
Chorion Type, Birthweight Discordance and Tooth-Size Variability in Australian Monozygotic Twins

Jonathan P. Race, Grant C. Townsend, and Toby E. Hughes

Dental School, The University of Adelaide, Australia

Chorion type is an important variable that can affect the prenatal environment of monozygotic (MZ) twin pairs, leading to differences in growth and development. In particular, vascular anastomoses between monochorionic (MC) MZ twin pairs can lead to an imbalance in nutrition between co-twins. One objective of this study was to determine whether maternal reports, hospital records or birthweight discordances found in MZ co-twins provide reliable indications of monochorionicity. The other objective was to test the hypotheses that in MZ twin pairs of known chorion type, MC twin pairs would show greater birthweight differences and greater within-pair variability in permanent tooth size than dichorionic (DC) twin pairs, reflecting greater differences in intrauterine environment between MC MZ pairs. Birthweights and tooth size data were recorded for 170 pairs of MZ Australian twins enrolled in an ongoing study of dentofacial growth and development. Chorion type based on maternal reports was compared with that based on hospital records for a subsample of 68 pairs of these MZ twins. Maternal reports were found to be unreliable for determining chorion type and hospital records often did not provide enough information to be certain about chorionicity. For 27 twin pairs with confirmed chorion type, associations were tested between birthweight discordances in MZ twin pairs and chorion type, and also between intrapair variances for tooth size and chorion type. A significant association was noted between birthweight discordance and chorion type ($p < .05$), with greater discordances occurring more often in MC twin pairs. Although significant heterogeneity of intrapair variances for tooth size was found in only 5 of 48 comparisons, intrapair variances for crown dimensions were greater significantly more often in MC pairs than DC pairs ($p < .05$). Our findings indicated that neither maternal reports nor often hospital records can be relied upon for information on chorion type. However, when analyses were performed on data for MZ twin pairs of known chorion type, we found evidence of a significant association between intrapair birthweight differences and chorion type and also between intrapair variances of dental crown measurements and chorion type. Consistent with our hypotheses, large birthweight discordances were

found to occur more often in MC twin pairs than DC twin pairs, and intrapair variances for tooth size in MC twin pairs exceeded those in DC twin pairs more often than expected due to chance.

Monozygotic (MZ) twinning arises when a single zygote cleaves at an early stage of embryological development and the timing of cleavage determines the characteristics of the placental membranes. Early cleavage within the first 3 days postconception leads to dichorionic (DC) placentation, whereas cleavage after the chorion has begun forming (day 4) results in monochorionic (MC) placentation (Boklage, 1981; Hall, 2003). Approximately 60% to 75% of MZ pregnancies are MC whereas 20% to 30% are DC, with the two placentas being either fused or separate (Blickstein, 1990; Hall, 2003; Robertson & Neer, 1983; Townsend & Richards, 1990).

Chorionicity has been used to explain certain variations that can be observed between MZ twin pairs who are assumed to be genetically identical. Chorion type has been linked with discordant birthweight, dermatoglyphic patterns, schizophrenia, brain morphology and variations in tooth size (Blickstein, 1990; Burris & Harris, 2002; Davis et al., 1995; Reed et al., 2002; Reed et al., 1997). Unfortunately, there are few studies of twins where chorionicity has been determined with certainty. Instead, researchers have either used reported placenta type or birthweight discordance to retrospectively infer monochorionicity (Hay & Howie, 1980). Chorionicity is an environmental factor that is potentially very important during the prenatal growth and development of twins. How chorionicity actually affects general growth and development, and more specifically dental development, is still far from clear, but differences in placental blood flow to MC co-twins who share a

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Address for correspondence: Professor G. C. Townsend, Professor of Dental Science, Dental School, The University of Adelaide, Adelaide SA, Australia. E-mail: grant.townsend@adelaide.edu.au

single placenta have been reported by some researchers (Denbow et al., 2000; Seng & Rajadurai, 2000). A recent study by Burris and Harris (2002) reported on differences in tooth dimensions due to the chorionicity of MZ twins. Their study analyzed the mesiodistal and buccolingual tooth crown diameters of 28 permanent teeth in 50 pairs of MC and 50 pairs of DC MZ twins to determine whether there were differences due to chorionicity. Although mean crown size was not directly associated with zygosity or chorionicity, intrapair variance differed significantly between MC and DC groups.

