DIFFERENCES IN HLA DQ2/DQ8 HAPLOTYPE AND FOXP3 GENE EXPRESSION BETWEEN CELIAC AND NON-CELIAC DOWN SYNDROME PATIENTS

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Introduction

- Down syndrome (DS) is a neurogenetic aneuploid disorder resulting from an extra copy of human chromosome 21.

- Celiac disease (CD) is an immune-mediated enteropathy with a strong genetic predisposition; 95% of the patients carries the HLA-DQ2, the remainder 5% the HLA-DQ8 haplotype

- While the prevalence of CD is 0.5-1% worldwide in the normal population, its prevalence rises up to 5-15% among DS patients

(I.Sanchez-Albisua, W.Storm, I. Wascher, M. Stern: How frequent is celiac disease in Down syndrome? Eur J Pediatr (2002) 161: 683–684)

Regulatory T cells in gut homeostasis and CD

- Regulatory T cells (Tregs) play a pivotal role in the maintenance of self-tolerance within the immune system

- We hypothesize that number and/or function of Treg may be abnormal in CD

FoxP3

- FOXP3 is a transcriptional factor expressed by Treg cells and critically involved in their development and function.
- A impaired expression of FOXP3 has been associated to different autoimmune diseases as Type 1 Diabetes and Rheumatoid arthritis

(A Kivling,L Nilsson, K F¨alth-Magnusson,S S¨ollvander,C Johanson,M Faresj: Diverse FOXP3 Expression in Children with Type 1 Diabetes and Celiac Disease, Immunology of Diabetes V: Ann. N.Y. Acad. Sci. 1150: 273–277 (2008); W Wang,S Shao , Z Jiao, M Guo, H Xu, S Wang:The Th17/Treg imbalance and cytokine environmentin peripheral blood of patients with rheumatoid arthritis. Rheumatology Int)

- Four domains characterize FOXP family's members:

1. Repressor domain

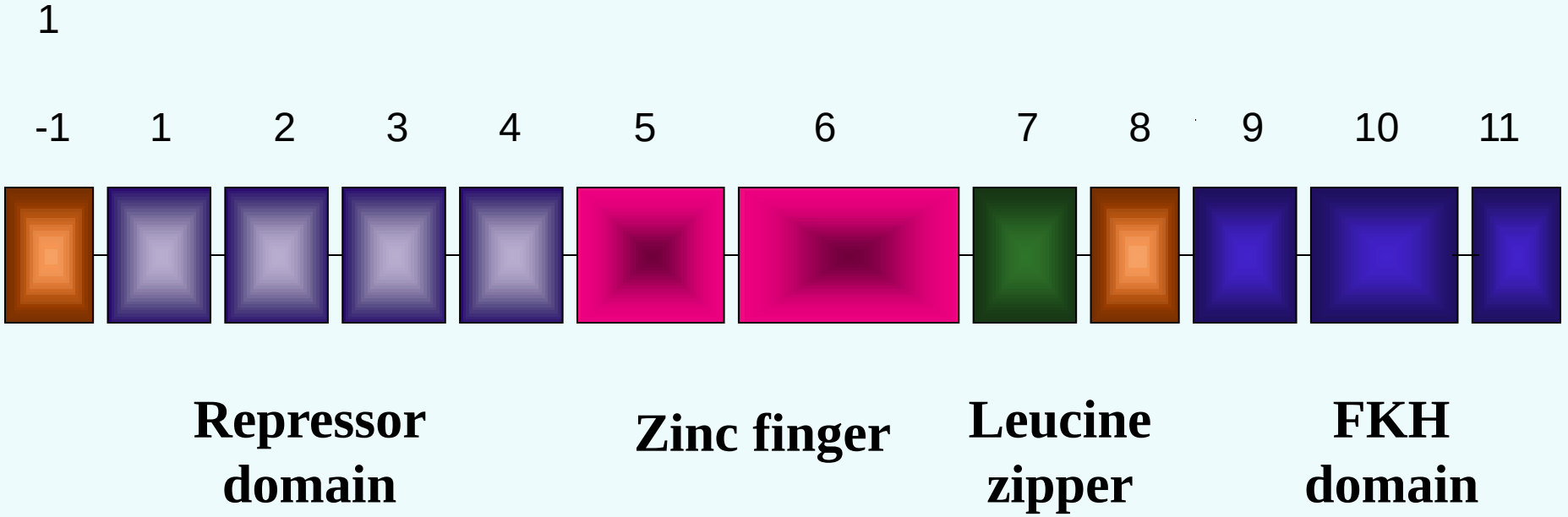
2. Zinc finger
3. Leucine zipper

} Homodimerization

4. FKH domain for the DNA binding

FOXP3 is expressed in two different isoforms:

