8. ——, *Pfluegers Arch.* **376**, 223 (1978).
9. H.-G. Zimmer and H. Ibel, *Experientia* **35**, 510 (1979).
10. E. Gerlach, B. Deuticke, R. H. Dreisbach, *Pfluegers Arch.* **278**, 296 (1963).
11. H.-G. Zimmer, C. Trendelenburg, H. Kammermeier, E. Gerlach, *Circ. Res.* **32**, 635 (1973).
12. W. Isselhard, D. H. Hinzen, E. Geppert, W. Mäurer, *Pfluegers Arch.* **320**, 195 (1970).
13. R. B. Jennings, H. K. Hawkins, J. E. Lowe, M. L. Hill, S. Klotman, K. A. Reimer, *Am. J. Pathol.* **92**, 187 (1978); J. Schaper, J. Mulch, B. Winkler, W. Schaper, *J. Mol. Cell. Cardiol.* **11**, 521 (1979).
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Origin of chi46,XX/46,XY Chimerism in a Human True Hermaphrodite

Abstract. *Using chromosome heteromorphisms and blood cell types as genetic markers, we demonstrated chimerism in a chi46,XX/46,XY true hermaphrodite. The pattern of inheritance of the chromosome heteromorphisms indicates that this individual was probably conceived by the fertilization, by two different spermatozoa, of an ovum and the second meiotic division polar body derived from the ovum and subsequent fusion of the two zygotes. This conclusion is based on the identification of the same maternal chromosomes 13, 16, and 21 in both the 46,XX and 46,XY cells of the patient. In the two cell lines of the chimera, chromosomal markers showed different paternal No. 9 chromosomes and sex chromosomes, as well as the same paternal chromosome 22.*

A chimera is an individual with two or more genetic cell types resulting from the fusion of different zygotes (*1*). Chimeras can be readily classified as whole-body or partial chimeras according to their mode of origin. Partial chimeras can arise by placental cross-fertilization between dizygotic twins, maternal-fetal transplacental exchange, transfusions, or grafting (*2, 3*). Because of lack of suitable studies, the origin of whole-body chimeras is less clear. Theoretically, they can arise by (i) early fusion of different embryos, (ii) fertilization of an ovum and any polar body by two different sperm and subsequent fusion of the zygotes, (iii) fertilization of a haploid ovum or polar body and subsequent fusion with a diploid polar body or ovum, or (iv) fusion of a diploid sperm with an embryo (*2*).

The origin of whole-body chimeras may be learned by comparing markers between the cell lines of the chimera and those of the parents and explaining their presence by oogenesis and spermatogenesis. Because most chimeras have been investigated with use of genetic markers that frequently cross over in meiosis, such as blood groups and cell types, and most cytogenetic studies have been done with nonbanding techniques, the specific origin for any whole-body chimera has not been established (*3, 4*). Almost all cases have had the karyotype chi46,XX/46,XY, but some diploid/triploid individuals may also be chimeras (*5, 6*). Thus, most reported chimeras have sexual abnormalities such as clitoral hypertrophy or true hermaphroditism. Chimeras of the type chiXX/XX or chiXY/XY probably exist but are clinically normal and only fortuitously discovered (*7*).

By using banding techniques cytogeneticists have demonstrated that there is considerable interindividual heteromorphism of satellites and certain bands among human chromosomes (*8*). These heteromorphisms are inherited in a Mendelian fashion, and meiotic crossing-over rarely occurs (*9*). Using chromosome heteromorphisms, we studied a true hermaphrodite who probably arose by fertilization of an ovum and its second meiotic division polar body.