One objective of this study was to evaluate whether maternal reports, hospital records or birthweight discordances found in MZ co-twins can provide reliable indications of monozygosity. The other objective was to test the hypotheses that, in MZ twin pairs of known chorion type, MC twin pairs would show greater birthweight differences and greater within-pair variability in permanent tooth size than DC twin pairs, reflecting greater differences in intrauterine environment between MC MZ pairs.

Materials and Methods

This study involved analyses of data derived from MZ twin pairs who are enrolled in an ongoing study of dental and facial development being carried out at the Dental Schools in Adelaide and Melbourne (Townsend & Richards, 1990). Questionnaires completed by the mothers of each twin pair were examined and information about each twin's zygosity, chorionicity and birthweight was recorded. Initially, surveys of 269 MZ twin pairs were examined, but 106 pairs were excluded due to either lack of information regarding the type of chorion (99 pairs) or about the twins' birthweight (7 pairs). Information regarding birthplace was available for 137 of these twin pairs and the twins were born at over 50 different hospitals. For logistical reasons, only the hospitals where two or more twin pairs were born were contacted for the collation and viewing of hospital records. Hospital records were finally viewed for 68 twin pairs.

All twin zygosity was confirmed by the analysis of DNA obtained from buccal cells, which has an accuracy of greater than 99% (Apps et al., 2004; Dempsey et al., 1999; Hughes et al., 2001). Chorionicity, according to maternal report, was assessed by asking mothers whether their twins had a single placenta, fused placentas or two separate placentas. Maternal responses were then checked against the twins' hospital records to determine the validity of the reported placentation.

The dental data comprised maximum mesiodistal and buccolingual crown diameters for all the permanent teeth (except second and third molars) obtained from dental models, using a pair of digital callipers with tapered beaks. The models were poured immediately after impressions had been obtained to minimize any distortion. Teeth that were not fully erupted, or those with obvious wear or other defects that

obscured determinations, were not measured. Recordings were made to an accuracy of 0.1 mm. At least one study has shown that errors of measurement are actually reduced by measuring from models rather than intraorally (Hunter & Priest, 1960). Double determinations showed that there was no systematic error in the measurement method, based on paired *t* tests, and also confirmed that the random errors, quantified according to Dahlberg (1940), were small (0.1 mm) and therefore unlikely to bias results.

Descriptive statistics, including mean values and standard deviations, were computed for 48 tooth-size variables. Mean values were compared using Student's *t* tests. Intrapair variances (s^2) were calculated for the tooth-size variables according to the formula ($s^2 = \Sigma d^2 / 2n$) where s^2 was the intrapair variance, d , was the difference in tooth size between co-twins, and n was the number of twin pairs (Osborne & DeGeorge, 1959).

For the 27 MZ twin pairs for whom chorion type was confirmed, two types of associations were tested: between birthweight discordance and chorionicity and between intrapair variability for tooth size and chorionicity. Birthweight discordances were expressed as percentages and categorized as being between 0% and 10%, 11% and 20%, and 21% and 30%. Chi-square analysis and Fisher's exact test were used for these analyses, with a *p* value of less than .05 being considered significant. For the tooth-size data, comparisons of intrapair variances between MC MZ twin pairs and DC MZ twin pairs were made using an *F* test with statistical significance set at *p* < .05.

Results

Of the 68 maternal reports of placentation, only 20 (29.4%) were confirmed by hospital records and seven (10.3%) were contradicted. The hospital records for the other 41 cases (60.3%) were either inconclusive or unavailable. For the subsample of 27 MZ twin pairs for whom chorion type was confirmed on the basis of the hospital records (14 MC and 13 DC), there were three cases where mothers reported that their twins were DC when they were, in fact, MC but no cases where maternal reports of dichorionicity were contradicted by hospital records.

The frequencies of birthweight discordances, expressed as percentage differences, in the 27 pairs of twins of known chorionicity are given in Table 1. Fisher's exact test demonstrated a significant association between chorion type and birthweight discordance, with a greater proportion of the MC twin pairs showing larger birthweight discordances.