1) full length; composed by 11 exons
2) Δ2; lacking of exon 2



- The exon 2 presents the binding site for the transcription factor RORγt
- The interaction between FOXP3 and RORγt balances the differentiation of naïve CD4+ T cells into a regulatory phenotype (Tregs) or a pro-inflammatory one (Th17)
- The lack of the exon 2 may be associated to an increase of the number of pro-inflammatory cells

Methods

- Venous blood was drawn from 89 DS patients and 16 DS patients with CD (DS-CD)

- HLA DQ2/DQ8 haplotype was evaluated using DQ-CD Typing Plus Kit of BioDiagene

- RNA was extracted from a subset of the blood samples (19 DS, 5 DS-CD) with the Midi Rneasy Lipid Tissue Kit (Qiagen)

- Real-time RT-PCR (SYBRgreen) was run to detect gene expression of total FoxP3, its full length (*FL*) and *Δ2* isoform

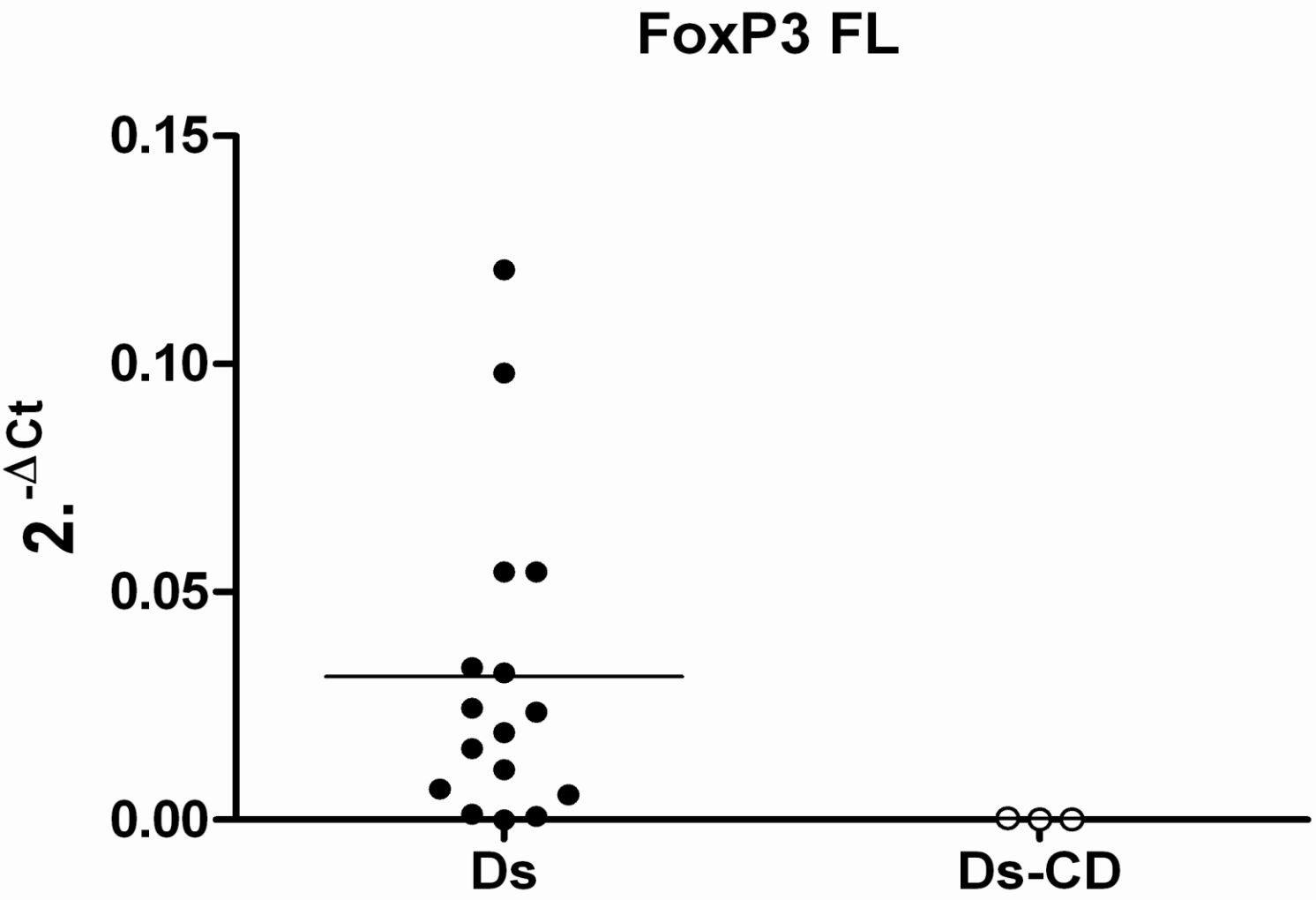
Results

HLA evaluation

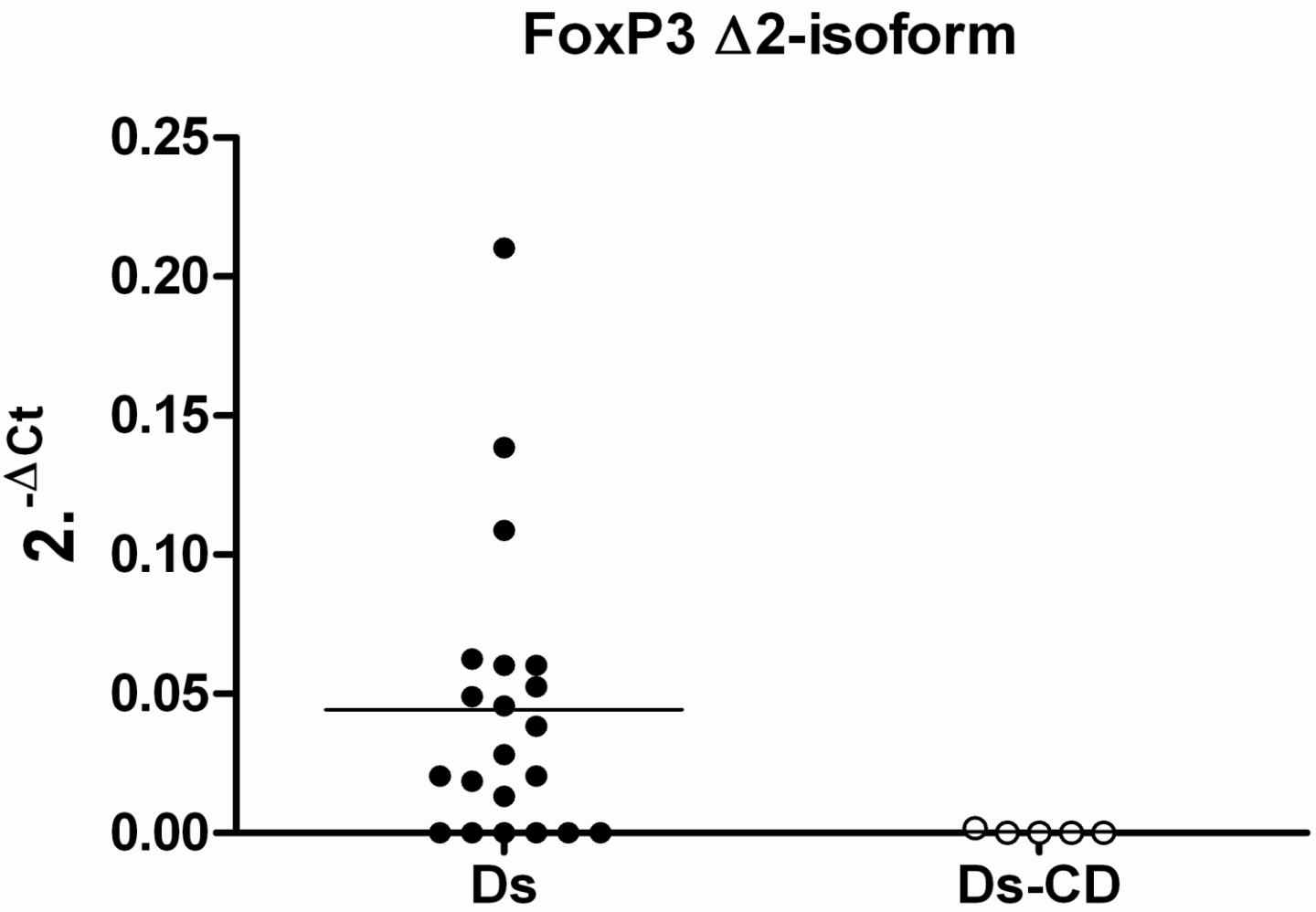
	DQ2	*DQ8*	*DQ2/DQ8*	*DQ2/DQ8 (%)*	*n*
Down/ Celiac	7 (43.8%)	0	2 (12.5%)	**56.3%***	16
Down	25 (28.1%)	5 (5.56%)	2 (2.2%)	**35.9%***	89

