

# CONGENITAL HYPOTHYROIDISM IN TWIN COUPLES AND TRIPLETS

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**Introduction** Over the years special screening procedures for preterm and twin babies (re-screening at 2-4 weeks of life) have been adopted by many screening laboratories worldwide. However, no extensive studies have been performed to verify how many co-twins with negative test at first screening (3-5 days of life) become positive at re-screening, and the utility of a long-term follow-up also in co-twins with negative test at screening and re-screening

## Objectives

- 1) to estimate the **concordance rate for CH** within the first month of life in twin couples/triplets discordant for CH at the first screening;
- 2) to verify whether a **long-term follow-up** of co-twins with negative test at screening and re-screening may be useful to verify the occurrence of thyroid hypofunction in these children during development;
- 3) to characterize probands and co-twins by next generation sequencing (NGS) analysis of a panel of candidate genes (**NKX2-1, FOXE1, PAX8, GLIS3, JAG1, TSHR, SLC26A4, DUOX2, DUOXA2, TPO, TG**)

**Methods** Thirty-eight twin couples and 4 triplets discordant for CH at first screening (42 CH probands) were recruited for the study. The range of the long-term follow-up in the couples/triplets was 3-21 years (median 8.1 yr). Pairwise concordance rate (PWCR) was calculated as the proportion of concordant pairs over the sum of concordant and discordant pairs. Case wise concordance rate (CWCR) is the probability that one twin in a pair is affected, given that his/her co-twin is affected. Survival analysis using Kaplan-Meier method was performed to describe the occurrence of thyroid hypofunction in co-twins.

## Results

Among the couples/triplets discordant at first screening 5 co-twins resulted positive at re-screening. PWCR and CWCR for CH confirmed at re-screening are reported in **FIG.1**

During the long-term follow-up a thyroid hypofunction was observed in 4 co-twins and a treatment with L-thyroxine was started at the age of 2 months for two co-twins, 9 months, 12 years. PWCR and CWCR for permanent thyroid hypofunction are reported in **FIG.2**. Details of concordant twin pairs are reported in **TABLES 1-2**. Kaplan-Meier survival curves concerning the long term follow-up are shown in **FIG.3**

The systematic NGS analysis revealed variations consistent with the observed phenotype in 50% of concordant twin couples. Most of the discordant MZ cases remain unexplained by NGS analyses (**TABLES 3-4**).