For 24 of the 27 MZ twin pairs of known chorion type, there were dental data available for most of the permanent teeth. In these twins, there was no evidence of a systematic difference in average tooth size between MC and DC twins. Figures 1 and 2 show the intrapair variances for tooth-size variables in MC and DC twins. Significant heterogeneity of intrapair variances was noted in only 5 of the 48 comparisons (*p* < .05), with

Table 1

Frequencies of Percentage Birthweight Discordance and Placentation Type in Twins

Birthweight discordance (%)	Placentation	
	Monochorionic (<i>n</i> = 14 pairs)	Dichorionic (<i>n</i> = 13 pairs)
0 < BWD ≤ 10	5 (36%)	10 (77%)
10 < BWD ≤ 20	5 (36%)	3 (23%)
20 < BWD ≤ 0	4 (28%)	0 (0%)

Note: Table probability .008 (Fisher's exact test).

three of the five dimensions involving the first molars. However, 33 of the 48 intrapair variances were greater in MC than DC twins, more than would be expected due to chance ($p < .05$).

Discussion

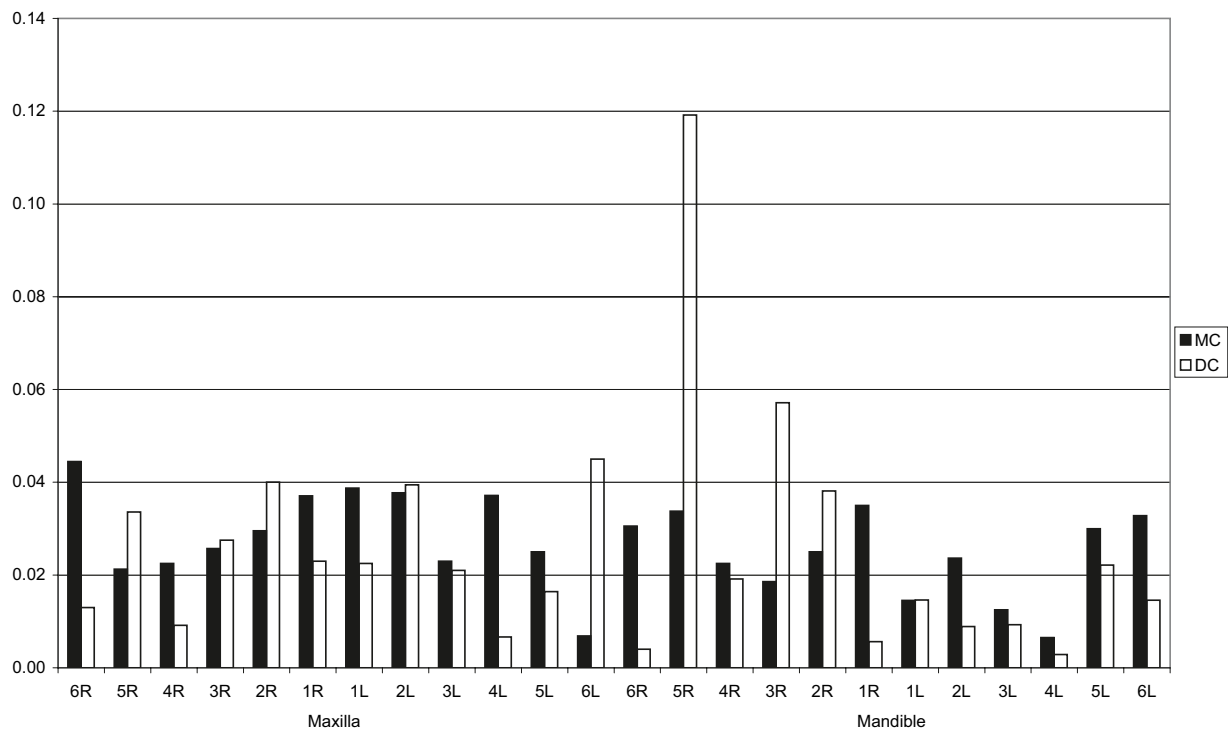
The present study examined chorion type, based on maternal reports and hospital records, birthweight and tooth-size variation in a sample of Australian MZ twins. Sixty-one per cent of the MZ twins were reported by their mothers to be MC whereas 39% were reported to be DC. This ratio is similar to previous reports by Corney (1978) and Benirschke (1995).

