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Long-term effect of placental type on anthropometrical and psychological traits among monozygotic twins: a follow up study

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The long-term effect of differences in placentation in MZ twins is a controversial subject. An effect has been clearly established for birth weight but data on psychological traits are still under debate. We studied 20 pairs of monochorionic MZ (MCMZ) and 24 pairs of dichorionic MZ (DCMZ) twins. A chorion effect was observed for Block Design (WISC-R) confirming a previous report: MC MZ co-twins were more similar that DC MZ co-twins. For anthropometrical measures, an expected effect in the opposite direction was found. No chorion effect was significant for the other variables. A follow up was undertaken 3 years later using cognitive, national academic evaluations, and personality variables. The sample included 16 pairs of MC MZ and 22 pairs of DC MZ twins. Again a chorion effect was observed on anthropometrical variables but results on the Block Design were not replicated. However, the MC MZ co-twins were more similar than the DC MZ co-twins for two other cognitive variables: Perceptual Organization Index from the WISC-R and Global Visualisation from a Belgian reasoning test. Among the personality variables only one was sensitive to a chorion effect. The discussion focuses on the need for larger samples to achieve adequate power in statistical comparisons.

Keywords: twins, placenta, intelligence, weight, height, body mass, personality, uterine environment

Introduction

Within-pair similarity is generally greater in monozygotic than in dizygotic pairs, leading to the interpretation that the trait under study is controlled by genetic variance. This interpretation assumes equality of pre and postnatal environmental influences within the two types of twin. The debate on the equality of environments, however, still continues and no consensus has yet been reached, especially on the issue of prenatal influences, see Spitz et al¹ and Spitz and Carlier² or Carlier and Spitz.³ One of the most studied prenatal environmental variables that may induce differences within twin pairs is the type of placentation.

Placental differences in monozygotic twins are well known. ^{4,5} The type of placentation results from the timing of zygotic division. If the division occurs within 72 hours of fertilisation each of the two MZ twins develops its own foetal membranes, ie chorion and amnion, and as such is classified as dichorionic diamniotic. If the division occurs between days 4

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Received 25 November 1998; accepted 27 April 1999

and 7, the two embryos share the same chorion but have different amnion (monochorionic diamniotic twins). In the case of even later division, the two embryos share the same two membranes (monochorionic monoamniotic twins). The sharing of the same chorion is the most frequent case in MZ twins (about two thirds). DZ twins, in contrast, all have dichorionic diamniotic placentation as a result of the fertilisation of separate eggs.

In MZs the effect of different placentation on birth weight is best known: the within pair differences are greater in monochorionic MZs (MC MZs) than in dichorionic MZs (DC MZs). In Vlietinck et al's recent paper⁶ the chorion effect accounts for 12% of the birth weight phenotypic variance. Foetofoetal blood transfusion due to vascular communications between the foetuses may be the cause of weight discordance in a twin pair. This type of communication is often present in monochorionic placentation.7,8 Regarding the effect of placental type on psychological measures, the trend seems to be reversed, the MCs being more similar within the pair than the DCs. Table 1 summarises data already published. Due to the weak validity of the retrospective assessment of the placental type, only data from designs assessing chorion type by direct placenta examination were included in this table. Five studies satisfied this criteria and tested positive for chorion effects on cognitive and/or personality

Table 1 Summary of the published data on chorion effect on cognitive and personality measures. Papers are arranged according to the age of the twins (from the youngest to the oldest).