**Table 3. Details of discordant MZ twins with permanent CH**

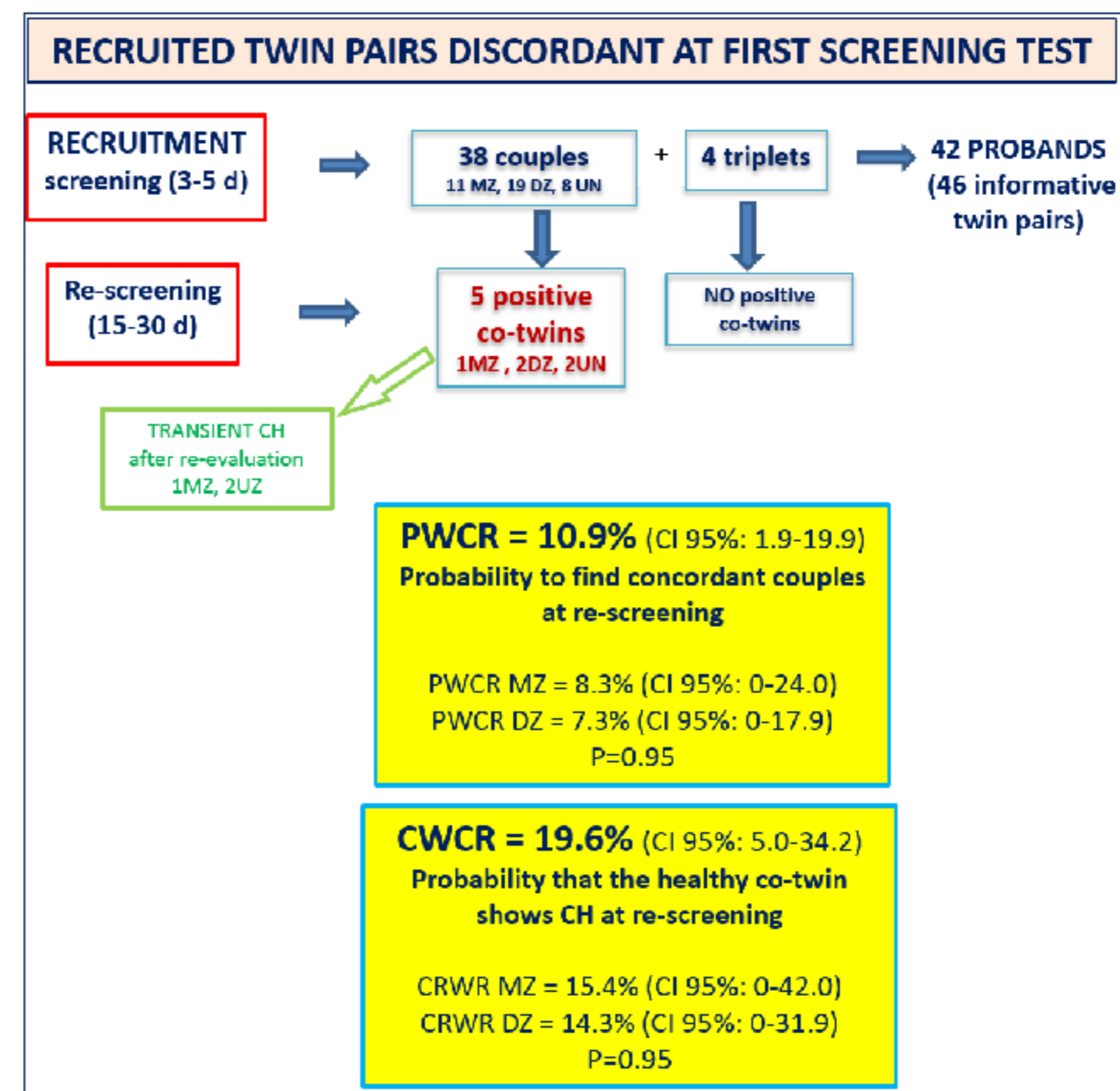
Discordant twin pairs	Sex	Zigosity	CH diagnosis	Perm/Trans	NGS analysis
Proband DL1 Co-twin DL1 (follow-up 18 yr)	F F	MZ MZ	Agensis Normal thy	Perm CH Healthy	DUOX2:p.Q556X, COSM 4055126 DUOX2:p.Q556X, COSM 4055126
Proband DL2 Co-twin DL2 (follow-up 5 yr)	F F	MZ MZ	Agensis Normal thy	Perm CH Healthy	WT WT
Proband DL3 Co-twin DL3 (follow-up 21 yr)	M M	MZ MZ	Agensis Normal thy	Perm CH Healthy	WT WT
Proband DL4 Co-twin DL4 (follow-up 14 yr)	F F	MZ MZ	Hypopl thy Normal thy	Perm CH Healthy	WT WT
Proband DL5 Co-twin DL5 (follow-up 15 yr)	F F	MZ MZ	Ectopic thy Normal thy	Perm CH Healthy	WT WT
Proband DL6 Co-twin DL6 (follow-up 8 yr)	F F	MZ MZ	Ectopic thy Normal thy	Perm CH Healthy	SLC26A4:p.T41DM SLC26A4:p.T41DM
Proband DL7 Co-twin DL7 (follow-up 11 yr)	F F	MZ MZ	Ectopic thy Normal thy	Perm CH Healthy	NO DNA NO DNA

