Am. J. Hum. Genet. 49:120–130, 1991

Twinning and Mitotic Crossing-over: Some Possibilities and Their Implications

Gilbert B. Côté and Jolanda Gyftodimou

Genetics Division, Institute of Child Health, Athens

Summary

Mitotic crossing-over does occur in man and is much more frequent and important than generally assumed. Its postzygotic occurrence before an embryo differentiates into MZ twins is theoretically predicted to have disrupting effects on genomic imprinting and cis-acting sequences, with consequences ranging from early lethality to MZ twin discordance. Some predictions are at odds with classical views on twinning and include a high discordance rate of MZ twins for some genetic diseases. A review of MZ twin discordance and an attempt at explaining some of the data lead one to hypothesize both the existence of a sex difference in the rate of mitotic crossing-over and the impossibility for crossed X chromosomes to undergo inactivation. The close interrelationship of twinning and midline malformations further suggests a major role of mitotic crossing-over in the induction of the twinning process itself. The model can be tested with molecular methods and provides a new approach for the gene mapping of so-called multifactorial diseases and of rarer disorders with apparently irregular inheritance.

Introduction

In his admirable editorial essay on human twinning, Boklage (1987) affirms that much of current twin theory is based on erroneous assumptions. He remarks that, contrary to generally held beliefs, several developmental aberrations have an increased incidence in both MZ *and* DZ twins, as well as in their maternal *and* paternal relatives; moreover, infant mortality in like-sex DZ twins is twice that in unlike-sex twins and may even exceed that of MZ twins. This implies that unlike-sex twins are not representative of all DZ twins as is usually assumed, that MZ and DZ twinning processes share part of their causes, and that fathers must also contribute to the DZ twinning process.

The following discussion offers a solution to several apparent paradoxes brought to light by the reappraisal of twin data, if one is ready to abandon even more basic assumptions. The argumentation stems from a theoretical study of the possible pathological consequences of meiotic and mitotic crossing-over in man (Côté 1989*a*, 1989*b*, 1990). It is extended and applied to several types of abnormalities presenting with various modes of inheritance, with special reference to mitotic crossing-over occurring prior to differentiation of the embryo into MZ twins.

Received October 10, 1990; revision received February 20, 1991.

Address for correspondence and reprints: Gilbert B. Côté, Department of Genetics, Sudbury General Hospital, 700 Paris Street, Sudbury, Ontario P3E 3B5 Canada.

0002-9297/91/4901-0015$02.00

Mitotic Crossing-over in Man

The occurrence of mitotic crossing-over in man is well documented (Craig-Holmes et al. 1975; Passarge and Bartram 1976; Therman and Kuhn 1976, 1981; Kurnit 1979; Raizis et al. 1985; Kipps and Herzenberg 1986; Dao et al. 1987; Henry et al. 1987*a*, 1989; Sarto et al. 1987; Groden et al. 1988; Morley et al. 1990). Its chromosomal consequences are also well known (Stern 1936; Pontecorvo and Käfer 1958; Passarge and Bartram 1976; Therman and Kuhn 1976; Chandley 1989). Most important, a cis-trans change of configuration always occurs. The ensuing random chromosomal segregation has two equiprobable outcomes: In half of all cases each daughter cell inherits one crossed and one uncrossed chromosome, with consequent uniparental homozygosity at all loci distal

to the point of exchange. This is detectable as a loss of heterozygosity (LOH) when the distal genes happen to be heterozygous in the mother cell. In the other half of cases, there is no uniparental homozygosity; one daughter cell inherits both crossed chromosomes while the other is identical to the mother cell with two uncrossed chromosomes.

Experience with cancer shows that mitotic crossing-over is often aberrant. It can be unequal (in which case it may generate alleles that differ in length from the original two) or incomplete (identified cytogenetically as incomplete symmetrical quadriradials) and can lead to serious imbalances (Therman and Kuhn 1976; Mannens et al. 1988; Chandley 1989).

There is no theoretical reason to believe that mitotic crossing-over cannot take place in the early embryo. Indeed, it is bound to happen every now and then, and this is a sufficient reason to want to appraise what its possible effects might be. Thought experiments of this kind can help uncover important general principles.

Theoretical Considerations

It is easy to imagine that, if mitotic crossing-over takes place at an early postzygotic division, before each daughter cell eventually develops into a different co-twin, then pathological conditions will occasionally be induced, depending on the genes involved, the precise point of exchange, the specific imprinting pattern, the effects of random chromosomal segregation, and the developmental stage of the embryo.

Cis-trans disruption of the differential parental imprinting necessary for normal embryogenesis (Cattanach and Kirk 1985; Surani et al. 1987; Searle et al. 1989) can be expected, with consequences ranging from early lethality and gross structural anomalies to discordance of MZ twins for diseases thus produced (Côté 1989*a*, 1989*b*). Differential cis-trans effects on cis-acting sequences can also be expected (Côté 1989*a*, 1990). LOH at any locus distal to the point of exchange will also lead to discordance of MZ twin embryos, each embryo becoming homozygous for a different allele at this locus.

There is no doubt that most MZ twins are remarkably similar, but the chance occurrence of an early postzygotic mitotic crossing-over involving susceptible genes can conceivably contribute to the known association of twinning with several congenital defects and malformations (Schinzel et al. 1979; Layde et al. 1980; Nance 1981; Uchida et al. 1983; Källén 1986; Boklage 1987; Little and Bryan 1988). Concurrent crossing-over of several chromosomes can also be expected to cause further discordance, to the point of mimicking DZ twinning, especially if differences in blood groups, face shape, and eye color are involved (this in turn would increase the likelihood of erroneously assuming dizygosity). In such cases, an increased concordance of some "DZ" twins is expected for near-centromeric markers of both maternal *and* paternal origin, in accordance with observations otherwise difficult to explain (Van Dyke et al. 1977; Boklage 1987).

