementation of standardization of serum/plasma total thyroxine measurements (UGent/WG-STFT/IFCC).

Action-10: Provide 1st draft of an "open letter" to providers of EQA/PT surveys (UGent/WG-STFT/IFCC).

Postponed, actions should be concerted

- Agreement to standardize among clinic, profession, manufacturers, (regulation)
- Elaboration of implementation with timelines and responsibilities.

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Status of phase I manuscripts

Action-01: Revision of 3 manuscripts: 2nd draft; 2nd review process; submission to *Clin Chem*

- Second draft circulated, April 23
- Deadline for comments, June 26
- Received replies positive and editorial of nature
- Still some replies missing

Next step:

- submission to IFCC SD (3 weeks response time)
- Submission to *Clin Chem*

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Clinical relevance of TSH and FT4 standardization

Paolo Beck-Peccoz



*Department of Medical Sciences
University of Milan*



*Endocrinology and Diabetology Unit
Fondazione Policlinico IRCCS
Milan, Italy*

paolo.beckpeccoz@unimi.it

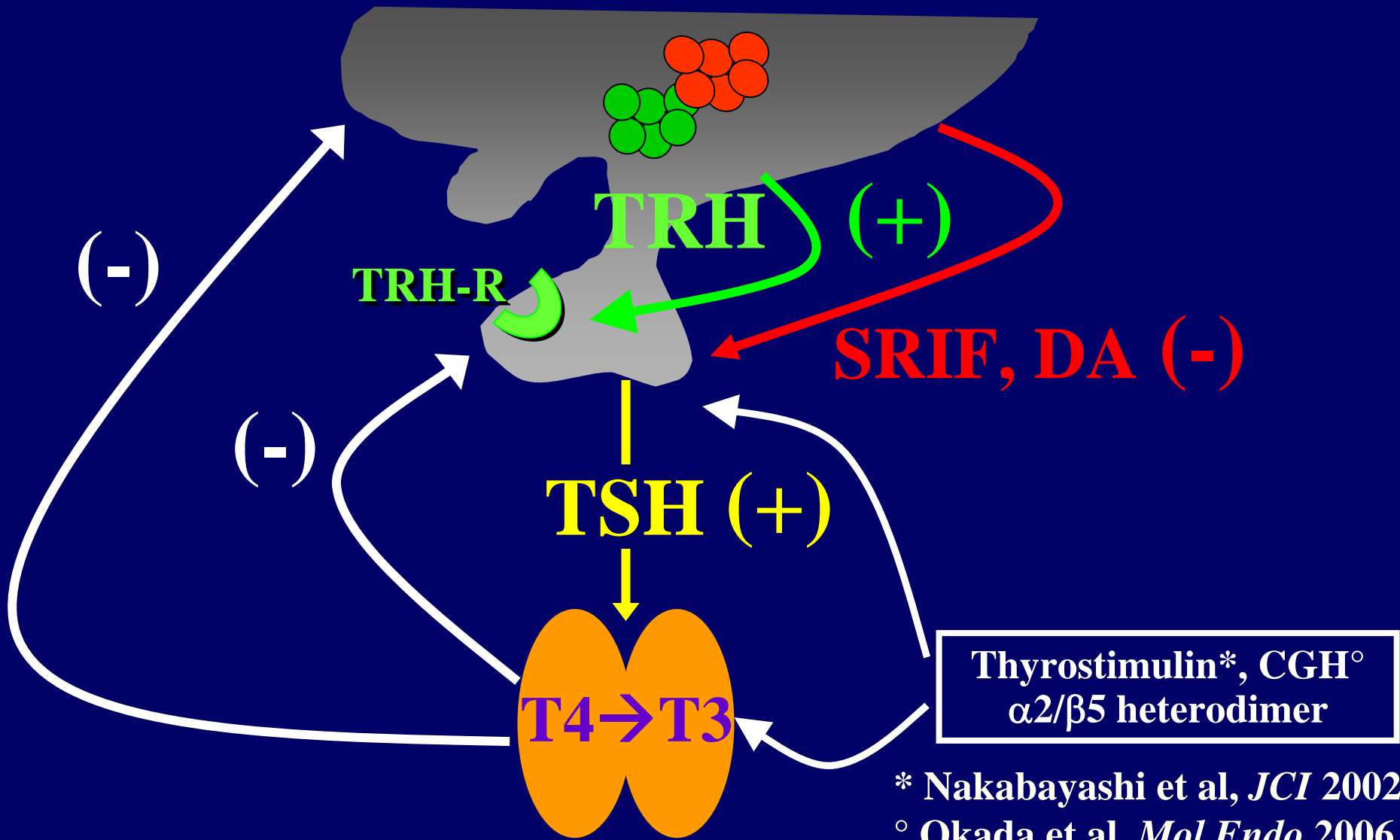
Outline

- **New players in the hypothalamic-pituitary-thyroid axis.**
- **Reformulation of the “free hormone hypothesis”.**
- **Tools in the diagnosis of thyroid disorders.**
- **Free *vs* total TH measurement.**
- **Situations in which FT4 and TSH must be highly reliable, i.e. well standardized.**

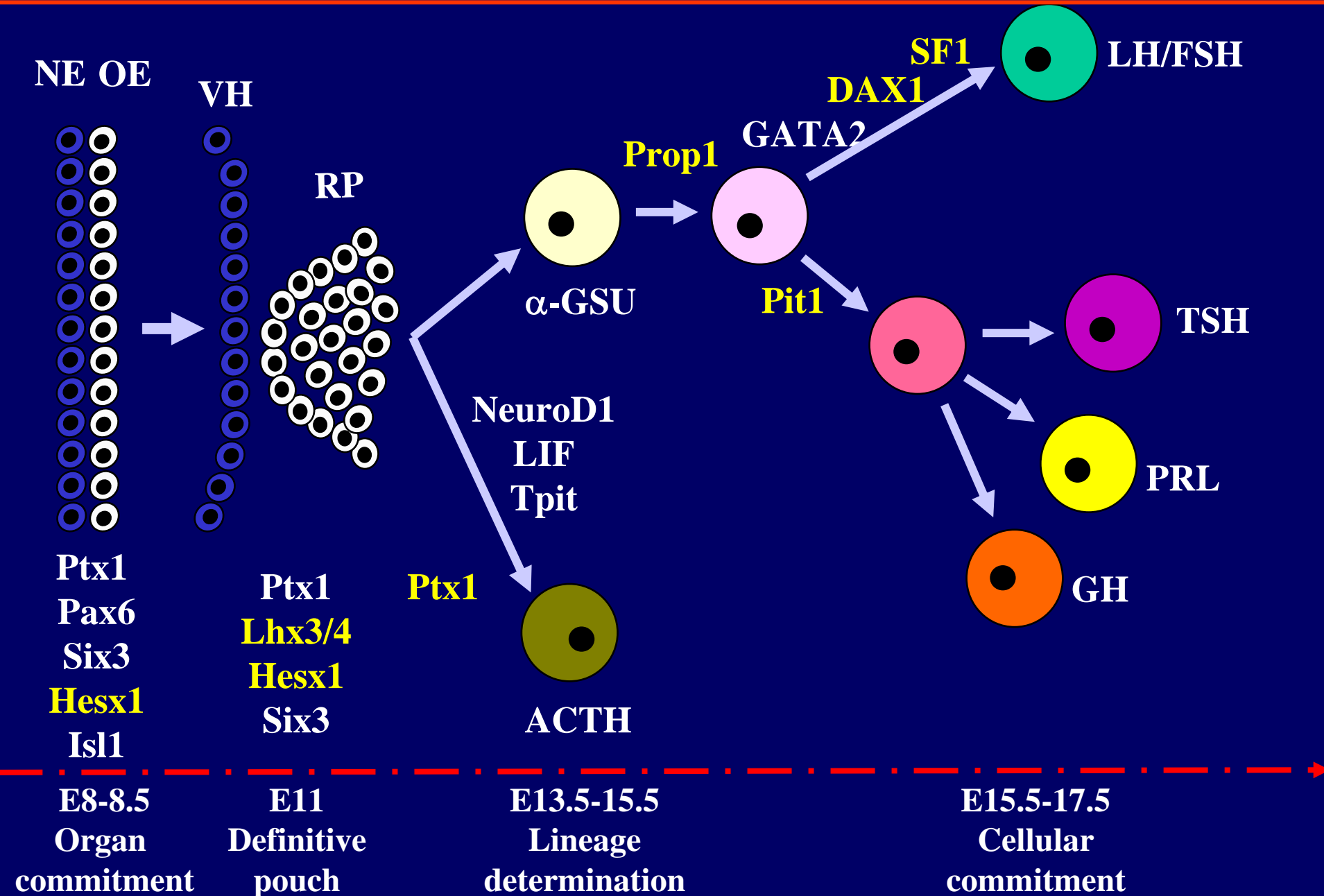
Outline

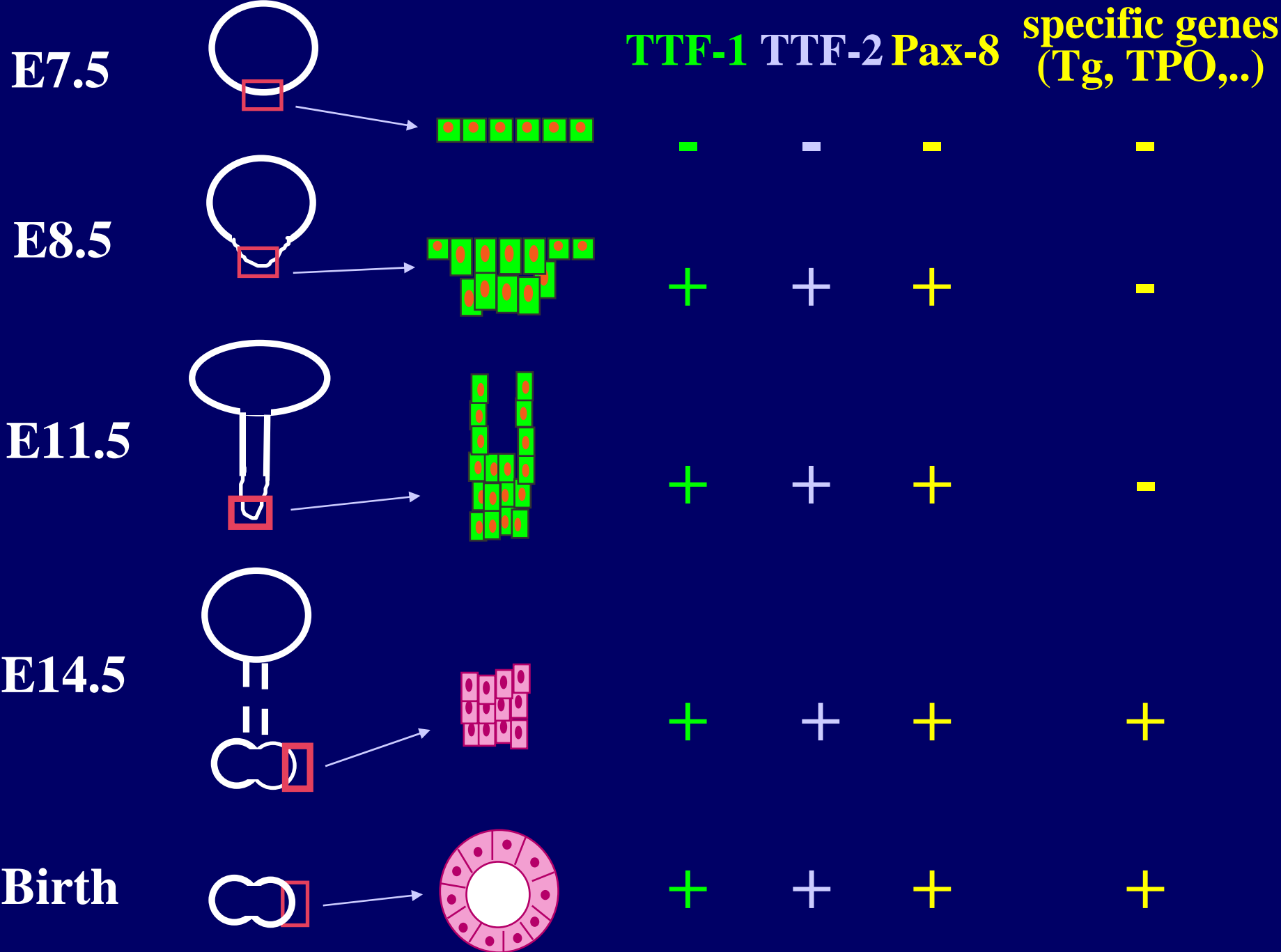
- **New players in the hypothalamic-pituitary-thyroid axis.**
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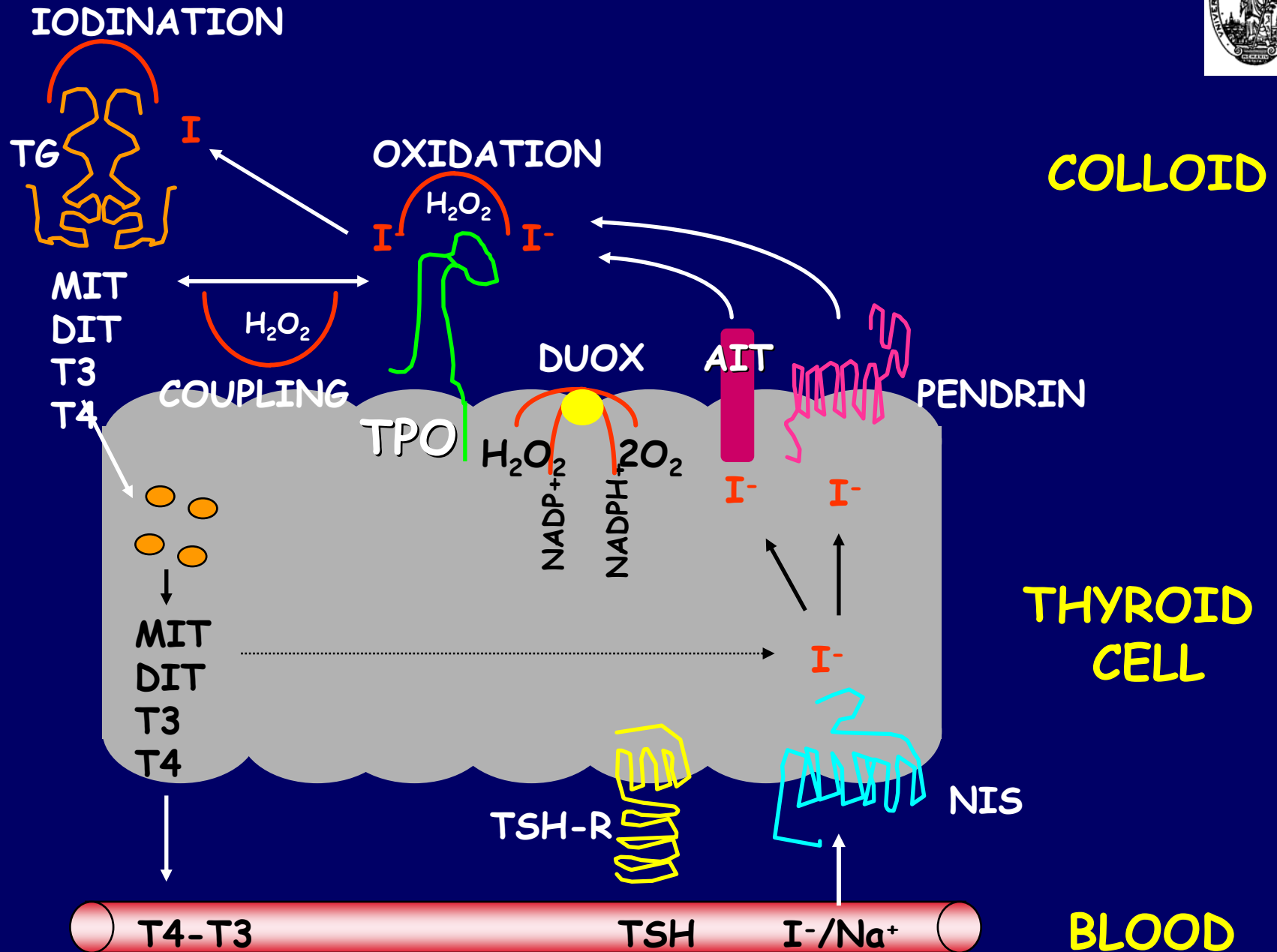
Hypothalamic-pituitary-thyroid axis

