MONITORING MUTATIONS IN PEOPLE: AN *IN VIVO* STUDY OF PEOPLE ACCIDENTALLY OR OCCUPATIONALLY EXPOSED TO IONIZING RADIATION

Barry W. Glickman
Director, Centre for Environmental Health
University of Victoria
Victoria, B.C.

Recent developments in molecular biology and medicine now permit the monitoring of mutation in people *in vivo*. While several approaches are possible, the most practical, and currently most commonly used, is the study of mutations at the hypoxanthine phosphoribosyl transferase *(hprt)* locus in peripheral T-lymphocytes. This approach has several advantages. Blood samples are relatively easy to obtain and the T-cells can be readily processed, frozen, and transported until they are needed for analysis. T-cells can be efficiently grown with the help of a mitogen such as phytohaemoglobin (PHA) and growth simulators such as human interleukin (IL-2). Once the T-cells are growing, HPRT mutants can be effectively selected by their resistance to the analogue 6-thioguanine. Because the *hprt* gene is X-linked, mutants can be selected without requiring that the subjects be heterozygotes. Moreover, because of selective X-inactivation, mutation frequencies can be measured in both men and women. Of equal importance is that the gene has been well characterized and is well suited for mutational analysis. It is not an essential gene so that all classes of mutations can be isolated, including complete deletions of the gene. The gene is not too large, about 45 KB, for study by Southern blotting or multiplex PCR, yet large enough to be representative as a mutational target. The small size of the CDNA, less than 800 bp, makes it convenient for sequence analysis. Finally, because of the properties of the T-cell receptor (TCR), the clonal origins of mutations can be examined and mutant frequencies can be recalculated as actual mutation frequencies.

There are also other aspects of the assay that make the *hprt* system attractive. These include the fact that the assay can be carried out *in vitro* using a diverse range of cell types, and that there is a considerable data base on both *in vitro* and *in vivo* mutation in this gene. Perhaps an additional attraction is that reasonably extensive deletions, including the loss of the entire coding region, have been recovered.

Our laboratory has been examining the question of how the *hprt* locus could be used for monitoring the genetic consequences of exposure to ionizing radiation. To study this question two different exposed populations have been examined. The first is a group of about 40 people who were accidentally exposed to caesium-137. The second group is a series of Soviet cosmonauts with extensive space flight experience. In addition, we have been studying mutation in a set of monozygotic twins with the view towards estimating the genetic contribution to background mutation frequencies. This report summarizes what we have learned by the application of the *hprt* clonal assay to these populations.

The Radiological Accident of Goiania, Brazil

In September of 1987 during the demolition of a private cancer clinic, an abandoned radiotherapy unit containing over 3,000 Ci of ^{137}Cs was unlawfully

removed and sold for scrap metal. The dismantling of the unit led to the rupture of the cesium canister and to the subsequent contamination of a number of foci in Goiania. Several hundred people were unknowingly exposed to radiation. They were subjected to a range of external and internal contamination. Four people died as a consequence of acute radiation exposure.

We have applied the *hprt* assay to a number of the survivors who were exposed to between 0.1 and 8 Gy. This includes people ranging from 5 to 70 years of age at the time of exposure. As controls we have selected non-exposed members of the same families, neighbours and workers of the health organization with which we have been co-operating. The use of local people and family members as controls is an attempt to minimize the effects of life style, diet, and other environmental and potential genetic factors. We have followed mutations in these subjects for the past five years. Our report can be summarized as follows:

1. Spontaneous mutation at the *hprt* locus increases with age and is higher in smokers than in non-smokers.

2. In adults we found a linear dose response to radiation with a doubling dose at this locus of between 0.75 and 1.0 Gy.

3. The mutational spectrum from the exposed adults did not reveal an obvious "radiation-induced" mutational fingerprint. In other words, large deletions were not recovered from the exposed individuals. Overall, the mutational spectrum was not unlike the unexposed controls. There is some suggestion that the distribution of mutations within the gene might be different and there may be an excess of frameshift events. This tendency was also observed in patients who received radio-immune-therapy (RIT) as part of their cancer therapy (in press). The results are summarized in the Table below:

