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METHODS AND MEASURES

The discordant MZ-twin method: One step closer to the holy grail of causality

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Twin studies are well known for their value in quantifying the contribution of genes to population variation in behaviors and personality traits. Twin studies also provide a unique opportunity to untangle the contribution of environmental experiences to emotional and behavioral development. This is particularly true when examining monozygotic (MZ) twins since they represent a pair of individuals naturally matched on both their genetic background and their shared environment, thus allowing the identification of environmental experiences unique to each twin which may impact developmental outcome. This article presents two analytical strategies based on the discordant MZ-twin method. It stresses the power of this method to establish plausible causal pathways between environmental factors and developmental outcomes, and provides examples from the socio-developmental literature to illustrate its application. It also describes the limitations of this method and its requirements for optimal utilization.

Keywords: causality; methods; twins

Developmental psychologists and psychopathologists have been searching for new methods to test causal effects of environmental variables on children's and adolescents' developmental outcomes. Testing for causality implies, essentially, testing that: (i) the variation of the environmental variable precedes the variation of the outcome measure; and (ii) the co-variation between an environmental variable and an outcome variable cannot be explained by a third factor (i.e., the confounders) (Susser, 1991). The most convincing method to achieve such high standards of internal validity is by experimentally manipulating the environmental variable while keeping the confounders constant through random assignment of the participants into different levels of the environmental variable (i.e., the experimental conditions; Campbell & Stanley, 1963; Shadish, Cook, & Campbell, 2002). While desirable, this strategy is not always possible for either ethical or practical reasons. For example, it would be unethical to expose children to peer victimization to examine its impact on their development.

A vast array of non-experimental methods has been proposed to maximize internal validity without direct manipulation of the environmental variable. These approaches rely on methodological and statistical strategies to control for possible confounders (Rutter, Pickles, Murray, & Eaves, 2001; Shadish et al., 2002). Part of this arsenal of methods to achieve control over possible confounders is the discordant monozygotic (MZ)-twin method (Dick, Rose, Viken, Kaprio, 2000; Jinks & Fulker, 1970; Kaprio et al., 1993; Pike, Reiss, Hetherington, & Plomin, 1996). Although not equivalent to true experiments, the discordant MZ-twin method may be among the best methods to achieve strong internal validity because it controls for a wide range of confounding factors, either genetic or environmental. The first goal of this paper is to describe and illustrate the use of the discordant MZ-twin method. The second goal is to explore the conditions, extensions, and limitations of its applicability.

Overview of the discordant MZ-twin method

MZ twins share 100% of their genetic background and they grow up sharing several factors in their environment, such as prenatal exposure to maternal smoking, poverty, maternal depression, or criminality in the neighborhood. Yet, even MZ twins growing up together do not share all possible environmental experiences; some experiences are unique to each twin (i.e., non-shared). Examples of such unique experiences that may only concern one twin in a pair can be a visit to the hospital, being bullied by a classmate, or having an inspiring teacher. By focusing on the within-twin pair differences with respect to these unique experiences, and by linking them to within-twin pair differences in psycho-behavioral functioning, the discordant MZ-twin method offers a unique tool to establish a probable causal pathway between exposure to non-shared environmental experiences and each twin's psycho-behavioral development. This assumption of probable causality rests on the fact that genes and shared environmental experiences cannot explain differences between the two members of an MZ pair, because these factors are shared (i.e., constant) within a given twin pair. In other words, the potential influence

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of genes and of shared environmental experiences on psychobehavioral functioning is methodologically controlled in the discordant MZ-twin method. When left uncontrolled, as is the case in studies using only one child per family, these factors may be confounded with the non-shared environmental experiences of interest through gene–environment correlations or through environment–environment correlations. A gene–environment correlation could occur, for example, when a child's genetic disposition to aggressiveness increases the child's risk of becoming the victim of a school bully. Any association observed between exposure to school bullying and subsequent aggression might thus be explained, at least in part, not by the bullying experience but by the child's genetic disposition to aggressiveness – unless such genetic influence is controlled. Similarly, an environment–environment correlation could occur, for example, when a high general level of criminality in the neighborhood increases the risk of a child becoming the victim of an attack. Any association observed between the experience of the personal attack and subsequent aggression might thus be explained, at least in part, not by this attack experience (which is unique to the child) but by the high general level of criminality in the neighborhood (which is shared by the members of a family) – unless such shared environmental influence is controlled.

