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A Leu402Pro mutation of the non-HLA gene *IL18RAP* in agressive neonatal type 1 diabetes mellitus

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Background

Neonatal diabetes mellitus (NDM) is defined by severe hyperglycemia appearing before six months of age. It occurs in about 1 in 200,000 live births and most cases are known to be of monogenic origin. Classical autoimmune type 1 diabetes mellitus (DM) is exceptional in this age group (<1%). Single genes defects leading to type 1 DM associated with complex autoimmune phenotypes have been identified in cases of *AIRE*, *FOXP3* and *SIRT1* genes mutations.
Recently non-HLA type 1 DM susceptibility genes, such as *IL18RAP*, influencing the rate of progression to diabetes among children with multiple autoantibodies have been described.

Methods

Genetic analysis were performed by Sanger sequencing of selected genes known to cause NDM and by a targeted next-generation sequencing assay. This assay was performed by sequencing (PGM, Ion Torrent) DNA selected for all coding and splicing region of 323 genes using the Haloplex technology (Agilent technologies, Santa Clara, USA). Sequencing raw data were analyzed using a locally developed pipeline integrating BWA, SAM, PINDEL and ANNOVAR algorithms. Most prominent variants were selected according to the score of prediction of damaging effects on the respective proteins using SIFT, Polyphen-2 and confirmed by Sanger sequencing.

Case report & Results

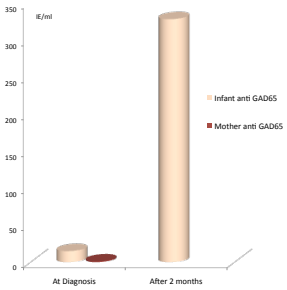
We report the case of a three months old boy, born at term with a normal birth weight of 3080 g, who was thought to have sepsis. The first blood results however showed severe ketoacidosis (Table1) and fluid replacement and *Iv* insulin therapy was started rapidly. The continuous subcutaneous insulin infusion (1U/kg/d) with diluted insulin 1:10 could be introduced after 48h. The child grew regularly and psychomotric millstones were acquired normally during his follow-up.

Table 1: Blood results

Glycemia	33 mmol/l
PH	6.9
BE	-26 mmol/l
PCO2	1.9 kPa
HCO3-	3.4 mmol/l
Sodium	152 mEq/l
Ketones (β-OHButyrate)	6.0 mmol/l

HbA1c was 6.4%. The anti-GAD65 antibody (14.9 EI/mL, N<10) was positive and increased to 328 IE/mL after two months. The mother's anti-GAD65 antibodies were negative (Fig.1). Pancreatic ultrasound and fecal elastasis level were within normal limits, as well as thyroid function tests. HLA genotyping confirmed a high risk for type 1 DM with homozygosity for HLA DR3-DQ2. No mutation was found by direct sequencing of *KCNJ11*, *ABCC8*, *INS* and *FOXP3* genes. The targeted next-generation sequencing assay revealed a p.Leu402Pro mutation of *IL18RAP,* confirmed by direct sequencing (Fig.2). The mutation was damaging (SIFT: 0.15; Polyphen-2: 0.9).

Figure 1: The anti-GAD65 antibodies



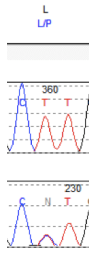


Figure 2:
PCR sequencing. Exon 10:c.T1205C:p.L402P

Discussion & Conclusion

We concluded that our patient has a type 1 DM, based on increasing anti-GAD antibodies and high-risk HLA genotyping. Maternal antibody transfer could be eliminated. A monogenic form of NDM secondary to a K_{ATP} channel or an *INS* mutation could be excluded, as well as a mutation of *FOXP3 or AIRE*.
Current genetic testing are restircted to small number of genes and multiple gene analyses in a single test use panels covering up to hundred of genes, but here we used massive parallel analysis of more than 300 genes, which revealed the Leu402Pro mutation in the *IL18RAP* gene.

Type 1 DM is characterized by T-cell-mediated autoimmune destruction of pancreatic β-cells and IL18 primarily produced by macrophages is associated with such a Th1 response (Fig.3). The receptor is composed of IL18R1 and the IL18R accessory protein (IL18RAP). The functional effect of this Leu402Pro mutation on *IL18RAP* probably involve the IL18 pathway, IFNγ production and T-cell helper 1 differentiation. This case illustrates an extremely early and aggressive onset of diabetes in infancy in the presence of a novel mutation in the type 1 diabetes susceptibility gene *IL18RAP* combined with a high risk HLA genotype.

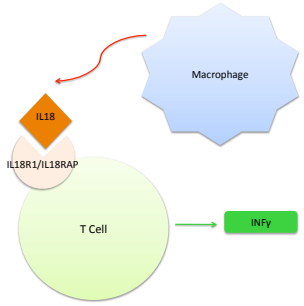


Figure 3:
IL18R is composed of IL18R1 and the IL18R accessory protein (IL18RAP).
Cytokine IL18 produced by macrophage promotes IFNγ production by T cells and is asssociated with a Th1 response.

References: E.Bonifacio, Acta Diabetologia 2014. P. Achenbach, Diabetologia 2013. C.B. Myhr, Journal of autoimmunity 2013