Authors, year, type of variables	Test used	Number of twin pairs, mean age	Results: comparison of average absolute intrapair differences between MC and DC MZs ^a		
Welch et al 11 Cognitive variables	Bayley Scales of Infant Development	20 MC MZ 12 DC MZ 18 months	No significant chorion effect		
Karras Sokol et al ¹⁰ Cognitive variables Personality variable	McCarthy Scale for Children Abilities Personality Inventory	23 MC MZ 21 DC MZ 6 years	Differences in 3 of the 19 subtests: Number questions, Tapping sequence: DC>MC. Draw a Design: DC <mc 3="" 6="" chorion="" dc="" differences="" effect="" for="" global="" in="" no="" of="" pic="" scales:="" significant="" the="">MC and in 8 of</mc>		
Melnick et al 12 Cognitive variables	for Children (PIC) WISC IQ	23 MC MZ 9 DC MZ	the 12 clinical scales: DC>MC Difference in IQ: Greater within pair mean square in DCs than MCs		
Cognitive variables		7 years	DOS (Hall IVIOS		
Spitz et al ¹ Spitz ¹³ Cognitive variables	Vocabulary and Block Design of the WAIS, K-ABC, Attention tests	20 MC MZ 24 DC MZ 10 years	Differences in Block Design (DC>MC) and not in other tests		
Rose et al ¹⁴ Cognitive variables	Vocabulary and Block design of the WAIS	17 MC MZ 15 DC MZ Adults	Differences in Block Design (DC>MC) and not in vocabulary		

^aDC>MC: the average absolute intrapair differences is larger in DC than MC MZs

measures. 10-14 Placental type was not systematically associated with cognitive measures. In all cases but one when the two groups of MZs differed, the placental difference in cognitive traits tended towards an increase in the average absolute difference within the DC MZ pairs. Only one study included personality variables. 10 Again when the two groups of MZs differed, the DC MZs pairs displayed greater average absolute difference.

Greater discordance in MC MZs than in DC MZs for anthropological measures at birth are easily understood, as vascular communications between the two foetuses are common in MC MZs (see above). The reverse trend for psychological traits, ie greater discordance in DC MZs than in MC MZs, are more intriguing. One may consider that DC MZs having their own placenta may receive different qualities of nutriment from the mother, and/or more different stimulations from the outside world through the mother's uterus. However, to the best of our knowledge no data are available to support this hypothesis.

The GNC-EFTC project (Génétique Neurogénétique Comportement – Etude Française du Type de Chorion chez les Jumeaux), the Genetics Neurogenetics Behavior - Chorion Type Twin Study, was designed in 1992 to test for the effect of placentation in MZ twins. The data obtained in 1996 were rather puzzling and it was decided to reassess the children. At the time of the first evaluation the twins were 10.44(± 0.14) years old. Anthropological (weight, height) and psychological traits were assessed. Weight and height at birth were also available. The sample included 20 pairs of MC and 24 pairs of DC MZs. The psychological battery consisted of several cognitive traits: two subtests from the Wechsler Intelligence Scale for Children (WISC-R; Block Design and Vocabulary), the two processing scales from the Kaufman Assessment Battery for Children (K-ABC, sequential and simultaneous scales), two perception tests from the Educational Testing Service kit (Identical Pictures and Hidden Patterns), an adaptation of the Shepard and Metzler Mental Rotations test, the Cornblat attention test and a semantic categorisation test (for more details see Spitz et al¹ and Spitz¹³). Laterality was also assessed.15

Differences between the two types of MZ were significant for weight at birth. Surprisingly, the differences remained significant for weight, height and body mass index when the twins were about 10 years old. In each case the within-pair difference was greater in MC than in DC MZs.1 These data refuted the common belief that chorion effect on anthropometrical measures disappears during early childhood. For laterality variables, there were no significant differences between the two types of twin. 15 For cognitive variables, a significant chorion effect was observed only on Block Design. The within-pair difference was greater in DC than in MC MZs.¹ Although this difference confirmed results from an earlier publication 14 it might be the result of a false positive due to the large number of compar-