**Table 4. Details of discordant DZ twins with permanent CH**

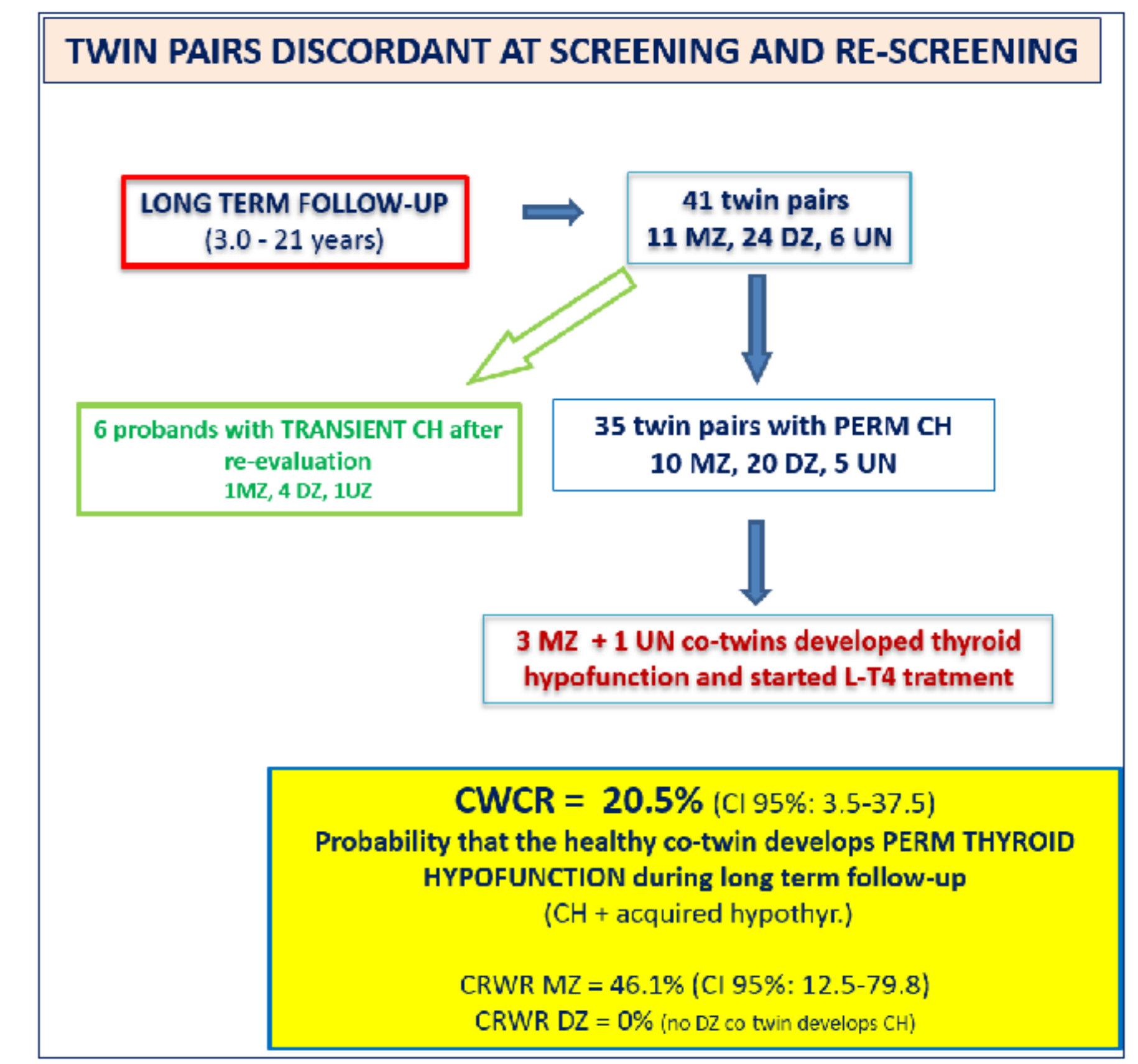
Discordant twin pairs	Follow-up yr	Sex	Zigosity	CH diagnosis	Perm/Trans	NGS analysis
Proband DL8 Co-twin DL8	15	F F	DZ DZ	Agensis Normal thy	Perm CH Healthy	WT WT
Proband DL9 Co-twin DL9	3	F F	DZ DZ	Agensis Normal thy	Perm CH Healthy	NO DNA NO DNA
Proband DL10 Co-twin DL10	20	F F	DZ DZ	Ectopic thy Normal thy	Perm CH Healthy	TG:p.R588H (15683332,MAF 0.0537) TG:p.R588H (15683332,MAF 0.0537)
Proband DL11 Co-twin DL11	19	F F	DZ DZ	Ectopic thy Normal thy	Perm CH Healthy	DUOX2:p.N74W; SLC26A4:p.T59V; SLC26A4:p.T59V
Proband DL12 Co-twin DL12	15	F F	DZ DZ	Ectopic thy Normal thy	Perm CH Healthy	WT WT
Proband DL13 Co-twin DL13	6	M M	DZ DZ	Hemigenesis Normal thy	Perm CH Healthy	NO DNA NO DNA
Proband DL14 Co-twin DL14	3	F F	DZ DZ	Hypopl thy Normal thy	Perm CH Healthy	TPO:p.F135A; SLC26A4:p.M20T WT
Proband DL15 Co-twin DL15	11	F F	DZ DZ	Hypopl thy Normal thy	Perm CH Healthy	TG:p.R2152L WT
Proband DL16 Co-twin DL16	11	F F	DZ DZ	Hypopl thy Normal thy	Perm CH Healthy	WT WT
Proband DL17 Co-twin DL17	4	F F	DZ DZ	Hypopl thy Normal thy	Perm CH Healthy	NO DNA NO DNA
Proband DL18 Co-twin DL18	4	F F	DZ DZ	Hypopl thy Normal thy	Perm CH Healthy	NO DNA NO DNA
Proband DL19 Co-twin DL19	6	M M	DZ DZ	Normal thy Normal thy	Perm CH Healthy	WT WT
Proband DL20 Co-twin DL20	3	M M	DZ DZ	Normal thy Normal thy	Perm CH Healthy	NO DNA NO DNA