MUTATIONAL SPECTRUM OF *hprt* cDNA			
	SPONTANEOUS		EXPOSED
Mutation Class	Database	Brazil	Brazil
Total Base Subst.	43%	39%	44%
Transitions	21%	24%	29%
Transversions	22%	15%	15%
Exon Skipping	29%	41%	25%
Frameshifts	9%	3%	12%
Complex	4%	9%	6%
Insert./Duplicat.	1%	0%	4%
Deletion	15%	9%	8%
No Mutation	0%	0%	2%
Total Mutants	100% (161)	100% (34)	100% (52)

4. In addition to having low mutational backgrounds which facilitate the detection of induced mutation in children, it appears that children are particularly sensitive to ionizing radiation. Children exposed to as little as an estimated 0.1 Gy had significantly higher levels of mutation than expected. No dose response was observed. This is not surprising considering the low doses received and the uncertainties surrounding dose. No specific "radiation-like" mutational fingerprint was detected.

5. The level of mutation in those exposed to radiation dropped each subsequent year following the accident. This probably reflects the natural T-cell turn over. However, this observation means that measurements of people exposed several years ago or over extended periods, likely underestimate the real mutation frequency.

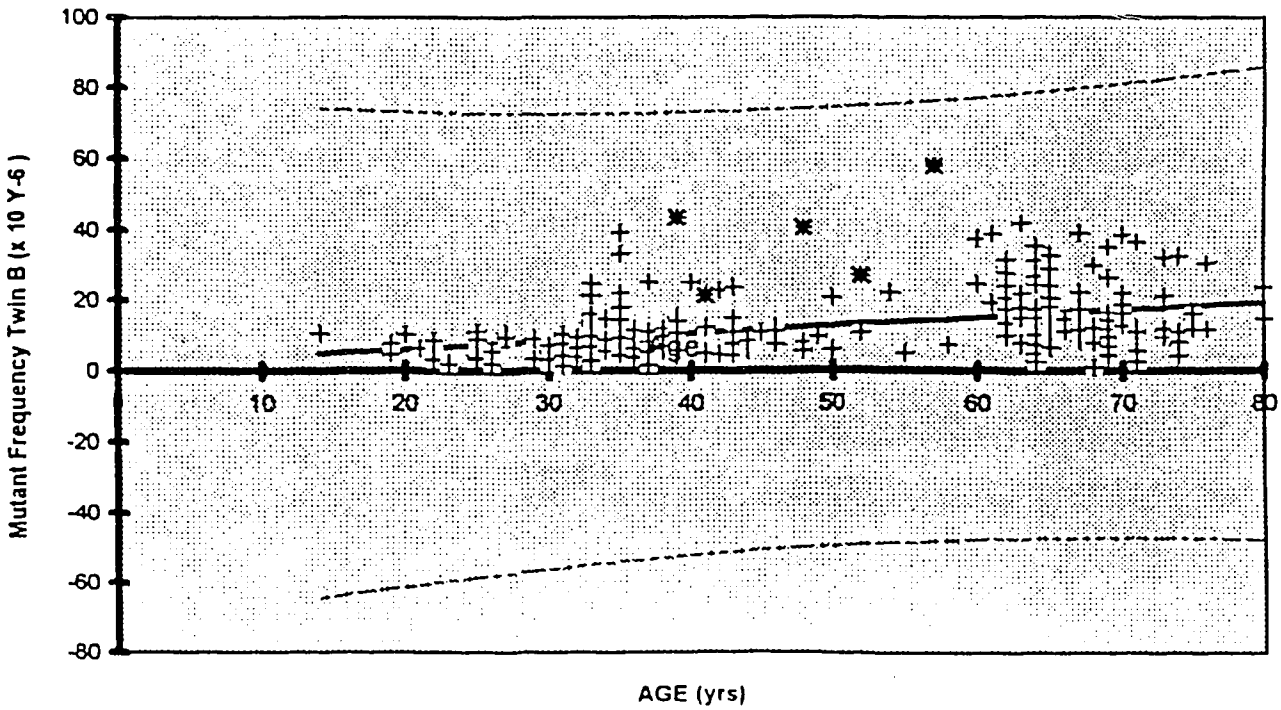
Mutation in Experienced Soviet Cosmonauts

People flying extended missions in space are subjected to types of radiation not normally experienced on earth. As such, long-term space flight carries with it an increased risk of health effects due to prolonged low-level exposure to radiation. A hint of the potential health problems can be seen in the increased incidence of brain tumours in airline pilots flying polar routes. One Soviet cosmonaut is known to have died from brain cancer.