Studies that retrospectively examine data contained in hospital records rely on the medical staff to

accurately document all required information. In this study, chorion type could not be determined for 41 of the 68 pairs of twins for whom hospital records were viewed because the records were either too vague in their description or provided no information regarding placentation. The plea made by Benirschke in 1961 that is summarized in the paper's title — 'Accurate recording of twin placentation: A plea to the obstetrician' — is as valid today as it was 40 years ago.

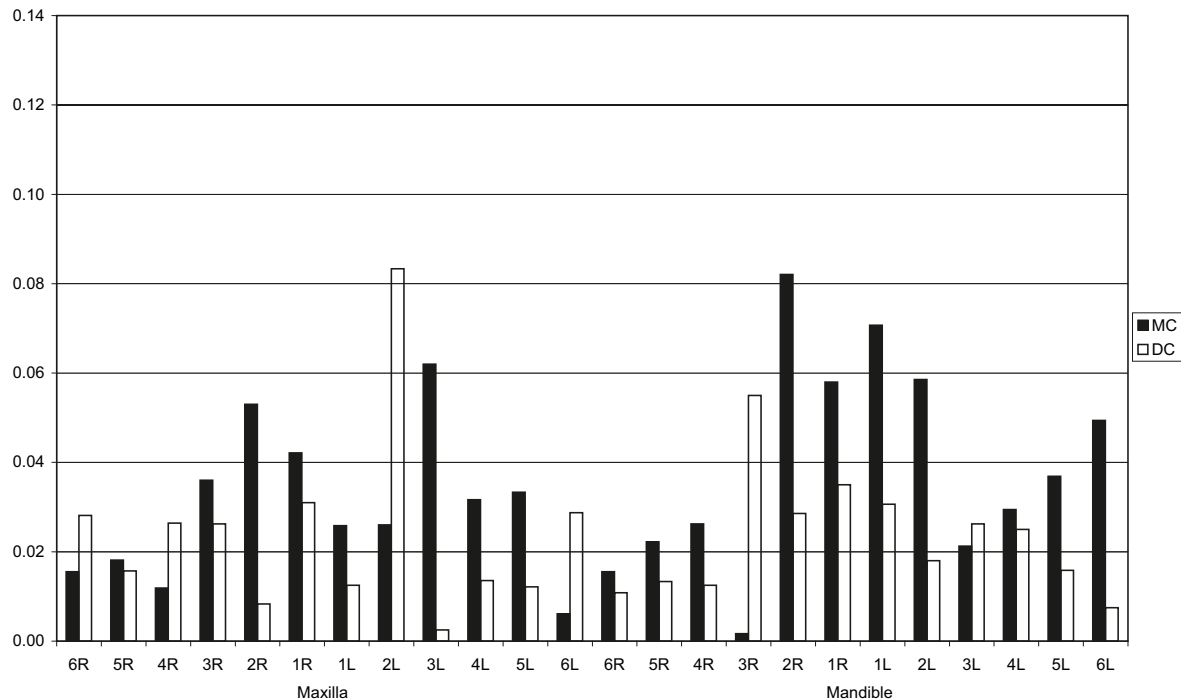
Many of the records requested for viewing (20 of the 68 records) could not be retrieved from either hospital records storage units or various government archive departments. The high number of records that were unavailable raises concern about archiving practices in Australian hospitals, especially as most of the twins were born in either the late 1980s or early 1990s. Furthermore, it poses a considerable problem for future researchers attempting retrospective studies of chorion type on twins.

No maternal reports of a single placenta or two separate placentas were found to contradict the hospital records. This encouraging fact may be because it is relatively easy to identify a single placenta or two separate placentas. In this study, all maternal reports contradicting the hospital records were due to the mothers reporting fused placentas. Eighteen cases of fused placentas were reported by mothers (out of 68), whereas the hospital records did not report one case

**Figure 1**

Intrapair variance of MC and DC twins for mesiodistal crown dimensions.

Note: 6, 5, 4, 3, 2, 1 refer to first molar, second premolar, first premolar, canine, lateral incisor and central incisor respectively. R refers to right and L refers to left. Teeth displaying significant intrapair variance ($p < .05$) between MC and DC twins were maxillary 6L and mandibular 1R.

**Figure 2**

Intrapair variance of MC and DC twins for buccolingual crown dimensions.