Shortly after birth, this patient was referred for chromosome analysis because of ambiguous genitalia. Genital abnormalities included a prominent phallus, scrotalization of the labial-scrotal swelling, slight posterior fusion of the labia minora, and a single orifice at the base of the phallus. The plasma, electrolytes, and glucose were normal. The concentrations of 17-hydroxyprogesterone in the plasma and 17-ketosteroid and 17-ketogenic steroids in the urine were normal. Pregnanetriol was undetectable. At laparotomy, a rudimentary unicornate uterus, fallopian tubes, and a streaklike gonad containing oocytes were found on the left side and removed. On the right side, a fetal testis, vas deferens, and epididymis were found and removed. Thus, this individual showed characteristics of a true hermaphrodite. Clitoral reduction was performed.

For the cytogenetic studies we used GTG-, QFQ-, and C-banding techniques on cultures of peripheral blood lymphocytes and biopsy specimens of the skin, fetal testis, and ovarian gonad from the patient and on peripheral blood specimens of the parents(*10*). All tissues from the patient showed both 46,XX and 46,XY cells (Table 1). Heteromorphisms of chromosomes 9, 13, 16, 21, and 22, as well as the Y chromosome, proved to be informative (Fig. 1). One of the paternal No. 9 chromosomes stands out because its centromere is located so that about one-third of the C band is on the p arm and about two-thirds is on the q arm. One maternal chromosome 16 is different because it has a larger C band than its own homolog or either of the paternal No. 16 chromosomes.

The satellites on the paternal No. 13 chromosomes are similar to one another but distinct from those of the mother: their stalks are very short, with relatively dull fluorescent terminal chromatin, and there is a bright QFQ band at their bases. One of the maternal No. 13 chromosomes has satellites with relatively longer stalks and a larger terminal chromatin mass than the other. One maternal chromosome 21 is conspicuous because of rather large, bright fluorescent satellites. Its homolog has just a faint satellite. One of the paternal No. 21 chromosomes has relatively large satellites, but it does not fluoresce as brightly as the conspicuous maternal chromosome 21. The other paternal chromosome 21 has small satellites. One paternal chromosome 22 is distinguished by large, bright fluorescent satellites. Its homolog has small satellites, and the maternal No. 22 chromosomes have even less satellite material.

In the XY line of the chimera, the Y chromosome and chromosomes 13 and 21 are of paternal origin and the homologs are of maternal origin. In the XX line, the paternal markers 9, 13, and 21 can be identified, as can the maternal markers 13 and 21. The maternal marker 16 and paternal marker 22 are not evident in either of the cell lines of the patient.

We performed ABO, Rh, MNSs, Duffy, Kidd, and lymphocyte HLA typing concomitantly on cells from the patient and the parents with use of the same serum and incorporation of appropriate positive and negative controls. Of these, only the Kidd system proved informative. Five different serum samples were used for the Jk^a (Kidd antigen) typings, and results were confirmed twice. All red cell typings were examined microscopically for the presence of "free" or unagglutinated cells mixed with large agglutinates (so-called mixed-field typing). In the Jk^a typing of the patient's cells, virtually the same degree of mixed-field

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typing was seen with each of the five serum samples. About 70 to 80 percent of cells were negative for Jk^a and 20 to 30 percent were positive. It is interesting that this is similar to the ratio of XX (77 percent) to XY (23 percent) cells in this patient's lymphocytes.

The paternal Y chromosome and marker 9 are present in only one of the cell lines of the patient, and there are two blood-cell populations as shown by the Kidd system. This establishes chimerism in this individual. Mosaicism is unlikely because there are too many differences between the cell lines. Since there is a paternal and a maternal haploid set of chromosomes in each of the cell lines of the chimera, there were apparently two separate fertilization events. Evidently, an egg and a polar body from the first meiotic division were not involved because the maternal marker 16 was not present in either of the chimera's cell lines. Instead, its homolog was present in each cell line. If the ovum and second meiotic division polar body derived from the ovum were each fertilized by a different sperm, then the same maternal markers should be present in both cell lines of the chimera. This interpretation is consistent with the cytogenetic findings, inasmuch as the same maternal chromosomes 13, 16, and 21 are present in both the XX and XY cell lines. The same findings could occur if two different ova were involved, but since this would involve two independent gametogenic processes, there is a 1-in-2 chance that each cell line would contain any one of the markers. There would be a 1-in-8 chance that the same three maternal markers would be present in both cell lines.