Another expectation is that the close chromosome pairing required for mitotic crossing-over will increase the probability of chromosome lagging and nondisjunction. This in turn would positively contribute to the known association of twinning with chromosomal abnormalities (Nielsen 1967; Benirschke 1972). In cases where one of the resulting twins is not viable as an early embryo, the surviving co-twin will erroneously be accounted for as a singleton, either normal or affected. This concords with the view that the rate of twin inception in man must be much higher than reported numbers of twin pregnancies and deliveries would lead one to believe (Landy et al. 1982, 1986; Boklage 1990).

If an early postzygotic mitotic crossing-over capable of inducing discordance between two MZ twins occurs without being followed by twinning, the two parts of the resulting individual may be discordant instead, this being most easily noticeable in cases of gross asymmetry and whenever it affects genes involved in the determination of eye color and thus leads to heterochromia iridis. The occasional occurrence, in only one of MZ twins, of such heterochromia (Edwards et al. 1966; G. B. Côté, unpublished data) or other asymmetric conditions (Campbell et al. 1975; Ornoy et al. 1980; Boklage 1987; Boles et al. 1987; Samn et al. 1990) provides further support for the suggestion that mitotic crossing-over may be associated with the twinning process.

Finally, it is interesting to note that, if such an association exists, it should be influenced by various factors and conditions. If the probability of a mitotic crossing-over turns out to be increased in certain circumstances (such as the presence of a microdeletion, the occurrence of maternal hyperthermia, an exposure to abnormal oxygen pressure, or after the fertilization of an overripe ovum, to mention only a few possibilities), the conclusions reached in the present paper will fully concur and tie in with other theories, such as Witschi's (1952; Witschi and Laguens 1963) and Jong-

bloet's (1968, 1971, 1980, 1988) theory of overripeness ovopathy and Boklage's belief (1987) that MZ and DZ twinning processes share part of their causes. Let us now apply these theoretical considerations to actual data.

X-linked Disorders

Discordance of MZ twins for X-linked disorders has been reported in cases of Duchenne muscular dystrophy on Xp21 (Gomez et al. 1977; Pena et al. 1982, 1987; Burn et al. 1986; Chutkow et al. 1987; Richards et al. 1990*b*), Christmas disease on Xq26.3-q27.1 (Revesz et al. 1972; Kitchens 1987), the fragile X chromosome on Xq27.3 (Tuckerman et al. 1985), colorblindness on Xq28 (Koulischer et al. 1968; Philip et al. 1969), and glucose-6-phosphate dehydrogenase deficiency on Xq28 (Phelan et al. 1980). As can be seen, all the genes involved are grouped in two *specific chromosomal regions,* one in the midportion of the short arm (Xp21) and the other on the distal part of the long arm (Xq26–28). All twins involved are *females,* and in all well-documented cases *both* twins have been shown to be genetically *heterozygous* at the gene concerned, no LOH having ever been reported. However, in each pair, one twin is severely affected while her co-twin is clinically normal.

An extremely asymmetric pattern of *random* lyonization is usually invoked to explain these findings, despite a close to zero probability and the contradictory evidence provided by the absence of a full range of intermediate cases in MZ twins. (*Mild* cases of X-linked diseases due to slightly skewed X inactivation would certainly have been reported if they had been observed.) The hypothesis of *random* lyonization nevertheless gained substantial support after detailed investigations showed (*a*) the normal X to be consistently inactive in all (or nearly all) the affected twin's cells and (*b*) the mutated X to be inactive in all (or nearly all) cells derived from the normal twin (Tuckerman et al. 1985; Burn et al. 1986; Richards et al. 1990*b*). Moreover, skewed lyonization has also been reported recently in two of 20 normal female MZ twin pairs (Richards et al. 1990*a*). But, since the absence of recorded intermediate cases in twins suggests that all this is very unlikely to be due to chance, it is certainly worth considering the alternative explanation provided by the predicted consequences of an early embryonic mitotic crossing-over.

An additional argument against *random* lyonization is that, if it were solely responsible for the inactivation of the normal gene in the affected twin, it should occasionally lead to discordance of diseases mapped along the *entire* length of the chromosome, without excluding proximal and near-centromeric locations. On the contrary, discordance due to the effects of crossing-over is expected to be associated with diseases mapped away from the centromere—and in regions prone to such exchanges, as must be the case for the distal Xq27–28 (Davies et al. 1985; Purello et al. 1985; Laird et al. 1987) and the Duchenne giant gene on Xp21 (Hu et al. 1989, 1990; Daiger et al. 1990).

If we first consider the possible effects of an early postzygotic mitotic crossing-over distal to a particular gene and make the sole hypothesis that, after the completion of mitosis, crossed chromatids become chromosomes that cannot be efficiently inactivated, then it is clear that, if the two daughter cells are destined to become co-twins, then the random segregation of the four chromatids (two crossed and two uncrossed) leaves us with two (and only two) possibilities:

1. If each daughter cell (and eventual twin) receives one crossed and one uncrossed chromatid, then the uncrossed one will necessarily form the Barr body when lyonization is induced at a later stage. The crucial point is that in one twin the crossed and active chromosome carries the abnormal allele while in the other twin it carries the normal one. The two twins must then be discordant, with a precisely determined (i.e., *non*random) pattern of opposite lyonization.
2. If the two crossed chromatids segregate to the same daughter cell, then no Barr body will be possible in the eventual twin. This condition is lethal (Takagi and Abe 1990) and will leave the co-twin to be born as an apparent carrier singleton, with two ordinary uncrossed X chromosomes one of which carries the harmful mutation.

A crossover proximal to the gene concerned would lead to LOH, but this has not yet been observed. It may soon be reported if more MZ female twins are investigated; but, if it is not found, then its persistent absence would become indicative of the mechanism of mitotic crossing-over involving a normal and a defective allele—the crossing point being always distal to the region involved.