Note: 6, 5, 4, 3, 2, 1 refer to first molar, second premolar, first premolar, canine, lateral incisor and central incisor respectively. R refers to right and L refers to left. Teeth displaying significant intrapair variance ($p < .05$) between MC and DC twins were maxillary 3L and 6L and mandibular 6L.

of a fused placenta. This is of considerable concern, as a recent study by Loos et al. (2001) highlighted the fact that a significant proportion (55%) of DC MZ twins have fused placentas. When maternal reports were compared with hospital records for the 27 twin pairs whose chorionicity was confirmed, the maternal reports were all correct for dichorionic twins, but there were some discrepancies for monozygotic twins, presumably where there were two placentas.

Derom et al. (2003) and Carlier and Spitz (2004) both reason that it is highly probable that inaccuracy in maternal reporting of chorion type is because many obstetricians may be relaying the wrong chorion type to mothers. This unfortunate situation arises because there is still a general misunderstanding about the fact that DC twins are born in two chorionic sacs and that approximately half of DC twins placentas are fused while the other half are separate (Derom et al., 2003).

Growth and development may be affected by certain environmental stressors unique to the twin pregnancy. One of the most important variables that may affect perinatal morbidity and mortality of MZ twins is chorionicity. Phung et al. (2002) describes chorionicity as the 'variable that most strongly affects twin outcome', although its significance in terms of its relationship to outcomes is often not appreciated. DC and MC twins experience markedly different environments that present different challenges for the growing fetuses.

Monozygotic placentation significantly increases the risk of complications during pregnancy, including early pregnancy loss (before 24 weeks), preterm birth, congenital anomalies, decreased birthweight, discordant birthweight, perinatal death and the twin-twin transfusion syndrome (TTTS; Grennert et al., 1980; Hoskins, 1995; Layde et al., 1980; Machin et al., 1996; Minakami et al., 1999; Sebire et al., 1997; Seng & Rajadurai, 2000; Victoria et al., 2001; Wee & Fisk, 2002). TTTS is a serious and often fatal complication of MC MZ twinning, resulting from transplacental vascular communications (Blickstein, 1990). The incidence of TTTS has been reported to be between 5% and 15% of MC twin births; however, there seems to be some placental vascular connection in approximately 90% of MC twins (Seng & Rajadurai, 2000). This hostile environment that may affect general growth and development is almost exclusively unique to MC twins and differs significantly from that of DC twins.

The etiology of birthweight discordance seems to vary depending on whether twins are MC or DC. Hemodynamic imbalance caused by placental vascular anastomoses is the most commonly cited cause of birthweight discordance in MC twin pairs (Denbow et al., 2000; Eberle et al., 1993). Explanations for discordant growth in DC twins have included varying genetic potential, congenital anomalies, crowding *in utero*, unequal sharing of placental mass, placental insufficiency and placental vascular thrombotic lesions

and pathological conditions affecting either one or both of the placentas (Blickstein et al., 1999; Eberle et al., 1993; Rizzo et al., 1994; Wenstrom et al., 1992).

In the past, there has been an underlying assumption that large birthweight discordance between MZ twin pairs is an indication of the TTTS found almost exclusively in MC MZ twin pairs (Hay & Howie, 1980). Although we found a significant relationship between birthweight discordance and chorion type in our relatively small sample of MZ twins of known chorion type, the association is not strong enough to enable retrospective assessment of chorion type to be performed reliably.

Birthweight discordance is, by definition, a measurable developmental condition representing the absolute birthweight difference between the twin fetuses. However, this definition of birthweight discordance gives no understanding of what etiological factors are responsible for the differences seen in birthweight between twin pairs. It does, however, indicate that 'normal' growth and development has been interrupted in one or both twins that may lead to a significant increase in the risk of one or both twins developing a serious morbidity or even worse, mortality.

Little is known about whether birthweight discordance can affect other less obvious structures such as the developing dentition. Can a highly coordinated and complex process such as odontogenesis that is under strict genetic control (Cobourne & Sharpe, 2003; Thesleff, 2000) be influenced by the same environmental factors that are responsible for birthweight discordance?