Notably, despite the control for genetic and shared environmental influences, there may be other measured and non-measured factors that may differ between two MZ twins and that may operate as confounders when examining the role of a specific non-shared environmental influence on an outcome. For example, twins can affiliate with different friends whose aggressiveness differs. These differences in friends' aggressiveness can contribute to twins' differentiation with respect to aggressiveness as much as differences in experience of school bullying can. It may thus be important to control for correlated non-shared experiences that may operate as confounders with the main environmental variable of interest. The plausibility of a causal role of non-shared environmental experiences can be augmented further by showing that these non-shared environmental experiences predict *increases* in MZ twin differences in psychological or behavioral functioning through the use of a longitudinal approach; i.e., by controlling for initial within-pair differences with respect to the outcome variable. Although the discordant MZ-twin method can be applied to concurrent data, a longitudinal approach is particularly important to disentangle directionality between environmental effects on the person and person effects on the environment (Kendler & Baker, 2007). Such an approach requires appropriate timing (in developmental terms) of the measurement of the environmental experiences, and sufficient change in the outcome variable over the assessed time period.

Methodological considerations of the discordant MZ-twin method

Analytical strategies using discordant MZ twins rest on the variability between two twins in a pair with respect to given environmental and outcome variables. To maximize "true" intra-pair variability, several issues need to be considered. First, because MZ twins are assumed to be genetically identical, few differences in the variables are observed if the measure is largely explained by genetic factors. In the domain of psychopathology, this requirement is met relatively easily. Indeed, even with so-called "heritable" behaviors such as aggression, anxiety, and depression, 40% or more of the variance is explained by non-shared environmental factors (Happonen et al., 2002; Rhee & Waldman, 2002; Thapar & McGuffin, 1995). Importantly, a similar requirement also refers to the environmental variable of interest. A related point refers to the possibility that heritability estimates may vary with age, perhaps because external constraints are minimized and gene–environment correlations are maximized as children grow older (Rushton & Bons, 2005). One example in this regard would be the active selection of friends with certain characteristics (e.g., aggressiveness) based on the children's own genetic propensity for such behavior. Because older children and adolescents can be more autonomous in their friendship choices than younger children, genetic influence on friends' aggressive characteristics may increase with age and thus yield fewer differences between MZ twins in this regard. Therefore, it may be more promising to examine MZ twins' discordance in social experiences at the earliest age when these experiences become relevant for child development.

Second, the decision to use dimensional or categorical measures of the environmental and the outcome variables is influential when attempting to maximize variability between twins. Generally, continuous measures provide optimum variance as opposed to categorical measures. More specifically, when categorical measures are used (e.g., presence or absence of a clinical diagnosis of depression), the likelihood of finding sufficient numbers of MZ pairs who are discordant on these variables can be low. As a result, statistical power may be compromised unless a very large sample of MZ twins is available. In contrast, dimensional measures of the environmental or the outcome variables are likely to generate sufficient within-pair variability even in relatively small samples and may thus be preferable. However, the choice between dimensional and categorical measures ultimately needs to be informed by theoretical considerations rather than by mere methodological constraints.

