where J_A is the one-way flux (in moles per square centimeter per second), $^{14}C^{T}$ is the amount of tracer (in counts per minute) entering the front compartment during the sampling time t (in seconds), A is the surface area of the membrane (in square centimeters), and SA^{R} is the specific activity of tracer in the rear compartment (in counts per minute per mole).

11. T. E. Andreoli, V. W. Dennis, A. M. Weigl, *J. Gen. Physiol.* **53**, 133 (1969); R. Holz and A. Finkelstein, *ibid.* **56**, 125 (1970); O. H. LeBlanc, Jr., *Biochim. Biophys. Acta* **193**, 350 (1969).
12. From Fick's first law

$$J_{HA} = D_{HA}[HA]/\delta$$

we estimated the rate of diffusion of $10^{-4}M$ HA across the unstirred layer of water 10^{-2} cm thick to be about 8×10^{-11} mole cm^{-2} sec^{-1}. The diffusion coefficient for HA in water is 8×10^{-6} cm^2 sec^{-1}.
13. The membrane resistance in the presence of high concentrations of A$^-$ and HA was 10 to 50 times lower than the control level, which ranged from 10^7 to 10^8 ohm-cm^2. We will not discuss in detail these changes in membrane resistance, because in all of our experiments 99.9 percent of the one-way flux or net flux, or both, was electrically silent. The effect of salicylates on the electrical properties of phospholipid bilayers has been discussed by S. McLaughlin [*Nature* **243**, 234 (1973)].
14. The appropriate equation is

$$J_{A^-} = \frac{R\,T}{Z^2\,F^2} G_{A^-}$$

where J_{A^-} is the one-way flux of salicylate ions, R is the gas constant, T is the absolute temperature, Z is the ionic valence, F is the faraday, and G_{A^-} is the conductance of the membrane to A$^-$. Because the total membrane resistance was always greater than 10^5 ohm-cm^2, the upper limit for G_{A^-} is $< 10^{-5}$ mho cm^{-2} and the upper limit for J_{A^-} (or $J_{HA_2^-}$) is $< 2.5 \times 10^{-12}$ mole cm^{-2} sec^{-1} [see A. L. Hodgkin, *Biol. Rev. (Cambridge)* **26**, 339 (1951)]. A second argument against the possibility of an ionic diffusion process is that an applied membrane voltage of +50 mv had no effect on the one-way flux.
15. However, if *both* the ionic and nonionic forms of the weak acid are highly permeant, then the electrical properties of the membrane may be very dependent upon chemical reactions within the unstirred layers [see, for example, LeBlanc (*3*) and B. Neumcke, *T.I.T. J. Life Sci.* **1**, 85 (1971)].
16. J. Gutknecht, L. J. Bruner, D. C. Tosteson, *J. Gen. Physiol.* **59**, 486 (1972).
17. From electrostatic considerations it is not surprising that the permeability to the neutral species (HA) is orders of magnitude higher than the permeability to the charged species (A$^-$) [see Finkelstein and Cass (*2*)].
18. Our model also provides a reasonable explanation for some "anomalous" observations on the diffusion of labeled weak acids across an amphibian epithelium. H. Rosen, A. Leaf, and W. Schwartz [*J. Gen. Physiol.* **48**, 379 (1964)] found that the permeability coefficients of nonionic forms of lipophilic species appear to increase markedly with increasing *p*H. However, as the *p*H increases, the ratio [A$^-$]/[HA] increases, and A$^-$ provides a parallel pathway for tracer diffusion through the unstirred layers. Thus the permeability coefficient for HA appears to increase with increasing *p*H, whereas the true permeability coefficient for HA remains constant.
19. We assumed, as before, that the reacting species are in chemical equilibrium throughout the bulk solutions and unstirred layers. The values that we used for the permeability coefficients (in centimeters per second) were as follows:

$$P^{M}_{HA} = 0.7,\ 2P^{UL}_{A^-} = 2P^{UL}_{HA} = 1.2 \times 10^{-3},$$

and

$$2P^{UL}_{H^+} = 1.3 \times 10^{-2}.$$

The factor 2 is used because in this case we consider each unstirred layer separately.
20. Supported by PHS grant HE 12157. We thank D. Hastings, J. Graves, and D. Benos for constructive comments.
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6 March 1973; revised 24 August 1973

Mapping Human Autosomes: Assignment of the MN Locus to a Specific Segment in the Long Arm of Chromosome No. 2

Abstract. *The locus for MN blood groups,* MN, *is tentatively assigned to a specific region near the centromere of the long arm of chromosome No. 2 on the basis of a demonstrable deletion of band 2q14 from a No. 2 chromosome in a boy previously reported to be hemizygous* (M/−) *at that locus.*

A mildly retarded boy with microcephaly, facial dysmorphia, and an abnormal gait was reported in 1968 by German, Walker, Stiefel, and Allen (*1*), with evidence (*2*) that the MN blood group locus of only one chromosome was being expressed. The father's blood type was N, but the child's was M. Dosage tests with antiserums to M and N (anti-M and anti-N) suggested that the boy was not homozygous for *M*; the father, however, gave double-dose reactions with antiserum to N, confirming that he was homozygous (*N/N*). (The mother and both sibs of the propositus, who were type MN, gave single-dose reactions with anti-M and anti-N). The child, therefore, appeared to be hemizygous at the *MN* locus, or to have a condition that could not be distinguished from hemizygosity by serological methods.