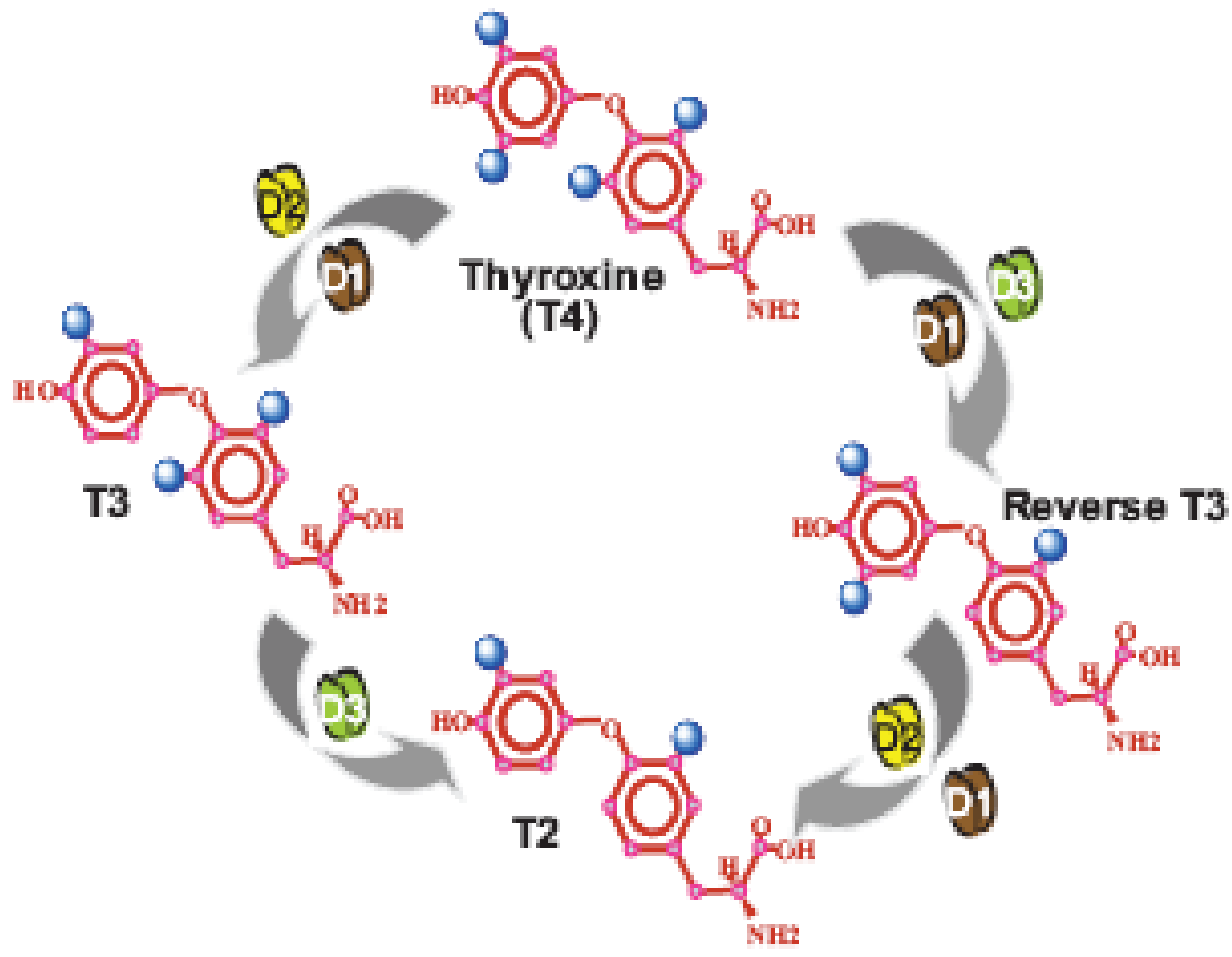


Organogenesis of the pituitary gland





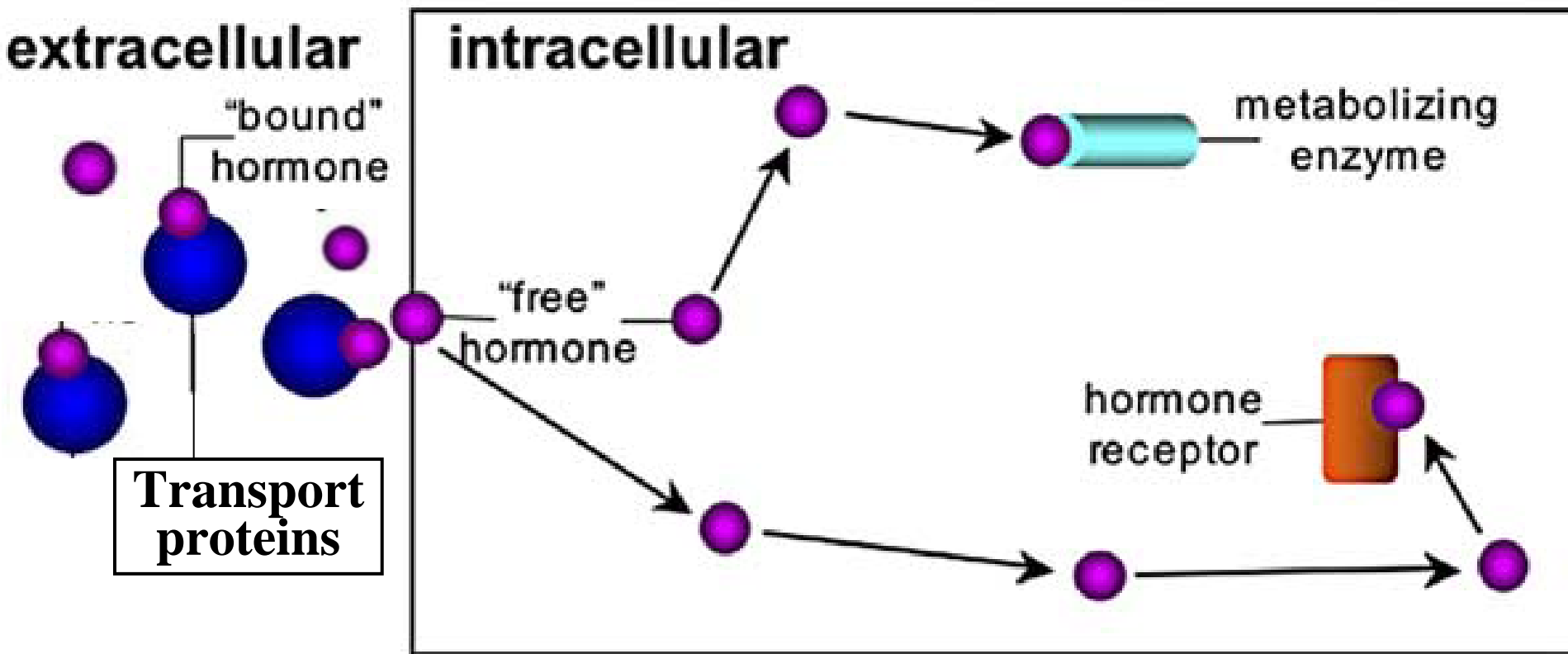




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Free hormone hypothesis



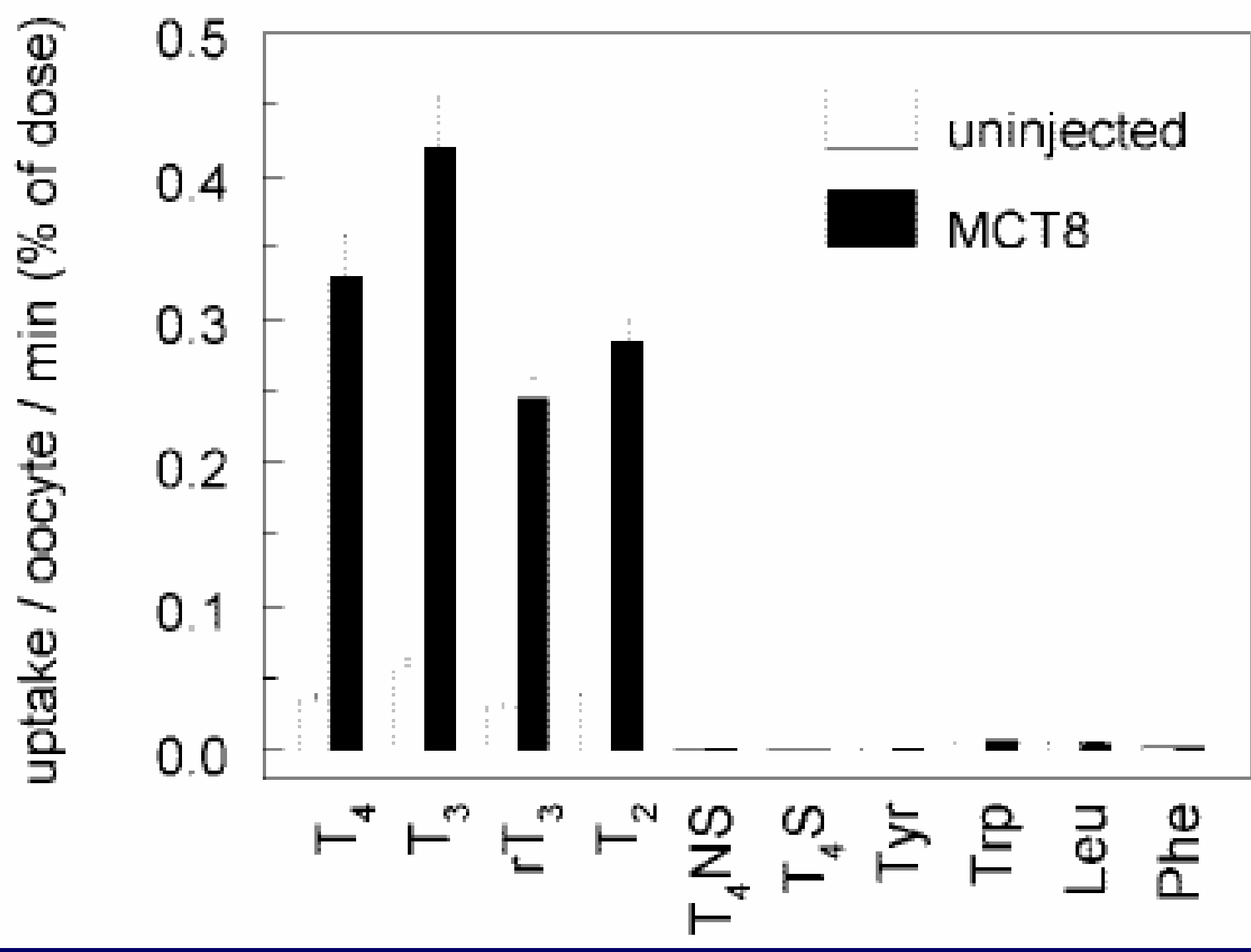
Identification of Monocarboxylate Transporter 8 as a Specific Thyroid Hormone Transporter*

Received for publication, January 28, 2003, and in revised form, July 10, 2003
Published, JBC Papers in Press, July 18, 2003, DOI 10.1074/jbc.M300909200

Edith C. H. Friesema‡§, Sumita Ganguly§¶, Amal Abdalla‡, Jocelyn E. Manning Fox¶**,
Andrew P. Halestrap¶, and Theo J. Visser‡ ‡‡

From the ‡Department of Internal Medicine, Erasmus University Medical Center, Rotterdam 3015 GE, The Netherlands and the ¶Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom

Monocarboxylate transporter 8
(*MCT8*) gene is located on the
X chromosome and encodes a
613-amino acid protein with
12 predicted transmembrane
domains.

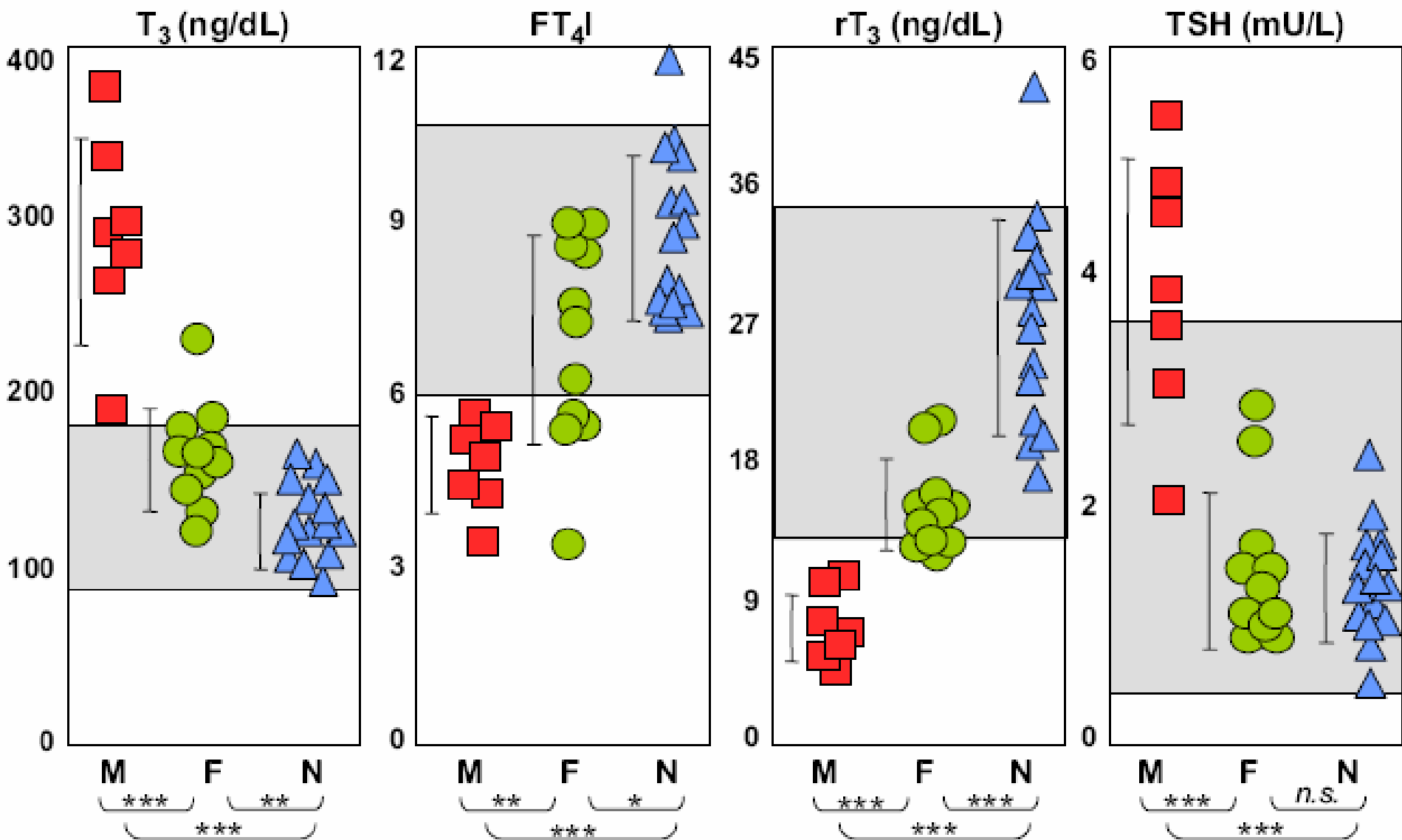


Report

A Novel Syndrome Combining Thyroid and Neurological Abnormalities Is Associated with Mutations in a Monocarboxylate Transporter Gene

Alexandra M. Dumitrescu,¹ Xiao-Hui Liao,² Thomas B. Best,⁵ Knut Brockmann,⁶
and Samuel Refetoff^{2,3,4}

¹Departments of Human Genetics, ²Medicine, and ³Pediatrics, and ⁴the Committee on Genetics, University of Chicago, Chicago; ⁵Saskatoon, Saskatchewan, Canada; and ⁶Department of Pediatrics and Neuropediatrics, University of Göttingen, Göttingen, Germany



Refetoff&Dimitrescu
Best Practice & Research Clinical Endocrinology & Metabolism
Vol. 21, pp. 277–305, 2007

Neurological abnormalities in the 1st wk consisted of dystonia, irritability, feeding problems, and rotary nystagmus.

Subsequent motor and mental development was severely delayed.

At the age of 2 years, the boy was unable to sit, crawl, or stand and had paroxysmal dystonia.

No seizures were observed, and electroencephalogram results and magnetic resonance imaging were normal.

Allan-Herndon-Dudley syndrome was among the first of the X-linked mental retardation syndromes to be described (in 1944) and among the first to be regionally mapped on the X chromosome (in 1990).



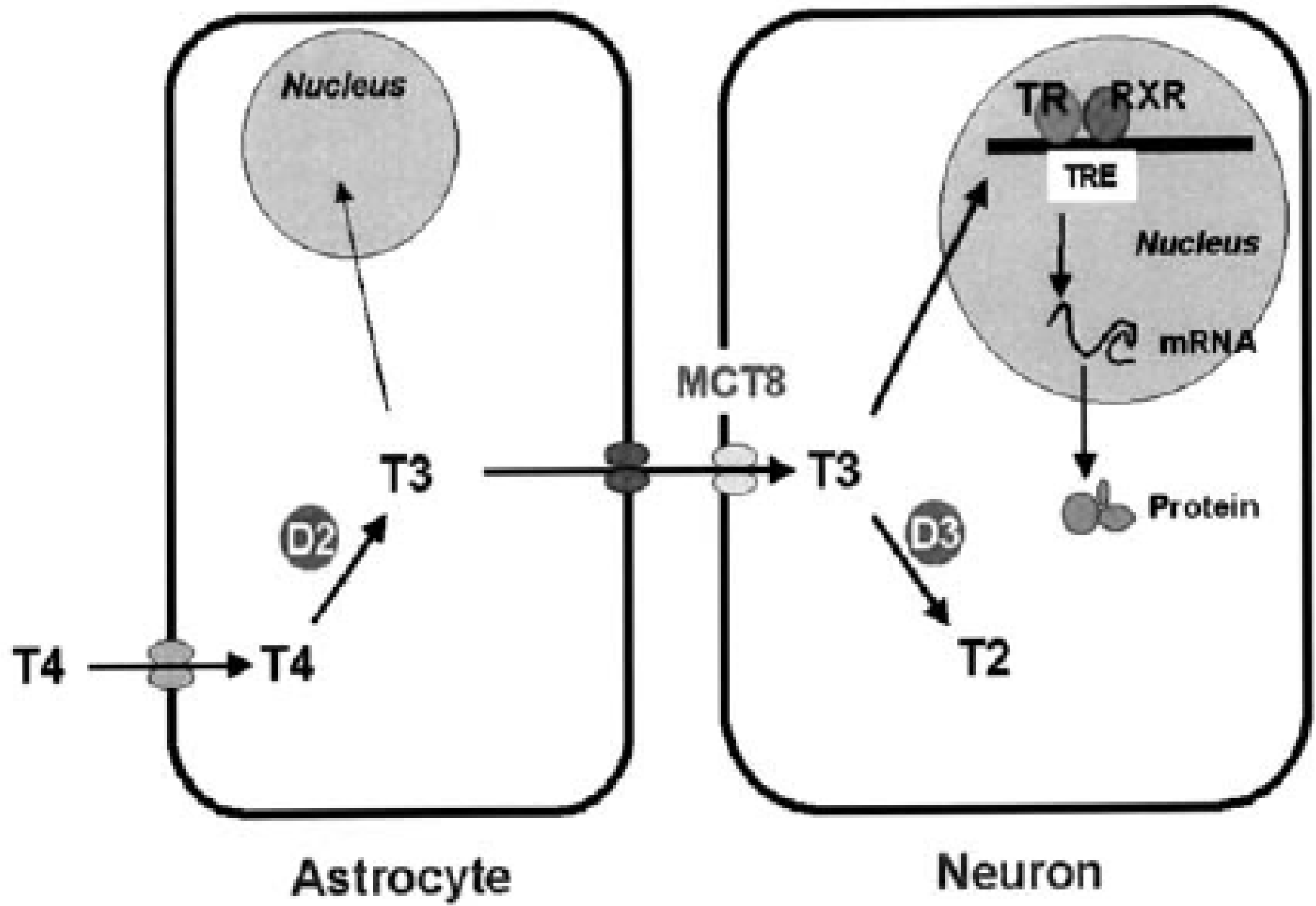
A, IV-1 at age 1 year, showing a normal face.

B, III-11 at age 14 years, showing an elongated and myopathic face.



C, III-3 at age 28 years, showing synophrys and prominence of the lower lip.

D, II-8 at age 39 years, showing an elongated face with prominence of malar areas and an open mouth.



Role of Endocytosis in Cellular Uptake of Sex Steroids

Annette Hammes,^{1,6} Thomas K. Andreassen,^{4,5,6}
Robert Spoelgen,^{1,6} Jens Raila,² Norbert Hubner,¹
Herbert Schulz,¹ Jochen Metzger,³
Florian J. Schweigert,² Peter B. Lippa,³
Anders Nykjaer,^{4,5,*} and Thomas E. Willnow^{1,*}

¹Max-Delbrueck-Center for Molecular Medicine
13125 Berlin

vators
transp
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steroid

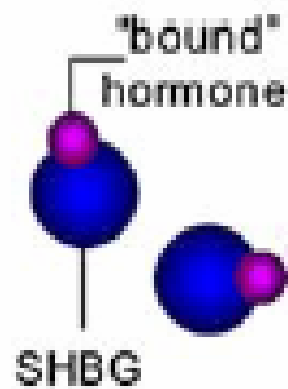
and D.

