

## Kabuki Syndrome: Novel Genes and Molecular Mechanisms

### Background

Kabuki syndrome (KS; OMIM 147920, 300867) is a classic, clinically well investigated malformation syndrome with characteristic craniofacial features: long palpebral fissures; eversion of the lateral third of the lower eyelids; broad, arched and (in the lateral third) sparse or interrupted eyebrows; short columella and depressed nasal tip; large, prominent or posteriorly rotated ears. These features can occur in combination with intellectual disability of a varying degree, postnatal growth retardation, persisting finger pads and urogenital anomalies. Genetically, mutations in *KMT2D* account for Kabuki syndrome in about 50% of patients, while about 13% of the patients who are negative for *KMT2D* mutations carry causative changes in *KDM6A*.

### Novel Kabuki genes: *RAP1A*, *RAP1B*

Apart from the known KS genes *KMT2D* and *KDM6A*, we found mutations in the genes *RAP1A* and *RAP1B* in individual patients with Kabuki syndrome; further in vitro and in vivo investigations in cell systems and in a zebrafish model demonstrated that these mutations play a causative role in the pathogenesis of Kabuki syndrome.

### Unravelling the molecular mechanisms

*KMT2D* and *KDM6A* are components of a multiprotein complex that effects transcriptional gene activation by epigenetic modification [1,2,3]. Within this complex, *KMT2D* places activating methylation signals on histone 3 lysine 4 (H3K4), whereas *KDM6A* catalyzes demethylation, thus removing repressive signals on H3K27 [4]. Together with our collaboration partners, we performed extensive functional studies in patient fibroblasts, embryonic fibroblasts of a *Kmt2d* knockout mouse and a zebrafish morpholino knockout. They showed that *RAP1A* and *RAP1B* play a causative part in the development of Kabuki syndrome and that mutations in those two genes and in the previously known KS genes bring about alterations of the MEK-ERK signaling pathway. Furthermore, morpholino knockdown of *RAP1A*, *KMT2D* and *KDM6A* in zebrafish resulted in defects in early embryonic development, morphological changes in the cranioskeletal architecture and disturbed cell interaction. This cellular effect may explain not only the skeletal anomalies, but also some of the other organ involvement as for example heart defects and neurological issues.

### Our publications on Kabuki syndrome

Mutation Update for Kabuki syndrome genes *KMT2D* and *KDM6A* and further delineation of X-linked Kabuki syndrome subtype 2.

Bögershausen N, Gatinos V, Riehmer V et al. *Hum. Mutat.* 2016, doi: 10.1002/humu.23026. [Epub ahead of print].

An unusual presentation of Kabuki syndrome: orbital cysts, microphthalmia, and cholestasis with bile duct paucity.

Bögershausen N, Altunoglu U, Beleggia F, et al. *Am. J. Med. Genet. A* 2016, doi: 10.1002/ajmg.a.37931 [Epub ahead of print].

*RAP1*-mediated MEK/ERK pathway defects in Kabuki syndrome.

Bögershausen N, Tsai IC, Pohl E et al. *J Clin Invest* 2015, 125(9):3585-99.

*CHARGE* and Kabuki syndromes: a phenotypic and molecular link.

Schulz Y, Freese L, Mänz J et al. *Hum Mol Genet* 2014, 23(16):4396-405.

Skirting the pitfalls: a clear-cut nomenclature for H3K4 methyltransferases.

Bögershausen N, Bruford E, Wollnik B. *Clin Genet* 2013, 83:212-214.

Unmasking Kabuki syndrome.

Bögershausen N, Wollnik B. *Clin Genet* 2013, 83:201-211.

A mutation screen in patients with Kabuki syndrome.

Li Y, Bögershausen N, Alanay Y et al. *Hum Genet* 2011, 130:715-724.

### Current research: Kabuki syndrome and obesity

Mutations in *KMT2D* and *KDM6A* provide the genetic cause of Kabuki syndrome only in about 60% of patients. We therefore assume that (apart from differential diagnoses in some patients) further genes exist that are associated with Kabuki syndrome. We are searching for these novel genes and causative mutations by whole-exome sequencing. In addition, we aim to elucidate important aspects of the pathogenesis of Kabuki syndrome. As an example, patients typically show progressive obesity. Against this background, we plan to perform further research into adipocyte differentiation and metabolism in Kabuki syndrome patients using induced pluripotent stem cells and cell systems generated by CRISPR/Cas9 technology.

### Contact

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### References

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2. Issaeva I, Zonis Y, Rozovskaia T et al. Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. *Mol Cell Biol.* 2007;27:1889-1903.
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