The child's blood lymphocytes had a unique chromosome complement, in which a reciprocal translocation had occurred between the long arm of a chromosome No. 2 and the long arm of a No. 4; by the nomenclature of the Chicago Conference (*3*) his complement was 46,XV,t(2q−;4q+). Each of the 70 metaphase cells examined had the translocation, and no evidence of mosaicism was found. The rearrangement was considered to have occurred de nova, because the complements of the child's parents were normal. An autoradiographic study of the translocation made when it was first detected showed that the breaks, at least one in each chromosome, had occurred near the middle of 2q and near the end of 4q. Measurements of the affected chromosome segments suggested that a short segment of chromosome had been lost during the rearrangement (*2*). The maldevelopment of the child supported the conclusion that the translocation was not completely balanced, that is, that either deficiency, duplication, or both had occurred; but, position effect was considered to be another possible explanation. Similarly, loss of a segment of chromatin seemed to be the most likely explanation for the child's hemizygosity (*M/−*), but translocation of the gene to a new position near heterochromatin (leading to inactivation) was also a reasonable possibility, especially because 4q consists of prominently late-replicating chromatin.

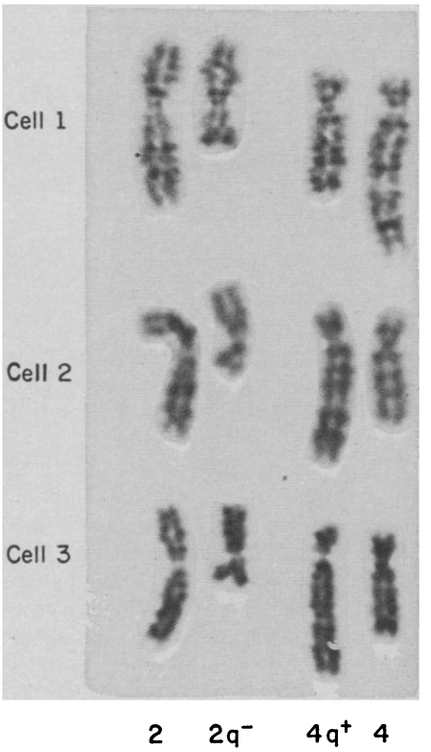

Fig. 1. G-banding of the two chromosomes No. 2 (normal and rearranged) and the two chromosomes No. 4 (normal and rearranged) in three cells.

The observations reported suggested to us that *MN* is normally located in either the 2q or the 4q, but neither confirmatory nor contradictory reports have appeared since our report. [Blood grouping in one other patient studied in our laboratory (*1*) suggested that the locus was not in the 4q.]

A fibroblast cell line, identified as HG 406, had been derived from a fragment of skin taken from the patient in 1968 and has been maintained since in liquid nitrogen. It has now been activated, and the translocation has been characterized by G-banding techniques (*4*, *5*) with the use of cells harvested at the tenth subculture generation.

We analyzed 57 cells with chromosomes showing good band patterning.