Table 1. Frequency of XX and XY cells in peripheral blood and fibroblast cultures of various tissues from a chimera.

Tissue	46,XX (%)	46,XY (%)	Cells counted (*N*)
Lymphocyte	77	23	100
Skin	51	49	50
Right gonad*	69	31	100
Left gonad†	83	17	100

*Fetal testis. †Streaklike gonad with oocytes.

The paternal cytogenetic data indicate that two different spermatozoa were involved. It would not be possible for two sperm of the same gametogenic process to contain different No. 9 markers and sex chromosomes and at the same time have the same chromosome 22. It follows that since the paternal marker 22 was not present in either cell line of the chimera, its homolog must have been present in each cell line. This combination of paternal chromosomes could occur only by random chromosome segregation, which occurs when different gametogenic processes are involved. It is not surprising that two different sperm were involved, considering the large number of sperm in an ejaculate.

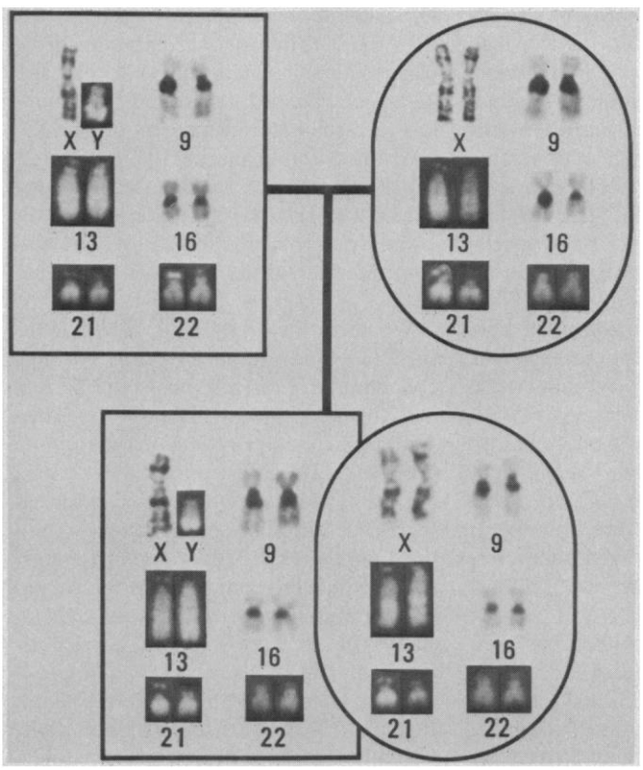

Fig. 1. Representative marker chromosomes from the father (rectangle), mother (oval), and chimera (rectangle merged with oval). For convenience, wherever clear identification was possible, the paternal marker chromosomes of the patient were placed as the right member of the homologous pair of chromosomes. See text for description of markers.

We are not aware of any report of a whole-body chimera in which there has been a sufficient number of genetic markers to demonstrate conclusively the specific mechanism by which the individual arose. Specific mechanisms have been suggested in two cases studied by blood typing (*11*, *12*), but these genetic markers are subject to meiotic crossing-over and the results are not conclusive. De La Chapelle *et al.* (*13*) identified one paternal and maternal marker in each of the cell lines of a chimera; they suggested that the chimera originated from fusion of two embryos. However, this also could result from fertilization, by two different sperm, of an ovum and a first-division polar body. More recently, Fitzgerald *et al.* (*14*) identified two maternal chromosome heteromorphisms that were both present in each cell line of a chimera. They suggested that this could occur by fertilization of the ovum and its second meiotic division polar body but that the fertilization and fusion of two ova could produce similar results. The fusion of two embryos requires double ovulation, fertilization of both ova, and then the coming together and merging of two embryos. Any one of these events by itself would be relatively uncommon, and the possibility that all three events would occur together is extremely remote.