Under the sole hypothesis of the impaired inactivation of crossed X chromosomes, it would thus seem that the mechanism involving an early postzygotic mitotic crossing-over is much more likely than is random lyonization alone to explain the observed MZ twin discordance of X-linked disorders: although both

mechanisms exclude the discordance of male pairs, only mitotic crossing-over *always* causes a *severe* condition in the affected female twin—leading to *complete* discordance of two heterozygotes and involving some *chromosomal regions* more than it does others. Moreover, mitotic crossing-over can also occur in autosomes and may help resolve the mystery of MZ twin discordance of autosomal traits.

Autosomal Mendelian Traits

In consideration of the above discussion on X-linked traits, it may not come as a surprise to realize that, with no reported exception, autosomal recessive disorders are always concordant in MZ twins, thus minimizing the possibility of an association of twinning with LOH in the case of autosomal Mendelian traits as well as in the case of X-linked disorders. A most likely explanation is that, because of the paramount importance of differential parental imprinting during embryogenesis (Cattanach and Kirk 1985; Reik et al. 1987; Surani et al. 1987), at least one of the co-twins with LOH will not be able to survive. If ever due to mitotic crossing-over and accompanied by twinning, such a LOH would lead to early lethality of at least one co-twin and to the occasional birth of an apparent singleton homozygous with a double contribution from only one parent. (This is known to be possible and has been reported in several cases of uniparental disomy, by Spence et al. [1988], Nicholls et al. [1989], Voss et al. [1989], Malcolm et al. [1990], and Schinzel et al. [1990].) In the absence of carrier testing, there would be no easy way of recognizing the contribution of this unexpected mechanism. With traits for which carrier testing is available (but without the advantage of a full DNA analysis), the occasional discovery of inheritance discrepancy would undoubtedly be kept silent and be attributed to a laboratory error or to illegitimacy, even—and perhaps especially—if the mother refused to concur with the "scientific evidence."

The possibility of biased underreporting is corroborated by the existence of (*a*) only one report of a concordant but "inconsistent" inheritance of HLA and blood groups in an MZ twin pair described because of discordance for Russell-Silver syndrome (Campbell et al. 1987) and (*b*) only two reports of MZ twin discordance of autosomal codominant traits (the Duffy blood group and glutamate pyruvate transaminase) disclosed, without satisfactory explanation, in articles written, respectively, because of discordance for Goldenhar syndrome (Yovich et al. 1985) and tuberous sclerosis (Primrose 1975), the latter resulting in a misclassification as DZ twins that was later corrected by DNA fingerprinting (Sampson et al. 1989). In both cases, one twin was phenotypically heterozygous for the codominant trait while the co-twin appeared to be homozygous, thus presenting a pattern identical to that seen with the discordance of X-linked disorders (i.e., the inactivation or silencing of an allele present in the genome of a heterozygous MZ twin).

To complete the comparison with X-linked traits, it would seem that, if early postzygotic mitotic crossing-over ever leads to the discordant expression of autosomal disorders in living MZ twins, it will generally not be because of LOH but, more likely, through a cis-trans effect on contiguous DNA sequences. The mechanisms already proposed (Côté 1989*a*, 1989*b*) include the interaction of closely linked cis-acting DNA sequences as well as the disruption of differential parental imprinting, a phenomenon resembling X inactivation but less massive and thus more difficult to observe.

The disruption, inactivation, silencing, or deletion of a very large autosomal segment may not be compatible with life, but smaller regions may be involved if included within a double crossing-over. It is worth noting at this point that the two randomly determined breakpoints of a double crossing-over occurring in a particular region may involve a different length of DNA in each affected individual; such differences would be likely to accompany syndromes characterized by variable expressivity and by phenotypic overlap with closely linked disorders. This feature has already been recognized for the "contiguous gene syndromes" (Schmickel 1986) and is best exemplified by the "neurofibromatosis–cherubism–LEOPARD syndrome–Noonan syndrome–polyarticular pigmented villonodular synovitis–gingival fibromatosis syndrome" overlap (Gorlin 1990).

In MZ twins, there will be some abnormalities in which the segregation of both crossed chromatids to one daughter cell should be associated with *absolute* discordance in *all* pairs. For anomalies in which various alleles of the same gene are differentially affected by closely linked cis-sequences (Côté 1989*a*, 1990), both twins may show signs of a disorder—but to a different degree. In view of all the above, full concordance of a disorder due to the disrupting effects of crossing-over seems improbable.

Regular autosomal Mendelian traits are thus unlikely to be associated with twinning and crossing-

over. But, if the latter two are related, a twinning association can be expected—and is indeed observed—with *irregular* autosomal dominant disorders characterized by reduced penetrance and expressivity; with syndromes of unknown, obscure, or disputed aetiology; with so-called multifactorial diseases; as well as with sporadic congenital chromosomal aberrations.

Disorders with Irregular Inheritance

Four important characteristics of autosomal genetic disorders due to the cis-trans effects of mitotic crossing-over can be theoretically predicted and are in sharp contrast with the classical views on MZ twinning:

1. With a crossing-over etiology, MZ twin discordance is expected to be *high*. For some diseases, it should be virtually *constant* and *complete*.
2. An important consequence of the above is that the rate of MZ twin concordance for these disorders may be even *less* than the recurrence rate in regular sibs.
3. MZ twin discordance can be expected to be associated with disorders characterized by an increased rate of asymmetry in singletons.
4. Sex differences in the rates of meiotic crossing-over are well known. Under the assumption that sex differences also characterize mitotic crossing-over, disorders showing an unequal sex distribution of asymptomatic carriers will be expected to show a similar sex difference in the incidence of discordant MZ twin pairs (Côté 1989*a*).

