CENTOGENE
THE RARE DISEASE COMPANY

C26Cer is a highly sensitive screening biomarker for Farber disease based on dry blood test (DBS)

Claudia Cozma[1], Marina Hovakimyan[1], Jan Lukas [2], Susanne Zielke[2], Arndt Rolfs[1,2]

[1] Centogene AG, Rostock, Germany;
[2]AKOS Institute, University of Rostock, Rostock, Germany

Introduction: Farber disease is an autosomal recessive, extremely rare disease caused and characterized by a deficient acid ceramidase activity encoded by ASAH1 gene. Low ceramidase activity is resulting in accumulation of fatty substances, mainly ceramides. The typical clinical key features of Farber disease are periarticular nodules, lipogranulomas, swollen and painful joints and a hoarse voice or a weak cry. In about 40% of all cases we have a late-onset and monosymptomatic phenotype with hepatosplenomegaly or rapid neurological deterioration or developmental delay. **Materials and method:** We present a new method of diagnosis of Farber disease by determining the concentration of C26 ceramide isoforms using LC/MRM-MS followed by ASAH1 gene sequencing for confirmation. Moreover, we found that cis-isomer of the C26 ceramide is a specific biomarker for Farber disease, with pathological values in a range of 39.2-150.0 nmol/L blood (normal range 13.6-23.4 nmol/L blood, N=192, healthy individuals). **Summary:** The new biomarker can be determined directly in the dried blood spot (DBS) extract with low sample consumption, easy sample preparation, high reproducibility and it presents the possibility of being used in high throughput screenings.

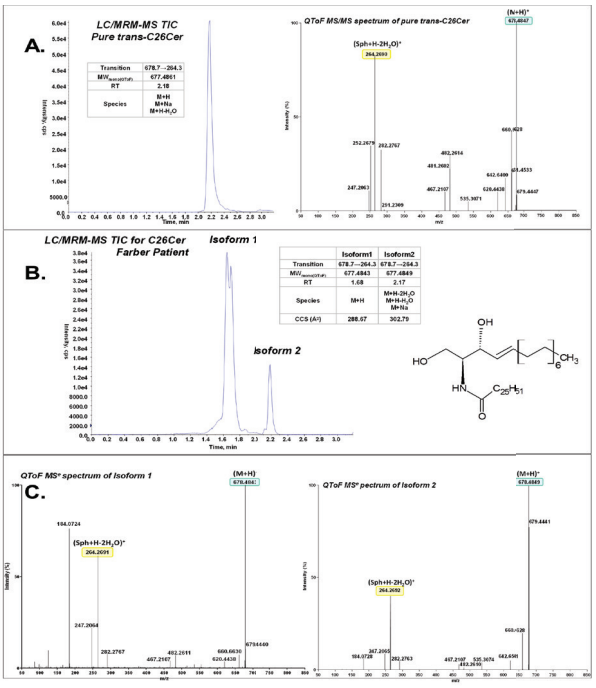

Figure 1: The fragmentation patterns of the two isoforms of ceramide C26 separable by LC/MS. We proposed here that they were the cis- and trans- isomers of the C26. The hypothesis was sustained by two facts: (i.) the analysis of pure trans-C26 ceramide revealed that it elutes at RT 2.17 min, and (ii.) the fragmentation pattern was identical for the two peaks.

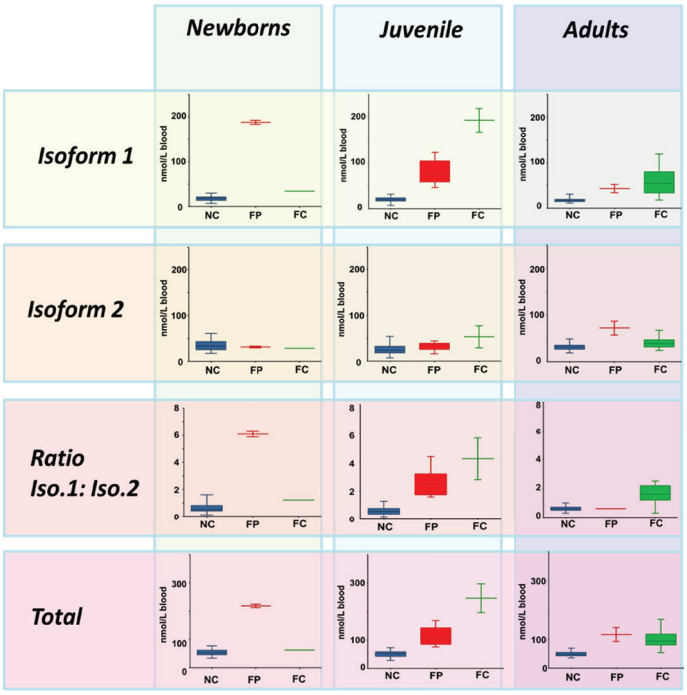

Figure 2: C26Cer isoforms levels in Farber patients (FP), Farber carriers (FC) and healthy controls (NC) in relation with the age of the donors. **Observation:** while in SMA-PME subtype (Farber adults) FC and FP have similar levels of C26Cer but distinguishable from NC; in Farber classical subtype (Farber juveniles) FP, FC and NC values are not overlapping.

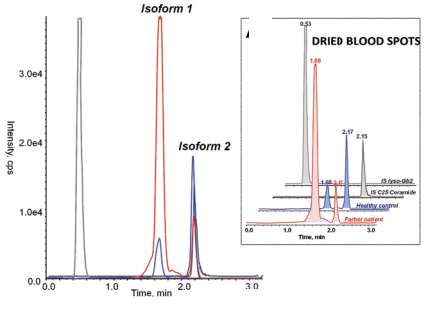

Figure 3: TIC (total ion chromatogram) profile of C26-ceramide isoforms in samples from genetically confirmed Farber patients' vs normal controls in DBS

Samples	N	Ceramide C26:0 (nmol/L) : mean ± STD					
		Isoform1	*p-value	Isoform2	Ratio	Total	*p-value
Cut-off	-	28.3	-	-	-	69.0	-
Farber Patients	10	94.6 ± 55.4	< 0.0001	40.1 ± 19.6	2.9 ± 2.0	134.8 ± 52.2	< 0.0001
Farber carriers	11	81.6 ± 62.8	-	41.6± 16.7	2.0 ± 1.4	123.1 ± 71.92	-
Healthy controls	192	18.5 ± 4.9	-	33.8 ± 8.6	0.6 ± 0.2	49.8 ± 9.6	-
Gaucher Patients	5	24.0 ± 1.4	0.002	35.6 ± 6.8	0.7 ± 0.2	59.3 ± 6.8	0.002
Niemann-Pick A/B Patients	5	19.5 ± 7.8	0.002	28.5 ± 9.9	0.8 ± 0.3	48.5 ± 13.3	0.002
Hunter Patients	5	23.3 ± 5.6	0.002	35.8 ± 7.0	0.7 ± 0.1	59.0 ± 10.8	0.002

*p-value found in Mann-Whitney test for Farber patients vs. patients affected by other LSDs

Table: Ceramide C26 levels in DBS healthy controls, Farber patients and Farber carriers and samples from patients suffering of other LSDs

References

1. Centogene AG, EP15 002 041.0 2.
2. Cozma et al 2017, Nat.Sci.Rep.,2017
3. www.centomd.com

Disclosure of conflict of interest:

This study was sustained in part by Centogene AG, Rostock, Author of the presentation, Claudia Cozma, and 2 co-authors are employees of Centogene AG, Rostock, Germany