***The percentage of DQ2/DQ8 haplotype in non-down celiac patients is ~ 100%, while the percentage of DQ2/DQ8 haplotype within the general population is ~ 30%

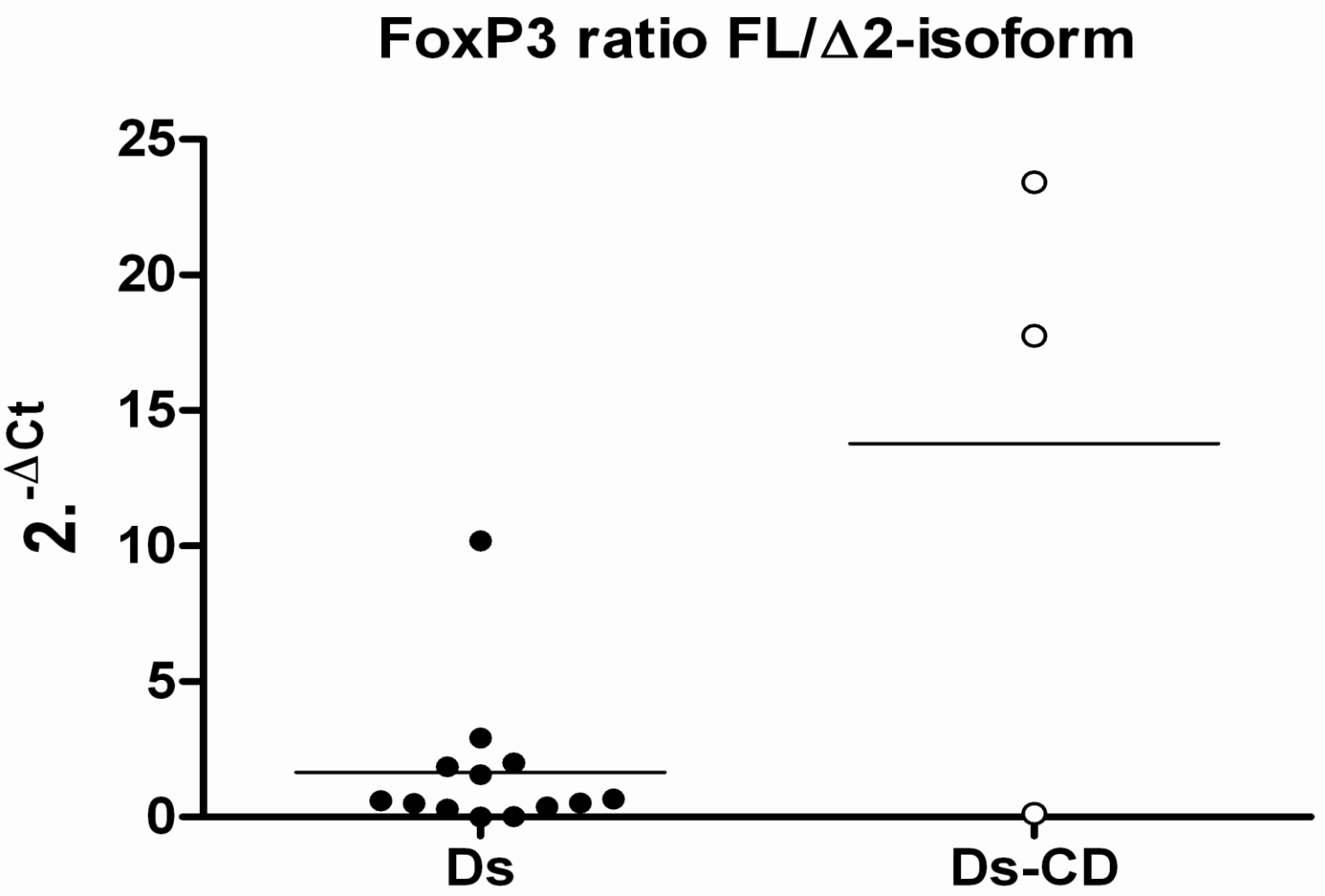
Expression FOXP3 full length



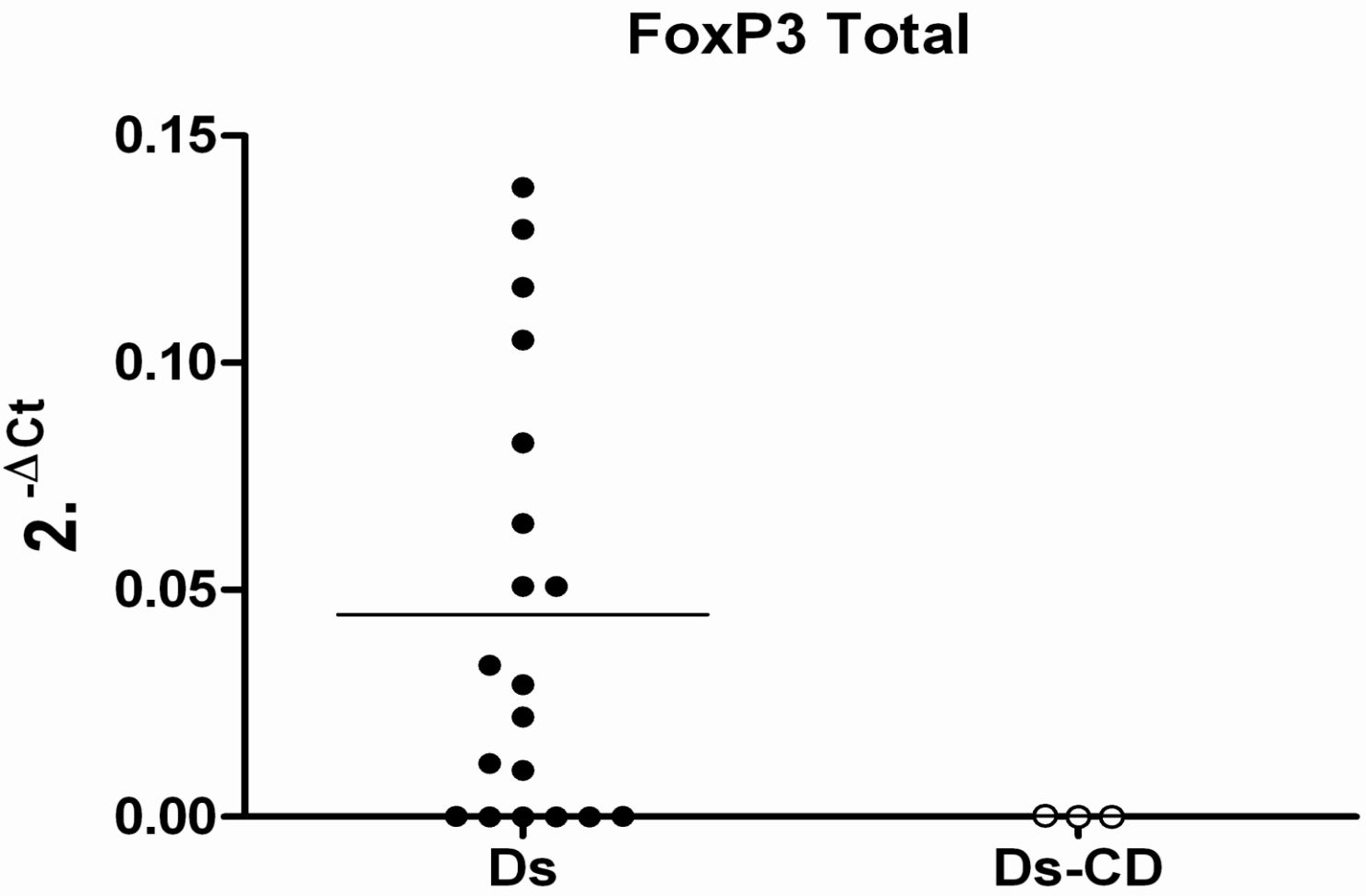
Expression FOXP3 Δ2



Expression ratio FOXP3 FL/FOXP3Δ2



Expression total FOXP3



Conclusions

HLA haplotype

- Frequency of HLA DQ2/DQ8 haplotype in DS patients was similar to that of the normal population (30%)

- HLA DQ2/DQ8 haplotype was less frequent in DS-CD patients compared to non-Down CD population worldwide population (~ 100%)

FoxP3 expression

- The real-time RT-PCR analysis showed that expression of total FoxP3 and isoforms was higher in DS patients *vs.* DS-CD.

Discussion

- The less frequent HLA-DQ2/DQ8 haplotype in DS-CD patients suggests the implication of an alternative HLA haplotype in DS-CD

- Our preliminary data, showing a higher FoxP3 (isoforms) gene expression in DS *vs.* DS-CD patients, suggests impaired functionality of immune regulation in DS-CD