It may be wise to refrain from indulging in speculation at this point, especially in the absence of unbiased data. Nevertheless, it may be worth briefly mentioning a few of the most characteristic disorders that undoubtedly fit one or more of the above four criteria, in order to identify potential candidates for a crossing-over etiology.

1. *Constant Discordance*

Constant discordance is well documented for amyoplasia (Hall et al. 1983), even in conjoined twins (Weston et al. 1990). This has led to the conclusion that "because only one of each pair of MZ twins is affected, a genetic cause of the amyoplasia seems highly unlikely" (Hall et al. 1983, p. 598). Clearly, and for the same reason, one can also argue that, on the contrary, a genetic cause is most likely. The association with MZ twin discordance is even more impressive for amyoplasia associated with gastroschisis, bowel atresia, and defects of the muscular layer of the trunk, for which one of 13 patients is a discordant MZ twin (Reid et al. 1986).

2. *Concordance Lower than Recurrence*

Diseases independent of twinning should have the same incidence in regular sibs and in the co-twins of MZ index cases. MZ concordance for Parkinson disease (PD) is extremely rare (Eldridge and Ince 1983; Eldridge 1984) with a rate of only 2.3%, a rate much lower than the 5.7% in sibs >60 years of age. More data and analyses are needed, but at present the general feeling that "the major contribution to the etiology of PD is nongenetic" (Campanella et al. 1984, p. 1398) could just as well be replaced by a feeling that the major contribution *is* genetic, since the simple occurrence of an early embryonic mitotic crossing-over may constitute the "unconventional process occurring early in life" (Eldridge 1984, p. 1399) and hypothesized to explain (*a*) the lifelong personality differences between the discordant co-twins, (*b*) the failure to demonstrate an environmental risk factor in most affected twins, and (*c*) the fact that the MZ-twin concordance rate is lower than the recurrence rate in non-twin sibs.

3. *Discordance and Asymmetry*

Goldenhar syndrome and hemifacial microsomia, variously claimed to be dominant, recessive, multifactorial, nongenetic, or, more appropriately, of disputed etiology, have been reported to be discordant in six DZ pairs and in 21 of 24 MZ pairs, the three concordant pairs being differently affected (Burck 1983; Connor and Fernandez 1984; Boles 1987; Ryan et al. 1988). Full concordance has thus never been reported for this asymmetric condition. Jongbloet (1968, 1980, 1987) has convincingly argued that Goldenhar syndrome and overlapping dysplasias are related to MZ twinning through overripeness ovopathy, a contention rejected by Yovich et al. (1987) on the grounds that this theory "has not been supported by other workers at this stage" and that this congenital anomaly has only been seen once in 2,000 IVF infants. In the absence of a better hypothesis, and in light of the fact that one of the only two abnormal children born after IVF and ever seen at this division in Athens had Goldenhar syndrome, their rejection may as well be turned into acceptance! Moreover, ovopathy and an increased probability of mitotic crossing-over soon after

fertilization are interestingly compatible and reinforce together the idea that such conditions are related to the twinning process.

4. *Extreme Sex Differences*

To date, MZ twins with the Wiedemann-Beckwith syndrome have all been discordant and females; and asymptomatic carriers are virtually only females too. This extreme example was the first one used to link twinning and crossing-over (Côté 1989*a*). It is not so clear whether the mode of action is through LOH, as originally suggested, or through another cis-trans effect; but the further association of this syndrome with Wilms tumor, itself most likely due to a disruption of imprinting following mitotic crossing-over (Côté 1989*b*), seems to indicate that the etiological search is proceeding in the right direction.

It is certainly worth mentioning here that since the publication of this theory (Côté 1989*a*) two cases of Wiedemann-Beckwith syndrome were seen at this division in Athens; while the pediatric records made no reference to twinning in either case, further questioning revealed that one of the two, the typically affected daughter of a carrier mother, had been shown to be a twin by ultrasonography in early pregnancy. The co-twin later vanished, leaving an apparent singleton to be born with the syndrome.

"Multifactorial" and Other Disorders

The unexplained cross-association, both between themselves and with twinning, of certain "multifactorial" and other conditions may perhaps be indicative of a common causal element. One example among others is the suggested association of congenital and/or acquired hypothyroidism with (*a*) twinning (Schinzel et al. 1979), (*b*) Down syndrome (Pozzan et al. 1990), and (*c*) X-chromatin positivity in women, XX males, and Klinefelter patients (Sarri et al. 1988). Another example is the interrelationship of twinning, neural tube defects, oral clefting, and congenital heart defects, well reviewed by Boklage (1987). These midline abnormalities are generally considered to be "multifactorial" in origin and are also characterized by puzzling sex ratios and asymmetries (e.g., see James 1975, 1976, 1979*a*, 1979*b*, 1980*a*, 1980*b*, 1988). The study of their surprising relationship with both nonrighthandedness and twinning led Boklage (1987, pp. 79–80) to conclude that "by means we cannot yet explain, 'splitting' is a consequence, and not a cause" of twinning and that "the underlying genetic factor(s) seem more likely to be few and pleiotropic."

It would seem that mitotic crossing-over ideally meets the requirements for such a factor. Its early embryonic effect on homeotic and other genes could very well alter the genetic blueprint and lead to both a "programmed splitting" and various fusion malformations. Mitotic crossing-over thus provides a common solution to both twinning and midline defects, through its regulating/disruptive action on the normal mechanism controlling the fusion or not of the two embryonic halves. Under this hypothesis, twinning induction by crossing-over and the actual development of the embryo into two are two completely different actions, with the gentle separation of two embryonic halves being the consequence of very precise twinning instructions.

The Twinning Process

In full agreement with Boklage (1981, 1987), the MZ twinning mechanism outlined above is at odds with the frequently entertained idea of a harsh "splitting" event or a traumatic embryonic "insult" similar to "teasing apart starfish blastomeres or tying hairs around early embryos of newts" (Boklage 1981, p. 155). Several authors talk of a "twinning event" as if it were instantaneous, but the implications of the crossing-over model are that there is no such event more than there is a "bladder event" or a "foot event."