Teeth are particularly useful for genetic studies because initial development takes place *in utero* with crown morphology being determined well before the emergence into the mouth. Apart from environmental factors such as functional wear, disease experience and restorative procedures, the external morphology of the teeth remains unchanged during life (Townsend & Richards, 1990). The embryological development of the primary dentition is initiated between the 6th and 8th week *in utero* and of the permanent dentition between the 20th week *in utero* and the 10th month after birth. The initial calcification of the permanent molars begins between birth (first molar) and the 7th to 10th year of life (third molar; Ten Cate, 1998). Overall, dental development continues for about 21 years postnatally, and finishes with the completion of the third molar roots. This extended time of dental development provides a good model for studying the nature and timing of developmental disturbances that may occur from *in utero* until late adolescence (Townsend & Richards, 1990).

It is well known that tooth development may be disturbed by certain postnatal environmental factors. Certain systemic conditions, such as high fever during early childhood, may produce enamel defects that may be seen after the teeth erupt into the mouth (Simmer & Hu, 2001). Most environmental factors do not disturb dental development in such an obvious

manner as a high fever or systemic disease. A less obscure example was revealed in a study by Alaluusua et al. (1996) who reported that extended breastfeeding may increase the risk of mineralization defects in the teeth of healthy children. Kieser et al. (1996) showed that parental smoking during pregnancy destabilizes dental development by delaying the maturation of the permanent teeth by up to 6 months.

Studies like these show the sensitivity of the developing dentition to certain environmental factors. Furthermore, they raise important questions about the role of the prenatal environment on the developing dentition. Given that the environment may influence dental development, it is not unreasonable to hypothesize that a stressed intrauterine environment may affect the developing dentition, both prenatally and postnatally.

Our study, like that of Burris and Harris (2002), showed no evidence of a systematic difference in average tooth size between MC and DC (fused or single) twin groups. However, when intrapair variance was examined in relation to chorion type it was found that variances for MC twin pairs exceeded those for DC pairs in 33 of a possible 48 comparisons. These results suggest that the prenatal environment may have an affect on the developing dentition. Furthermore, greater intrapair variance seen in MC than DC twins adds support to the hypothesis that, although MC twins share more similarities in their environment than DC twins, this sharing of the chorion actually increases the environmental stress during development thus increasing the discordance between MC MZ twin pairs.

An interesting observation to note regarding the MC and DC intrapair variances was that three of the five tooth-size variables that showed significant differences between MC and DC twin pairs related to the first molar teeth. These teeth are the first in the permanent dentition to begin to develop and they are also the first to commence to calcify at around birth. Given that a considerable amount of their crown formation occurs prenatally, more than any other permanent tooth, it is not surprising that they will be most likely to show an effect related to the intrauterine environment. Indeed, Dempsey and Townsend (2001) have shown previously that a significant amount (22%–27%) of the total phenotypic variation in first molar crown size can be attributed to common environment or so-called maternal effects.

Future studies are needed to clarify the extent to which tooth development is affected by chorionicity. The primary dentition should also be examined, as it is hypothesized that greater intrapair variation would be seen in the primary dentition than in the secondary dentition. This is because calcification of the primary dentition is initiated while the fetus is *in utero* and still dependent upon the placenta for survival. Stresses in the environment should have an immediate effect on the developing primary dentition. Examination of other physical features developing at the same time as

the dentition, such as dermatoglyphic patterns, would provide further evidence of the effects of chorion type.

With increasing knowledge about the importance of the chorion on general growth and development, it is essential that biologically based chorionicity reporting, such as histopathological examination or ultrasound, be performed routinely for all twin births. Ultrasound assessment of chorionicity is becoming popular and has been shown to have high sensitivity and specificity with increased accuracy if performed prior to 14 weeks' gestation (Scardo et al., 1995; Stenhouse et al., 2002). Stenhouse et al. (2002) found that chorionicity can be accurately determined in over 95% of cases when antenatal transabdominal ultrasound is used. This level of accuracy, if routinely used, would allow not only improved clinical care but also aid in unravelling the mystery that still surrounds the exact influence that the chorion has on development. Examining the association between chorion type and tooth-size variation in MZ twins would also help to improve our understanding of how environmental influences can affect the developing human dentition. The dentition, with its extended period of development, provides a unique window by which to view the influences of the intrauterine environment on general growth and development in both the early and later stages of life.

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