Immunologic phenotype of a child with the MEHMO syndrome

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SUMMARY

Background: MEHMO syndrome is a rare X-linked syndrome characterized by Mental retardation, Epilepsy, Hypogenitalism, Microcephaly, and Obesity associated with the defect of protein synthesis caused by the *EIF2S3* gene mutations. We hypothesized that the defect in protein synthesis could have an impact on the immune system.

Aims and Patients: We describe immunologic phenotype and possible treatment outcomes in patient with MEHMO syndrome carrying a frame-shift mutation (I465fs) in the *EIF2S3* gene.

Results: The proband (currently 9-year-old boy) had normal IgG and IgM levels, but had frequent respiratory and urinary tract infections. On subcutaneous immunoglobulin therapy achieving supra-physiological IgG levels the frequency of infections significantly decreased in Poisson regression by 54.5% (CI 33.2-89.7, $p = 0.017$).

Conclusion: The MEHMO patient had had frequent acute infections despite normal IgG and IgM serum levels and responded well to the immunoglobulin treatment.

INTRODUCTION

Primary immunodeficiencies (PID) are a heterogeneous group of inborn disorders characterized by dysfunction of immune system or absent function in one or more components of the immune system (McCusker and Warrington 2011). PID are rare diseases with an estimated prevalence of 1 in 1200 live births (Boyle and Buckley 2007, Modell *et al.* 2014). Most of them are genetic disorders with onset in early childhood, usually during the first year of life with recurrent or persistent infections (otitis, sinus infections, pneumonia, skin abscesses), developmental delay, failure to thrive, and predisposition for autoimmune diseases and tumors (Vozeh 2018). Early diagnosis and treatment are critical for preventing severe complications and early mortality (Shehata *et al.* 2010). PID are often associated with various syndromes.

MEHMO syndrome is a rare X-linked disorder characterized by mental retardation, epilepsy, hypogenitalism, microcephaly and obesity (Steinmuller *et al.* 1998, Skopkova *et al.* 2017). It is caused by the Ile465Serfs mutation in the *EIF2S3* gene encoding eukaryotic translation initiation factor 2 (eIF2) γ subunit, while missense mutations cause less severe clinical picture (Skopkova *et al.* 2017, Young-Baird *et al.*2020). Decreased initiation of proteosynthesis is one of the cellular responses to endoplasmic reticulum stress and triggers further actions that can eventually lead to apoptosis (Pavitt et Ron 2012). Therefore, impairment of eIF2 has the most important impact on cells with high protein synthesis rate or cells requiring stringent proteosynthesis regulation. This is supported by the observed deficiency of the *EIF2S3* mutation carrying patients in peptide hormones (Stanik *et al.* 2018). Therefore we hypothesized that protein synthesis defect could influence serum immunoglobulin levels or other protein components of naive and adaptive immune response and cause the phenotype of

primary immunodeficiency in the MEHMO patients. Here we describe the immunologic phenotype in a male proband with MEHMO and immunoglobulin therapy outcomes.

PATIENTS AND METHODS

We refer on previously described proband of Slovak origin (Skopkova *et al.* 2017, Stanik *et al.* 2018) with MEHMO syndrome caused by the causal Ile465Serfs mutation in the *EIF2S3* gene. Blood count, immunoglobulins levels and CRP were measured in the local labs by standardized methods. Flow cytometry was realized by Navios EX flow cytometer (Beckman Coulter) and immunoglobulins by Cobas C501 analyzer (Roche). By the retrospective analysis in proband we evaluated the frequency of febrile infections, respiratory tract infections, frequency of antibiotic therapy and serum immunoglobulin levels according to the immunoglobulin treatment (Gammanorm® 165mg/mL solution for subcutaneous injection, Octapharma, Belgium). Febrile infection was defined as an increase in body temperature with an increase of laboratory inflammatory markers (C-reactive protein, leukocytosis). Respiratory tract infection was defined as symptoms of rhinitis, bronchitis, cough or dyspnea.

Statistical analyses were performed with GraphPad Prism v7.0 software (GraphPad Software, San Diego, USA) and SPSSv25 software (IBM, NY, USA). Numeric data are displayed as mean ± SD. Poisson regression models were used to assess differences in incidence rates for acute infections and antibiotic use. Regression model included data since the 1st year of life until one month after stopping the therapy with immunoglobulins at the age of 9.2 years. As dependent variable was selected sequentially number of acute infections, number of respiratory infections, number of antibiotics, and number of systemic antibiotics used during the time of observation. Counts were calculated as whole numbers per year, and weighted for

the time period shorter than 1 year. Immunoglobulin therapy (yes, no) was the only factor included to the model. P values less than 0.05 were considered as statistically significant.