Attempts at timing the twinning process must thus deal separately with the occurrence of mitotic crossing-over and the period of embryogenesis when the crossed genes affected would normally be active. Boklage's (1981) observations led him to differentiate between the cellular "commitment" to twinning and the twinning "detection" that became possible later. According to him, a lot of "commitment" seems to take place during the time the embryonic inner cell mass (ICM) reorganizes itself to assume bilateral symmetry.

The vast majority of blastocyst cells form the extraembryonic membranes and placenta, without contributing to the tissues of the adult. The human fetus itself originates exclusively from precursor cells that appear in the primitive ectoderm of the ICM on the 15th day after fertilization, at the beginning of gastrulation, and it is at that stage that the number of individuals liable to develop from a single fertilized egg is finally fixed (Gardner 1990).

Mitotic crossing-over can certainly take place at the

very first postzygotic division and has no effect until the crossed genes are switched on, later on during embryogenesis. But the point can be made that it can also occur up to several days later and still affect *all* cells of the primitive ectoderm, if these cells all derive from the same precursor cell. A cross-over occurring in this mother cell could cause the presence of different alleles or imprinting patterns in the two halves of the embryo or in the two twins to come.

This is also in agreement with data on the armadillo, a mammal that normally and regularly produces four MZ yet nonidentical quadruplets. This led Storrs and Williams (1968, p. 914) to conclude >23 years ago that, according to their evidence, "it is erroneous to assume that monozygous human twins have identical inheritance." Few, if any, took heed.

A New Gene-mapping Method

One of the most interesting aspects of this model may be that it is amenable to testing with molecular methods. DNA analyses have already shown differences between MZ twins discordant for the Proteus syndrome (Schwartz et al. 1989, 1990) and tuberous sclerosis (Brilliant et al. 1990), and there can be no doubt that several new cases will soon come to light. Mapping the differences is the next logical step and offers much promise.

Correct zygosity determination is an essential prerequisite and can now be reliably ascertained (Derom et al. 1985; Hill and Jeffreys 1985; Motomura et al. 1987) even on macerated abortuses, although small differences should not be misinterpreted as dizygosity, concordance after superficial testing should not be confused with monozygosity, and due attention should be given to the methods' inability to detect very small differences. (For instance, Henry et al. [1987*b*] studied two brothers with adrenocortical carcinoma and reported strictly identical electrophoretic patterns after the use of Jeffreys probes on both lymphocytic and tumoral DNA, although LOH due to mitotic recombination was known to be present in the tumors.) It should also be remembered that blood cells and fibroblasts are subject to chimerism in twins and will not always constitute suitable material for investigations.

Finally, it should be emphasized that crossing-over is not always accompanied by a change in DNA sequence: its occurrence in completely homozygous alleles having different parental imprinting will produce alleles with an abnormal hybrid pattern not detectable by DNA analyses insensitive to imprinting. Given the additional difficulties inherent in such detection, the task will not be easy, but the crossing-over approach to twinning should nevertheless help identify not only the general mechanisms of some diseases but also the very genes or groups of genes responsible for much human suffering, an intricate problem almost impossible to address effectively with rare sporadic singletons alone.

Conclusion

The present article and the previous ones in the series (Côté 1989*a*, 1989*b*, 1990) do not constitute a full theory; there are still too many missing bits and pieces. Indeed, the very idea of giving such an important role to crossing-over may turn out to be nothing but a gross exaggeration, an oversimplification, or an outright error. But this is of little importance, as the model's merits do not lie in its veracity or accuracy. Indeed, its primary value is to point out once more (*a*) the inadequacies of the classical views on twinning and (*b*) the probable existence of a unified theory of genetics that will encompass the whole field and provide the long-awaited answer to the problems posed by penetrance, expressivity, discordance, twinning, sex differences, the so-called multifactorial common disorders, syndromes of unknown or disputed etiology, DNA repair, cancer, and the embryonic interactions of homeotic genes with imprinting.

Acknowledgments

Sincere thanks are due to Professors Judith G. Hall and John H. Edwards for an interesting discussion of this theory under the Greek sun of Corfu Island in May 1990. Professor Charles E. Boklage, Dr. Ann C. Chandley, Professor F. Clarke Fraser, Dr. Piet Jongbloet, and Professors Marion Lewis and Pierre Philippe commented on the first draught and contributed to its improvement. Their interest, agreement, disagreement, questioning, and encouragement are gratefully acknowledged. The final version owes a great deal to the much appreciated expert editing and incisive probing of Professor Charles E. Boklage.

References

Benirschke K (1972) Origin and clinical significance of twinning. Clin Obstet Gynecol 15:220–235

Boklage CE (1981) On the timing of monozygotic twinning events. Prog Clin Biol Res 69A:155–165