The rationale for choosing the psychological tests for the second evaluation was the following: first, a chorion effect had been shown once on the total IQ12 and twice on the Block Design, 1,14 but no data had



been published on the totality of the WISC battery. The use of the age-appropriate Wechsler intelligence scale was thus considered as a priority. As IQs are correlated with academic achievement, we collected national school evaluations. Second, we wanted to find what elements from the Block Design were sensitive to the chorion type. This Wechsler subtest is known to be a test of reasoning involving perceptual organisation, spatial visualisation and abstract conceptualisation. ¹⁶ Perceptual organisation and spatial visualisation were assessed in the first evaluation by two perception tests and a mental rotation test. As results of the two types of twin did not differ in these tests, it was assumed that the reasoning ability was the component from the Block Design which was sensitive to placental type. Therefore, a Belgian test, the Figurative Reasoning Test (TRF)¹⁷ was selected. This test has the advantage of being less dependent on spatial reasoning than Block Design, of being non-verbal and of assessing different forms of reasoning. Third, data showing chorion effect on personality variables¹⁰ were in press when we designed the follow up. Hence, we completed the battery with some personality traits and behavioral measures. Self-attribution questionnaires completed by the twins and evaluation by teachers were used. Finally weight and height were again recorded.

In summary, the aim of the follow up was threefold: to test the persistence of the chorion effect on anthropometrical measures within the two groups of MZ twins; to determine whether the chorion effect would appear on cognitive tests other than Block Design in our population especially on reasoning ability and to check the chorion effect on personality and behavioral measures.

Materials and methods

Sample

Among the 44 families with MZ twins, five refused the follow up. One moved without giving a forwarding address and one was not contacted because of psychopathological disorders detected during the first evaluation. A pair of MCs who were unable to participate at the first evaluation because of the K-ABC upper age limit was included here. Thus, the final sample of the follow up included 38 MZ twin pairs with 16 pairs of MCs and 22 pairs of DCs. Children ranged from 10 to 16 years old (mean age: 13.05 ± 0.043 years). The mean ages and the socioeconomic status of the head of the family did not differ between the two groups of MZs (F = 1.43, df = 31.43, P = 0.28; $\chi^2_{(3)}$ = 2.36; P = 0.50, respectively). The diagnosis of zygosity was ascertained using molecular genetic technique with DNA extracted from buccal epithelial cells, dermatoglyphic analysis and a questionnaire completed by the parents. ¹⁸ The diagnosis of chorion type was established by MCVacher Lavenu, a pathologist specialising in the study of the placenta. For more details on the sample selection and diagnoses see Spitz et al. ^{1,18}

Measures

Parents signed informed consent before coming with the twins to the laboratory for half a day. Co-twins were tested separately by two trained examiners. All tests were scored blindly by a child psychologist and by the two first authors.

Anthropometrical variables Weight, height and body mass index (weight, kg/length, m²).

Cognitive variables The WISC-III (1996)¹⁹ provides three IQs (Verbal, Performance and Full Scale score) and three indexes (Verbal Comprehension, Perceptual Organization, Processing Speed) using the same scaled score (mean = 100, standard deviation = 15). The TRF consists of 90 items of figurative reasoning in which the twins have to choose one picture from five. Four types of items (labelled as analysis, synthesis, analysis + synthesis, and visualisation) give four subscores. In respect of academic achievement: every French schoolchild sits standard exams in the third year of primary school and in the 6th grade. Each exam includes a test in French and in mathematics. National norms are available each year.

Personality and behavioral measures WAE-C,²⁰ the Weinstein self-evaluation questionnaire, includes 23 items covering different attributes such as intellectual ability, social skills, physical attractiveness, emotional stability, dynamism and discipline. The questionnaires were applied for self-evaluation and cross-evaluation, ie subjects answered the questions for themselves and for their co-twins. The Rutter children's behaviour questionnaire²¹ was also applied, and a French adaptation of the Devereux Elementary School Behavior (DESB)²² was completed by the child's school teacher.

