ESM Methods 2 – Timeline of the project and molecular studies

Characteristics of children with *GCK*-MODY and Wolfram syndrome were presented in earlier reports [1-4]. Children with neonatal diabetes caused by mutation of the *KCNJ11* and *ABCC8* genes (and who resided in the study regions) were recruited earlier in collaborative studies with the Peninsula Medical School (Exeter, UK) and Collegium Medicum of the Jagiellonian University of Cracow (Poland) [5-6]. Data on all types of MD were collected in the study centres, and the major milestones of the project recruitment activities are presented in ESM figure 1.

Since genetic studies for particular genes were initiated at different time-points, patients treated by the study centres were re-evaluated continuously for phenotypic characteristics suggestive of MD. The prevalence of specific types of MD was calculated retrospectively using patients' birthdate and age at onset of diabetes. Patients with CFRD were included in the analysis as a separate group to calculate the relative frequencies of either type of diabetes as genetic testing for the primary disorder was performed through other diagnostic initiatives. No patients with MD were diagnosed in the studied regions before January 2005.

Molecular studies

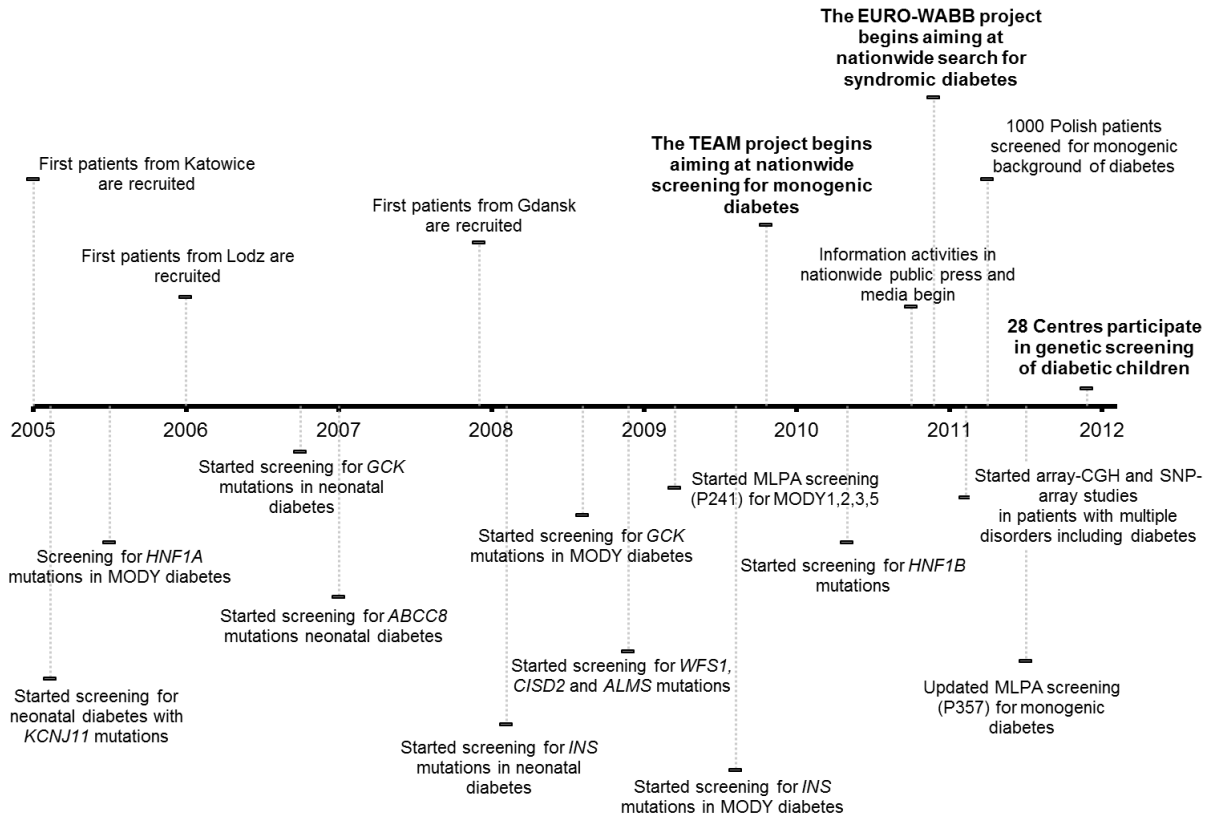
Sequencing of *GCK, INS, KCNJ11, HNF1A, HNF4A, HNF1B, WFS, CISD2* and *ALMS* was performed at the Laboratory of Immunopathology and Genetics of the Department of Paediatrics, Oncology, Haematology and Diabetology in Lodz (the principal laboratory of the project). Sequencing of *ABCC8* was performed in the genetics laboratory of Peninsula Medical School (Exeter, UK). Screening for copy number variants of the whole genes or their fragments was performed in the principal laboratory. Patients' DNA was tested for potentially pathogenic variants in genes associated with particular phenotypes of the respective types of MD. In ambiguous cases an expert panel of three members of the research team re-evaluated each case. All children with diabetes diagnosed before one year of age underwent testing of *KCNJ11, ABCC8, INS* and *GCK*. Patients with suspected Wolfram or Alström syndromes underwent testing only for *WFS1, CISD2* or *ALMS1* defects, respectively. Children with suspected MODY underwent sequencing of *GCK, HNF1A, HNF4A, HNF1B and INS*.

To counter the possibility of missing patients who aged beyond 18 years prior to introduction of particular genetic tests, since 2007 attempts were made to obtain DNA from all patients with a phenotype suggestive of MD and store the genetic material until genetic studies were possible. It is, however, possible that some individuals may have been missed during the

initial years of the project, which would result in underestimation of *HNF1A/HNF4A*-MODY and *HNF1B*-MODY - characterised by a progressive loss of beta cell function and age-dependent increase in incidence [7,8].

We have not yet performed studies on the rarest forms of MD, which probably may have contributed to the observed lower prevalence of transcription factor-dependent types of MODY, although we consider this as unlikely to have imposed a major bias.

ESM Figure 1 – Timeline of the genetic screening procedures launched in Poland between 2005 and 2011. Upper labels represent major recruitment actions of the three regions covered by the study and nationwide initiatives of the project. Fields below the timeline represent starting dates of particular genetic diagnostics procedures. MLPA – Multiplex Ligation-dependent Probe Amplification.



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