Finally, another way to maximize differences between the two twins in a pair with respect to the environmental and the outcome variables of interest is to use multiple informants for each member of the pair. When using a single informant for both twins in a pair, similarity between twins may be exaggerated by expectations that MZ twins should behave or be treated similarly, which may create inflated intra-pair correlations with respect to the measured variables. To avoid such biases, the use of multiple informants for each twin may be a solution. For example, the use of both mothers *and* fathers reporting on each child's behavior or the use of peer ratings, or of independent observers, would maximize intra-pair variability in the variables of interest. However, the use of multiple informants may also artificially increase within-pair variability. To overcome this dilemma, we propose two strategies: when a single informant is used to assess an environmental or outcome variable for both twins in a pair, objective measures that do not require a subjective assessment from the informant would be preferable. When subjective measures are unavoidable, the use of more than one informant for each twin would be ideal. This would enable the computation of latent scores across the informants for each twin in order to minimize bias and measurement error. In this case, it is also advisable to use informants from the same context (e.g., mothers and fathers, or teachers and classmates) to ensure sufficient inter-rater concordance (Achenbach, McConaughy, & Howell, 1987).

Illustrations of the discordant MZ-twin method

Although the method itself is almost 40 years old (Jinks & Fulker, 1970) and has been widely used in the medical field, reports using the discordant MZ-twin method to examine the association between environmental experiences and children's or adolescents' psycho-social development are relatively recent. Two different, albeit related analytical, strategies have been used in these studies. Each strategy is described in more detail in the next sections.

The difference score strategy

The first strategy refers to the *difference score strategy* and is based on the correlation between relative differences between members of an MZ dyad with respect to an environmental experience of interest and relative differences between members of an MZ dyad with respect to an outcome. In the following illustration, both the environmental and the outcome variables are based on continuous scores. The environmental and the outcome variables are treated separately, and relative within-pair difference scores are calculated by first randomly assigning one of the twins as Twin 1 and the other as Twin 2, and second by subtracting the score of one twin from the score of the co-twin. Correlations, regression analyses or structural equation modeling can then be used to assess the association between difference scores on the environmental factor and difference scores on the outcome. In the ordinary least square (OLS) regression framework, the application of the MZ method can be formulated in the following way:

$$E(\Delta Yi) = \beta w\ (\Delta Xi)$$

The coefficient βw gives the expected change in the difference between Twin 1 and Twin 2 on the outcome variable (ΔY) for each unit of change in the difference between Twin 1 and Twin 2 on the environmental variable (ΔX).

As already mentioned, differences in other correlated environmental variables (i.e., confounding variables) need to be controlled to avoid spurious effects. To illustrate, Caspi et al. (2004) used differences in observer-rated, maternal-expressed emotion to predict, both concurrently and longitudinally, differences in mother and teacher ratings of antisocial behavior in a sample of 565 five-year-old MZ pairs. Using a series of hierarchical multivariate regressions, the authors showed that differences in maternal warmth were associated with current and later within-pair differences in antisocial behavior, even after accounting for possible confounders such as differences in neurological status, as indexed through differences in birth weight.

Putative moderators can also be added to the analyses. Importantly, moderators do not necessarily have to be variables that are unique to each member of a twin pair but they can also be shared variables that are the same across the two twins in a pair. For example, Asbury, Dunn, Pike, and Plomin (2003) examined whether differences in negative parental feelings toward the child and differences in harsh parental discipline predicted differences in a series of behavioral outcomes in a large sample of 4-year-old MZ twins. The authors identified variables that moderated the co-variation between the environmental variables and the outcomes. These moderators referred to shared environmental experiences, namely family socio-economic status, family chaos, and maternal depression. Family chaos was particularly important as a moderator, exacerbating the relationship between differences in parental negative feelings and differences within a twin pair in both externalizing and internalizing behaviors. As illustrated by a recent study, mediators can also be added to the analyses. Specifically, Vitaro et al. (2008) examined whether differences in friends' aggressiveness in kindergarten predicted increased differences in MZ twins' aggressiveness from kindergarten to grade one and whether differences in friendship quality mediated these longitudinal relationships. To ensure that the observed associations were not spurious, differences in parent coercion and peer rejection were controlled because these variables were related to both friends' aggressiveness and twins' aggressiveness. Results showed that differences in friends' aggression during kindergarten significantly increased MZ twins' differences in aggression from kindergarten to grade one for both boys and girls. Differences in friendship quality mediated this association, albeit only for boys.