——— (1987) Twinning, nonrighthandedness, and fusion

malformations: evidence for heritable causal elements held in common. Am J Med Genet 28:67–84
——— (1990) Survival probability of human conceptions from fertilization to term. Int J Fertil 35:75–94
Boles DJ, Bodurtha J, Nance WE (1987) Goldenhar complex in discordant monozygotic twins: a case report and review of the literature. Am J Med Genet 28:103–109
Brilliant MH, Cleve H, Edwards JH, Waldinger D (1990) Cell lines from monozygotic twins discordant for tuberous sclerosis display protein and DNA differences. Am J Hum Genet 47 [Suppl]: A210
Burck U (1983) Genetic aspects of hemifacial microsomia. Hum Genet 64:291–296
Burn J, Povey S, Boyd Y, Munro EA, West L, Harper K, Thomas D (1986) Duchenne muscular dystrophy in one of monozygotic twin girls. J Med Genet 23:494–500
Campanella G, Idone M, De Michele G, Filla A (1984) Paternal preponderance in familial Parkinson's disease. Neurology 34:1398–1399
Campbell JA, Harley EH, McCord WK (1975) The Russell-Silver syndrome in discordant monozygotic twins. Birth Defects 51:326
Cattanach BM, Kirk M (1985) Differential activity of maternally and paternally derived chromosome regions in mice. Nature 315:496–498
Chandley AC (1989) Asymmetry in chromosome pairing: a major factor in de novo mutation and the production of genetic disease in man. J Med Genet 26:546–552
Chutkow JG, Hyser CL, Edwards JA, Heffner RR, Czyrny JJ (1987) Monozygotic female twin carriers discordant for the clinical manifestations of Duchenne muscular dystrophy. Neurology 37:1147–1151
Connor JM, Fernandez C (1984) Genetic aspects of hemifacial microsomia. Hum Genet 68:349
Côté GB (1989*a*) The cis-trans effects of crossing-over on the penetrance and expressivity of dominantly inherited disorders. Ann Genet 32:132–135
——— (1989*b*) Wilms' tumour and related syndromes: a unifying theory. Ann Genet 32:69–72
——— (1990) The cis-configuration effects of rare chromosomal fragile sites. Clin Genet 37:470–472
Craig-Holmes AP, Moore FB, Shaw MW (1975) Polymorphism of human C-band heterochromatin. II. Family studies with suggestive evidence for somatic crossing-over. Am J Hum Genet 27:178–189
Daiger SP, Reed-Fourquet LL, Blanton SH, Hejtmancik JF (1990) Multipoint linkage analysis of markers within the Duchenne muscular dystrophy (DMD) locus: evidence for recombination hot spots. Am J Hum Genet 47 [Suppl]: A176
Dao DD, Schroeder WT, Chao L-Y, Kikuchi H, Strong LC, Riccardi VM, Pathak S, et al (1987) Genetic mechanisms of tumor-specific loss of 11p DNA sequences in Wilms tumor. Am J Hum Genet 41:202–217
Davies KE, Mattei MG, Mattei JF, Veenema H, McGlade S, Harper K, Tommerup N, et al (1985) Linkage studies of X-linked mental retardation: high frequency of recombination in the telomeric region of the human X chromosome. Hum Genet 70:249–255
Derom C, Bakker E, Vlietinck R, Derom R, van den Berghe H, Thiery M, Pearson P (1985) Zygosity determintion in newborn twins using DNA variants. J Med Genet 22: 279–282
Edwards JH, Dent T, Kahn J (1966) Monozygotic twins of different sex. J Med Genet 3:117–123
Eldridge R (1984) Paternal preponderance in familial Parkinson's disease. Neurology 34:1399–1400
Eldridge R, Ince S (1983) The cause of Parkinson disease in twins. Am J Hum Genet 35 [Suppl]: 88A
Gardner RL (1990) Le destin des cellules dans l'embryon. La Recherche 21:1476–1485
Gomez, MR, AG Engel, GD Dewald, HA Peterson (1977) Failure of inactivation of Duchenne dystrophy X-chromosome in one of female identical twins. Neurology 27:537–541
Gorlin R (1990) Future aspects of syndromology. Lecture presented at Symposium 1990 of the European Society of Human Genetics, Corfu, Greece, May 16–19
Groden J, Nakamura Y, White R, German J (1988) Molecular evidence for somatic recombination in vitro in Bloom's syndrome cells. Am J Hum Genet 43 [Suppl]: A24
Hall JG, Reed SD, McGillivray BC, Herrmann J, Partington MW, Schinzel A, Shapiro J, et al (1983) Part II. Amyoplasia: twinning in amyoplasia—a specific type of arthrogryposis with an apparent excess of discordantly affected identical twins. Am J Med Genet 15:591–599
Henry I, Grandjouan S, Azoulay M, Huerre-Jeanpierre C, Couillin P, Junien C (1987*a*) Mitotic recombination with loss of heterozygosity distal to FSHB in a WAGR associated nephroblastoma. Cytogenet Cell Genet 46:628
Henry I, Huerre-Jeanpierre C, Azoulay M, Chaussain JL, Junien C (1987*b*) A recessive oncogene for familial adrenocortical carcinoma (ADCC) maps to 11p. Cytogenet Cell Genet 46:629
Henry I, Jeanpierre C, Grandjouan S, Brugieres L, Serre JL, Ahnine L, Antignac C, et al (1989) Loss of alleles for 11p regions in hereditary and sporadic childhood tumors. Cytogenet Cell Genet 51:1012–1013
Hill AVS, Jeffreys AJ (1985) Use of minisatellite DNA probes for determination of twin zygosity at birth. Lancet 2:1394–1395
Hu X, Burghes AHM, Bulman DE, Ray PN, Worton RG (1989) Evidence for mutation by unequal sister chromatic exchange in the Duchenne muscular dystrophy gene. Am J Hum Genet 44:855–863
Hu X, Ray PN, Murphy EG, Thompson MW, Worton RG (1990) Duplicational mutation at the Duchenne muscular dystrophy locus: its frequency, distribution, origin, and phenotype/genotype correlation. Am J Hum Genet 46: 682–695
James WH (1975) Sex ratio in twin births. Ann Hum Biol 2:365–378