.....lack of receptor expression in *megalin*, a member of the low-density lipoprotein receptor-related protein (LRP) family, knockout mice results in impaired descent of the testes into the scrotum in males and blockade of vagina opening in females.

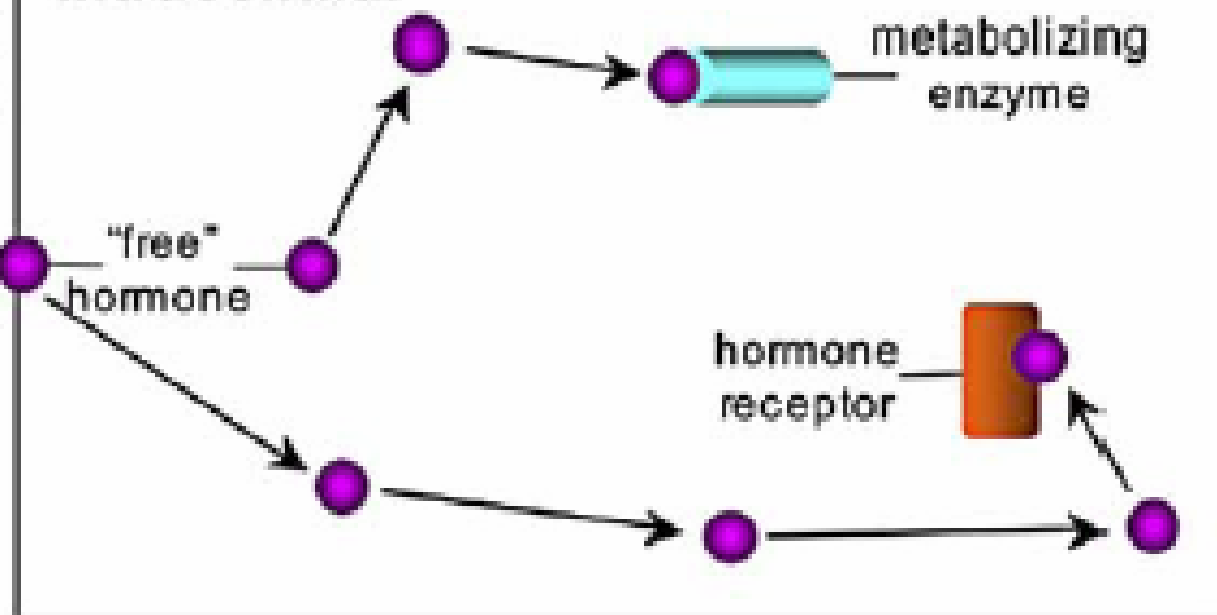
Both processes are critically dependent on sex-steroid signaling, and similar defects are seen in animals treated with androgen- or estrogen-receptor antagonists.

Thus, our findings uncover the existence of endocytic pathways for protein bound androgens and estrogens and their crucial role in development of the reproductive organs.

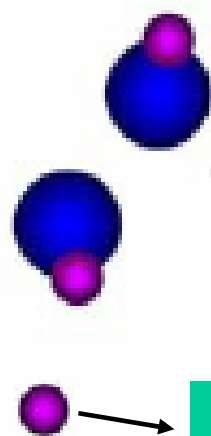
extracellular



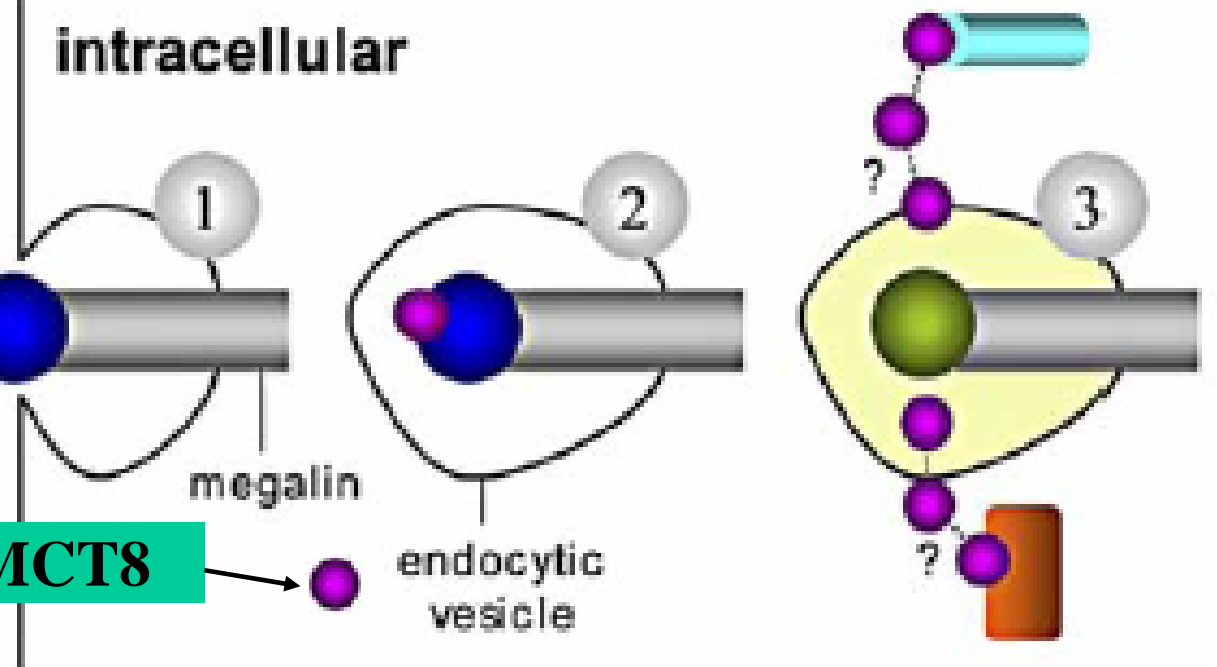
intracellular



extracellular



intracellular



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*GIOPPINO,
i.e. carnival
mask of
Bergamo
(Italy)*



BERNARDA
grandmother of
GIOPPINO

Thyroid function tests

1. MEASUREMENT OF CIRCULATING HORMONE LEVELS

Free T4 (v.n. 9-20 pmol/l)

Free T3 (v.n. 4-8 pmol/l)

TSH with ultrasensitive measurement methods (0.26-5 mU/l)

Thyroglobulin (Tg): marker thyroid carcinoma and thyrotoxicosis factitia (provided that no TgAb are present)

Autoantibodies: TgAb; TPOAb; TRAb.

2. *TRH STIMULATION TEST*

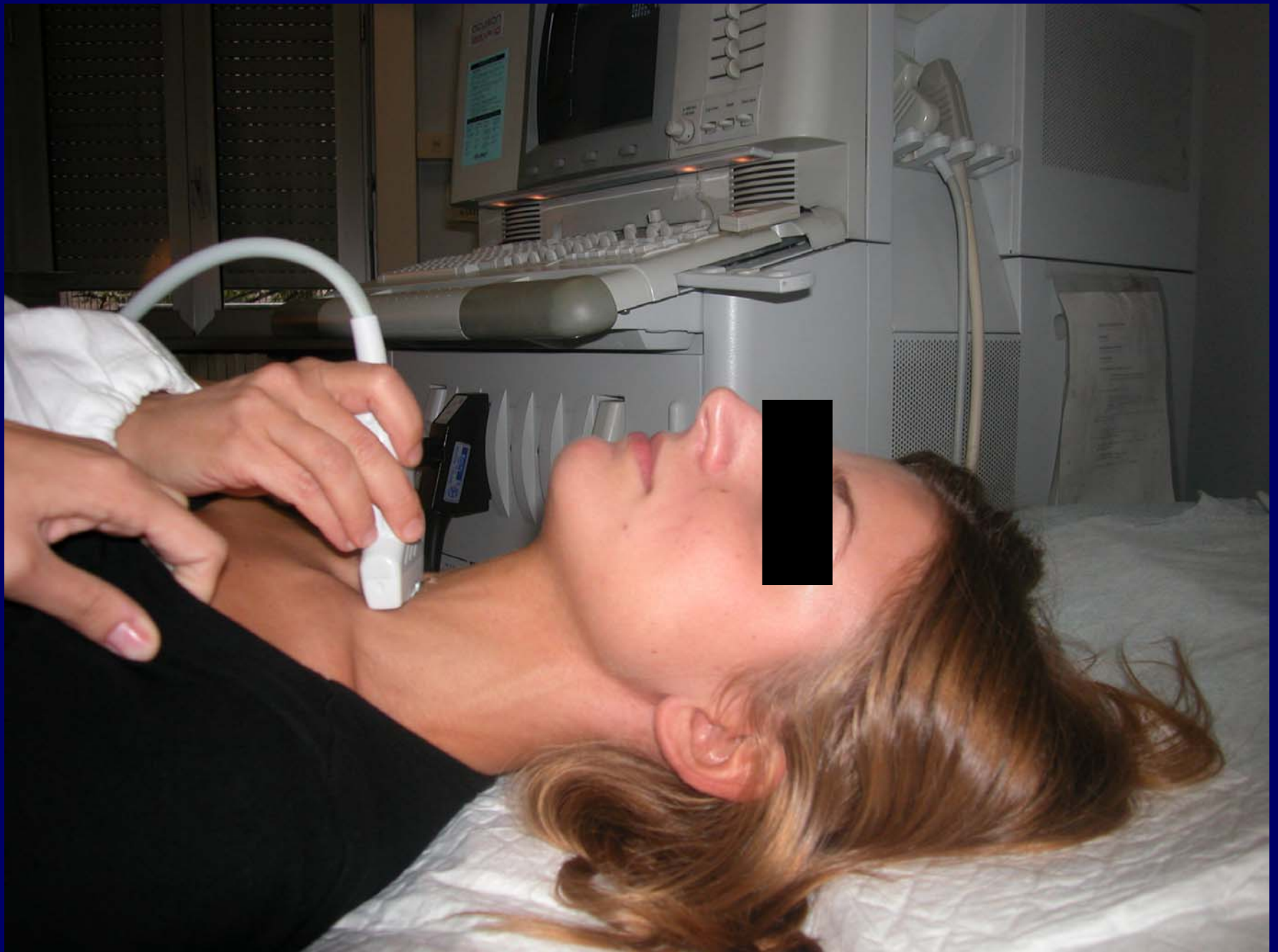
3. THYROID ULTRASOUND

4. THYROID SCINTIGRAPHY (^{131}I , ^{123}I , $^{99\text{m}}\text{Tc}$)

only in the presence of TSH levels <0.05

6. *RX, TAC, RM (retrosternal goiter)*

7. FNA-FINE NEEDLE ASPIRATION



Thyroid function tests

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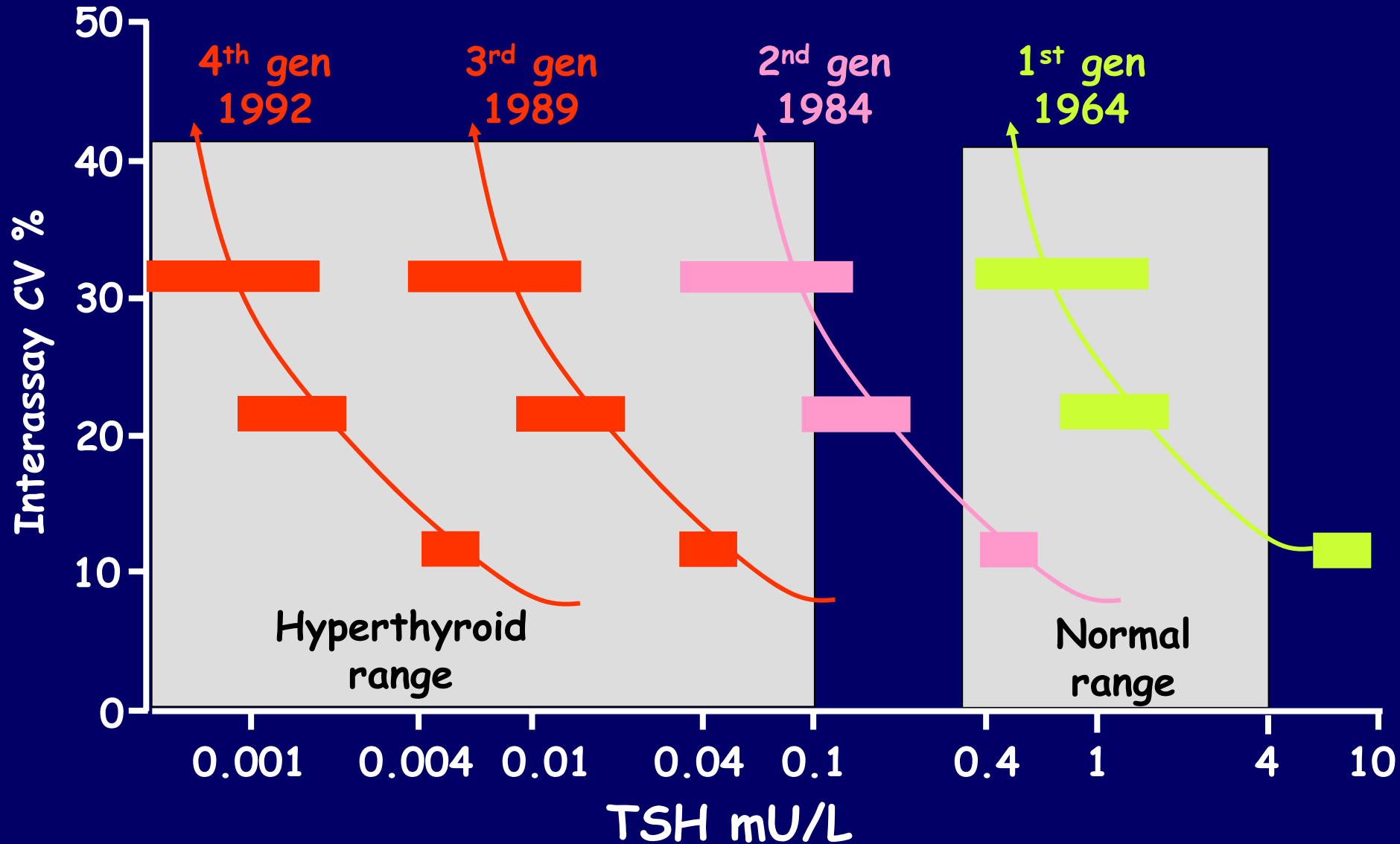
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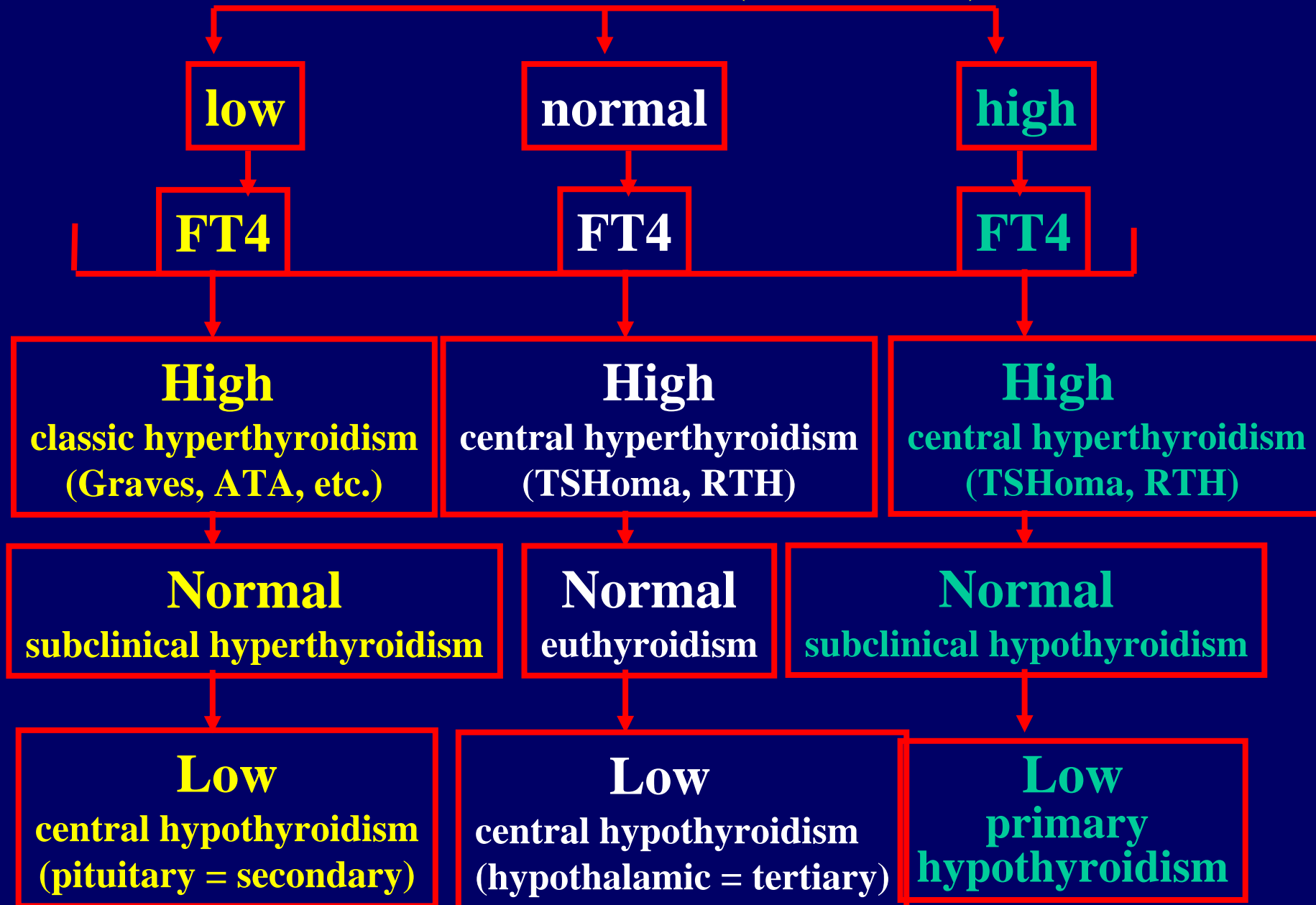
7. FNA-FINE NEEDLE ASPIRATION