Notably, instead of examining differences in social experiences as predictors, some studies investigated differences in personal characteristics or in behavior (e.g., age of menarche, birth weight, testosterone, depressive symptoms, early cannabis use, headaches) as predictors of differences in developmental outcomes (Dick et al., 2000; Eriksson, Kaprio, Pulkkinen, & Rose, 2005; Lynskey et al., 2003; Riese, 1994, 2001; Sihvola et al., 2008; van Os et al., 2001; Virtanen et al., 2004). These studies can be considered as other examples of the application of the difference score strategy since within MZ-twin pair differences in personal characteristics and behavior likely reflect influences from unique environmental experiences. For example, by examining how differences in cannabis use among adolescent twins are related to differences in later alcohol and drug abuse/dependence, Lynskey and colleagues (2003) were able to determine the potential causal effect of cannabis exposure as a unique environmental risk factor on later adjustment. Notably, the authors controlled for differences in tobacco and alcohol use as well as differences in psychiatric disorders because of their partial overlap with differences in cannabis use. They also speculated about a series of pharmacological and psycho-social mechanisms that could have accounted for these likely causal effects of cannabis use, but no mediation test was performed.

A variant of the difference score strategy rests on identifying discordant twin pairs with respect to the outcome of interest and comparing them on theoretically relevant predictors. Virtually all of the studies that used this variant relied on a dichotomous grouping procedure with respect to the outcome variable (i.e., they identified high/low twin pairs). To illustrate, Korhonen et al. (2008) compared adolescent MZ twins who were discordant with respect to cannabis use on a series of antecedent individual, family, and peer factors. The authors found that early smoking initiation, drinking to intoxication, teacher-rated disruptiveness, a high number of smoking peers, and having at least one acquaintance with drug experience was related to within-pair discordance in cannabis use. In a similar type of study, Asbury, Dunn, and Plomin (2006) identified 9-year-old MZ twin pairs who were discrepant on anxiety according to both teachers and mothers. The authors were interested in identifying which perinatal, parent–child relationship and peer-related factors distinguished the twins in the discordant pairs. They observed that negative school experiences, illness and accidents, traumatic neonatal life events, parent–child relationships, and peer rejection distinguished the anxious from the non-anxious members of the twin pairs.

Some authors went a step further and compared discordant MZ-twin pairs with concordant MZ twin pairs to identify both non-shared and shared influences. As already mentioned, non-shared factors refer to environmental experiences that are unique to each twin in a pair. In contrast, it is important to keep in mind that shared factors refer to either genetic or environmental factors that are shared by both twins in a pair. The shared factors can differ between different twin pairs only, whereas the non-shared factors can vary between twins from the same pair. To illustrate, Lehn et al. (2007) identified MZ twin pairs that were discordant with respect to hyperactivity-attention deficits (ADHD) problems and MZ twin pairs that were concordant for the presence or the absence of ADHD problems (termed affected and non-affected pairs, respectively). In accordance with the variant of the difference score strategy described previously, the authors first contrasted the twins from the discordant pairs and found that the affected twins experienced more adversity in infancy such as lower birth weight and more time in the incubator and delayed motor and physical development compared to their unaffected co-twin. In addition to these within-pair comparisons, the authors also conducted between-pair comparisons of concordant MZ twin pairs to identify shared environmental influences of ADHD. Specifically, they found that concordant-affected MZ pairs were exposed to mothers' smoking during pregnancy, and lived in single-parent families more often than concordant non-affected MZ pairs (for other illustrations of this method see Cath, van Grootheest, Willemsen, van Oppen, & Boomsma, 2008; Pearsall-Jones et al., 2008; van't Ent et al., 2007). Notably, the within-twin pair comparisons and the between-twin pair comparisons in these studies were performed in separate analyses. The analytical strategy we describe next offers an elegant way to combine these separate analyses.