——— (1976) Twinning and anencephaly. Ann Hum Biol 3:401–409
——— (1979*a*) The sex ratio in anencephaly. J Med Genet 16:129–133
——— (1979*b*) The sex ratio in spina bifida. J Med Genet 16:384–388
——— (1980*a*) Concordance rates in twins for anencephaly. J Med Genet 17:93–94
——— (1980*b*) Sex ratio and placentation in twins. Ann Hum Biol 7:273–276
——— (1988) Anomalous X chromosome inactivation: the link between female zygotes, monozygotic twinning, and neural tube defects? J Med Genet 25:213–214
Jongbloet PH (1968) Overripeness of the egg: considerations on the aetiology of Goldenhar's syndrome and related congenital dysplasias including chromosomal aberrations. Maandschr Kindergeneeskd 36:352–366
——— (1971) Diagnostic criteria for overripeness ovopathy. Maandschr Kindergeneeskd 39:251–285
——— (1980) Monozygotic twinning, structural defects, and syndromes of 'obscure etiology.' J Pediatr 97:868–869
——— (1987) Goldenhar syndrome and overlapping dysplasias, in vitro fertilisation and ovopathy. J Med Genet 24:616–620
——— (1988) Duchenne muscular dystrophy in one of monozygotic twin girls. J Med Genet 25:214–215
Källén B (1986) Congenital malformations in twins: a population study. Acta Genet Med Gemellol (Roma) 35:167–178
Kipps TJ, Herzenberg LA (1986) Homologous chromosome recombination generating immunoglobulin allotype and isotype switch variants. EMBO J 5:263–268
Kitchens CS (1987) Discordance in a pair of identical twin carriers of factor IX deficiency. Am J Hematol 24:225–228
Koulischer L, Zanen J, Meunier A (1968) La théorie de Lyon peut-elle expliquer la disparité exceptionellement observée de la perception colorée chez des jumelles univitellines? Comptes-rendus du 1er Congrès International de Neuro-génétique et Neuro-ophtalmologie. Karger, Basel, New York, pp 242–254
Kurnit DM (1979) Satellite DNA and heterochromatin variants: the case for unequal mitotic crossing-over. Hum Genet 47:169–186
Laird C, Jaffe E, Karpen G, Lamb M, Nelson R (1987) Fragile sites in human chromosomes as regions of late replicating DNA. Trends Genet 3:274–281
Landy HJ, Keith L, Keith D (1982) The vanishing twin. Acta Genet Med Gemellol (Roma) 31:179–194
Landy HJ, Weiner S, Corson SL, Batzer FR, Bolognes RJ (1986) "The vanishing twin": ultrasonographic assessment of fetal disappearance in the first trimester. Am J Obstet Gynecol 155:14–19
Layde PM, Erickson JD, Falek A, McCarthy BJ (1980) Congenital malformations in twins. Am J Hum Genet 32:69–79
Little J, Bryan EM (1988) Congenital anomalies. In: MacGillivray I, Campbell DM, Thompson B (eds) Twinning and twins. John Wiley & Sons, New York, pp 207–240
Malcolm S, Nichols M, Clayton-Smith J, Robb S, Webb T, Armour JAL, Jeffreys AJ, et al (1990) Angelman syndrome can result from uniparental paternal isodisomy. Am J Hum Genet 47 [Suppl]: A227
Mannens M, Slater RM, Heyting C, Bliek J, de Kraker J, Coad N, de Pagter-Holthuizen P, et al (1988) Molecular nature of genetic changes resulting in loss of heterozygosity of chromosome 11 in Wilms' tumours. Hum Genet 81: 41–48
Morley AA, Grist SA, Turner DR, Kutlaca A, Bennett G (1990) The molecular nature of *in vivo* mutations in human cells at the autosomal HLA-A locus. Cancer Res 50: 4584–4587
Motomura K, Tateishi H, Nishisho I, Okazaki M, Miki T, Tonomura A, Takai S, et al (1987) The zygosity determination of Japanese twins using a minisatellite core probe. Jpn J Hum Genet 32:9–14
Nance WE (1981) Malformations unique to the twinning process: twin research 3: twin biology and multiple pregnancy. Prog Clin Biol Res 69A:123–133
Nicholls RD, Knoll JHM, Butler MG, Karam S, Lalande M (1989) Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. Nature 342:281–285
Nielsen J (1967) Inheritance in monozygotic twins. Lancet 2:717–718
Ornoy A, Navot D, Menashi M, Laufer N, Chemke J (1980) Asymmetry and discordance for congenital anomalies in conjoined twins: a report of six cases. Teratology 22:145–154
Passarge E, Bartram CR (1976) Somatic recombination as possible prelude to malignant transformation. Birth Defects 12:177–180
Pena SDJ, Karpati G, Carpenter S, Fraser FC (1982) The clinical consequences of X chromosome inactivation: Duchenne muscular dystrophy in one of monozygotic twins. Neurology 32:83A
——— (1987) The clinical consequences of X chromosome inactivation: Duchenne muscular dystrophy in one of monozygotic twins. J Neurol Sci 79:337–344
Phelan MC, Morton CC, Swenson PM, Winter PM, Nance WE (1980) Evidence for lyonization of G6PD in a monozygotic twin pair. Am J Hum Genet 32 [Suppl]: 123A
Philip J, Andersen CH, Dreyer V, Freiesleben E, Gurtler H, Hauge M, Kissmeyer-Nielsen F, et al (1969) Colour vision deficiency in one of two presumably monozygotic twins with secondary amenorrhoea. Ann Hum Genet 33:185–195
Pontecorvo G, Käfer E (1958) Genetic analysis based on mitotic recombination. Adv Genet 10:71–104

Pozzan GB, Rigon F, Girelli ME, Rubello D, Busnardo B, Baccichetti C (1990) Thyroid function in patients with Down syndrome: preliminary results from noninstitutionalized patients in the Veneto region. Am J Med Genet [Suppl 6]: 57–58

Primrose DA (1975) Epiloia in twins: a problem in diagnosis and counselling. J Ment Defic Res 19:195–203

Purello M, Alhadeff B, Esposito D, Szabo P, Rocchi M, Truett M, Masiarz F, et al (1985) The human genes for hemophilia A and hemophilia B flank the X chromosome fragile site at Xq27.3 EMBO J 4:725–729

Raizis AM, Becroft DM, Shaw RL, Reeve AE (1985) A mitotic recombination in Wilms tumor occurs between the parathyroid hormone locus and 11p13. Hum Genet 70:344–346