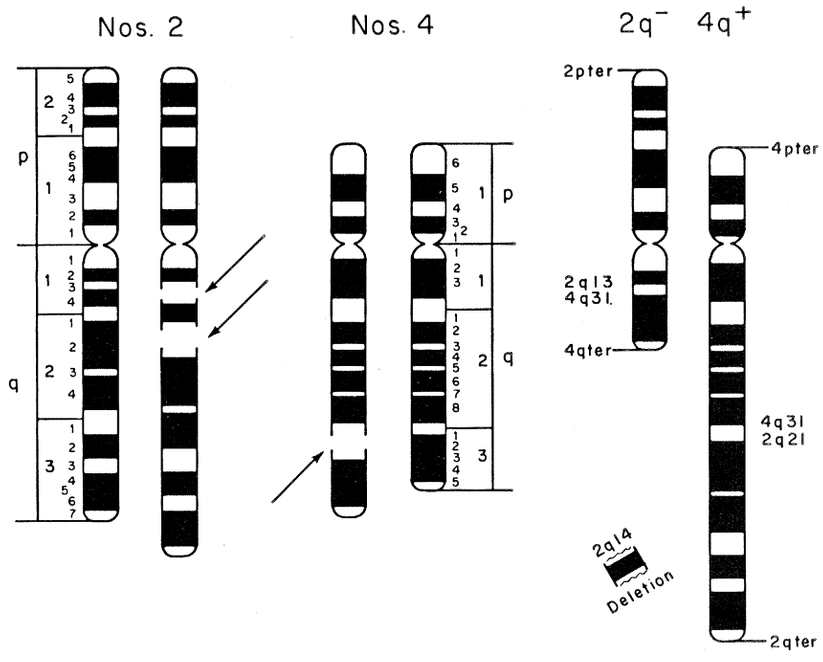

Fig. 2. The translocation in terms of the Paris Conference nomenclature (*5*). The chromosomes from left to right show: normal band pattern of chromosome No. 2; break points in chromosome No. 2 (q13 and q21); break point in chromosome No. 4 (q31); normal band pattern of chromosome No. 4; rearranged chromosome No. 2; and rearranged chromosome No. 4. The band pattern in the affected No. 2 was intact from the distal end of its short arm down past the centromere to a band in the proximal portion of the long arm (2q13). Beginning at that point, and continuing to the end of the chromosome, the band pattern was that of the distal end of the normal 4q, from band 4q31. The band pattern in the affected No. 4 was intact from the distal end of its short arm down past the centromere to the 4q31 band, near the end of the long arm. Beginning at that point and continuing to the end of this very long chromosome, the band pattern was that of the normal 2q, not from band 2q13 but from band 2q21. Thus, the segment of 2q between bands 2q13 and 2q21 was represented in neither aberrant chromosome; the segment lost comprised approximately band 2q14.

The chromosome complement of each of these included the two translocation chromosomes, 2q− and 4q+ (*6*). In 30 of the 57 cells, both the band patterns and the morphology of both the normal and abnormal No. 2 and No. 4 were excellent and permitted careful perusal of the patterns and measurement of the lengths of the relevant arms: 2q, 2q−, 4q, and 4q+.

The band patterns indicated that there were two breaks in 2q (in band 2q13 and band 2q21) whereas there was one break in 4q [in band 4q31 (*5*) (Figs. 1 and 2)]. The segment of 2q distal to the break in band 2q21 was translocated to 4q at its break point (band 4q31), while the segment of 4q distal to the break (in band 4q31) was translocated to the break in 2q at band 2q13. A segment of 2q approximately equal to band 2q14 was lost in the translocation.

The lengths of the long arms of the unaffected homologs, that is, 2q and 4q, were measured and added together, as were those of the long arms of the affected 2q− and 4q+. In 22 cells, (2q plus 4q) was greater than (2q− plus 4q+); in 5 cells, it was less; and in 3 cells, it was the same. Pooled measurements from all 30 cells indicated a deficiency in the length of (2q− plus 4q+), a deficiency equivalent to 12.8 percent of the length of the normal long arm of chromosome No. 2.

Thus, the banding patterns and the measurement of the affected chromosome arms reinforce one another in showing that a segment of the long arm of chromosome No. 2 was lost in the translocation. According to the nomenclature of the Paris Conference (*5*), our patient's chromosome complement is 46,XY,t(2;4) (2pter → 2q13:: 4q31 → 4qter; 4pter → 4q31::2q21 → 2qter). The cytogenetic observations and our interpretation of them are summarized in Fig. 1.

These banding studies, which supplement the previous autoradiographic studies, allow correlation of the loss of function of a genetic determinant with the loss of a short segment of an autosome. Thus, we tentatively assign *MN* to the area of band q14 in the proximal portion of the long arm of chromosome No. 2. Confirmation of this assignment may require the discovery of the appropriate chromosome aberration in another person from a family showing the appropriate segregation at this locus. Alternatively, it might eventually be possible to map a locus which is closely linked to *MN* (and thus *MN* also) with the use of cell hybridization techniques, or to map the DNA sequence of *MN* by in situ molecular hybridization.

Deletion mapping of chromosomes by means of phenotype-karyotype correlations is a method that has held great promise for gene assignment in man ever since the first human chromosome rearrangement was reported (*7*). Yet, it has been surprisingly and disappointingly nonproductive, and *MN* represents the first locus to be assigned to a well-defined autosomal region by this approach.

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5. Paris Conference (1971): Standardization in Human Cytogenetics, *Birth Defects: Original Article Series* (National Foundation, New York, 1972), vol. 8, No. 7.
6. In 6 of the 57 dividing fibroblasts available for study, an additional (and unexpected) structural aberration was found. In each cell, the No. 4 not involved in the translocation was deficient of almost its entire short arm. The significance of this deletion is unknown and, since it was not detected in lymphocytes, conceivably arose in vitro. That it is not of importance in relation to the lost *MN* allele in this case is suggested by the following evidence which indicates that *MN* is not on 4p. One patient of ours with the 4p— syndrome was blood type N_1 and quantitative studies showed that he is homozygous at the *MN* locus. In addition, R. A. Pfeiffer [*Z. Kinderheilk.* **102**, 49 (1968)] reported heterozygosity at this locus in a patient with the 4p— syndrome.
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8. We thank J. Fleming for expert technical assistance. Supported by PHS grants HD04134 and HL09011 and by a grant from the American Cancer Society.

27 August 1973