We favor the interpretation that the chimera described here arose by fertilization of an ovum by one sperm and fertilization of the polar body produced by the second meiotic division of the ovum by a second sperm. This would explain the presence of the same three maternal markers in both cell lines. Also, this interpretation requires fewer biological coincidences. The ovum second meiotic division polar body is produced at the time of fertilization and comes to lie adjacent to the ovum in the perivitelline space between the ovum membrane and zona pellucida (*15*). If a sperm were in this space at the time the ovum was fertilized, it could readily fertilize any polar body. Because of their proximity to each other, there is ample opportunity for these zygotes, or any cells derived from them, to mingle. Theoretically, if two sperm fertilized a first meiotic division polar body and its second-division polar body, then two zygotes could also be produced with the same maternal markers. Nevertheless, the occurrence of this seems unlikely, inasmuch as neither cell line would have sufficient cytoplasmic nutrients to sustain the embryo through the early stages of embryogenesis. Because previous reports of chimeras with chromosome heteromorphisms can be

explained by fertilization of a polar body and ovum, and because fewer embryologic processes are involved in this method, it may be that most chimeras are conceived by this mechanism.

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References and Notes

1. D. Anderson, R. E. Billingham, G. H. Lampkin, P. B. Medwar, *Heredity* **5**, 379 (1951).
2. C. E. Ford, *Br. Med. Bull.* **25**, 104 (1969).
3. R. R. Race and R. Sanger, *Blood Groups in Man* (Blackwell, Oxford, ed.6, 1975).
4. K. Benirschke, *Excerpta Med. Int. Congr. Ser. No. 250* (1971), p. 212.
5. W. Schmid and D. Vischer, *Cytogenetics (Basel)* **6**, 145 (1967).
6. G. Dewald *et al.*, *Clin. Genet.* **8**, 149 (1975).
7. W. R. Mayr, V. Pausch, W. Schnedl, *Nature (London)* **277**, 210 (1979).
8. Paris Conference (1971), Supplement (1975), *Birth Defects Orig. Artic. Ser.* **11** (No. 9), 1 (1975).
9. P. A. Jacobs, *Prog. Med. Genet. (n.s.)* **2**, 251 (1977).
10. G. Dewald, in *Current Methods of Autopsy Practice*, J. Ludwig, Ed. (Saunders, Philadelphia, ed. 2, 1979), p. 155.
11. S. M. Gartler, S. H. Waxman, E. Giblett, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 332 (1962).
12. W. W. Zuelzer, K. M. Beattie, L. E. Reisman, *Am. J. Hum. Genet.* **16**, 38 (1964).
13. A. De La Chapelle, J. Schröder, P. Rantanen, B. Thomasson, M. Niemi, A. Tilikainen, R. Sanger, E. B. Robson, *Ann. Hum. Genet.* **38**, 63 (1974).
14. P. H. Fitzgerald, R. A. Donald, R. L. Kirk, *Clin. Genet.* **15**, 89 (1979).
15. J. Langman, *Medical Embryology: Human Development—Normal and Abnormal* (Williams & Wilkins, Baltimore, ed. 3, 1975).
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Stereopsis in Human Infants

Abstract. *Stereoscopic depth perception was tested in human infants by a new method based on attracting the infant's attention through movement of a stereoscopic contour formed from a dynamic random-element stereogram. The results reveal that stereopsis emerges at 3 1/2 to 6 months of age, an outcome consistent with evidence for rapid postnatal development of the visual system.*

Although stereoscopic depth perception is typically present in adults, little is known about its ontogenetic development. Despite great interest, efforts to investigate stereopsis in infants have encountered formidable difficulties posed by labile attention and limited response repertoire. As a consequence, the results have been inconclusive (*1*). We now report an investigation of stereopsis in infants based on a new method that involves engaging an infant's attention through the apparent motion in visual space of a stereoscopic form contained within a random-element stereogram.

