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Quantitative estimation of trisomy on chromosome 6 in embryonic fibroblasts of mice carrying duplications obtained using CRISPR/Cas9 technology

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Motivation and Aim: The high efficiency of the CRISPR/Cas9 system used for editing human and animal genomes is achieved due to the sufficiently accurate recognition of the target DNA site by the guiding RNA of the CRISPR/Cas9 complex. However, editing errors can occur during the repair of double-stranded DNA breaks performed by the Cas9 protein. To date, there is increasing evidence of spontaneous gene mutations occurring not only away from the genome editing site, but also close to it. Previously, using the CRISPR/Cas9 technology in the zygotes, we obtained experimental mice that carry deletions with a size of 1137 kb, duplication of 2374 kb, and similar sized inversions in chromosome 6, involving only the *Cntn6* gene (encoding a protein contactin-6) [1]. Applying FISH analysis we unexpectedly found trisomy on chromosome 6 in 3.5–4.3 % of fibroblasts obtained from two adult founders (No. 1 and 20) heterozygous for the *Cntn6* duplication [2]. Taking into account that this is the first time when the phenomenon of trisomy in somatic cells is described in animals with CRISPR/Cas9 induced large-scale chromosomal rearrangements, we assumed that a possible factor provoking the onset of trisomy was a heterozygous duplication of the *Cntn6* gene in the genome.

Methods and Algorithms: Applying the method of fluorescent in situ hybridization (FISH) with a chromosome-specific probe for the entire mouse chromosome 6 we investigated the level of trisomy in 21 lines of mouse embryonic fibroblasts (MEF) obtained from 13-day-old embryos that are descendants of founders (No. 1 and 20).

Results: In 8 studied lines of MEFs heterozygous for *Cntn6* duplication the level of trisomy varied from 1 to 8 %, and in 5 homozygous lines – from 2 to 6 %. There were no statistically significant differences between these MEF lines (Mann–Whitney test, STATISTICA 10). In 3 out of 4 control MEF lines carrying the wild type allele of *Cntn6* gene, trisomy was detected in 1–2 % of cells. In order to "enhance" the heterozygosity of alleles of chromosome 6, and to see if the level of trisomy will increase, we generated and analyzed 4 MEF lines carrying heterozygous *Cntn6* duplication and *Cntn6* deletion in one genome. FISH with a chromosome-specific probe for the mice chromosome 6 showed the absence of trisomy in these 4 lines.

Conclusion: The obtained results demonstrate that the level of trisomy of chromosome 6 in MEF lines heterozygous and homozygous for *Cntn6* duplication is not significantly increased compared to that in control cell lines. Nevertheless, the variability of the trisomy level of chromosome 6 among heterozygous and homozygous for *Cntn6* duplication cell lines, as well as the level of total aneuploidy in these MEF lines indicates

increased genome instability. It is quite possible that trisomy on chromosome 6 in studied cell lines occurs at a high level, but is accompanied by rapid elimination of aneuploid cells in early embryo development [3]. Apparently, the presence of *Cntn6* duplication and *Cntn6* deletion on different homologues is a more stable modification for the genome than when the dose of the *Cntn6* gene in the genome is 1.5 or 2 times higher (in heterozygous and homozygous *Cntn6* MEFs).

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References

1. Korablev A.N., Serova I.A., Serov O.L. Generation of megabase-scale deletions, inversions and duplications involving the Contactin-6 gene in mice by CRISPR/Cas9 technology. *BMC Genet.* 2017;18:112.
2. Pristyazhnyuk I.E., Minina J., Korablev A., Serova I., Fishman V. et al. Time origin and structural analysis of the induced CRISPR/cas9 megabase-sized deletions and duplications involving the Cntn6 gene in mice. *Sci Rep.* 2019;9(1):14161.
3. Singla S., Iwamoto-Stohl L.K., Zhu M., Zernicka-Goetz M. Autophagy-mediated apoptosis eliminates aneuploid cells in a mouse model of chromosome mosaicism. *Nat Commun*. 2020;11(1):2958.