The mixed strategy

The second analytical strategy refers to the *mixed strategy* and offers the opportunity to simultaneously investigate the influence of unique and shared factors by assessing and comparing within-twin pair and between-twin pair effects (Carlin, Gurrin, Sterne, Morley, & Dwyer, 2005). As illustrated below, the within-twin pair coefficient estimates the effect of differences between the two members of the same twin pair with regard to a predictor variable X on an outcome variable Y. The between-twin pair coefficient estimates the effect of differences between different twin pairs in regard to the same predictor variable X on an outcome variable Y. Akin to multi-level models, the mixed strategy can thus inform on the effect of experiences that are unique to each twin within a pair as well as on the effect of factors, both environmental or genetic, that are common to the pair (but that may differ between different pairs). In contrast, the difference scores strategy documents solely the role of non-shared environmental factors. The basic equation that estimates the within- and the between-pair effects is the following (Carlin et al., 2005):

$$E(Yij) = \beta o + \beta w\ (Xij - \bar{X}i) + \beta b\ \bar{X}i$$

In this equation, (i) Yij represents the standardized estimate of the outcome variable for each twin, (ii) Xij represents each twin's score on the predictor variable, (iii) $\bar{X}i$ represents the average score of the two twins in a pair on the predictor variable, and (iv) $Xij–\bar{X}i$ represents the difference between each twin's score and the average score of the pair on the predictor variable. The βb coefficient represents the between-twin pair effect of the predictor variable X, whereas the βw coefficient represents the within-twin pair effect. For MZ twins, the βw coefficient is numerically similar to the $\beta w(\Delta Xi)$ coefficient yielded by the difference scores strategy described previously (see Carlin et al., 2005, for more details). In both strategies, this effect represents variation in the outcome that is explained by within-pair differences in the predictor variable, over and above the effect of between-pair differences. Only in the mixed strategy can the effect of between-pair differences be specifically estimated, however.

To illustrate the application of the mixed strategy, we refer to two studies which, to our knowledge, are the only studies to use this strategy in the field of behavioral development. In the first study, Arseneault and her colleagues tested whether variation in the experience of being bullied in a sample of 573 MZ twin pairs was associated with variation in internalizing problems (Arseneault et al., 2008). Measures of bullying for this study were categorical, identifying children who have been bullied versus those who have not. Findings indicated that the unique (i.e., non-shared) effect of being bullied by the age of 7 years was significantly associated with children's internalizing problems at age 10, over and above the significant effect of factors common to both twins in a pair. More specifically, the authors showed that twins who have been bullied had close to half a standard deviation more internalizing problems compared to their co-twins, who have not been bullied. This difference remained significant even after controlling for prior internalizing problems assessed when the twins were aged 7 years. This is strong support for a causal effect of being bullied on children's internalizing problems. First, these results indicated that the effect of being bullied on children's internalizing problems cannot be accounted for by a wide range of potentially confounding variables such as genetic makeup or family background. Second, the longitudinal analysis showed that the unique effect of being bullied remained significant after controlling for prior internalizing problems, thus demonstrating temporal priority between the bullying experience and changes in internalizing problems.

Wright and colleagues also utilized the mixed method to test whether differences in self-control, parenting, and number of deviant peers during adolescence predicted differences in delinquent and criminal behavior by early adulthood (Wright, Beaver, Delisi, & Vaughn, 2008). Using hierarchical linear modeling, the authors found that differences in regard to number of deviant peers, but not in regard to self-control or parenting, predicted variations in delinquent behavior within MZ dyads. Moreover, between-pair differences in the number of deviant peers predicted variations between twin dyads with respect to delinquent behavior. The authors, however, did not control for twins' initial level of delinquency; hence, it is not clear whether differences in the number of deviant peers predicted an increased variation in delinquent behavior either between or within-twin pairs.

