Abstract

Kabuki syndrome (KS) is a dominantly inherited rare disease caused mainly by *de novo* pathogenic variants (henceforward mutations) in the *KMT2D* (formerly *MLL2)* and *KDM6A* genes. It is rare multisystemic syndrome characterized by intellectual disability (ID) and typical facial dysmorphism. KS is clinically heterogeneous, which complicates its clinical diagnosis.

The first aim of this thesis was to introduce mutation testing of the two known KS causative genes in KS by Sanger DNA sequencing and by MLPA (Multiple Ligation Probe Amplification) at the Department of Biology and Medical Genetics of 2nd Medical Faculty of Charles University and University Hospital Motol, Prague followed by identification of underlying genetic mutations in *KMT2D/KDM6A* genes in 43 patients with phenotype typical for KS, who were indicated for this molecular genetic analysis by several collaborating genetic departments in the Czech Republic. We aimed to confirm or disprove of patient's clinical diagnosis, establish spectra of *KMT2D/KDM6A* mutations in the Czech population, render phenotype-genotype correlations and evaluate the phenotypic "MLL2-score" (published by Makrithanasis et al., 2013) utility as prediction tool for selection of cases for *KMT2D* sequencing.

Mutations in the *KMT2D* gene were detected by Sanger DNA sequencing in 40 % (17/43) of analyzed patients with the KS phenotype, vast majority of mutations were protein truncating and these were located in various exons. No causal mutation was found in the *KDM6A* gene and no intragenic rearrangements were found in either of the two genes using MLPA analysis. In addition, no correlation was found between a particular mutation and the KS phenotype. However, there was correlation between the KS phenotype and the presence vs. absence of a mutation in the *KMT2D* gene, expressed by the "MLL2-score". Patients with *KMT2D* mutation presented with average phenotypic score of 7,4 (range 6-9) and patients without a *KMT2D* mutation

with an average score of 5 (range 2-7). KS patients with score of 6 and higher are thus good candidates for targeted *KMT2D* testing.

The second aim of this study was to elucidate the molecular genetic cause of Kabuki phenotype in 18 patients lacking mutation in *KMT2D/KDM6A* genes using aCGH followed by Next Generation Sequencing (NGS) targeted to clinical exome (CES) and so determine genomic locuses that are responsible for rare diseases resembling KS – KS-like syndromes. Only in 18 patients out of the 26 *KMT2D/KDM6A* negative patients was granted consent with the NGS testing. Furthermore, we aimed to establish the most suitable testing algorithm in KS and KS-like cases.

In two patients with KS-like phenotype we found causal genomic imbalances (Copy Number Variations / CNV) at Xp21.1-Xp21.3 and 14q11.2 loci, respectively. In four patients with KS-like phenotype we found mutations in embryonically expressed genes, whose protein products participate in the regulation of transcription – gene *KMT2A*, in mRNA splicing – gene *EFTUD2*, in apoptosis – gene *HUWE1* and in signal transmission – gene *GRIN1*. In *KMT2D/KDM6A* negative patients are the aCGH followed by CES the methods of first choice, since the detection rate in our cohort is relatively high – 33 % (6/18 patients with NGS consent). Patients with the phenotypic score 5 and lower could be directly indicated to aCGH/CES testing, as in this cohort of patients no mutation in *KMT2D/KDM6A* genes was observed.

The last aim of this study was an examination of the CES utility in patients with rare disease characterized by syndromic ID (i.e. similar to KS) and elucidation of molecular pathogenesis in those clinicaly heterogeneous disorders. In 15 out of 60 patients tested we found likely pathogenic or pathogenic variants, increasing the diagnostic yield to 25% in patients with syndromic ID. In this group of patients we diagnosed two additional patients with a mutation in the *KMT2A* gene, although there wasn't original clinical suspicion of the Wiedeman-Steiner syndrome, i.e. similar as in the KS-like *KMT2A*-mutation

positive patient. Furthermore, we were able to make novel connection of histone demethylase *KDM6B* (from the same gene family as *KDM6A*) with ID. In addition, we had identified another patient with protein truncating mutation in the *EFTUD2* gene and a patient with mutation in the *GRIN2B* gene (from the same gene family as the *GRIN1* gene). In two other patients autosomal recessive (AR) molecular cause of ID disase was revealed, in two patients X-linked (XL) inherited cause was observed. The NGS method aimed at the clinical exome has a high detection rate in patients with syndromic ID and may provide rapid diagnosis especially in cases, where their clinical phenotype is ambiguous, often due to variable clinical / age-related expressivity of their features. Many of the studied patients, with syndromic ID, have mutations in histone metyltransferases (with SET domain) (*KMT2A, KMT2D*) and demetylases (*KDM6A, KDM6B*), in splisosomal GTPase (*EFTUD2),* in genes coding for neurotransmitter receptors activated by glutamate (*GRIN1, GRIN2B*) and in ubiquitin protein ligases with HECT domain (*HUWE1, HECT2*).