Statistical analyses

Different techniques were applied to adjust the data for covariables shared by the co-twins (age and gender). French normalised standard scores were available for the WISC-III only. 19 French normative data by age and gender (means and standard deviations) were available for anthropometrical 23 and for academic achievement measures. Raw data were

thus transformed into standard scores (classic z scores). For the other tests, the raw data were adjusted, when necessary, by regression analyses for age and gender using data from our sample. All available twin data (76 individuals if no missing data) were used to estimate regression coefficients. The adjusted score was classically defined as the difference between the raw and the expected

Standard and adjusted scores were analysed using a FORTRAN program TWINAN90,24 incorporating a test of equality of means and variances across twin types. The test of normality of the distributions proposed by the program is valid only for samples with at least 25 pairs, therefore it was not used here. Chorion effect was tested by comparing the average absolute within-pair differences between the two groups of twins (t test). A one-tailed test was chosen for anthropometrical measures as it is well known that the DC pairs are more similar than the MC ones. For psychological measures, a more conservative two-tailed test was used, since the direction of the chorion effect is not as clear. Note that the intraclass correlation coefficients are displayed in the result tables only as an indication of the intrapair similarity, but are not used in the statistical comparisons.

To balance Type I and Type II errors, 25 a hierarchical strategy was used for the Weschler scales analysis. First the six global scales (three IQs and three indexes) were examined. If there was a significant result for an global scale, then a further level of analysis occurred on the subtest included in this global scale.

Results

Anthropometrical measures

A chorion effect was significant for weight and body mass index (see Table 2): the MC MZ pairs were less similar than the DC MZ pairs. However, resemblance between the MZ co-twins was high irrespective of chorion type (see Table 2).

Cognitive traits

Results on the global scales of the WISC-III are presented in Table 3. A significant difference was observed in the Perceptive Organisation Index only: the MCs were significantly more similar than DCs (t = 2.44, 34.4 df, P = 0.02). This index includes four subtests from the Performance scale: Picture Completion, Picture Arrangement, Block Design and Object Assembly. No significant difference was observed in any of these subtests, including the Block Design. An analysis of variance with repeated

Table 2 Anthropometrical measures at the time of the second evaluation: means of the absolute intrapair differences and comparisons of MCs and DCs

	r	d±SE	df	t one-tailed, P			
Weight (after adjustment for age and gender)							
MC `	0.85	3.76±0.47	26.6	t=1.77			
DC	0.95	2.32±0.47		P=0.04			
Height (after adjustment for age and gender)							
MC `	0.95	1.82±0.34	30.9	t=0.86			
DC	0.98	1.45±0.27		P=0.20			
Body Mass Index (weight, kg/length, m²)							
МC	0.78 [`]	1.2 ±0.23	26.7	t=1.87			
DC	0.93	0.71±0.16		P=0.04			

r: intraclass correlation; d±SE: average absolute intrapair differences±standard error; df: degrees of freedom estimated according to the inequality of the variances

Table 3 WISC-III global scales: means of the absolute intrapair differences and comparison between MCs and DCs

	r	d±SE	df	t two-tailed, P
Verbal IQ M C DC	0.676 0.947	8.063±1.61 4.727±0.72	23.3	t=1.93 P=0.07
Performance MC DC	e IQ 0.846 0.756	5.250±0.84 6.864±1.20	38.0	t=1.09 P=0.28
Total IQ MC DC	0.814 0.925	6.125±0.93 5.409±0.64	31.4	t=0.53 P=0.60
Verbal Comp M C DC	orehension 0.601 0.920	index 7.813±1.74 5.636±0.83	25.7	t=1.20 P=0.24
Perceptive o M C DC	rganisatior 0.910 0.714	n index 3.625±0.69 6.909±1.23	34.4	t=2.44 P=0.02
Treatment ra MC DC	apidity ind 0.721 0.814	ex 7.375±1.71 6.045±1.07	29.2	t=0.73 P=0.47

r: intraclass correlation; d±SE: average absolute intrapair differences±standard error; df: degrees of freedom estimated according to the inequality of the variances

measures carried out on the population assessed twice led us to conclude that the non-replication of the chorion effect on the Block Design was not due to differential loss of participants (the interaction between placental type and evaluation time was significant, F = 7.34, 1.78 df, P = 0.01).

