MFISH Measurements of Chromosomal Aberrations Individuals Exposed in Utero to Gamma-ray Doses from 5 to 20 cGy
Final Report
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Our plan was to identify and obtain blood from 36 individuals from the Mayak in-utero exposed cohort, who were exposed in utero only to gamma ray doses from 5 to 20 cGy. Our goal was to do mFISH on these samples, to measure stable chromosome aberrations in these now adult individuals.

The results were compared with matched control individuals (same age, same gender) available from the large control population which we are studying in the context of our plutonium worker study (*1-3*). The long term goal was to assess the results both in terms of the sensitivity of the developing embryo/fetus to low doses of ionizing radiation, and in terms of different potential mechanisms (expanded clonal origin vs. induced instability) for an increased risk.

Our plan was to score samples from a total of 36 individuals, and in practice we were able to use samples from 30 (mean in utero dose 9.7 cGy) + 30 matched controls, matched by age and sex. Slides from all the individuals were hybridized using the mFISH muti-fluor technique, and metaphases from all of the in-utero-exposed individuals, and the controls were been scored.

Blood samples were collected and metaphase cell slide preparations made at the Southern Urals Biological Institute, Russia, using standard protocols. As is our standard practice, conventionally stained (Giemsa) slides are also prepared, which is useful for confirming chromatid-type aberration yields. The slide preparations was generally done at SUBI, in Russia, and the mFISH *in-situ* hybridization and aberration scoring is subsequently carried out in New York. Chromosome paints were obtained from MetaSystems GmbH, Germany. Microscopic analysis is performed using our Axioplan II imaging microscope (Carl Zeiss, Germany) with an HBO-103 mercury lamp and filter sets for FITC, Cy3.5, Texas Red, Cy5 and AQUA. Images are captured, processed and analyzed using Isis mFISH/mBAND imaging software (MetaSystems, Germany).

From the in-utero irradiated cells, we observed 29 translocations in 3,026 metaphases (0.96%), compared with 16 translocations in 2,860 metaphases in matched controls (0.56%). Using Fisher's exact test, the one sided p value is 0.053, and the two-sided p value is 0.10. So one might conclude there is marginally significant, but not conclusive, evidence for an increase in stable chromosome aberrations after a mean in-utero low-dose-rate dose averaging ~10 cGy. It may be pointed out that one of the in-utero exposed individuals had a quite dramatic clonally-based aberration, though a single such case is probably not unexpected (*4*).

(*1*) Hande MP, Azizova TV, Burak LE, Khokhryakov VF, Geard CR, Brenner DJ. Complex chromosome aberrations persist in individuals many years after occupational exposure to densely ionizing radiation: an mFISH study. Genes Chromosomes Cancer 2005;44:1-9.
(*2*) Mitchell CR, Azizova TV, Hande MP, Burak LE, Tsakok JM, Khokhryakov VF, et al. Stable intrachromosomal biomarkers of past exposure to densely ionizing radiation in several chromosomes of exposed individuals. Radiat Res 2004;162:257-63.
(*3*) Hande MP, Azizova TV, Geard CR, Burak LE, Mitchell CR, Khokhryakov VF, et al. Past exposure to densely ionizing radiation leaves a unique permanent signature in the genome. Am J Hum Genet 2003;72:1162-70.
(*4*) Nakamura N, Nakano M, Kodama Y, Ohtaki K, Cologne J, Awa AA. Prediction of clonal chromosome aberration frequency in human blood lymphocytes. Radiat Res 2004;161:282-9.