All steps of this study were approved by the Ethics Committee of National Institute of Children's Diseases in Bratislava, Slovakia. Written informed consent was signed by parents of the proband.

RESULTS

The results of the blood counts, CRP levels, flow cytometry and immunoglobulins of proband are summarized in Table 1.

Patient (MEHMO syndrome with frequent acute infections)

In our proband, the diagnosis of MEHMO was made at the age of 10 months. Currently, he is a 9-year-old boy. He was vaccinated with the first dose of the diphtheria-tetanus-pertussis vaccine, however, the vaccination was discontinued after epilepsy onset. The patient had the first upper respiratory tract infection at the age of 5 months. Subsequently, he was repeatedly hospitalized for pneumonia and urinary tract infections during the infant period and he required treatment with antibiotics. During the follow-up the patient was treated predominantly by a systemic antibiotic therapy of which 29% was beta-lactam antibiotics, penicilins, 29% cefalosporins, 17% chinolons, 16% macrolides a 9% of sulfonamides. During the follow-up (Table 1), the neutrophil counts and total lymphocyte counts were within the normal age-adjusted reference range. T and NK cell populations, including CD4+ and CD8+ T-cell subpopulations and naive CD4+ T cells corresponded to the patient's age as well. B cells, though initially normal, tended to decrease. Serum immunoglobulin M (IgM) and immunoglobulins G (IgG) levels were within the physiological range during the first 5

years of his life (Table 1), however, immunoglobulin A (IgA) levels remained very low during the whole monitored period. Patient has not been regularly vaccinated.

In regard to frequent infections, we initiated subcutaneous immunoglobulin therapy with a dose of 200 mg/kg/every 2 weeks at the age of 5.4 years. During the treatment, we have achieved supra-physiological serum levels of IgG (Figure 1). The incidence of acute infections per year was lower over the 3.8 years on the treatment compared with the time period since the 1st year of life until the therapy start (10.36±2.96 vs 5.89±2.90 acute infections per year without and on immunoglobulin treatment, in the Poisson regression was the incidence reduction by 54.5%, p=0.017). Similar results were obtained in reduction of frequency of respiratory tract infections (8.50±3.58 vs 4.68±2.93 respiratory infections per year, indicence reduction by 51.4%, p = 0.018), and systemic antibiotics use (7.45±2.82 vs 4.00±1.85 systemic antibiotics per year, rate reduction by 50.0%, p = 0.024). We observed the same trend in reduction of frequency of episodes of antibiotic treatment (8.59±2.96 vs 5.47±2.96 antibiotic therapies per year, rate reduction by 59.4%, p = 0.053) however, the differences were not significant. In Figure 1 are the rates displayed per month, as some time periods were shorter than 1 year.

We interrupted the subcutaneous immunoglobulin therapy after 3.8 years at the age of 9.2 years, as we thought that the risk for acute infections has decreased (age > 6 years). The effect of immunoglobulin treatment persisted also for the following 3 months, as the patient remained without any infection. However, subsequently he had several infections following each other during the period of 3 weeks requiring admitting to the hospital and a treatment with combination of intravenous antibiotics.

DISCUSSION

We have shown that our proband with MEHMO syndrome had normal IgG serum levels and partial abnormalities in the immune system, however, he had frequent acute infections and responded well to the treatment with immunoglobulins.

Our proband had the mutation of the *EIF2S3* gene encoding the γ subunit of eIF2 crucial for translation initiation. In agreement with this, we have previously shown that he had a deficiency of several peptide hormones (Skopkova *et al.* 2017, Stanik *et al.* 2018). However, some serum protein concentrations were normal in this patient, e.g. albumin, IgG and IgM levels. Therefore, the frequent infections in proband were not caused by a severe deficiency of immunoglobulin production. However, the quality of antibodies synthesized adaptively on-demand in acute infections could be defective. The patient had a deficiency of peptide hormones synthesized on demand (Stanik *et al.* 2018) and presented with decreased B cell counts. We could speculate, that deficiency of specific antibodies could explain the increased frequency of acute infections despite normal total IgG and IgM serum levels. This theory supports the good response on the immunoglobulin treatment and severe infections 3 months after interupting the immunoglobulin treatment. Another explanation could be decreased IgA levels, which should increase with age, although most people with IgA deficiency are asymptomatic. However, other regulatory or structural proteins of innate and adaptive immunity could also participate in frequent infection phenotype. Hypotonic muscles and decreased ability to expectorate may also partly explain frequent pneumonia, but it could not elucidate upper respiratory infection.