Reid COMV, Hall JG, Anderson C, Bocian M, Carey J, Costa T, Curry C, et al (1986) Association of amyoplasia with gastroschisis, bowel atresia, and defects of the muscular layer of the trunk. Am J Med Genet 24:701–710

Reik W, Collick A, Norris ML, Barton SC, Surani MA (1987) Genomic imprinting determines methylation of parental alleles in transgenic mice. Nature 328:248–251

Revesz T, Schuler D, Goldschmidt B, Elodi S (1972) Christmas disease in one of a pair of monozygotic twin girls, possibly the effect of Lyonization. J Med Genet 9:396–400

Richards CS, Hall JG, Palterman ML, Nance WE (1990*a*) Skewed X-inactivation in normal monozygotic twin pairs. Am J Hum Genet 47 [Suppl]: A105

Richards CS, Watkins SC, Hoffman EP, Schneider NR, Milsark IW, Katz KS, Cook JD, et al (1990*b*) Skewed X inactivation in a female MZ twin results in Duchenne muscular dystrophy. Am J Hum Genet 46:672–681

Ryan CA, Finer NN, Ives E (1988) Discordance of signs in monozygotic twins concordant for the Goldenhar anomaly. Am J Med Genet 29:755–761

Samn M, Lewis K, Blumberg B (1990) Monozygotic twins discordant for the Russell-Silver syndrome. Am J Med Genet 37:543–545.

Sampson JR, Scahill SJ, Stephenson JBP, Mann L, Connor JM (1989) Genetic aspects of tuberous sclerosis in the west of Scotland. J Med Genet 26:28–31

Sarri C, Côté GB, Mengreli C, Lambadaridis I, Pantelakis S (1988) Hypothyroidism and sex chromosomes. J Med Genet 25:247–249

Sarto GE, Kuhn EM, Therman E (1987) Segregation after mitotic crossing-over in isodicentric X chromosomes. Cytogenet Cell Genet 45:191–195

Schinzel A, Robinson W, Yargang X, Mächler M, Binkert F (1990) Molecular and cytogenetic studies on 21 Prader-Willi patients: analysis of deleted regions and identification of two cases of maternal isodisomy. Am J Hum Genet 47 [Suppl]: A235

Schinzel AAGL, Smith DW, Miller JR (1979) Monozygotic twinning and structural defects. J Pediatr 95:921–930

Schmickel RD (1986) Contiguous gene syndromes: a component of recognizable syndromes. J Pediatr 109:231–241

Schwartz CE, Der Kaloustian VM, McGill JJ, Saul RA (1990) Molecular evidence for somatic mutation as the cause of the Proteus syndrome. Am J Hum Genet 47 [Suppl]: A235

Schwartz CE, Sauer SM, Brown AM, Saul RA, Stevenson RE (1989) Detection of DNA fingerprint differences in monozygotic twins discordant for the Proteus syndrome. Cytogenet Cell Genet 51:1075

Searle AG, Peters J, Lyon MF, Hall JG, Evans EP, Edwards JH, Buckle VJ (1989) Chromosome maps of man and mouse. IV. Ann Hum Genet 53:89–140

Spence JE, Perciaccante RG, Grieg GM, Willard HF, Ledbetter DH, Hejtmancik JF, Pollack MS, et al (1988) Uniparental disomy as a mechanism for human genetic disease. Am J Hum Genet 42:217–226

Stern C (1936) Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics 21:625–730

Storrs EE, Williams RJ (1968) A study of monozygous quadruplet armadillos in relation to mammalian inheritance. Proc Natl Acad Sci USA 60:910–914

Surani MAH, Barton SC, Norris ML (1987) Influence of parental chromosomes on spatial specificity in androgenetic-parthenogenetic chimaeras in the mouse. Nature 326:395–397

Takagi N, Abe K (1990) Detrimental effects of two active X chromosomes on early mouse development. Development 109:189–201

Therman E, Kuhn EM (1976) Cytological demonstration of mitotic crossing-over in man. Cytogenet Cell Genet 17: 254–267

——— (1981) Mitotic crossing-over and segregation in man. Hum Genet 59:93–100

Tuckerman E, Webb T, Bundey SE (1985) Frequency and replication status of the fragile X, fra(X) (q27–28), in a pair of monozygotic twins of markedly differing intelligence. J Med Genet 22:85–91

Uchida IA, Freeman VCP, Gedeon M, Goldmaker J (1983) Twinning rate in spontaneous abortions. Am J Hum Genet 35:987–993

Van Dyke DL, Palmer CG, Nance WE, Yu P-L (1977) Chromosome polymorphism and twin zygosity. Am J Hum Genet 29:431–447

Voss R, Ben-Simon E, Avital A, Godfrey S, Zlotogora J, Dagan J, Tikochinski Y, et al (1989) Isodisomy of chromosome 7 in a patient with cystic fibrosis: could uniparental disomy be common in humans? Am J Hum Genet 45: 373–380

Weston PJ, Ives EJ, Honore RLH, Lees GM, Sinclair DB, Schiff D (1990) Monochorionic diamniotic minimally conjoined twins: a case report. Am J Med Genet 37:558–561

Witschi E (1952) Overripeness of the egg as a cause of twin-

ning and teratogenesis. Cancer Res 12:763–768
Witschi E, Laguens R (1963) Chromosomal aberrations in embryos from overripe eggs. Dev Biol 7:605–616
Yovich J, Mulcahy M, Matson P (1987) IVF and Goldenhar syndrome. J Med Genet 24:644
Yovich JL, Stanger JD, Grauaug AA, Lunay GG, Hollingsworth P, Mulcahy MT (1985) Fetal abnormality (Goldenhar syndrome) occurring in one of triplet infants derived from in vitro fertilization with possible monozygotic twinning. J in Vitro Fert Embryo Transfer 2:27–32