The advantage of studying cosmonauts for radiation effects is that they have been carefully monitored for radiation exposure. We have used the *hprt* assay to study mutations in a series of five Soviet cosmonauts who have experienced extensive space travel. Our results can be summarized as follows:

1. Each cosmonaut has a significantly "above average" level of mutation at the *hprt* locus. When corrected for the natural drop in mutant frequency due to T-cell turn over, the mutation levels are quite significantly enhanced. In Figure 1 mutant frequency is plotted against age.

Figure 1



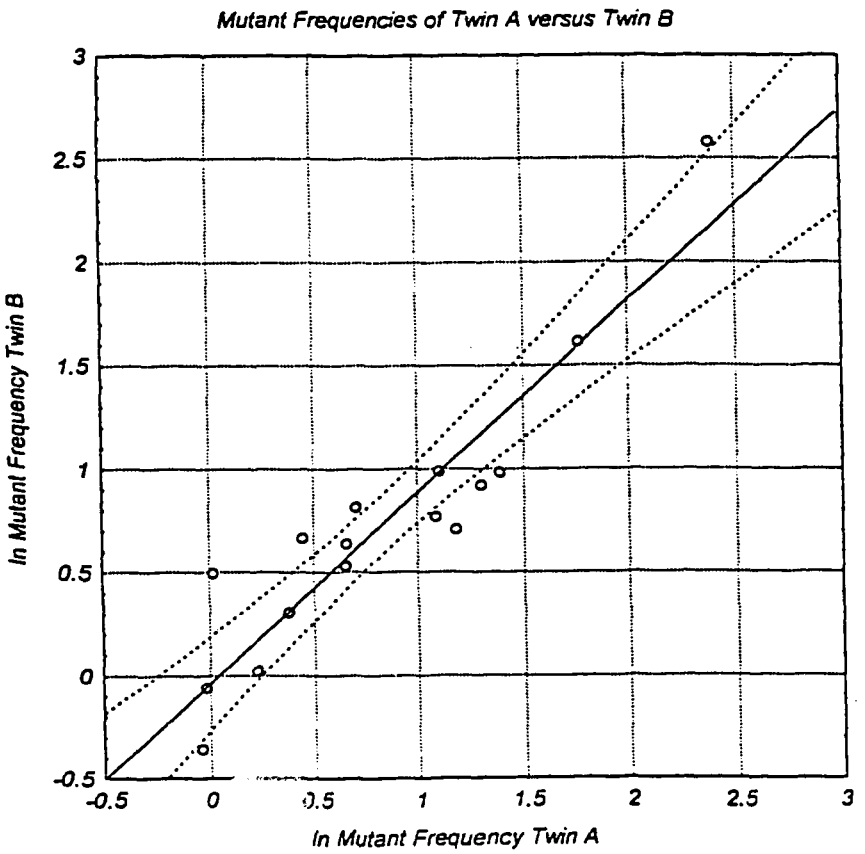
2. No "radiation-like" mutational fingerprint was uncovered. In addition, there was no obvious dose response.

3. It must be remembered that cosmonauts are exposed to low levels of protracted radiation in an environment of microgravity. They have also been exposed to enhanced oxygen containing environments and are subjected to above average levels of physical activities. It is thus not possible to conclude that the increased levels of mutation are due to their exposure to radiation in space.

Mutant Frequencies and Genetics

It is likely that mutation rates have been regulated by evolution. To examine this possibility in people, we have looked at the *hprt* mutant frequency in monozygotic twins. We make the following conclusions:

1. Mutation in monozygotic twins is much more similar than in dizygotic twins, other siblings and unrelated, but age-matched pairs. This indicates a major role for genetics in determination of natural mutation levels. The graph below displays the mutant frequency of twin A against the mutant frequency of twin B. The dashed line is the 95% confidence interval.



2. While this correlation is very strong in younger twin pairs (age 40 or less), this relationship deteriorates in older twin pairs. We assume this to be the result of environmental effects, i.e., as people age they accumulate mutations and this may be largely a question of environment and lifestyle.

3. The limited sequencing of mutations from twin pairs did not reveal any twin related patterns, at least at the level of resolution possible with the small numbers sequenced.

Conclusions

The ability to assay mutations in people *in vivo* is a valuable tool for studying the genetic and environmental factors that affect our genetic health. The production of mutational spectra provides a powerful tool for examining mutational responses as well as mutational mechanisms. The *hprt*, along with other future systems, are likely to be of increasing importance in monitoring human health.