TSH immunoassays



Adapted from Nicoloff & Spencer, JCEM 1990

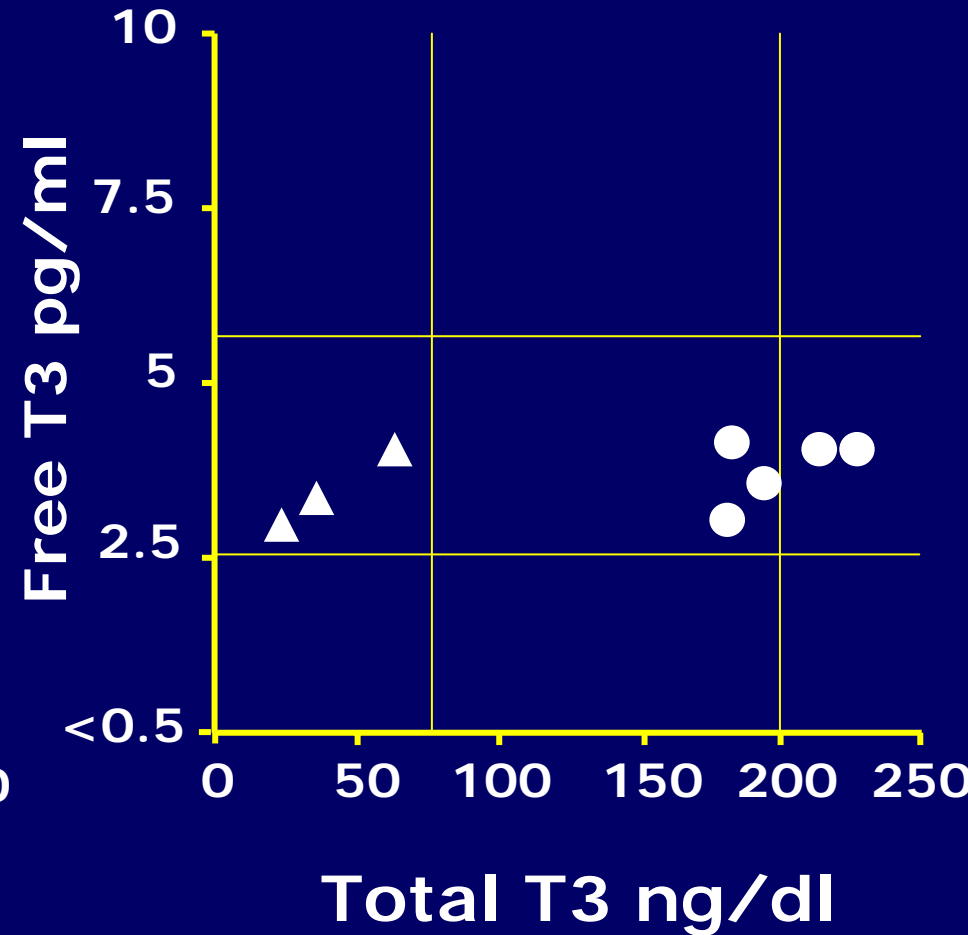
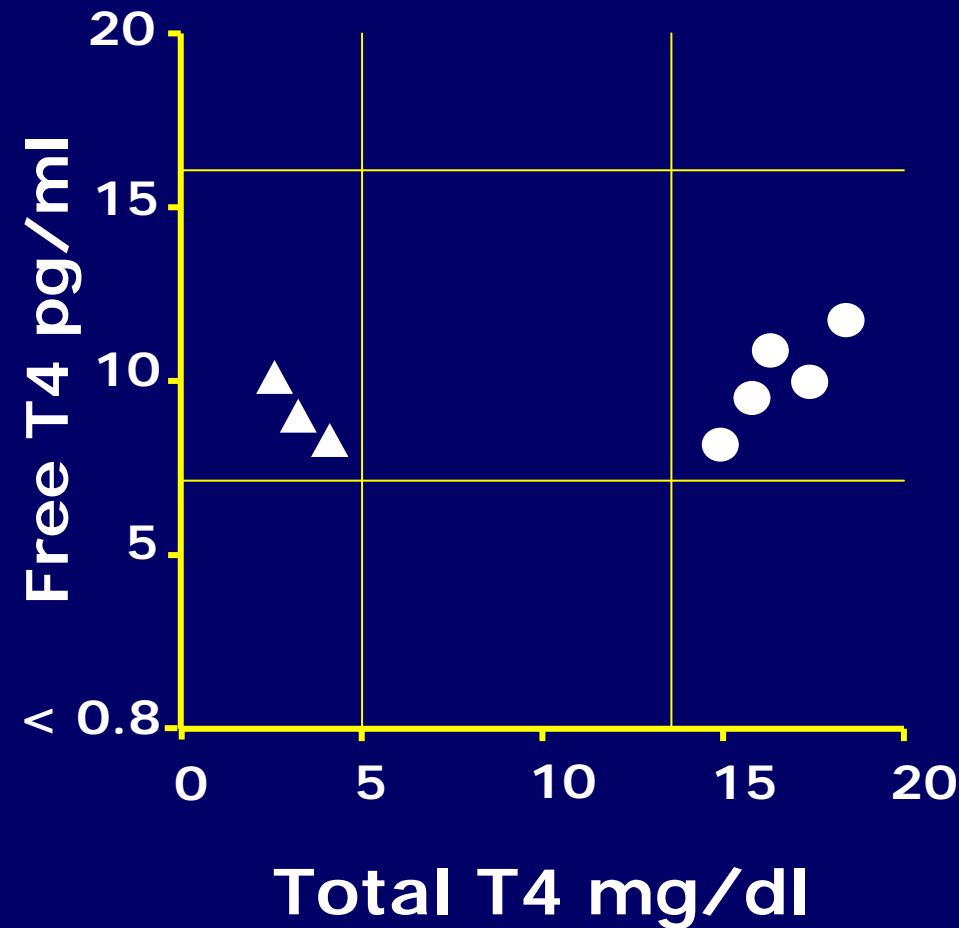
Ultrasensitive TSH (vn 0.3-4.0)



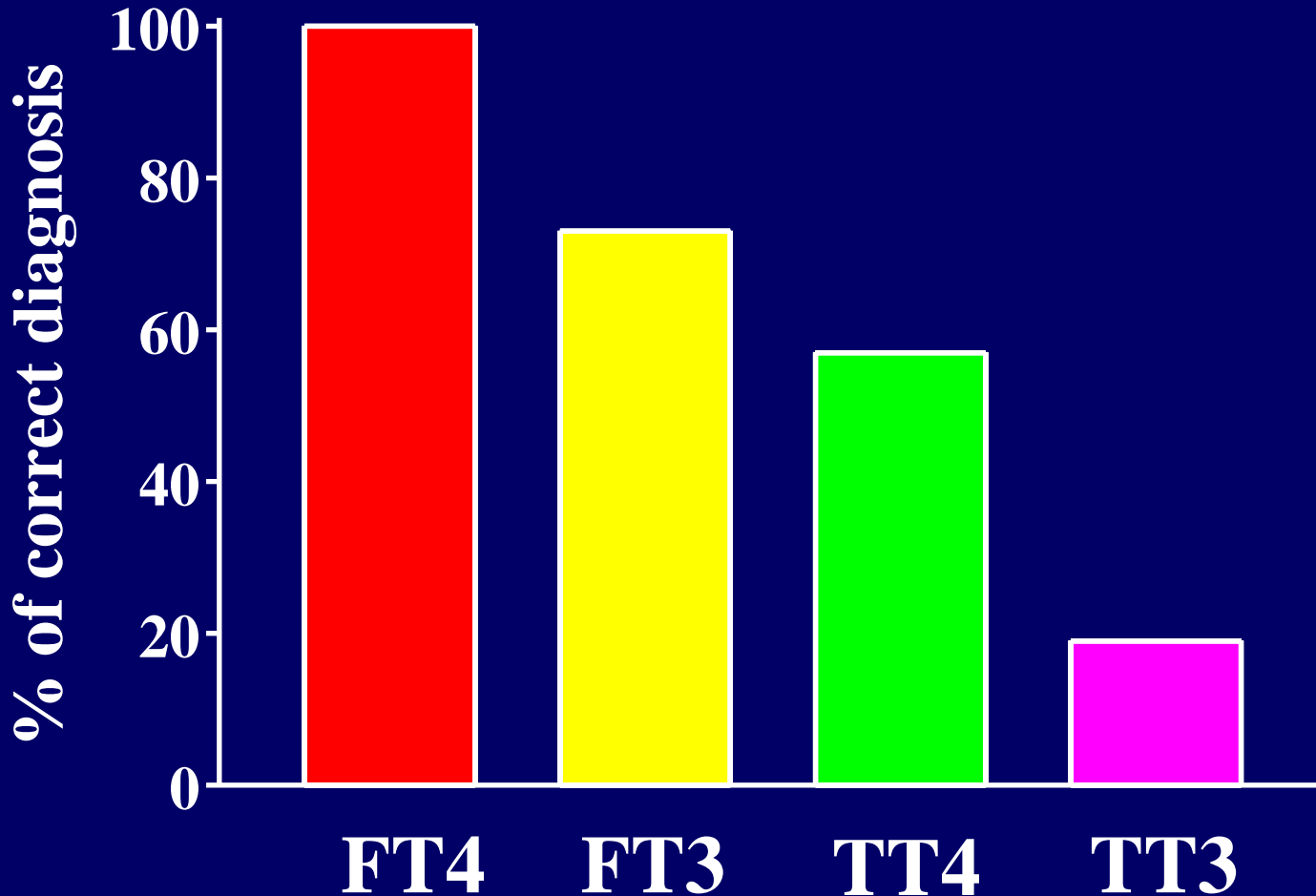
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Hereditary low/absent (\blacktriangle) and high (\bullet) TBG in euthyroid patients



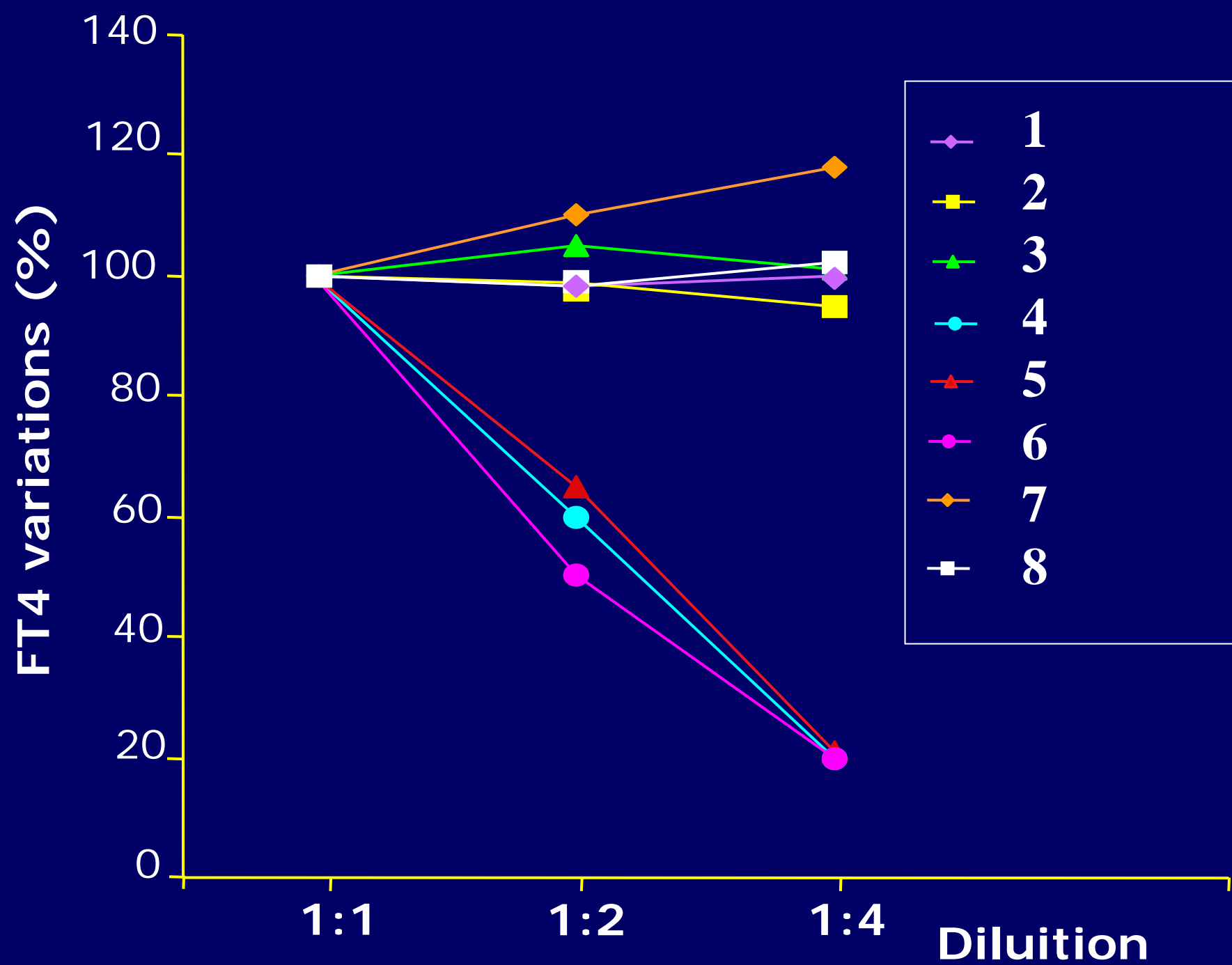
Diagnostic accuracy of thyroid hormone measurement (104 central hypothyroid patients)



By the law of mass action,

$$[FT4] = \frac{[bound\ T4]}{K [unbound\ TP]}$$

an equilibrium is reached between bound and free fractions of thyroid hormones, e.g. T4.



**CIRCULATING FACTORS MAY INTERFERE WITH MEASUREMENT
OF TSH OR TOTAL AND FREE THYROID HORMONES**



OVERESTIMATION OF SERUM LEVELS



CENTRAL HYPERTHYROIDISM?

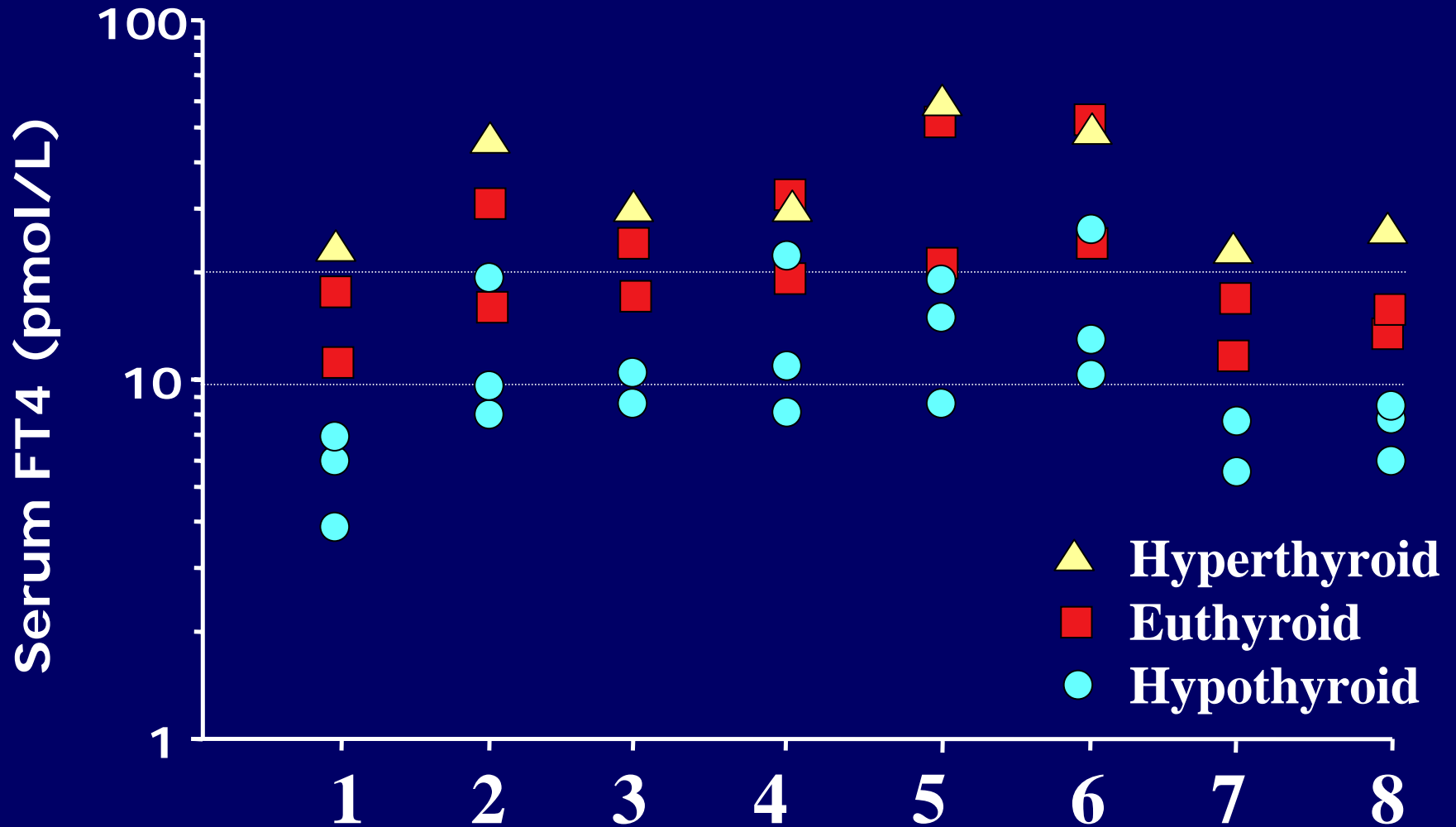
**Heterophylic Ab directed against
mouse γ -globulins: interference
with monoclonal Ab used in the IRMA**