Couples DL16, 17, 18, 20 show normal thyroid and no DNA

**FIG.1**



**FIG.2**



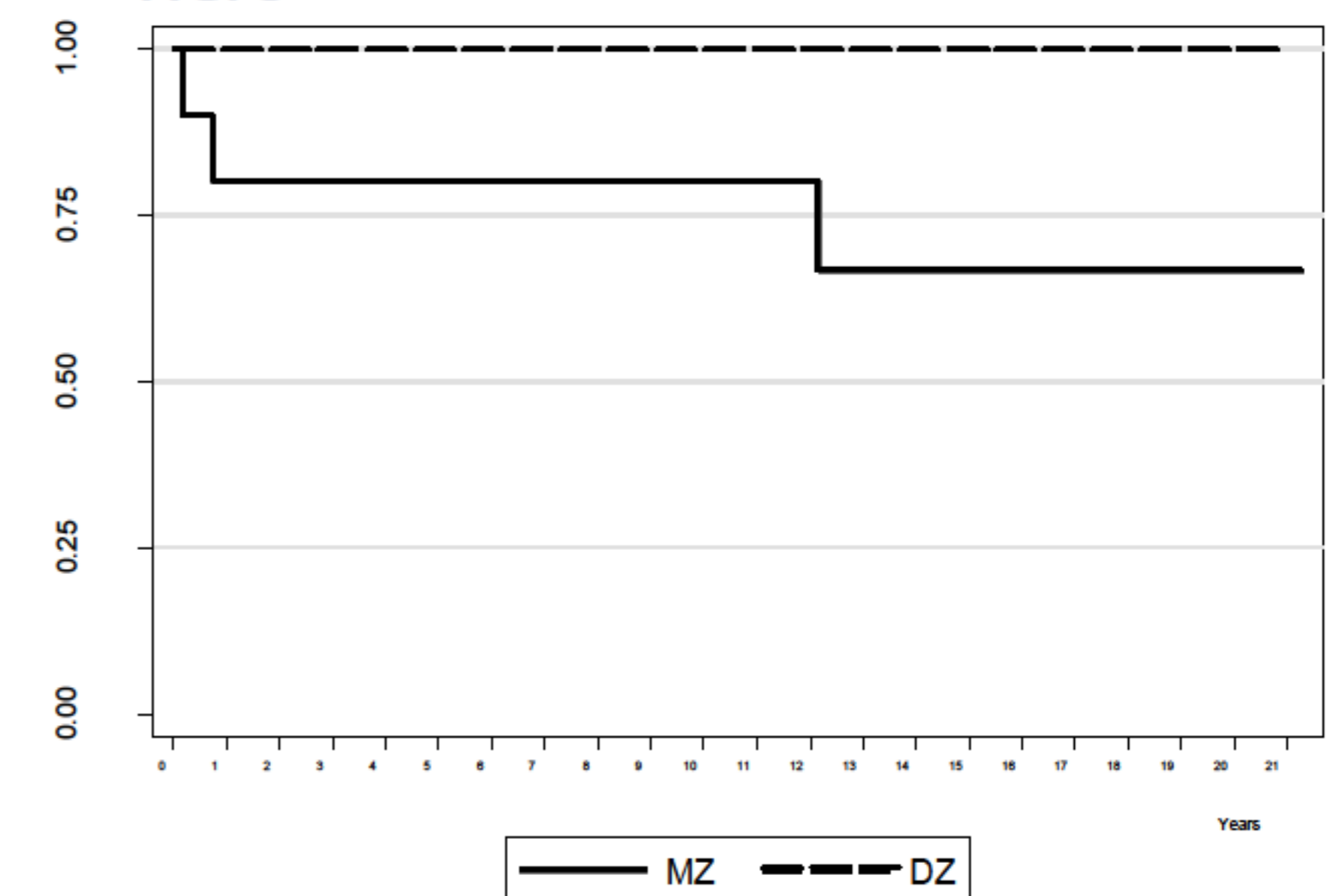
**Table 1. Details of twin pairs concordant at re-screening**

