type A MAO, it prevents qualitative changes (transformation) of the enzyme in hyperoxia. According to data in the literature [5], if catalase efficiency is present, marked stimulation of LPO takes place in the source of reactions catalyzed by transformed MAO. Chlorgyline, by preventing transformation of type A MAO, thereby exerts an antioxidant effect. Inhibition of MAO also reduces the formation of hydrogen peroxide, which intensifies peroxidation [13-15]. In view of the leading role of activation of LPO in the mechanism of oxygen poisoning, it can be tentatively suggested that the protective action of chlorgyline in hyperoxia is based on its antioxidant effect.

LITERATURE CITED

1. V. V. Vnukov, A. A. Krichevskaya, and A. I. Lukash, Byull. Éksp. Biol. Med., No. 6, 523 (1979).
2. I. A. Goroshinskaya and Z. G. Bronovitskaya, Izv. Severo-Kavkaz, Nauch. Tsentr., III, Estestv. Nauki, No. 3, 88 (1978).
3. I. A. Goroshinskaya, Vorp. Med. Khim., 25, 328 (1979).
4. Yu. L. Zakhar'in, Lab. Delo, No. 6, 327 (1967).
5. V. E. Kagan, A. V. Smirnov, V. M. Savov, et al., Vorp. Med. Khim., 30, 112 (1984).
6. A. V. Karakashov and E. P. Vichev, Micromethods in the Clinical Laboratory [in Russian], Sofia, Bulgaria (1973), p. 114.
7. V. G. Kolb and V. S. Kamyshnikov, A Guide to Clinical Chemistry [in Russian], Minsk (1982), pp. 202-203.
8. A. A. Pokrovskii (ed.), Biochemical Methods of Investigation in Clinical Medicine [in Russian], Moscow (1969), p. 349.
9. I. L. Balland and H. P. Koch, J. Chem. Soc., 7, 445 (1945).
10. E. G. Bligh and W. J. Dyer, Can. J. Biochem. Physiol., 37, 911 (1959).
11. A. S. Csallany and K. L. Ayaz, Lipids, 11, 412 (1976).
12. I. A. Goroshinskaya, Z. G. Bronovitskaya (Z. G. Bronovizkaya), and V. Z. Gorkin, Commun. Psychopharmacol., 1, 39 (1977).
13. J. S. Hothersall, A. L. Greenbaum, and P. McLean, J. Neurochem., 39, 1325 (1982).
14. G. R. Schacterle and R. L. Pollack. Anal. Biochem., 51, 654 (1973).
15. A. Seregi, P. Serfözö, L. Mergl, et al., J. Neurochem., 38, 20 (1982).

HIGH INTERINDIVIDUAL RESTRICTION FRAGMENT LENGTH AND COPY NUMBER OF POLYMORPHISM OF A TVRI FAMILY IN MODERATE HUMAN DNA REPEATS

E. I. Rogaev and Yu. A. Shapiro*

UDC 612.6.052.014.24:577.212.3

KEY WORDS: interindividual polymorphism; structure of genome; molecular marker

Studies of genome structure have revealed the existence of genetic elements with an increased frequency of aberrations in somatic or sex cells in some living organisms [1]. In man, these genetic elements may be families of multi-copy, dispersed DNA repeats [5-7]. However, it is impossible to establish the presence of essential interindividual polymorphism for distribution in the genome for the members of these DNA families, due to some extent to technical difficulties, caused by the high repetitiveness, but, at the same time, the dispersed distribution of these DNA families in the human genome.

An attempt was accordingly made to isolate other classes of sequences — with low or moderate copy numbers in the genome. In this paper we describe the selection of cloned human DNA sequences, with a copy number not exceeding 1000 copies per diploid genome, and their

*Deceased.

All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 57-58, January, 1987. Original article submitted May 19, 1986.

0007-4888/87/0103-0064$12.50

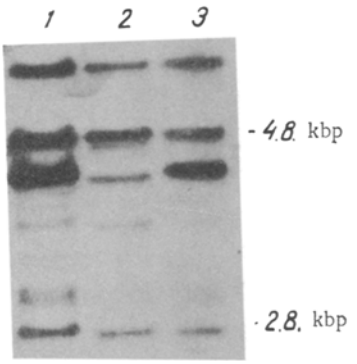

Fig. 1

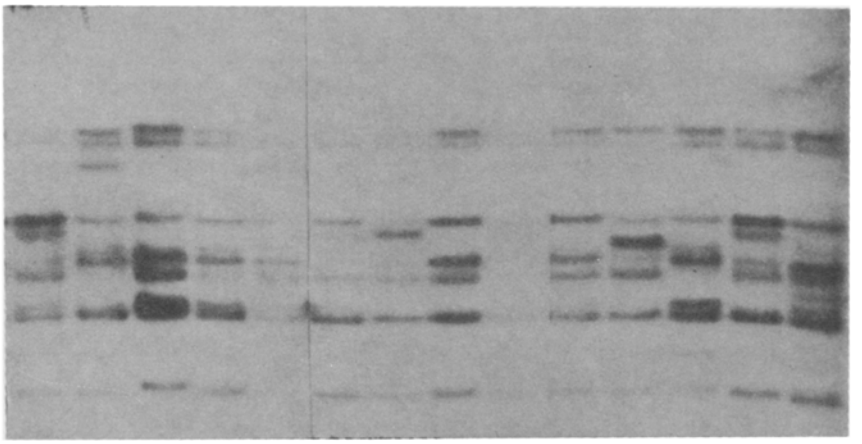

Fig. 2

Fig. 1. Autoradiograph of blot hybridization of ^{32}P-DNA pTVRI-6 with Eco81 digests of placental DNA from three individuals.