Concluding remarks

The discordant MZ-twin method is a powerful methodological tool for investigating the causal contribution of non-shared environmental experiences to children's development. This method naturally controls for a wide range of confounding factors, both genetic and environmental, that are shared between the two twins in a pair and, when applied to longitudinal data, can also test for temporal priority. The two strategies presented here afford high internal validity, especially if (i) a longitudinal design is used, (ii) correlated non-shared environmental experiences are controlled, and (iii) theoretically relevant mediators and moderators are included to support the theoretical coherence of the basic associations. The strategies are simple to use and they require no special statistical software. In sum, they can be used by anyone who has access to MZ twin data. However, they require that the MZ twins are sufficiently discordant with respect to the environmental and outcome variables of interest.

Because of the strict control of potential confounders in the discordant MZ-twin method, the percentage of explained variance is often modest (often less than 5%). Modest effect sizes are to be expected with this method especially when examining intra-familial environmental experiences. This is because MZ twins are often highly similar with respect to these experiences, and this similarity is even more pronounced if the same informant is used to rate both twins. Yet, these environmental effects may be closer to reality than those generated by singleton studies. The larger effect sizes found in singleton studies with respect to the effect of the intra-familial environment may actually be inflated because, in contrast to the discordant MZ-twin method, singleton studies do not control for the possible effect of genes on the environmental variables (i.e., gene–environment correlations) and on the outcome variables (Plomin, DeFries, & Loehlin, 1977). Indeed, many behavioral-emotional variables and also many environmental variables – especially intra-familial – are partially influenced by genetic factors. This influence is teased out in studies using the discordant MZ-twin method, often leaving relatively little intra-pair variance that can co-vary among the study variables (Oliver, Pike, & Plomin, 2008).

Given this context, the discordant MZ-twin method may be most successful when testing the role of non-shared extra-familial experiences. Indeed, extra-familial experiences are likely to differ more between the two members of a twin pair than intra-familial experiences because the former may be somewhat less influenced by genes than the latter. It should be noted, however, that even extra-familial experiences, such as peer rejection and victimization or friendship relations, may be at least in part under genetic influence (Ball et al., 2008; Brendgen & Boivin, 2009). Moreover, as mentioned earlier, the genetic influence on environmental experiences may become stronger with age as children's ability to actively select certain features of their social environment increases (Rushton & Bons, 2005).

The plausible causal role of differential environmental experiences could be enhanced further by examining the inter- and intra-personal mediating mechanisms that may account for their "effects". One interesting area of research in this context would be to link these differential social experiences to divergent patterns of DNA methylation that seem to emerge over the life span of MZ twins (Fraga et al., 2005). Methylation is a main component of epigenetics, which refer to chemical instructions for gene activity that do not alter DNA sequences (Tsankova, Renthal, Kumar, & Nestler, 2007). Differences in methylation, which may themselves be a result of non-shared environmental influences, may produce phenotypic differences between MZ twins (e.g., with respect to psycho-behavioral adjustment; Bruder et al., 2008; Kaminsky et al., 2009). Ignoring this issue might, therefore, lead to a false conclusion of direct environmental causes to explain phenotypic discordance in MZ twins (Wong, Gottesman, & Petronis, 2005). Two possible solutions can be considered. First, MZ twins could be regularly genotyped to see whether differences in methylation (or other epigenetic markers) correlate over time with specific non-shared environmental experiences and specific psycho-behavioral manifestations in a bi-directional, transactional manner. In this way, the possible epigenetic differences among co-MZ twins could be incorporated into explanatory models, both (i) as predictors of within-pair discordance at the psycho-behavioral level, (ii) as outcomes of specific non-shared environmental experiences and (iii) as possible mediators of the links between exposure to these specific non-shared environmental experiences and increased differentiation among co-MZ twins at the psycho-behavioral (i.e., phenotypic) level. The advent of new and relatively inexpensive technology to determine changes in gene expression makes this solution within reach. The second solution would be to study relatively young MZ twin pairs, because epigenetic differences between MZ twins only seem to become apparent later in life (Fraga et al., 2005).