**Anti-TSH Ab or
Ab cross-reacting
with TSH**

**Anti-T4 and/or
anti-T3 Ab**

**Abnormal forms of albumin
or transthyretin (FDH)**

Anti-T4 antibodies



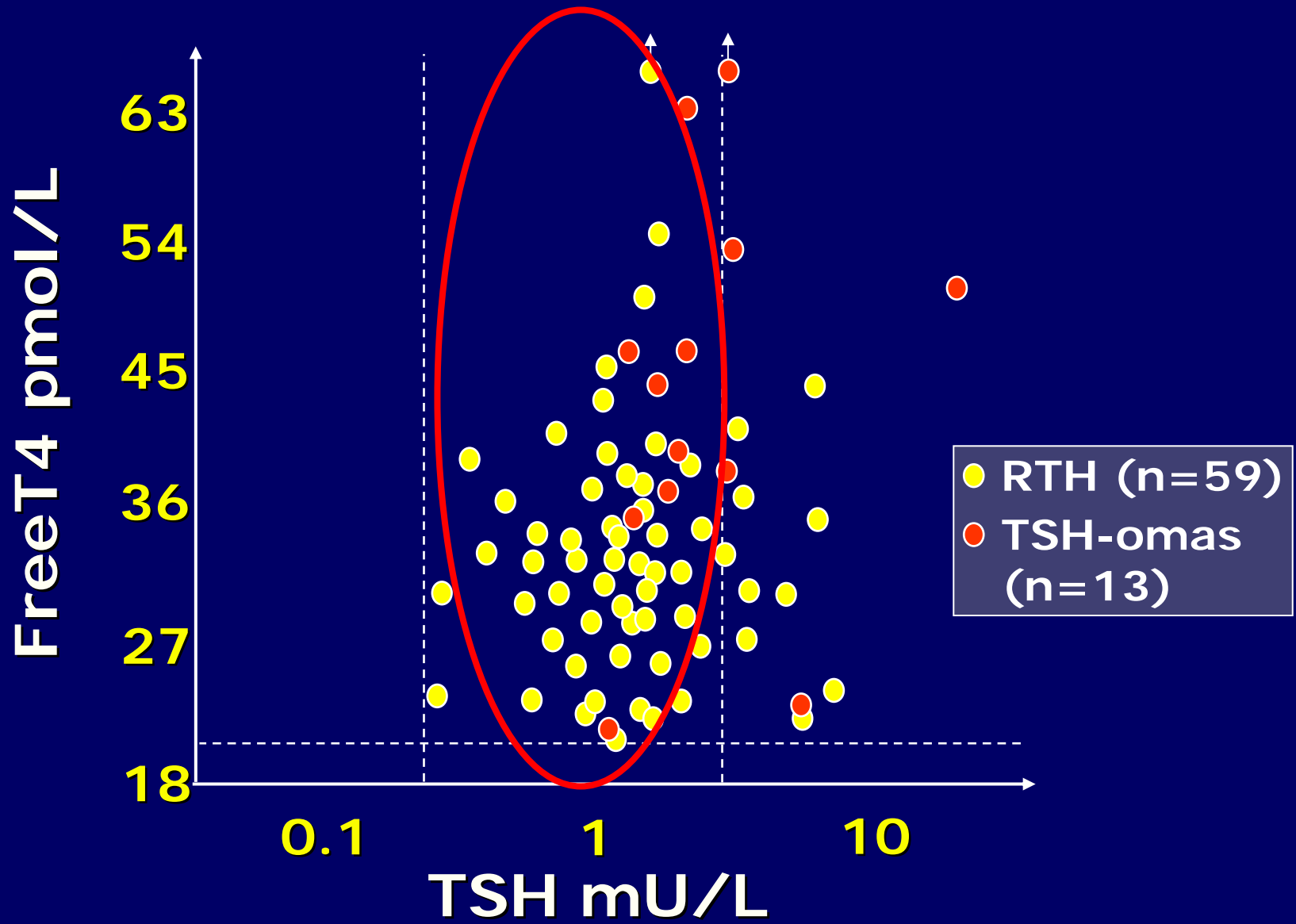
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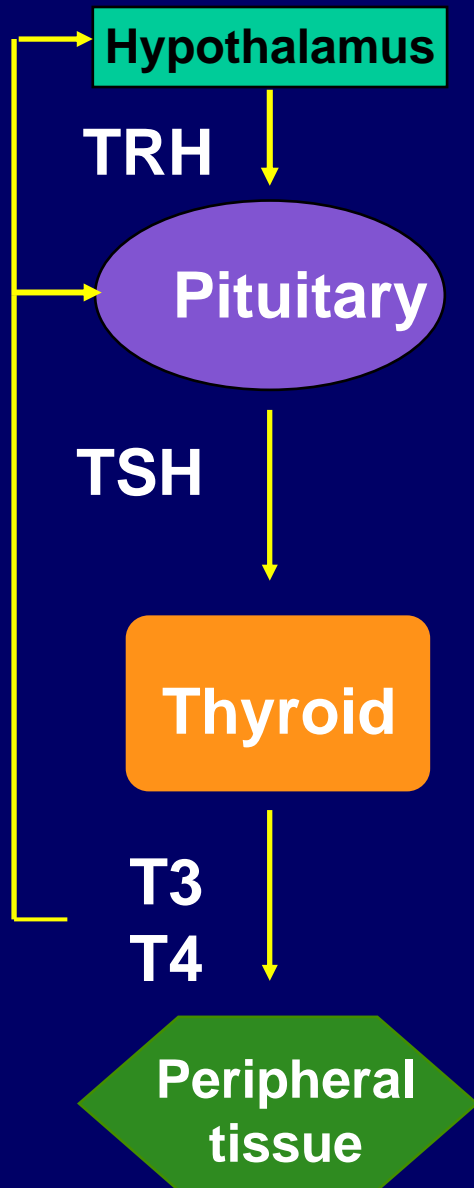
Central Hyperthyroidism:

Serum concentrations of TSH and FT4

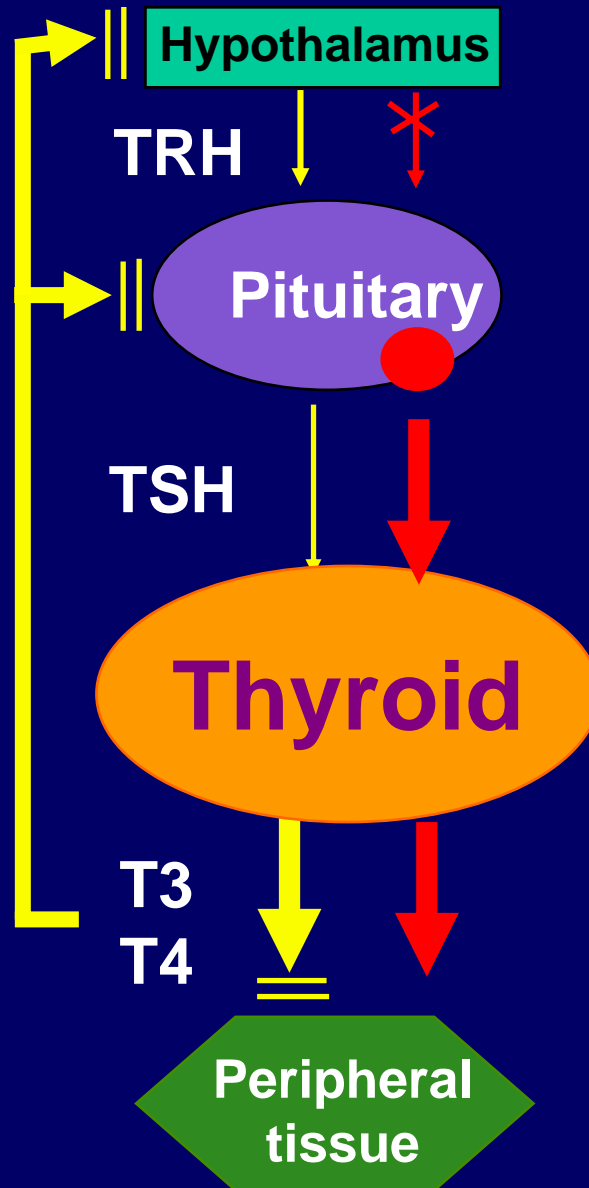
(data from Beck-Peccoz & Persani, in DeGroot's Endocrinology 2005)



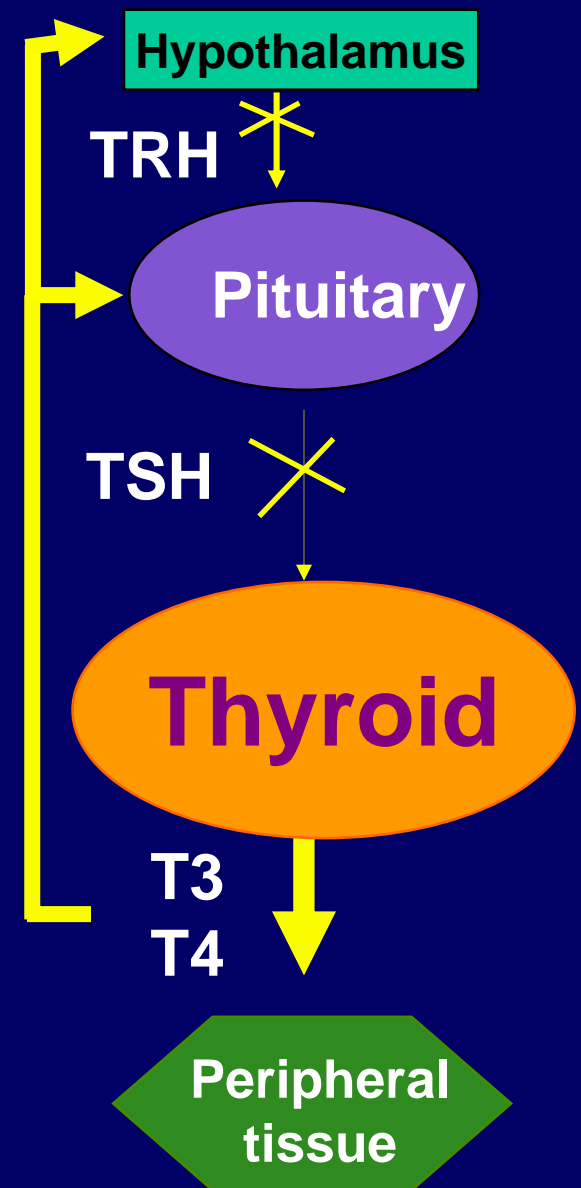
Euthyroid



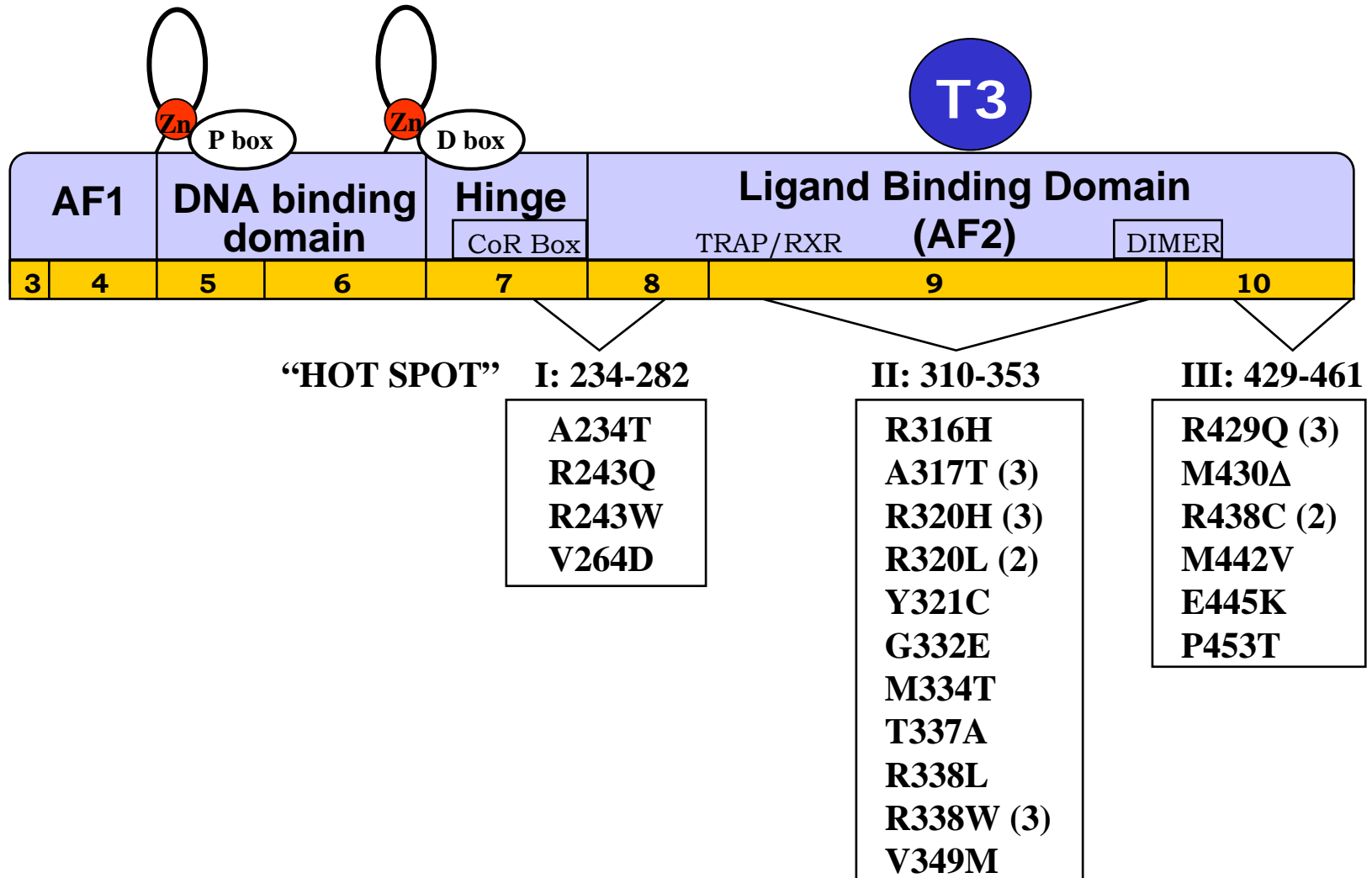
RTH & TSH-oma



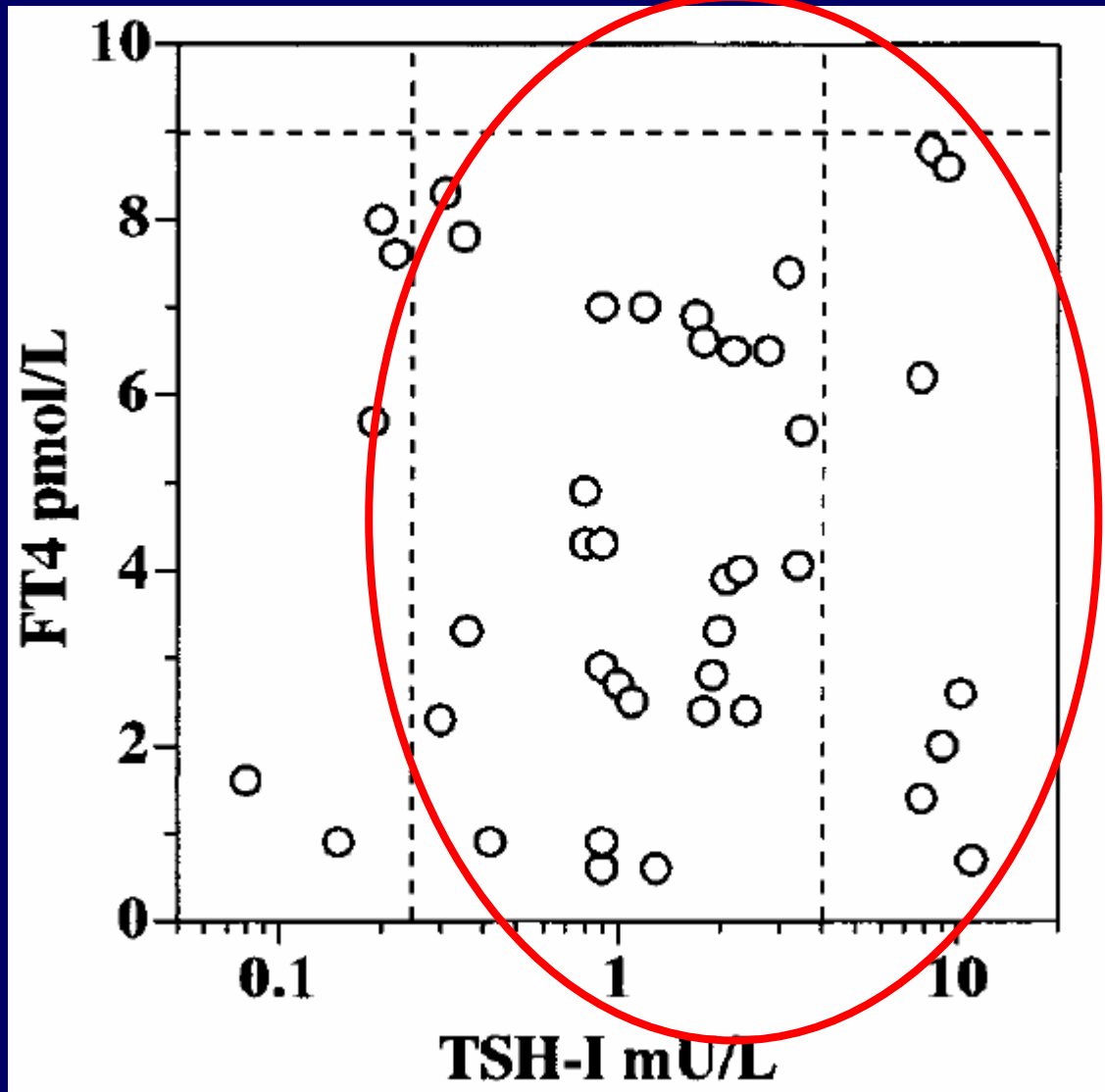
Hyperthyroid



Functional domain of TR β and “hot spot” of mutations in RTH

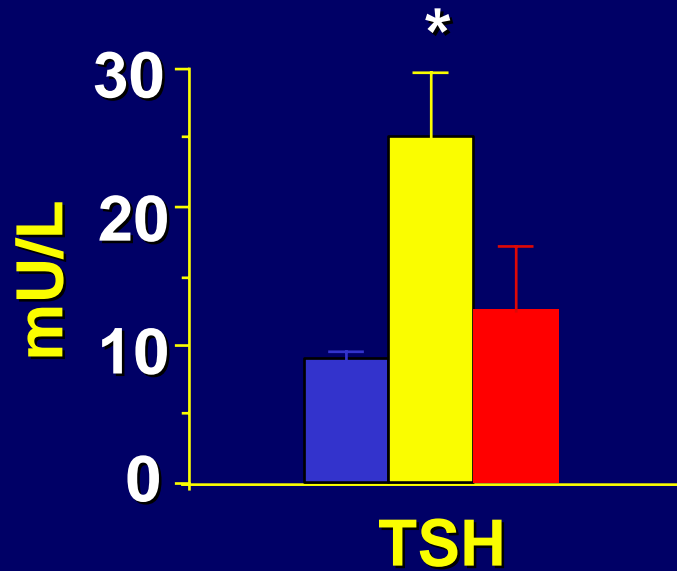


Serum TSH and free T4 levels in CH

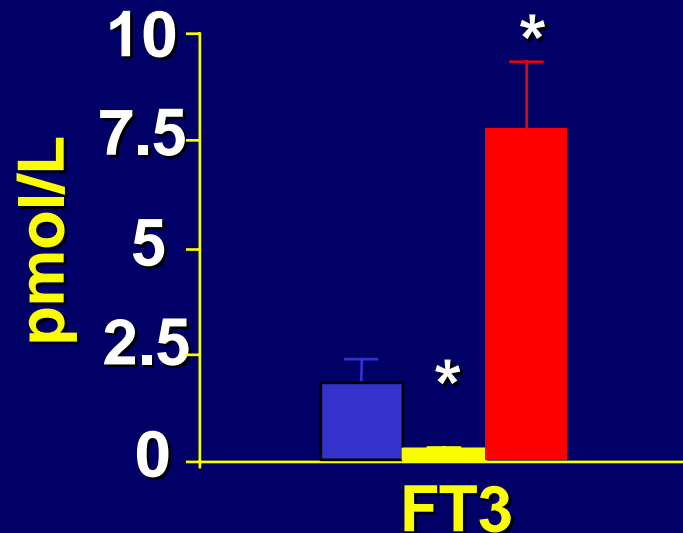
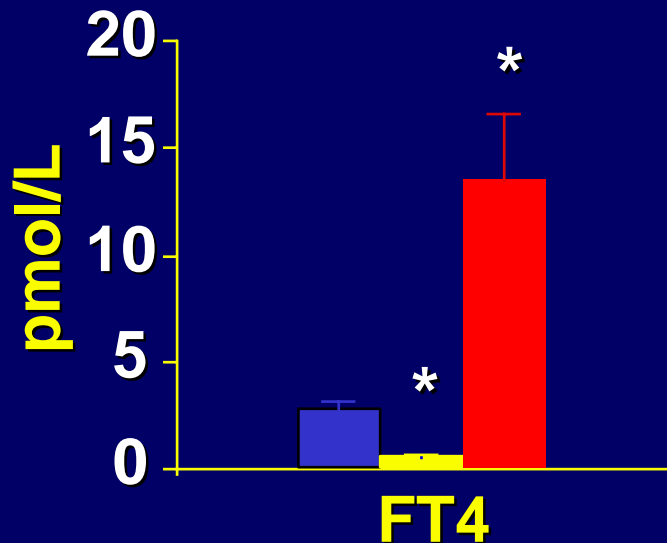


