Molecular Changes in the Offspring of Liquidators Who Emigrated to Israel from the Chernobyl Disaster Area

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The primary goal of this research was to reveal de novo mutations in the liquidators (cleanup personnel) who emigrated to Israel from the Chernobyl disaster area. We used genome fingerprinting simple sequence repeat-anchor polymerase chain reaction (PCR) amplification and random amplified polymorphic DNA PCR (RAPD PCR). The methodology involved a combination of RAPD PCR, polyacrylamide gel electrophoresis, and silver staining, with arbitrarily primed PCR. Use of microsatellite markers appears to be the most promising technique for high sensitivity analysis. The analysis involved DNA isolated from the blood of experimental and control subjects (including both offspring who were born before or after the disaster and their parents). Our studies have reproducibly detected new bands that appeared in the children born after the disaster. No such bands appeared in the children born in the same family before the accident or in the children of control families who had not been exposed to radiation. — Environ Health Perspect 105(Suppl 6):1479–1481 (1997)

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Introduction

The Chernobyl accident occurred in April 1986 (1,2). Radiation events such as the atomic bomb on Hiroshima and Nagasaki reportedly have not yielded genetic defects (3–5). Recently, Dubrova et al. (6) reported germine mutation at human microsatellite loci that were studied among children born in heavily polluted areas of the Mogilev district of Belarus after the Chernobyl accident. Baker et al. (7) also found high levels of genetic mutations in rodents in the vicinity of Chernobyl. Ranmaiya et al. (8) presented a comparative estimation of the frequencies of genetic disorders induced in germ cells of male mice by a single or long-term exposure to incorporated 137Cs or to external gamma radiation. The accident at the Chernobyl power plant created a different type of long-term exposure to low radiation doses (P Montague, unpublished data), although Bauchinger et al. (9) report that there is no absolute definition of a small dose of ionizing radiation. In terms of microdosimetry concepts, it can be assumed that biological effects are caused predominantly by one or two charged particles (10).

The primary goal of this research was to reveal molecular genetic changes (e.g., de novo mutations) that occurred in individuals exposed to ionizing radiation in the Chernobyl area. About 180,000 repatriates were living in the significantly contaminated region. The analysis involved DNA isolated from unrelated individuals and families (both offspring born before and after the disaster and their parents). Because of the small size of the experiment and control samples, tools enabling simultaneous testing of hundreds of genomic regions must be used. DNA fingerprinting based on microsatellite markers may be a suitable molecular genetic tool to address this problem.

Several molecular genetic methods for genome screening have been proposed for mutation detection (11,12). Genomic analysis based on polymerase chain reaction (PCR) appears to be the most promising technique for high sensitivity analysis (13). Recent advances in PCR applications make it possible to score individuals at a large number of loci. DNA fingerprinting by inter-simple sequence repeat (SSR) PCR can be used to identify the presence of the repeated elements targeted by the primers and to evaluate their distribution within different genomes. The examination of the amplification products of DNA from different individuals of the same species (including human) makes it possible to identify some individual-specific differences. These intraspecies polymorphisms open the possibility of using inter-SSR PCR as a system of multiple-locus DNA markers (14). random amplified polymorphic DNA (RAPD) is an attractive complement to the conventional DNA fingerprinting (14). RAPD includes the ease and rapidity of analysis, the use of a general set of universal primers, and a minimal amount of DNA. The profile of amplification products depends on the template–primer combination. The amplification products usually are resolved on agarose gels, whereas polymorphisms serve as dominant genetic markers, which are inherited in a Mendelian fashion (14,15). RAPD and AP PCR are not widely used in human and mammalian genome analysis. Here we report the first results of our efforts to reveal de novo mutations in the progeny of the irradiated liquidator (cleanup personnel).

Materials and Methods

Blood samples were collected from 150 unrelated liquidators and 47 individuals from 13 families of liquidators (both parents and one or two children) who are now living in Israel. For controls (not exposed to radiation) 2.5-ml blood samples were collected from three emigrant families who came from East and West Ukraine from a noncontaminated zone about 300 to 600 km from Chernobyl.
These three control families were screened with the aforementioned primers. High-quality DNA was extracted from the blood samples according to protocol of John et al. (16). Internal control within families (children born before and after the accident) guaranteed that the results obtained were reliable and allowed discrimination between somatic and germ line sources of new bands (see "Discussion").