Additional areas of research also remain unexplored. First, it would be informative to explore each twin's possible differential perceptions and reactions to the same experience as additional sources of differential-unique experiences. Indeed, it is possible that some environmental experiences that are assumed to be shared between the two twins in a pair (e.g., parental unemployment or divorce) are perceived by each member of a MZ dyad as different at the subjective level (Turkheimer & Waldron, 2000). Unless assessed and treated as differential experiences, these potentially different subjective reactions to shared environmental experiences can attenuate findings. Second, although the use of dizygotic (DZ) twin pairs is not informative in and of itself for assessing the role of non-shared environments, it could prove useful to see whether the same pattern of results applies to same-sex DZ twin pairs and to MZ twin pairs. If differences in environmental experiences predict discordance in psycho-social functioning equally well in MZ and same-sex non-genetically identical DZ twin pairs, it is possible to conclude that the mechanism whereby the environmental factor affects psycho-social functioning is not only likely causal, but also likely entirely environmental (i.e., no genetic programming is involved; Cederlof, Friberg, & Lundman, 1977; van Os et al., 2001). Hence, zygosity could be treated as a potential moderator of the linkages between within-pair differences on the predictor variables and within-pair differences on the outcome variables. Finally, it would be interesting to test transactional models whereby differential experiences could both influence and be influenced by differences in twins' psycho-social functioning. For example, differences in friends' aggressiveness can predict an increase in twins' discordance with respect to their own aggression scores (a peer socialization effect); in turn, differences in twins' aggressiveness can foster an increased difference in friends' aggressiveness (a selection effect). Examining these possible bi-directional effects over time could reveal interesting developmental patterns while clarifying the dynamic interplay between uniquely experienced environmental factors and personal adjustment. Including methylation effects or micro-social processes at the inter- and intra-individual levels would make the model even more comprehensive as already argued.

A final point to consider when using either of the MZ twin strategies is that, although internal validity may be high, external validity might be limited. Although twins' psychobehavioral characteristics are similar to those of non-twins (Gjone & Novik, 1995; Kendler, Martin, Heath, & Eaves, 1995; Pulkkinen, Vaalamo, Hietala, Kaprio, & Rose, 2003), some findings may only apply to twins and cannot be generalized to other populations. To bolster external validity, researchers may need to demonstrate that MZ twin samples are comparable to population-based samples of singletons with respect to the environmental and the behavioral-emotional variables under study, as well as with respect to potential confounding factors. Alternatively, they may use a sample of singletons for comparative purpose. For example, in a study of MZ-twin pairs discordant for autism, Kates et al. (2004) compared the non-affected twins with matched singletons, in addition to their affected co-twins. Besides cross-validating the findings, such comparisons may very well reveal that the true effect of environmental variables on children's psycho-social functioning lies in between the conservative effects produced by MZ-twin studies and the liberal effects generated by singleton studies.

To conclude, the limitations of twin studies in general, and of studies using the discordant MZ-twin method in particular are well documented (Oliver et al., 2008; Rutter et al., 2001). Unless addressed, they can limit the importance and relevance of results generated by the MZ-twin method. However, when all the precautions are taken, the discordant MZ-twin method offers a unique opportunity to maximize internal validity in non-experimental studies while exploring the role of nonshared (and shared) environmental experiences. Researchers from the medical field made abundant use of the discordant twin method over the past decades to uncover likely causal relationships. It is time for researchers in behavioral development to catch up and use this method to get one step closer to the holy grail of causality with respect to the explanatory factors and mechanisms central to our field.

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