Net increments after TRH injection

* $P < 0.01$ vs controls

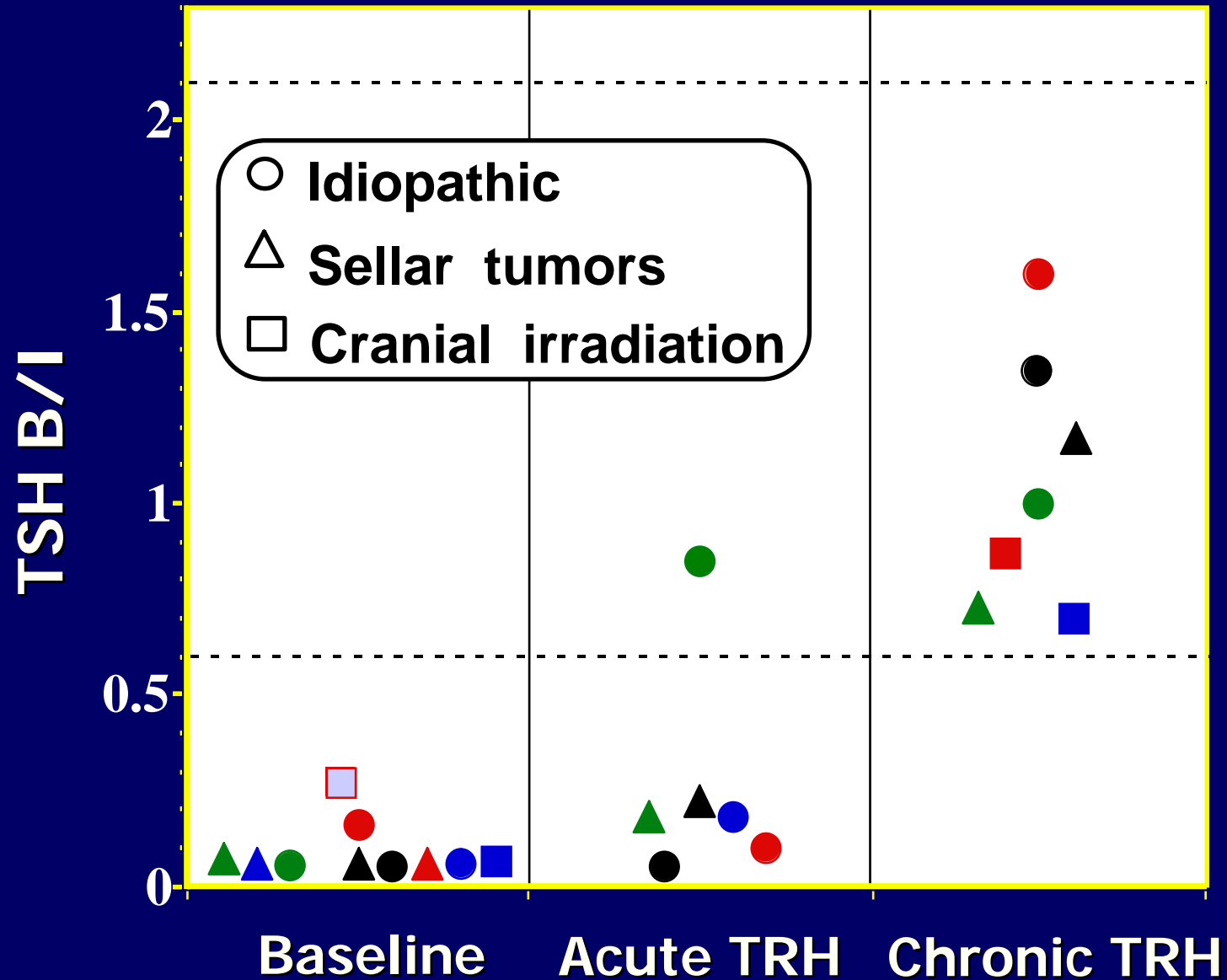


Controls
Hypothalamic hypothyroidism
RTH

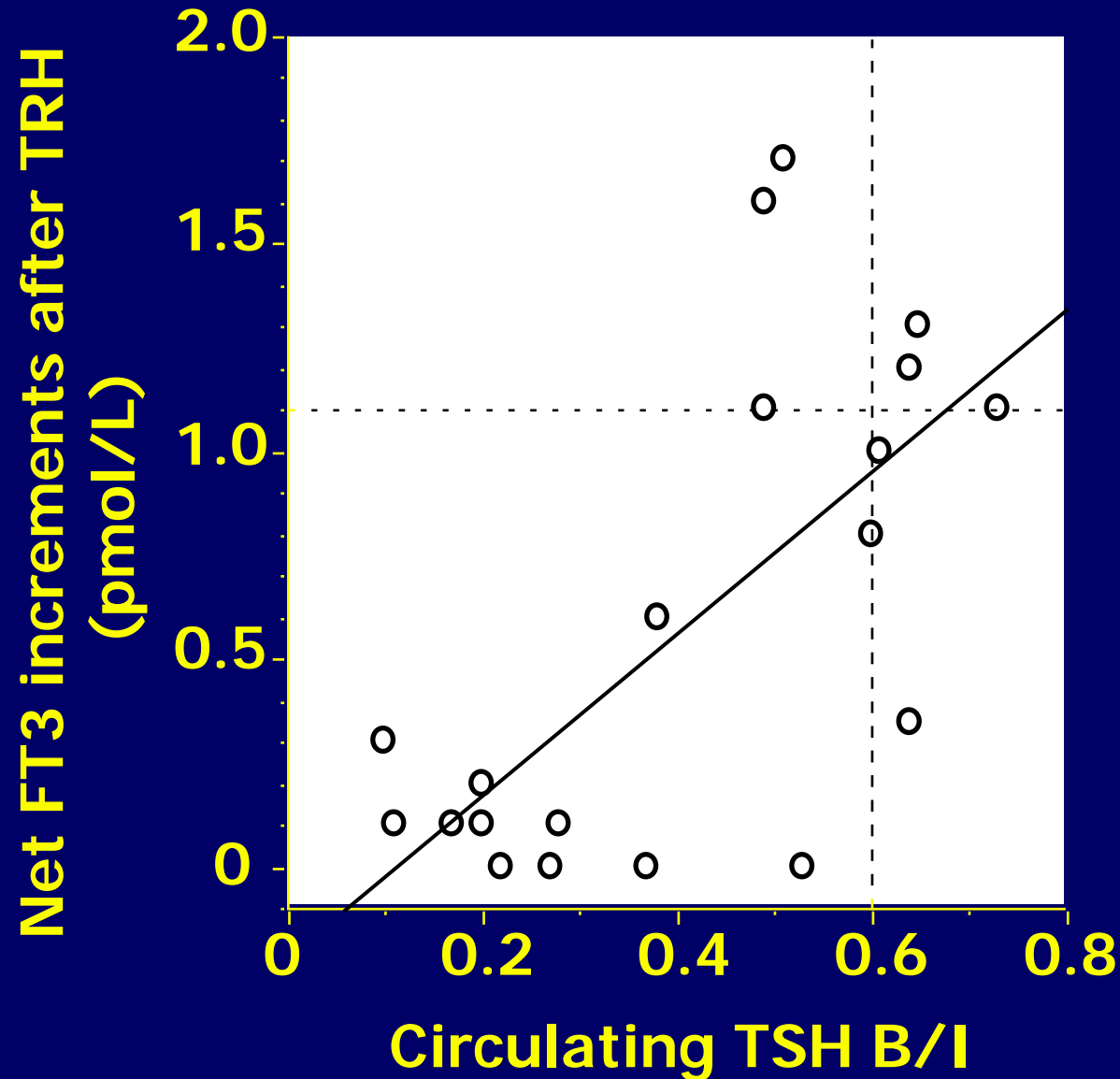


CENTRAL HYPOTHYROIDISM

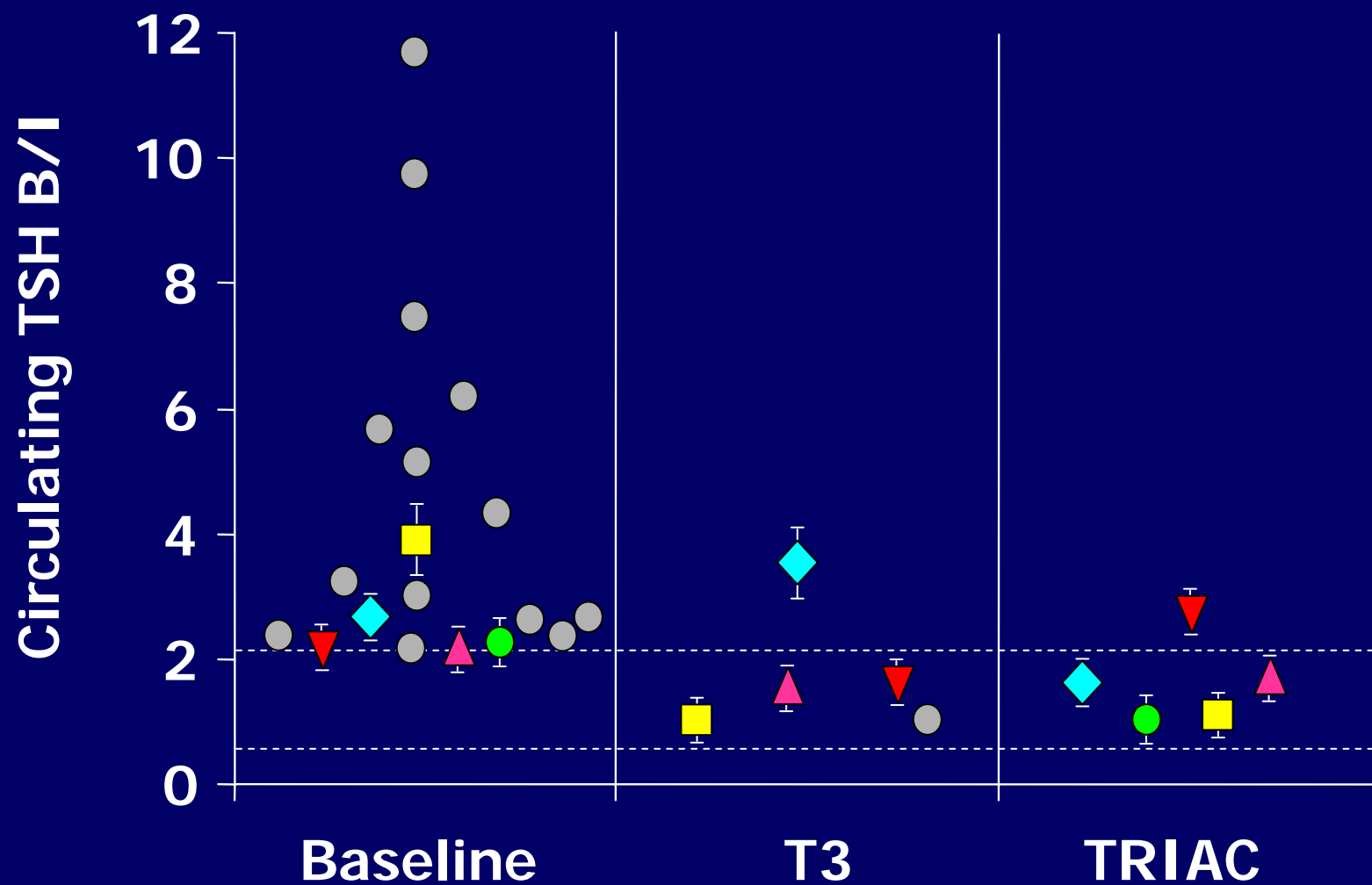
TSH bioassay in CHO-R cells



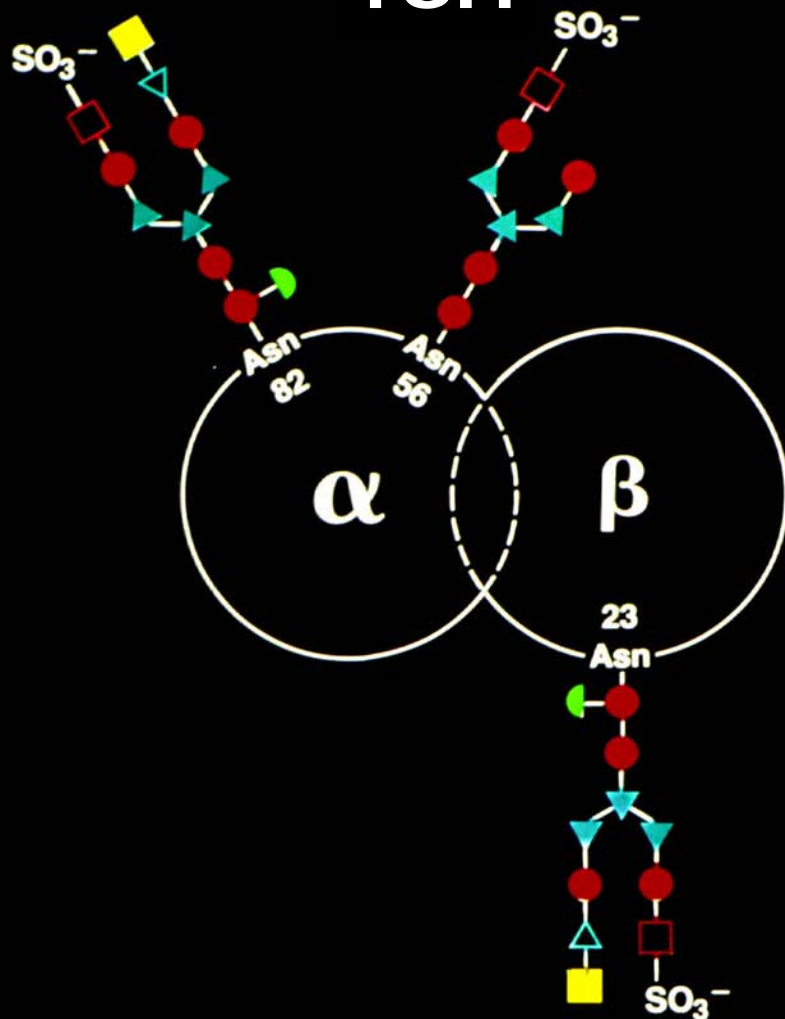
Central Hypothyroidism



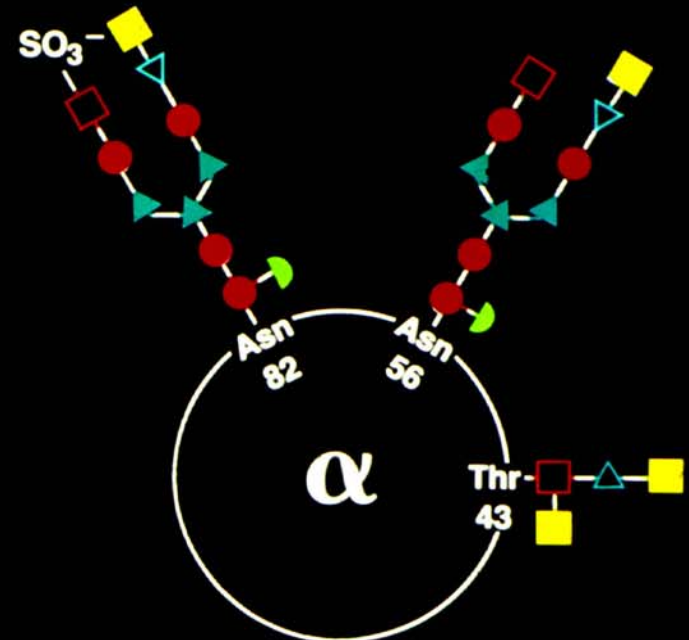
Resistance to Thyroid Hormone



TSH



α -GSU



- △ Galactose
- ▽ Mannose
- ◐ L-fucose
- N-acetylglucosamine
- N-acetylgalactosamine
- Sialic acid

What is the role of oligosaccharides in glycoprotein hormones?