Two PCR methods were used in our study: AP PCR and RAPD PCR. The separation of the amplification products was conducted using polyacrylamide gel electrophoresis and silver staining (SS). Band pattern was detected by SS to increase the sensitivity as compared to that of ethidium bromide staining. Various experiments were performed. In the first step approximately 400 PCR primers were screened. Three hundred of these primers were 10 nucleotides used for RAPD PCR amplifications; the remaining 100 primers were microsatellite primers used for AP PCR amplification. About 100 primers from both groups appeared to give good resolution (40–50 clear bands) and reproducibility. Dozens of RAPD reactions for AP PCR were conducted for the foregoing families in an effort to improve resolution and reproducibility.

Two RAPD PCR programs were compared in this work; they differed in denaturation time, annealing time, and temperature regime. The program profile was as follows: An initial denaturation step of 94 or 92°C was followed by 45 cycles of a) 94°C/1 min or 92°C/0.5 min, respectively, annealing; b) 37°C/1 min; and c) 42°C/1 min, with extensions of 72°C/2 min and 72°C/0.5 min. The final extension step of 72°C differed, with 2 min between the two programs.

The inter-SSR PCR (hot start) program profile involved an initial denaturation step of 94°C/6 min. Samples included a mixture of all reagents except enzyme, which was added to the reaction mix samples after 6 min denaturation. The second step included 27 cycles as follows: a) 94°C/30 sec, b) 52°C/45 sec (for most primers), c) 72°C/78 sec, 72°C/10 min.

Results

New bands were detected in some children of the irradiated families, as compared to their parents' band pattern. Only children born after the disaster manifest this pattern. In the 13 families studied (with one offspring born before the disaster and one after) new bands were detected only in the child born after the exposure of the parent to radiation. New bands were also revealed in families with only one offspring born after the disaster (Table 1). The same primers were used to screen DNA samples of the control families not exposed to radiation. In total, 37 new bands were detected in the offspring who were born in nonexposed areas (after the parents' employment as liquidators ended). No such bands appeared either in the children born in the same family before the accident or in the children of control families who had not been exposed to radiation (Figure 1). Changes in the DNA profiles were tested for reproducibility and confirmed in three independent PCR runs. The results with microsatellites show the bands detected in the children born after the explosion (Figure 2).

Discussion

Lee and Chang (13) reported that the RAPD method could be used for comparing PCR fingerprinting of humans and animals.

Table 1. Preliminary results of liquidators’ families.

| Family no | Parents' sex | Duration (days) | Offspring sex | RAPD PCR new bands, no | Inter-SSR PCR new bands, no | Dose, erg |
|-----------|--------------|----------------|--------------|------------------------|-----------------------------|-----------|
| 1         | M            | 240            | F            | 4                      | 0                           | 5         |
| 2         | M            | 10             | M            | 2                      | 1                           | 6         |
| 3         | M            | 2764           | M            | 4                      | 1                           | ND        |
| 4         | M            | 210            | F            | 2                      | 2                           | 10        |
| 5         | M            | 7              | F            | 3                      | 0                           | 5         |
| 6         | M            | 34             | M            | 6                      | 2                           | 25        |
| 7         | M            | 10             | M            | 1                      | 1                           | 5         |
| 8         | M            | 1684           | F            | 0                      | 0                           | 5         |
| 9         | F            | 210            | F            | 3                      | 0                           | 5         |
| 10        | M            | 150            | M            | 5                      | 1                           | 10        |
| 11        | M            | 150            | M            | 0                      | 0                           | 7         |
| 12        | M, F         | 2870           | F            | 6                      | 1                           | 3 (F), 2.5 (M) |
| 13        | M            | 2              | M            | 2                      | 1                           | ND        |

Total new bands 38 10

Abbreviations: F, female; M, male; ND, no data. *Dose reconstruction kindly provided by I Lichearev (Research Center for Radiation Medicine, Kiev, Ukraine). Liquidator families from Chernobyl with one child (born after the accident). These families are a part of the internal control group.

