A NOVEL N-GLYCAN ANALYSIS METHOD USING DRIED BLOOD SAMPLE (DBS) PAVES THE WAY FOR REMOTE TESTING AND NEWBORN SCREENING OF CDGs

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Background: Congenital Disorders of Glycosylation (CDG) are a group of inborn errors of metabolism that result in multisystem disease due to defects in the biogenesis of glycoproteins or other glycoconjugates. To date, more than 170 different CDG types are known. Over 90 of them have deficient N-linked protein glycosylation. Among them, several CDG can be treated effectively with monosaccharide therapy, including MPI-CDG, SLC35C1-CDG, PGM1-CDG and some of the CDG patients, including SLC39A8-CDG, SLC35A2-CDG, FUT8-CDG, were reported to partially respond to Mn, galactose and fucose therapy respectively. Importantly, new therapies for PMM2-CDG are also emerging. Thus, it is increasingly recognized that early diagnosis of CDG is often essential to achieve the best clinical outcome of these patients. In addition, Dried Blood Sample (DBS) based test is increasingly used for remote testing of CDG. Here we report a robust method to screen for CDG using N-glycan analysis of glycoproteins recovered from DBS.

Methods: We assessed N-glycans released from DBS through a highly sensitive, high-resolution and accurate mass ESI-QTOF method. The released N-glycans are derivatized, enriched through HILIC plate and are semi-quantified using a C13-labelled glycopeptide to obtain their abundance as a percentage of total glycans.

Results: For 29 diagnostic plasma N-glycans, the average recovery from DBS was at 110%, ranging from 76%-113%. Imprecision studies were performed on two level controls, and N-glycans analyzed on 10 different punches from the same DBS card over 5 days showed a coefficient of variation between 3-17%. N-glycans were stable on DBS kept at room temperature for over 4M. The N-glycan reference ranges that we collected from over 40 non-CDG normal controls are comparable to plasma N-glycan reference ranges. The diagnostic N-glycan profiles of a variety of type I and type 2 CDG on DBS are also consistent with those done on plasma, including PMM2-, PGM1-, SLC35A2-, FUT8-, ATP6AP1-, ALG3-CDG etc. In addition, we retrieved newborn screening DBS punches of a 3-year-old child with a known diagnosis of PMM2-CDG, and successfully identified her diagnosis with marked increase of mannose deprived tetrasaccharide, along with increased $Gal_1GlcNAc_2$, $Man_3GlcNAc_2$ and $Man_4GlcNAc_2$ providing evidence that CDG can be potentially diagnosed at birth.

Conclusions: In conclusion, we report a robust, rapid, and precise N-glycan profiling method using DBS cards which will facilitate remote lab testing for CDG and pave the way for CDG NBS in the near future.