- **Conferring tertiary & quaternary conformation**
- **Protection from intra-cellular degradation**
 - **Helping secretion from the cell**
 - **Modulation of biological activity**
- **Control on tissue fate, clearance and degradation**

Clinical situations

TSH B/I

Physiological

- **Nocturnal peak**
- Fetal life (>31 weeks of gestation)

↓
↑↑

Pathological

- **Central hypothyroidism**
- TSH β gene mutations
- **Primary hypothyroidism**
- **Pituitary TSH-secreting adenomas**
- Resistance to thyroid hormones
- **Resistance to TSH**
- Sleep deprivation in patients with major depression
- **Low T3 syndrome**

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DIFFERENTIAL DIAGNOSIS OF MILD (“SUBCLINICAL”) HYPOTHYROIDISM

Mild unrecognized thyroid gland failure

Chronic autoimmune thyroiditis

External beam neck radiation

Transient TSH elevation

Nonthyroidal illness (euthyroid sick syndrome)

Exposure to amiodarone, iodine-containing substances, lithium

Use of antidopaminergic agents

Post-partum and lymphocytic thyroiditis

De Quervain’s thyroiditis

Assay error due to interfering substances

Under-treatment of overt hypothyroidism

Over-treatment of overt hyperthyroidism

Euthyroid outliers

2.5% of healthy population falling outside reference range

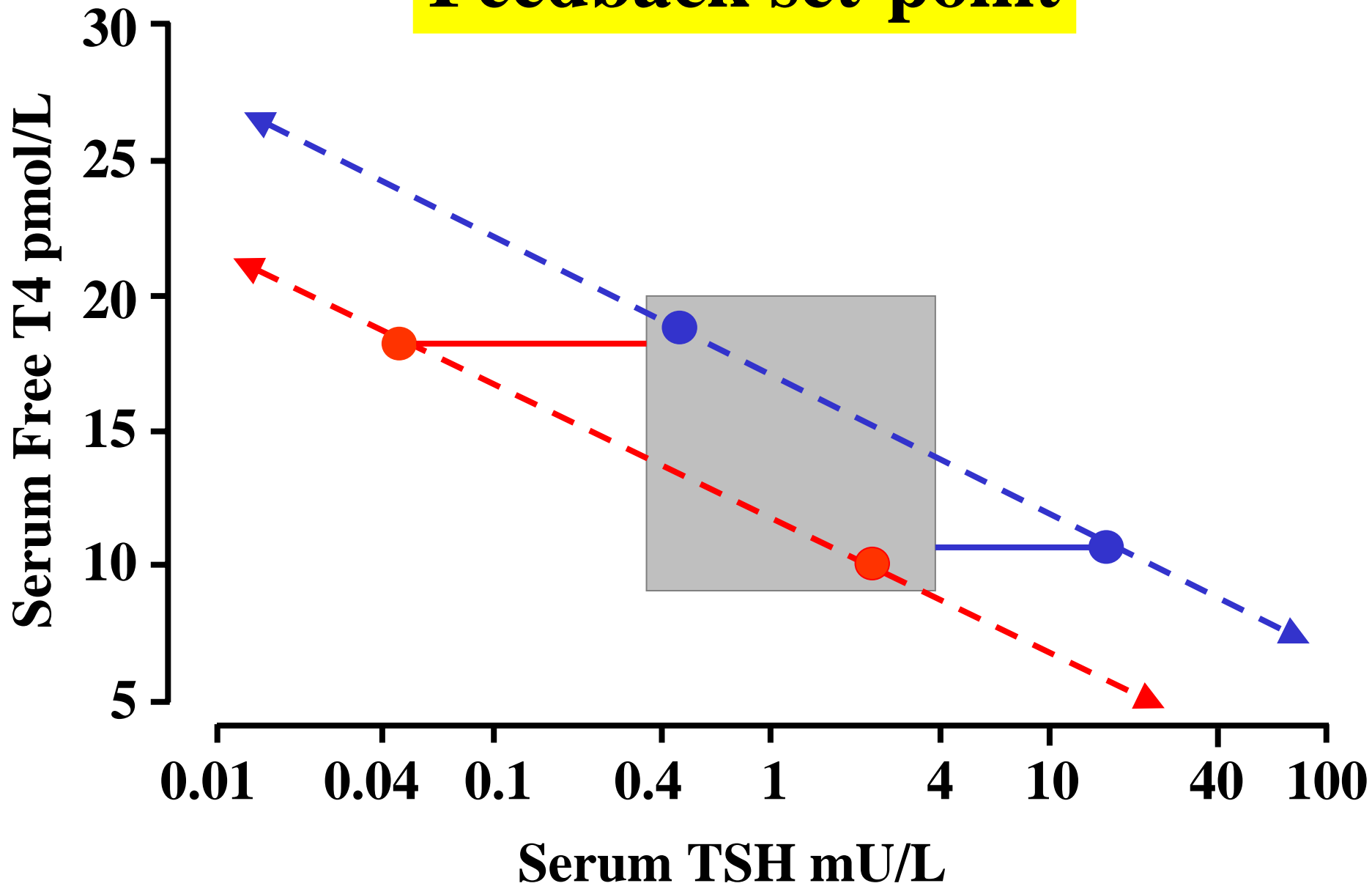
♀

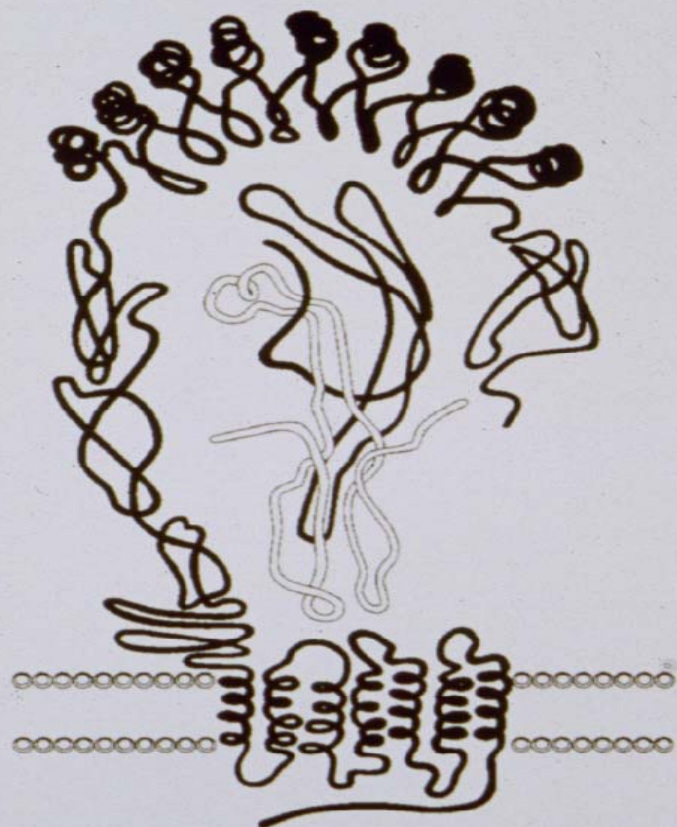
Thyroxine

AGE IN YEARS

**Hypothyroidism:
Effects on growth in
prepubertal age**

Feedback set-point





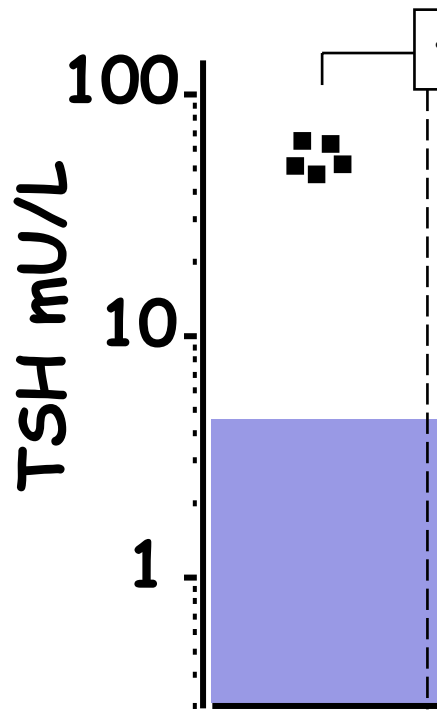
Resistance to TSH action: clinical and biochemical phenotype

- **Elevated TSH with normal response to TRH and T3-suppression tests**
- **Low/normal FT4/FT3**
- **Absent anti-thyroid autoantibodies**
- **Normal bioactivity of circulating TSH**
- **Thyroid volume: low/normal**
- **Pituitary MRI: normal**

Classification of the syndromes of resistance to TSH action :

- 1. Complete resistance to TSH (hypothyroidism)**
- 2. Partial resistance TSH (euthyroidism):**
 - a. in homozygous or compound heterozygous subjects**
 - b. in simple heterozygous subjects**

Resistance to TSH action



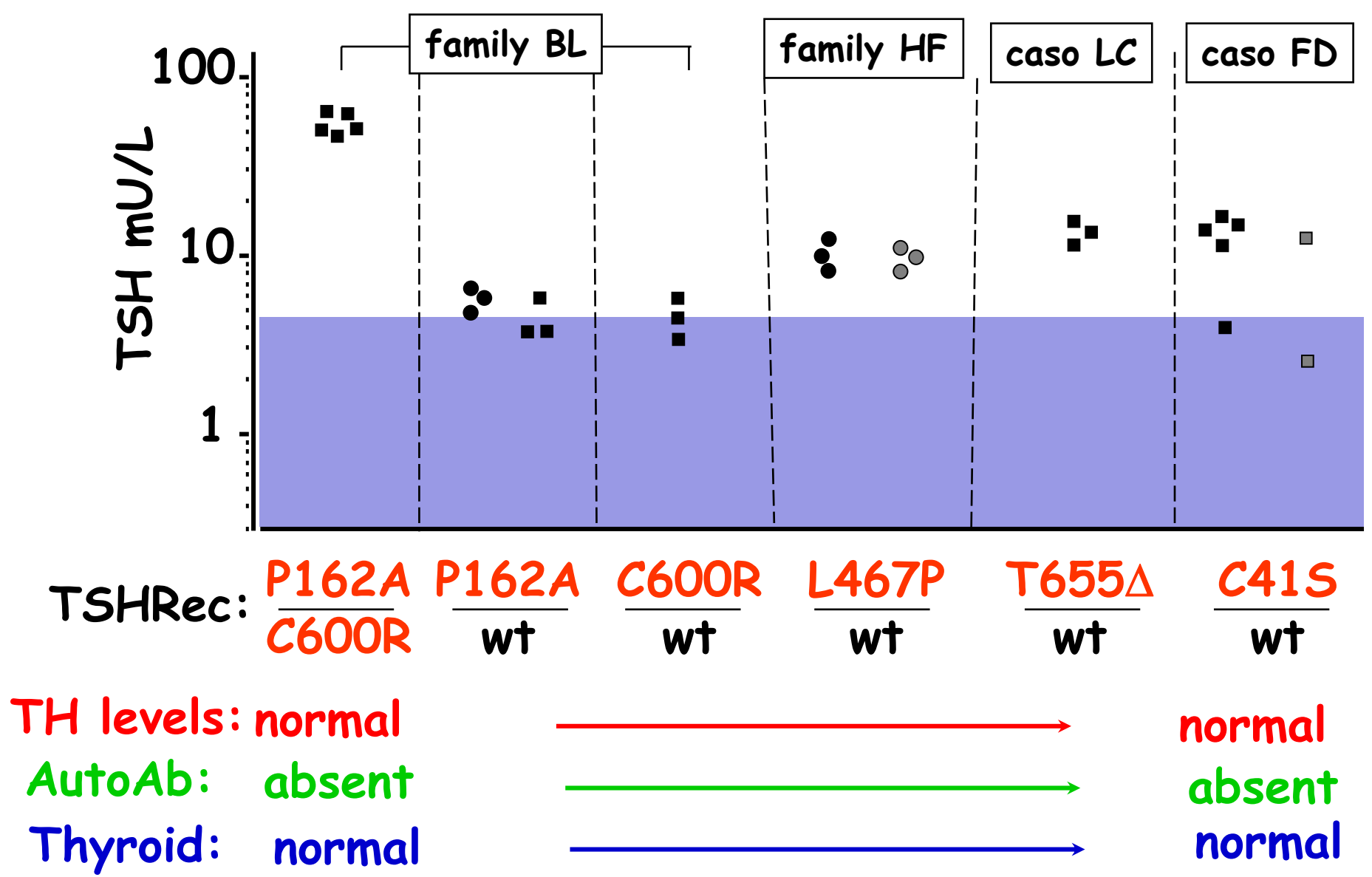
TSHRec: P162A
C600R

TH levels: normal

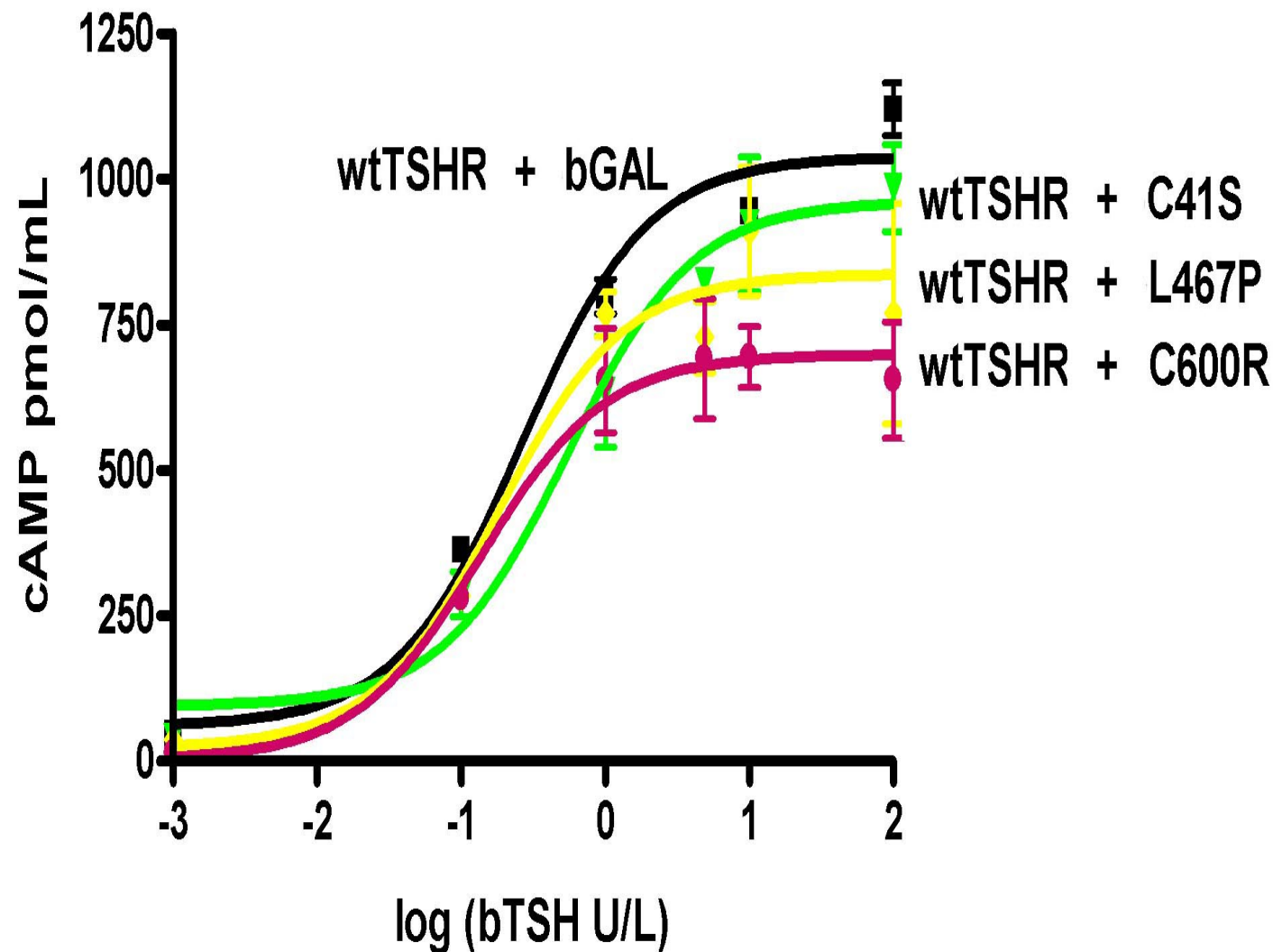
AutoAb: absent

Thyroid: normal

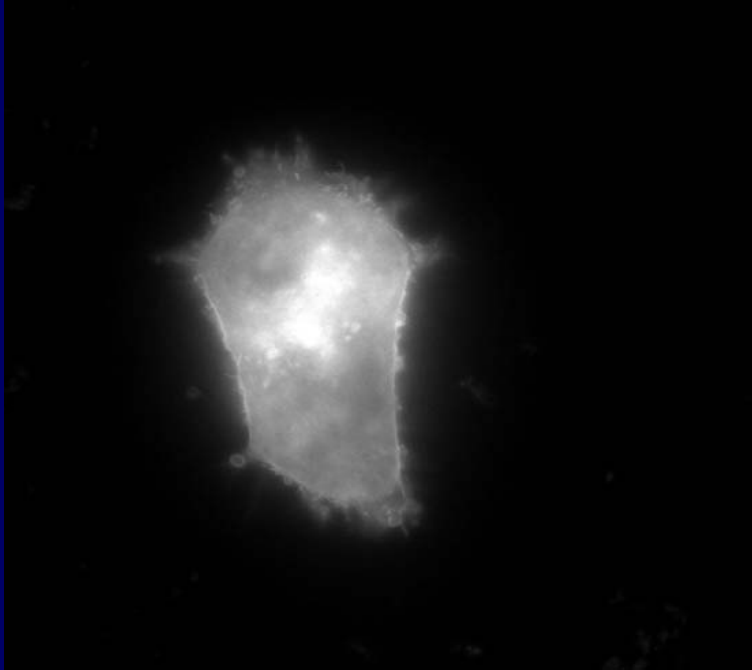
Partial resistance to TSH action



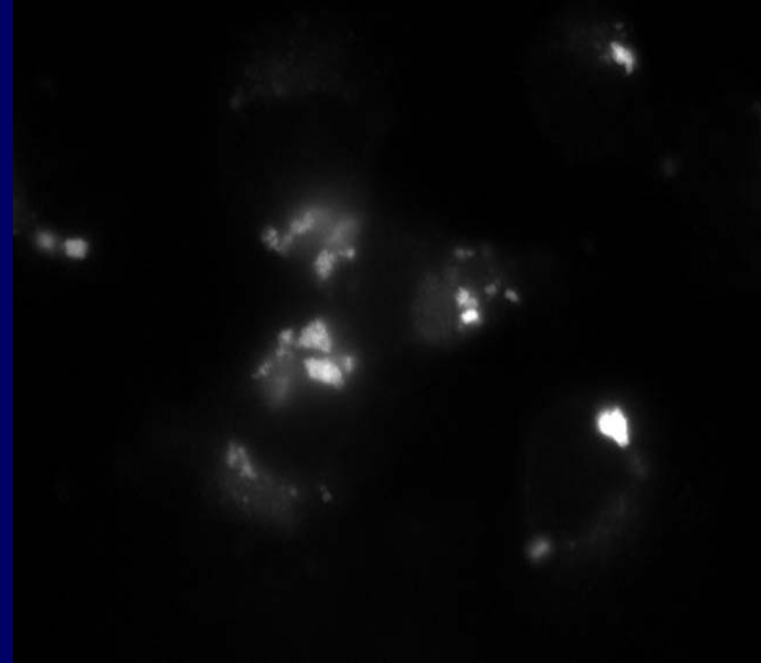
Dominant negative effect of mutant TSHR on cAMP production