Figure 1. RAPD detection of new amplimer in experiment and control families in offspring born after the Chernobyl accident. RAPD PCR products were separated on 5% polyacrylamide (PAA) and stained with silver. C, negative control, which included all the reaction reagents except DNA. Ma corresponds to pGEM (promega) marker restricted by Hinf1, RsaI and SmaI. Lanes 1 to 4 correspond to external control family members: F, father; M, mother; DB, daughter before; DA, daughter after. Lanes 8 to 11 correspond to Chernobyl liquidator family members: DB, SA, son after, with RAPD primer OPT16; F, M. Asterisks indicate the new band that was revealed in the offspring born after the disaster. Note that no such new band appeared in his F, M, or sibling.

Figure 2. AP PCR detection of new amplimer in experiment and control families in offspring born after the Chernobyl accident. AP PCR products were separated on 5% PAA and stained with silver. Ma lane 9 corresponds to 1 kb ladder marker. Lanes 1 to 4 correspond to external control family members: F, M; DB, SA, son after, with microsatellite primer BC 867. Lanes 5 to 8 correspond to Chernobyl liquidator family members: F, M; DA, SB, son before. Asterisk indicates the new band that was revealed in the offspring born after the disaster. Note that no such new band appeared in her F, M, or sibling.
such as cows, goats, pigs, rats, dogs, rabbits, chickens, and ducks. RAPD was also analyzed in population research in Spalax blind mole rats (17,18) to compare the PCR fingerprinting of four species and 13 populations in Israel. The RAPD results obtained paralleled those of isozymes and mitochondrial DNA in their geographic patterns. All of these markers responded similarly to increasing adversity stress. The radiation exposure following the accident in the Chernobyl nuclear power station in 1986 is generally considered a low-dose exposure (2). However, it has not been convincingly demonstrated in humans that exposure to low-dose radiation could cause heritable changes in germ cells and lead to increased load of de novo mutations in the progeny. Because of low mutation rates per locus enormous sample sizes are needed to address this question when classical genetic methods are employed (6). Hence, the possibility of detection of these changes in this study was enhanced by a large number of genome sites (about 3270 in all) amplified using the selected set of about 100 PCR primers (out of 400 primers screened).

The results presented here consistently demonstrate the appearance of new bands in children born after the exposure, as compared to their siblings. In contrast to the experiment results, no new bands appeared in the control families in children born after 1986. An important question related to the interpretation of these results is the nature of the new bands. Are they a manifestation of somatic changes in the tested children or are they changes that occurred in the germline cells of the parent(s) and were transferred to the next generation through the gametes? It would be premature to argue that we have a final answer to this question. Nevertheless, our strategy in establishing the tested material, by using siblings born before and after parental exposure to radiation, allows us to assume rather confidently that the new bands are a manifestation of genetic changes in the germline. Indeed, we could take the opposite assumption—that the origin of the registered changes is somatic mutation in the tested children, e.g., because they lived in the contaminated territory. But how could we then explain the absence of such changes in their siblings born before the accident who were subjected to the same environmental conditions during the same period and even before? If environment were the inducing factor, then one would expect these children to manifest the same phenomenon as well. The fact that this is not the case supports our contention that the new bands reflect genetic changes in the germline. Moreover, this consideration explains why it was so important to use the proposed family structure for this project.

These results point to a possible influence of low-dose radiation stress on the genetic material. We increased the number of families tested and we plan to increase the number of controls. We also tested a new PCR technique, the inverse sequence-taggged repeats method in collaboration with W Rohde from Max Planck Institute in Koln, Germany (19). Future plans include identification of de novo mutations in the unrelated liquidators, using the monomorphic markers identified in previous screens. Current work includes cloning of the new bands detected in the progeny of liquidator parents, with subsequent sequencing, to assess their molecular nature.

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