It is hard to compare our findings with previously reported patients with MEHMO, as the majority of other studies did not deal with the immune phenotype. Only Moortgat et al (Moortgat *et al.* 2016) reported on a patient with MEHMO, carrying the same mutation as our

proband, who died of respiratory failure and multiple organ dysfunction syndrome. Among other syndromes involved in eIF2 function or regulation influencing the protein synthesis, recurrent infections were reported only in patients with Wolcott-Rallison syndrome with a mutation of the *EIF2AK3* gene encoding eIF2 alpha kinase (Julier and Nicolino 2010, Huang and Wei 2019). Similarly to MEHMO patients, serum immunoglobulin levels are in the normal range in people with Wolcott-Rallison syndrome.

The major limitation is the impact of age on the frequency of acute infections - the younger children are more prone to infections, however, at least proband was treated excellently by his parents with very low microbial exposure. Also, his long-term stagnation in psychomotor development (corresponding to the age of 2-3 months) substantially decreased environmental exposures. Our patient is probably one of the longest surviving patients with this syndrome.

In conclusion, the defect in protein synthesis in MEHMO syndrome has a strong impact on several organs, potentially including the immune system. Our patient had frequent acute infections responding well to immunoglobulin treatment. Further case reports with more detailed analyses will be needed to confirm the benefit of immunoglobulin treatment in children with MEHMO syndrome.

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Contribution statement

All authors contributed to the study design and reviewed the manuscript critically and approved the final version. I.T. wrote the manuscript; D.S., M.S., and K.H. researched data, D.G. and J.S. reviewed/edited the manuscript, and P.C. researched data and reviewed/edited the manuscript.

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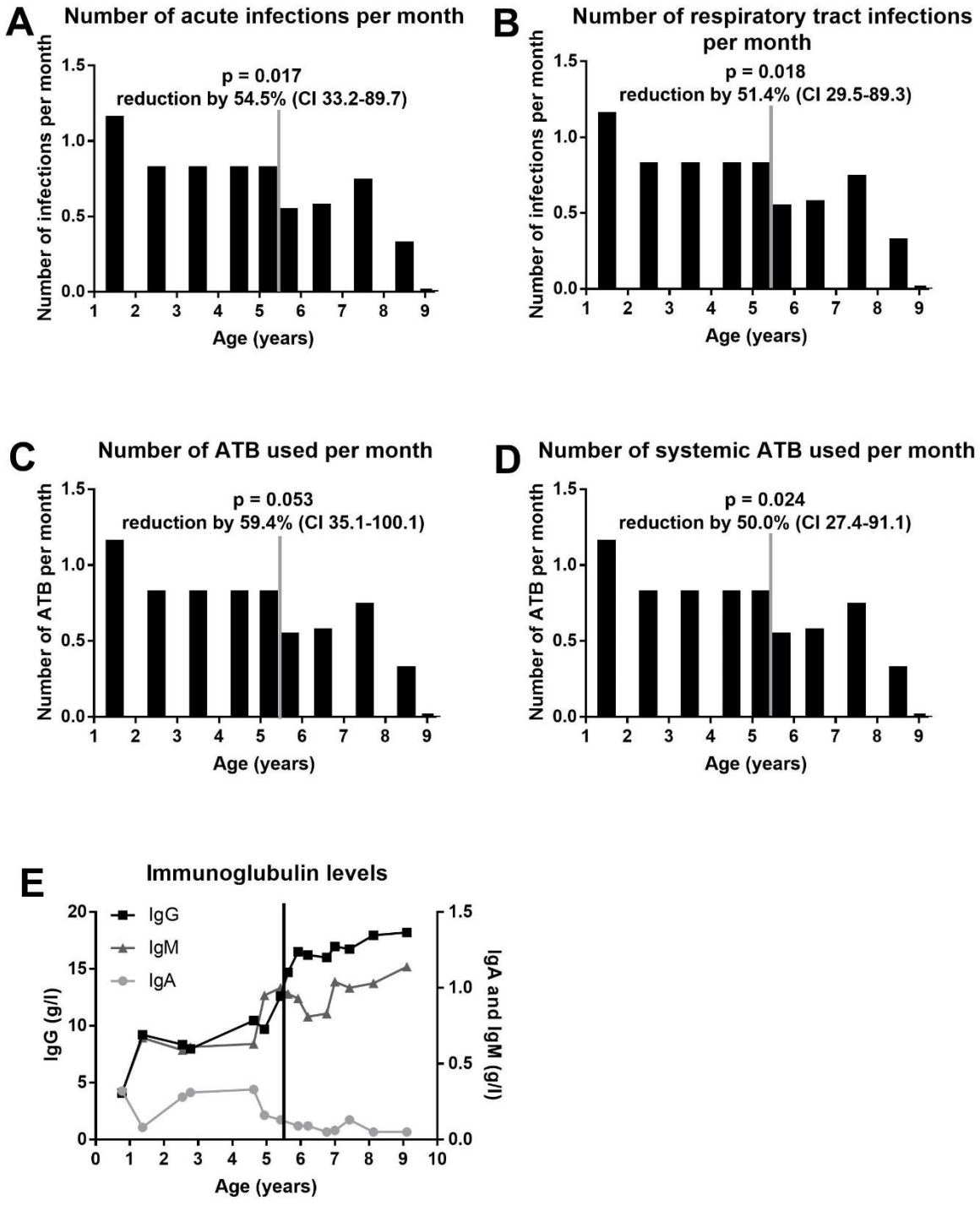
Figure 1