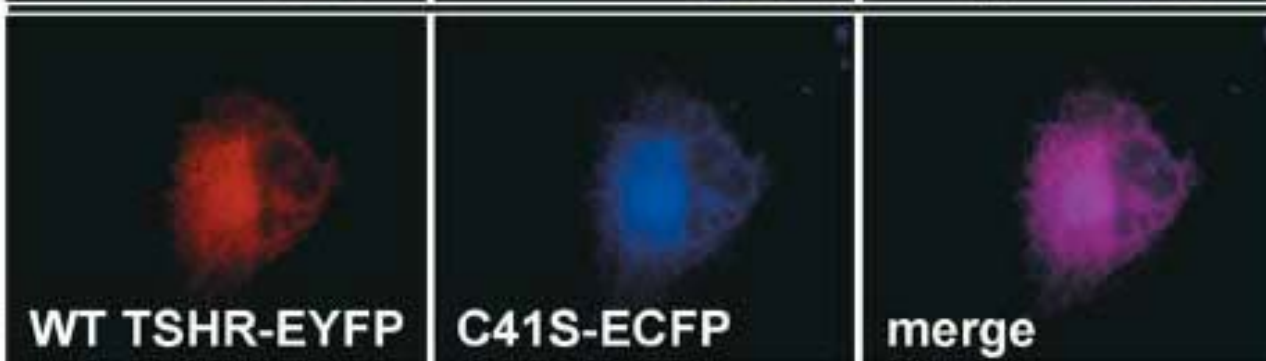
Single transfections



wtTSHR

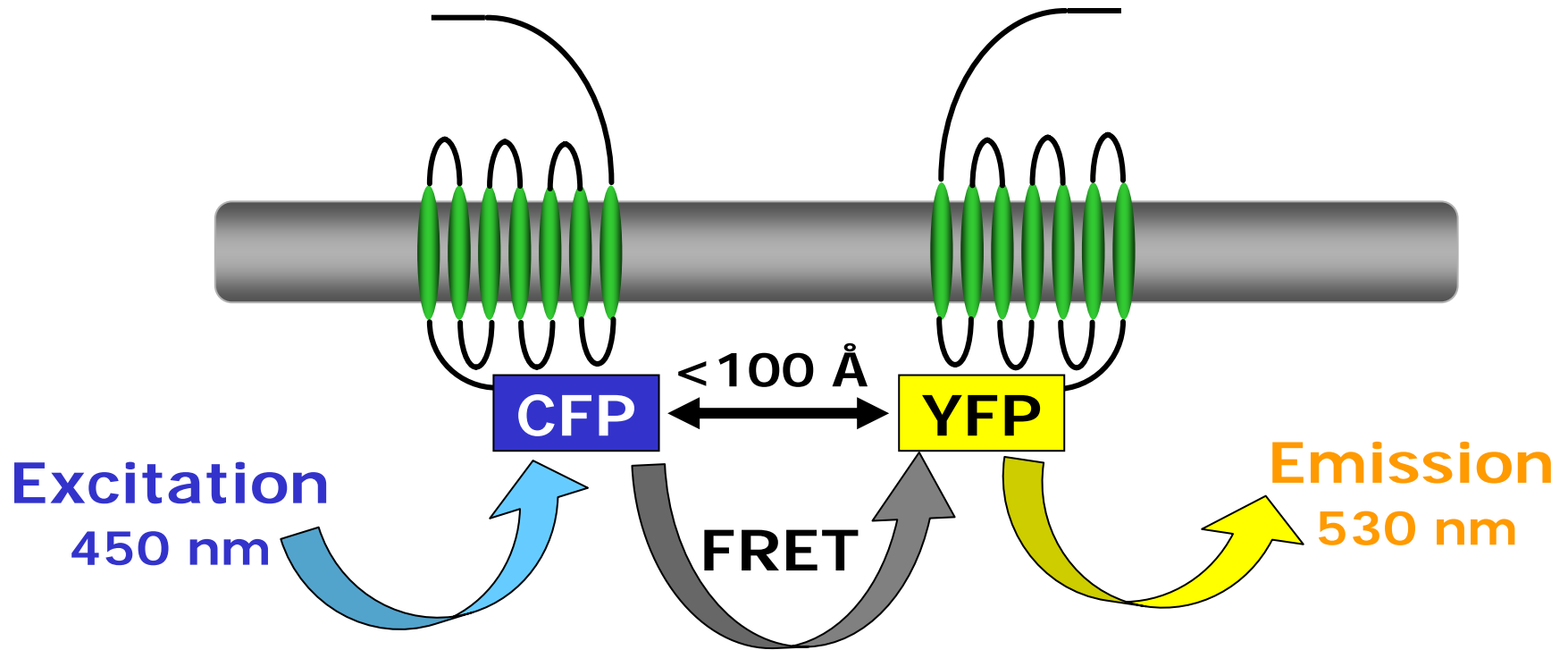


C41S



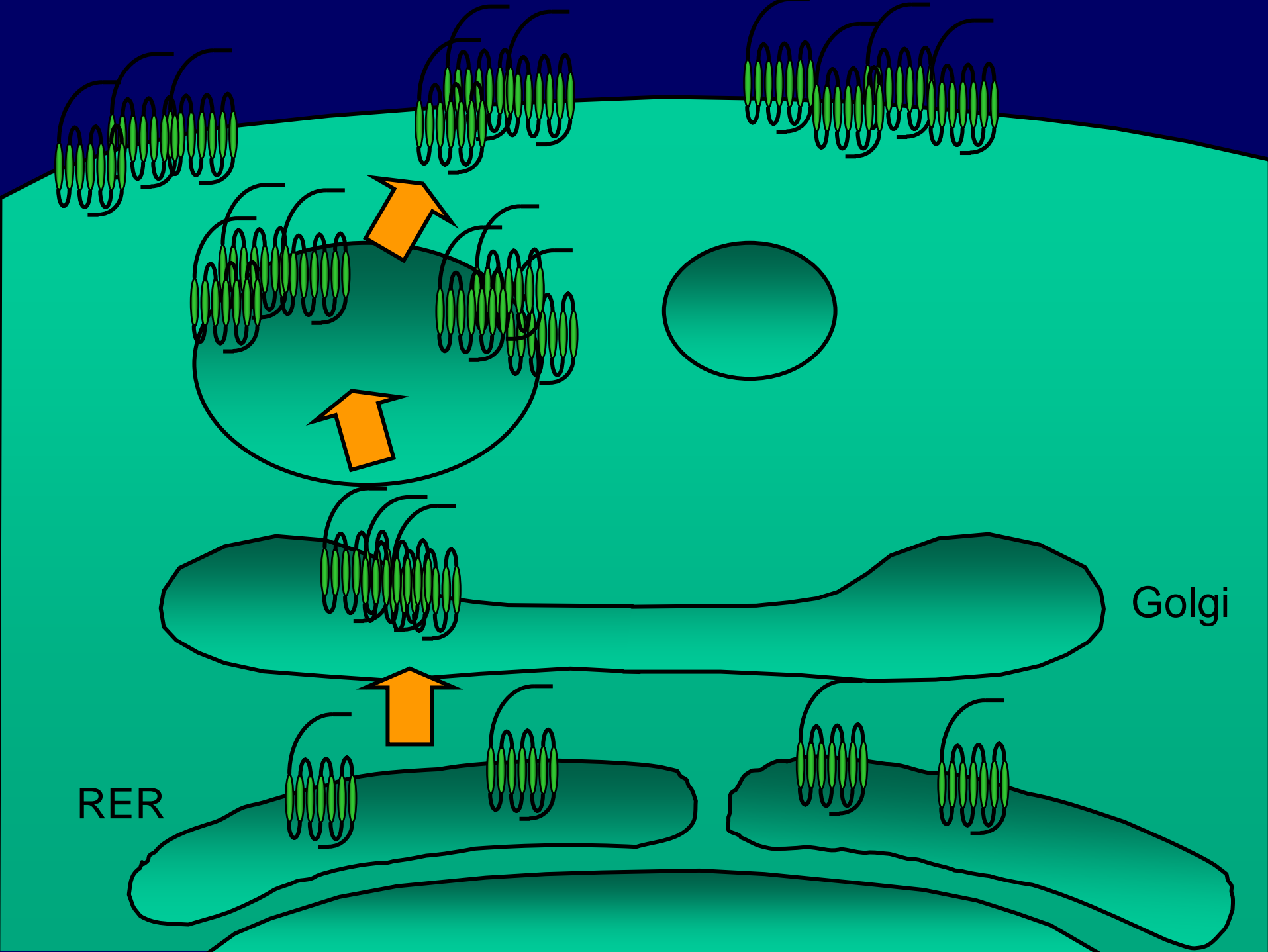
*Calebiro, Persani et al.
Human Molecular
Genetics,
2005;20:2991*

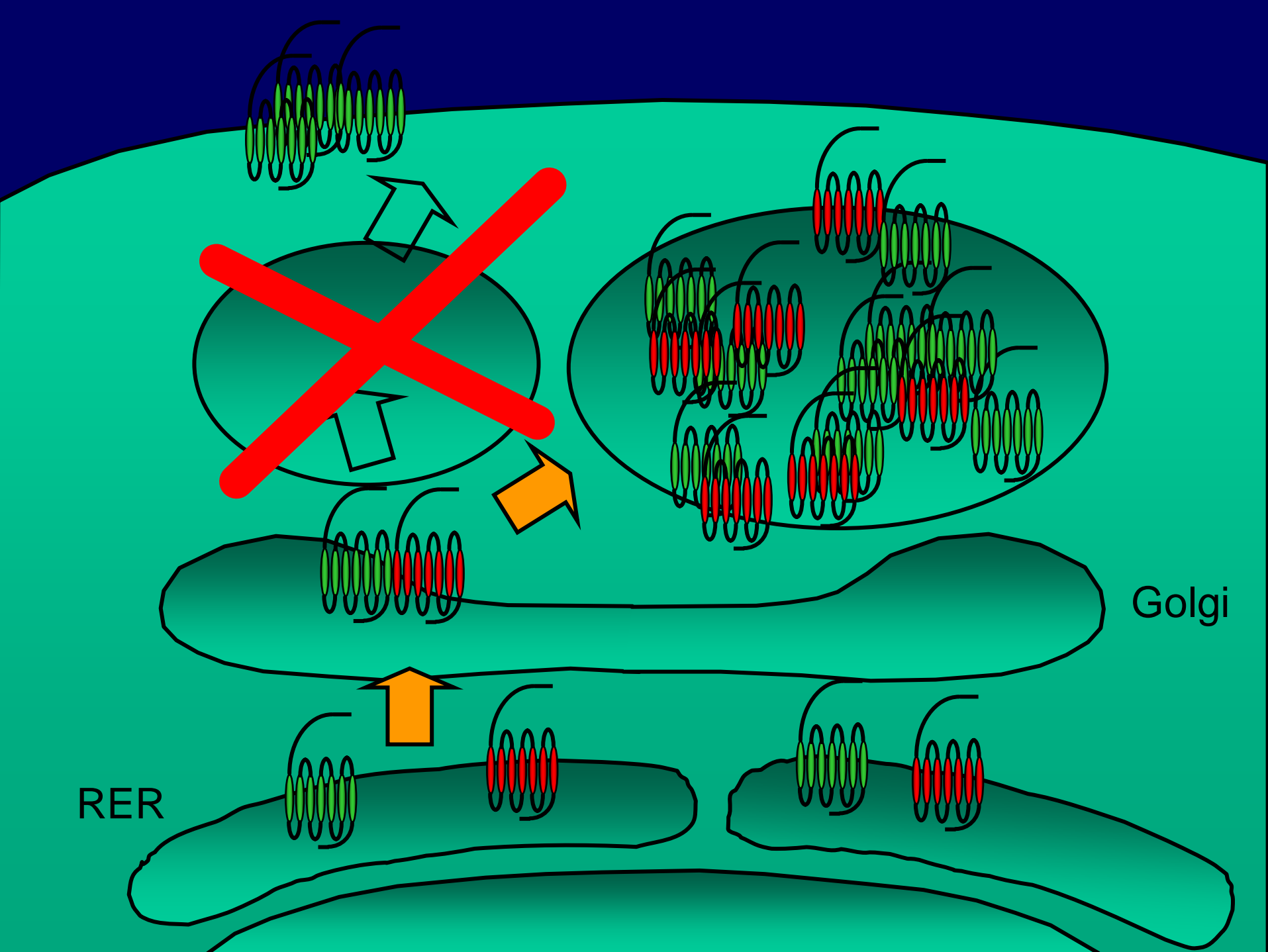
Fluorescence Resonance Energy Transfer

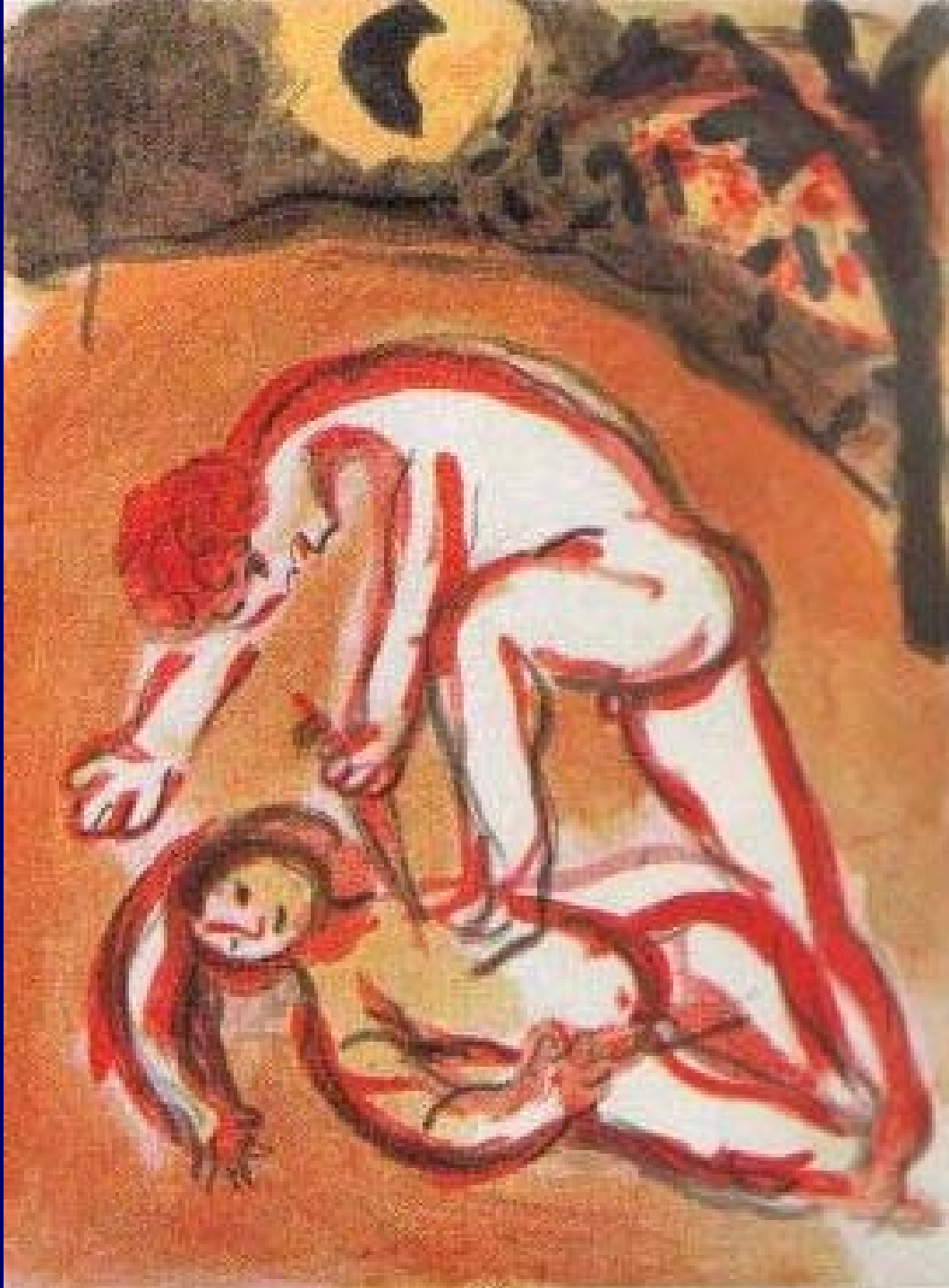


Using FRET technology:

- hTSHRs exist as oligomers (*Latif et al, JBC 2001*)
- hTSHR oligomers dissociate upon TSH stimulation (*Latif et al, JBC 2002*)

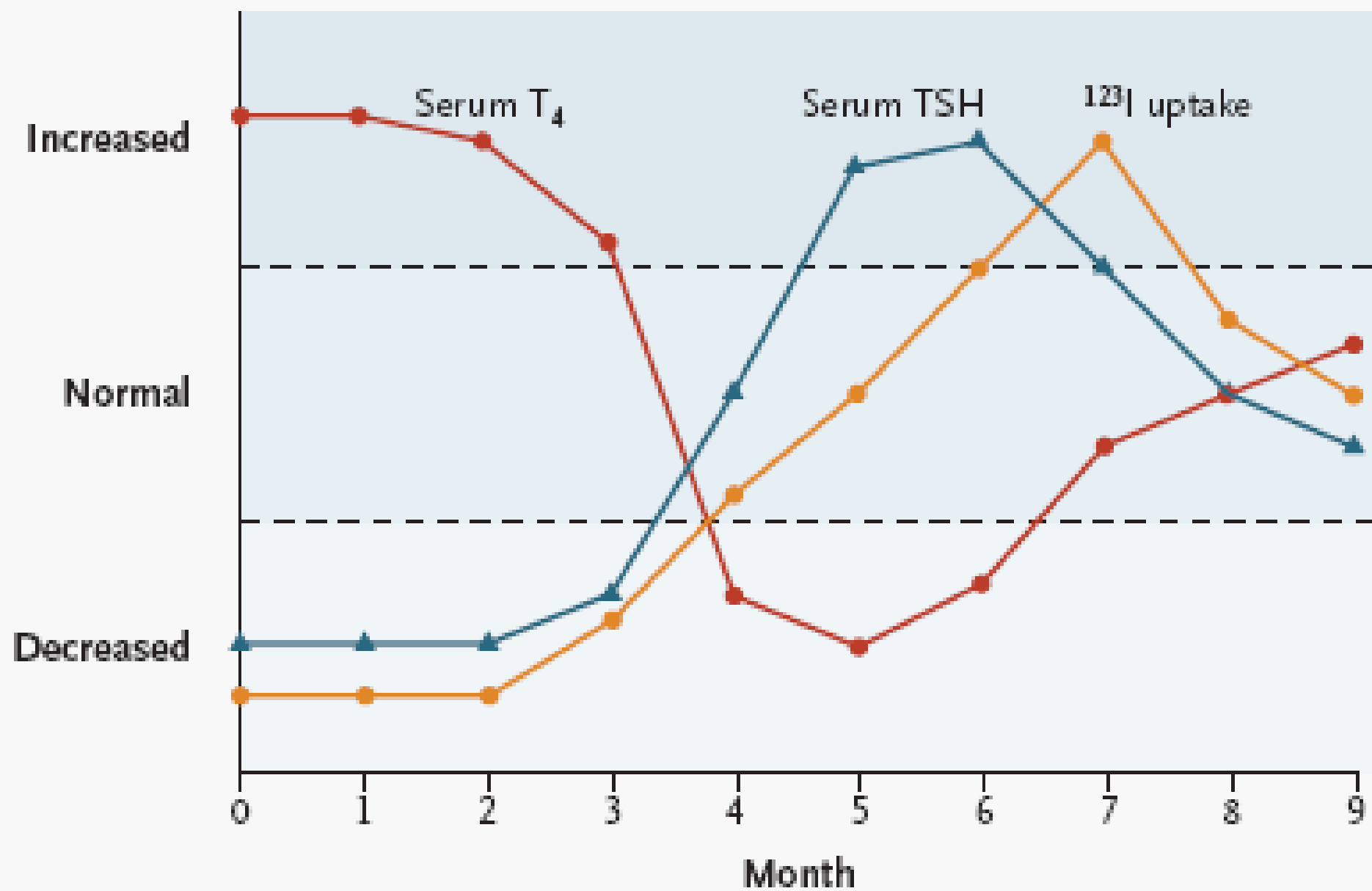






Cain & Abel

Marc Chagall





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Marie-Jeanne Papandreou

Marco Bonomi

Stefano Borgato

Elisabetta Ferretti

Carmela Asteria

Deborah Mannavola

Irene Campi

Gabriella Medri

Krishna Chatterjee

Samuel Refetoff

Catherine Ronin

Gilbert Vassart

Bruce D. Weintraub