Fig. 2. Autoradiograph of blot hybridization of ^{32}P-DNA pTVRI-6 with Pst I digests and with human placental DNA. Samples of DNA from different individuals applied to each lane.

testing for interindividual restriction fragments lengths and copy number of polymorphism (RFLCP). As a result of this investigation a DNA clone was found (TVRI-6), about 2.8 kilobase-pairs (kbp) in size, for which an unusually high level of interindividual RFLCP was discovered.

EXPERIMENTAL METHOD

The TVRI-6 sequence was obtained from a bank of Pst I restriction fragments of human placental nuclear DNA, cloned in pBR 322. Nuclear DNA from various human tissues was isolated by a modified method in [2]. Plasmid DNA was obtained by the standard alkaline method. The bank was analyzed by hybridization of colonies with ^{32}P-labeled human nuclear DNA. The copy number of the sequence in the genome was determined by the dot hybridization method [4], using serial dilutions of pBR 322 DNA as the standards. Hybridization of the ^{32}P-probe with human DNA restriction fragments was carried out by the standard blot hybridization method [8].

EXPERIMENTAL RESULTS

The bank of Pst I restriction fragments of human DNA was analyzed by the "colony hybridization" method with ^{32}P-labeled human nuclear DNA. Clones not giving a signal or giving a weak signal on the autoradiograph were selected. Cloned DNA fragments were isolated from the selected clones and their size determined. The copy number of the isolated DNA fragments in the human genome was next estimated more accurately by the method of dot-blot hybridization with a Pst I restriction digest of total human DNA.

As a result of these procedures DNA fragments whose copy number did not exceed 1000 copies per diploid genome, and giving a pattern in the form of several discrete bands on blot hybridization with a Pst I digest of total human DNA, were selected. Each of these DNA fragments was labeled with ^{32}P isotopes and hybridized with Msp I digest of lymphotytic DNA from four individuals. One clone, called TVRI-6, was hybridized with restriction fragments. Some of them were specific in length and copy number for each of the four individuals. Specificity of this kind also was found on hybridization of TVRI-6 with other digests of total DNA from different individuals, for example, an Eco81 digest (Fig. 1).

On comparison of Pst I digests of placental DNA from a large number of individuals it was found that all the individuals tested differed in the length and copy number of their restriction fragments (Fig. 2). This unusually high level of interindividual polymorphism can evidently be attributed to the high frequency of structural changes in the TVRI family of DNA repeats. These structural changes are probably not strictly specific for particular so-

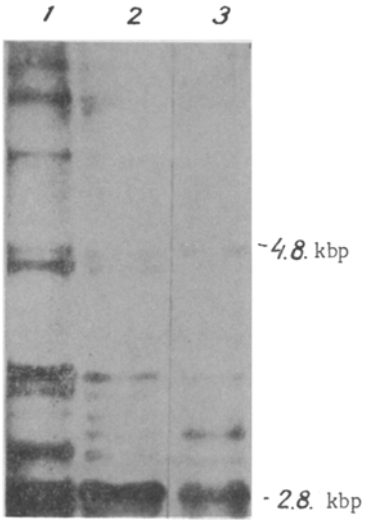

Fig. 3. Autoradiograph of blot hybridization of ^{32}P-pTVRI-6 with Pst I digests of different tissues from three different individuals. 1) Placental DNA; 2) spermatozoal DNA; 3) DNA from brain of a 15-week embryo.

matic cells of any given tissue, like structural changes in immunoglobulin genes during maturation of lymphocytes [3], since RFLCP is discovered both when DNA samples from the same tissues of different individuals (lymphocytes and placentas) and when different tissues from different individuals (Fig. 3) are compared.

In agreement with the experimental data a family of moderate DNA repeats with high interindividual polymorphism for structural organization was found in the human genome. By using a member of this family, namely the cloned TVRI-6 sequence, interindividual differences can be identified highly effectively in human DNA samples from different individuals. This is evidence that the TVRI-6 sequence can be used as a molecular marker for determining the zygosity of twins, for resolving disputed cases of paternity and maternity, and even in criminology.

LITERATURE CITED

1. R. B. Khesin, Inconstancy of the Genome [in Russian], Moscow (1984), p. 6.
2. N. Blin and D. W. Stafford, Nucleic Acids Res., 3, 2303 (1976).
3. D. I. Bentley, Nature, 307, 77 (1984).
4. F. C. Kafatos, C. W. Jones, and A. Efstratiadis, Nucleic Acids Res., 7, 1541 (1971).
5. I. Pan, I. T. Elder, C. H. Duncan, and S. M. Weissmann, Nucleic Acids Res., 9, 1151 (1982).
6. K. E. Paulson, D. Niren, C. W. Schmid, et al., Nature, 316, 359 (1985).
7. S. S. Potter, Proc. Natl. Acad. Sci. USA Biol. Sci., 81, 1012 (1984).
8. E. M. I. Southern, J. Mol. Biol., 98, 503 (1975).