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244 **Figure 1. Phenotype of the proband prior and on the immunoglobulin treatment.** A.

245 Number of acute infections per month, B. Number of acute respiratory tract infections per

month, C. Number of infections treated with antibiotics (ATB) per month, D. Number of infections treated with systemic antibiotics per month, and E. Immunoglobulin levels. The grey line symbolizes the start of immunoglobulin therapy. Diffrences between periods with and without immunoglobulin therapy were calcuted by Poisson regression.

251 **Table 1.** Laboratory parameters of immune system in boy with MEHMO syndrome.

Prameter	**Proband**									
Age (years)	0.5	1	2	3	4	5	6	7	8	9
Weight kg	4,32	7,85	11,89	13,8	16	15,3	16,5	16,7	19,5	22,7
Height cm	55	69		87	87	88	90	90	105	105
Leukocytes x 10*9/l (N: 4-10)	7.10	**12.80**	**15.10**	7.75	7.30	9.10	**12.90**	7.94	9.00	8.39
Lymphocytes x10*9/l (N: 1.5-7.0)	5.00	**7.78**	5.41	4.30	1.80	3.20	3.65	4.14	4.70	2.89
Neutrophils x10*9/l (N: 1.8-8)		3.53	**8.85**	2.63	4.80	5.30	**8.31**	2.96	3.80	4.55
Hemoglobin g/dl (N: 11,5-15,5)	**9,60**	13,80	11,6	14,00	12,2	13,6	12,3	**11,1**	**10,1**	**10,1**
IgG g/l (N: 6.00-13.00)		**4.10**	8.35	7.98	10.40	12.60	**16.50**	**16.00**	**17.96**	**18.21**
IgA g/l (N: 0.51-2.97)		**0.32**	**0.28**	**0.31**	**0.33**	**0.13**	**0.09**	**0.05**	**0.05**	**0.05**
IgM g/l (N: 0.40-1.50)		**0.31**	0.59	0.61	0.63	1.00	0.93	0.83	1.03	1.14
C3 g/l (N: 0.88-2.01)			1.60				1.50	1.30	1.50	1.45
C4 g/l (N: 0.16-0.47)			0.31				0.20	0.16	0.17	0.21
CD3 x10*9/l (N: 1.80-3.00)					**3.20**				2.50	1.73
CD19 x 10*9/l (N: 0.70-1.30)					0.90				**0.60**	**0.61**
CD16+56 x 10*9/l (N: 0.20-0.60)					**0.70**				**1.02**	0.23
CD3+CD4+ x10*9/l (N: 1.00-1.80)					1.50				1.40	**0.97**
CD3+CD8+ x10*9/l (N: 0.80-1.50)					1.30				0.96	**0.59**
CRP mg/l (N: <5)	2.60	0.00	3.90	3.00	**47.00**	**28.70**	5.00	4.50	**37.00**	**5.70**
Cholesterol mmol/l (N:3,21-4,80)	**4,83**		**9,49**	**9,61**	**9,31**	**8,14**	**6,00**	**5,49**	**6,36**	**6,11**
Triacylglycerol mmol/l (N:0,0-1,30)			**6,05**	**6,36**	**6,96**	**2,84**	**3,15**	**3,77**	**2,44**	**1,98**

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