Random-element stereograms, unlike conventional ones, contain no discrete contours or other monocular cues. Only viewers with stereopsis can perceive stereoscopic contours—without stereopsis only a random distribution of minute dots or elements is perceived (*2*). The stereogram display we used consists of a large array of red and green dots generated on a projection-type color television receiver. The red and green dots stimulate separate eyes when the array is viewed through red and green filters; this is the well-established anaglyph method of stereoscopic presentation. All stereogram dots are replaced randomly 60 times per second, which produces apparent random motion of individual dots but does not impair perceptibility of the stereoscopic form, at least for normal adults; however, it does camouflage local changes in dots that, under static conditions, might serve as a nonstereoscopic cue to changes in position of the stereoscopic form (*3*).

We used the capability of the stereogram generation system to produce moving stereoscopic forms in order to exploit the tendency of infants to track moving objects visually. Visual tracking of a random-element stereoscopic form would be compelling evidence of the possession of stereopsis, since stereopsis is a precondition for the perception of such a form. In the testing procedure, each infant was held by a parent approximately 130 cm in front of a large rear-projection screen upon which the stereogram was displayed. A spectacle frame containing one red and one green filter (Wratten 29 and 58) was placed on the infant's face. The stereoscopic form, a 10° by 15° vertically oriented rectangle, was positioned in the center of the screen at the beginning of each stereoscopic test trial (*4*). Whenever the infant's attention appeared to be directed toward the center of the screen, a concealed observer signaled the operator of the stereogram generator. The operator then moved the form laterally, left or right, in accord with a random schedule unknown to the observer. After 2 seconds, the operator returned the form to screen center and signaled the observer to make a forced-choice judgment as to the direction of form movement, a judgment based solely on the infant's visual behavior (*5*). To minimize bias, the observer received no feedback from the operator about the direction of movement, the operator received no feedback about the observer's judgment, and the parent could not detect the location of the stereoscopic form.

Before starting the stereoscopic test trials, the attentive state of each infant was assessed by a series of trials in which a physical analog of the stereoscopic form was the stimulus (*6*). If the observer could correctly detect direction of movement of the physical form at least 75 percent of the time, the infant was deemed suitable for stereoscopic testing. For stereoscopic trials the infants were tested for 40 trials, or fewer if they became uncooperative. Infants were excluded from the data analysis if they were not attentive for at least the first ten stereoscopic trials (*7*).

In experiment 1, infants were recruited to form three age groups—2 1/2, 3 1/2, and 4 1/2 months (*8*) (Fig. 1A). Performance of the 2 1/2-month group did not differ from chance [t (14) = 1.79, $P > .10$]. Performance was greater than chance for both the 3 1/2-month group [t (14) = 5.02, $P < .001$] and the 4 1/2-month group [t (9) = 11.61, $P < .001$]. The age trend across groups was significant [F (2, 37) = 8.37, $P < .0013$].

The performance of the older infants strongly suggests that they possess stereopsis, since the random-element stereogram prevents the use of nonstereoscopic cues. It is logically possible, however, that the lateral position of the form was detected even though it was not perceived in depth. To check on this we ran a second experiment with five disparity values, including two very large values that exceed adult fusional limits and do not induce stereopsis. Seventeen infants were tested (seven at 3 1/2 months and ten at 4 1/2 months); ten infants had served in experiment 1 (Fig. 2). The above-chance performance on the two intermediate disparities, 45 minutes [t (13) = 3.12, $P < .01$] and 134 minutes [t (15) = 7.21, $P < .001$], and the chance performance on the two largest disparities, 313 and 447 minutes [combined t (7) = −2.34, $P > .05$], suggest that performance is related to the stereoscopic depth position of the form, a result con-

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