Concordant at re-screening	Follow-up yr	Sex	Zigosity	CH diagnosis	Perm/Trans	NGS analysis
Proband R1 Co-twin R1	3	F M	DZ DZ	Ectopic thy Normal thy	Perm CH Perm CH	NO DNA NO DNA
Proband R2 Co-twin R2	6	F M	DZ DZ	Normal thy Normal thy	Perm CH Perm CH	TPO:p.R584W; TG: p.D1312G TPO:p.R584W; TG: p.D1312G
Proband R3 Co-twin R3	3	F F	MZ MZ	Hypopl thy Hypopl thy	Trans CH Trans CH	TG: IVS45-9bp T>G TG: IVS45-9bp T>G
Proband R4 Co-twin R4	9	M M	UN UN	Normal thy Normal thy	Trans CH Trans CH	NO DNA NO DNA
Proband R5 Co-twin R5	4	M M	UN UN	Normal thy Normal thy	Trans CH Trans CH	NO DNA NO DNA

**Table 2. Details of twin pairs concordant during the long term follow-up**

Concordant at long term follow-up	Sex	Zigosity	CH diagnosis	Time of diagnosis	Perm/Trans	NGS analysis
Proband F1 Co-twin F1 (follow-up 5 yr)	M M	MZ MZ	Hemigenesis Hypopl thy	2 mo	Perm CH Perm CH	WT WT
Proband F2 Co-twin F2 (follow-up 12 yr)	F F	MZ MZ	Normal thy Normal thy	9 mo	Perm CH Perm CH	DUOX2:p.V1078M; TG: p.S523 DUOX2:p.V1078M; TG: p.S523
Proband F3 Co-twin F3 (follow-up 21 yr)	F F	MZ MZ	Ectopic thy Normal thy	12 yr	Perm CH Perm CH	WT WT
Proband F4 Co-twin F4 (follow-up 4 yr)	M M	UN UN	Hypopl thy Normal thy	2 mo	Perm CH Perm CH	NO DNA NO DNA

**FIG. 3**



## Conclusions. These results show:

1. the importance of the **re-screening at 2-4 weeks** of life in **both MZ and DZ** twins;
2. the possible **benefit of a long-term follow-up** also in co-twins with negative test at screening and re-screening, especially if **MZ**;
3. the need of further studies in order to uncover the largely unexplained pathogenesis of CH.

The authors declare no conflict of interest