In the Figurative Reasoning Test, the average intrapair differences of the two groups differed on the visualisation score (MC < DC, t = 2.04, 38 df, P = 0.05). The two groups did not significantly differ in the other three abilities.

The national academic evaluations did not discriminate between the two groups of twins. However, these results must be considered with caution since we failed to obtain scores of the evaluation for many individuals (more than half of our sample). It



should be noted, however, that the intra-class correlations were very high (between 0.75 and 0.92 in most cases), irrespective of type of twin. This indicates that this type of measure is reliable even if a correlated measurement error effect might be expected (due to the fact that most of the co-twins from the same pairs were tested on the same day, in the same school).

Personality and behavioral variables

Several school teachers refused to complete the questionnaires, others did not answer every question. Consequently, our sample was reduced to 10 MC pairs and 15 DC pairs for some comparisons. Moreover, the Rutter questionnaire was not valid for our groups since the variability of the scores was very low, whatever the type of chorion, indicating that the twins very rarely displayed 'anti-social' or 'neurotic' behaviour. Hence the non-significant differences between the two groups of MZs on these questionnaires are not very informative.

Every twin completed the Weinstein self-evaluation questionnaire. The two groups of twins differed in only one of the five subscores: the perceived difference between the co-twins was greater in DCs than in MCs when the twins were asked to evaluate their intellectual ability and the ability of his or her co-twin (t = 2.24, 30.1 df, P = 0.03).

Discussion

A chorion effect was replicated on weight and body mass index: the MC MZ pairs were less similar than the DC MZ pairs. These results concord with the literature and our data from the first evaluation when the twins were 8–12 years old. Hence placental type has a long term effect on anthropometrical measures.

Significant placental type effects on within pair similarity were observed on two cognitive variables in relation to perceptual organisation: Perceptual Organization Index from the WISC-III and Visualization from the TRF. For all other personality and behavioural variables save one, no significant chorion effect was observed. The differences were of the nature described by previous papers (see Table 1 and Bleckher et al²⁶): the MCMZ pairs were more similar than the DC MZ pairs. It is noteworthy that the size of our sample did not allow conformity with the statistical model in each case (equality of means and variances in the two MZ groups) for the psychological variables. Moreover, as a number of variables were tested, the few significant differences found in psychological traits between the two MZ groups could be due to random fluctuations. Nor would a sample effect be ruled out.

Conclusion

In every available study dealing with placental differences and cognitive traits the group sample sizes were small. Therefore, the sample sizes were probably not large enough to detect adequately a small chorion effect. Using our data on Block Design from the first evaluation Wahlsten (personal communication 1996) came to the conclusion that we had achieved a detectability power of only 60%.1 This explains the non-replication of our results. Since to our knowledge it is not possible to assess chorion type retrospectively it is preferable to develop a prospective study, as did the Leuven team: the East Flanders Prospective Twin Survey has registered all twin pairs born in East Flanders since 1964.27 Another solution might be to assemble a consortium of all researchers who have collected data on that topic or to follow up the twins of the European Multiple Birth Study (EMBS).²⁸

Acknowledgements

This research was supported by grants to M Carlier from Sciences de la cognition 1992 and CNRS (UPR 9074), Ministry for Research and Technology, Région Centre, Préfecture de la Région Centre, University of Orléans and Institut Universitaire de Formation des Maîtres (Orléans-Tours). We thank the parents and the children who have collaborated in the study, CdeLongevialle for her participation in collecting data, F Perez-Diaz and A Cherfouh for their patience during statistical analysis. We are grateful to ECPA who allowed us to use the last version of the WISC before its publication. Without the help of physicians in three obstetric departments, this research could not have been undertaken - Gynecology and Obstetrics of the Hôpital International de l'Université de Paris, Dr H Cohen, CHU Cochin, Gynecology and Obstetrics, Port Royal - Baudelocque (Professors R Henrion, E Papiernik-Berkhauer, C Tchobroutsky), Neonatal Medicine (Professor JP Relier and Dr O Bethmann).

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