REPORT ON EDUCATION IN GENE SEQUENCING FOR PRIMARY IMMUNODEFICIENCIES

The purpose of my education in East-Central-European Infectious and Pediatric Immunology Centre for Training and Research, Department of Infectious and Pediatric Immunology at the University of Debrecen Medical and Health Science Centre, Hungary under supervision of Prof. Laszlo Marodi was to get introduced to the gene sequencing methods, to participate in the routine laboratory practice in the Centre, to learn gene sequencing methodology in the field of primary immunodeficiencies during 1 month and to analyze agreed number of available samples from Croatian PID patients.

During my stay I analyzed 14 samples of patients and parents.

Tested for *BTK* gene:

PATIENT 1. Male, 9 years old. There was suspicion on X-linked agammaglobulinaemia. Immunoglobulin level was low and CD19 was 0%. I found hemizygote nonsense mutation in exon 15 c.1558 C>T, p.R520X (*Hagemann (1994) Hum Mol Genet 3, 1743*). I also tested his mother and I did not find any mutation in exon 15 so I concluded that this was mutation *de novo*, but there can always be a possibility that mother is having mosaicism.

PATIENT 2. Male, age unknown. Immunoglobulin level was low and CD19 was 0%. I found hemizygote nonsense mutation in exon 8, c.763 C>T, p.R255X (*Bradley (1994) Hum Mol Genet 3, 79*). I also tested his mother and found that she was a carrier of that mutation.

PATIENT 3. Female, age unknown. Tested for carrier of X-linked agammaglobulinaemia. She wanted the test because her nephew has XLA, 7.5 kb deletion in exon 19 of *BTK* gene (*Richter (2001) Pediatr Allergy Immunol 12, 107*), and her sister was the mutation carrier. I did not find that mutation in her specimen.

Tested for *RAG1* gene:

PATIENT 4. Male, 2 years old. In family there was mutation in exon 13, c.2599_2600delGAinsTT, p.R829_K830delinsSX (*Santagata (2000) Proc Natl Acad Sci USA 97, 14572*). One of his brothers was heterozygote for that mutation, and the other one was homozygote and died in 2001. Both parents are carriers of above mentioned mutation because they had common ancestors (third great-grandparents). I did not find mutation in exon 13 in patient's sample.

Tested for *CYBB* gene:

PATIENT 5. Male, 6 years old. He had 4 bacterial pneumonias, 2 purulent lymphadenitis (axillary and inguinal) and severe febrile states with neutrophilia. Respiratory burst was absent, so there was suspicion on CGD. I did not find any mutation in *CYBB* gene.

Tested for *IL2RG* gene:

PATIENT 6. Female, 33 years old. Tested for carrier of common γ-chain deficiency, *IL2RG* gene. Her son died in 2010. at the age of 6 years from SCID (T-/B+/NK-), but he wasn't genetically tested. He had bone marrow transplantation in 2005. from his father, but got GVHD and died few years later. I did not find any mutation in this gene.

Tested for *ELA2* gene:

PATIENT 7. Male, 15 years old. He had pneumonias, otitis, sepsis and absolute granulocyte number <1000. He was diagnosed for cyclic neutropenia. I found heterozygote missense mutation in intron 4, c.597+1 G>T, that has not been published yet. I tested his parents also, and did not find that mutation with them.

PATIENT 8. Male, 17 years old. He had respiratory tract infections and absolute granulocyte number <500. His brother and sister had neutropenia and both died from it. I found heterozygote missense mutation in exon 2, c.137 C>T, p.S46F (*Bellanne-Chantelot (2004) Blood 103, 4119*). I also tested his parents and did not find above mentioned mutation in their samples.

As I would like to specialize in my career in gene sequencing and genomic primary immunodeficiency diagnostics, my study stay in Debrecen was very valuable. I learned how to analyze 5 genes: *BTK*, *RAG1*, *CYBB*, *IL2RG* and *ELA2*. Furthermore, I learned principles of gene sequencing as a final step in completing the patients' diagnosis.

I will introduce and apply the acquired knowledge in the routine laboratory practice in Clinical Unit for Molecular Diagnostics, Department for Laboratory Diagnostics, University Hospital Centre Zagreb (Croatia). Also I would like to continue collaboration with Department of Infectious and Pediatric Immunology at the University of Debrecen, Prof. Marodi and his co-workers and to establish collaboration with other ESID Pediatric Immunology Centers and other ESID junior and senior members.

And finally, I would like to express my sincere gratitude to ESID board for giving me this fellowship, and the opportunity to learn more and to improve my knowledge. Also I would like to thank Prof. Marodi, Dr. Darko Richter and Prof. Jadranka Sertic for giving me opportunity and support to attend education in gene sequencing in Debrecen.

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