X Inactivation as a Source of Behavioural Differences in Monozygotic Female Twins

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Although members of monozygotic twin pairs are identical in genome sequence, they may differ in patterns of gene expression. One early and irreversible process affecting gene expression, which can create differences within pairs of female monozygotic twins, is X inactivation — one twin can express mainly paternally-received genes on the X chromosome while the other twin expresses mainly maternally-received genes. It follows that non-identical X chromosome expression may cause female monozygotic twins to correlate less strongly than male monozygotic twins on complex behavioural traits affected by X-linked loci. We tested this hypothesis using data from around 4000 same-sex twin pairs on 9 social, behavioural and cognitive measures at ages 2, 3 and 4. Consistent with our hypothesis, monozygotic males were generally more similar than monozygotic females. Three of four significant differences were in traits showing higher correlations in males than females, and these traits — prosocial behaviour, peer problems, and verbal ability — have all been proposed previously in the literature as being influenced by genes on the X chromosome. Interestingly, dizygotic twins showed the reverse pattern of correlations for similar variables, which is also consistent with the X inactivation hypothesis; taken together, then, our monozygotic and dizygotic results suggest the presence of quantitative trait loci on the X chromosome.

In the vast majority of cases, the genomic sequence of monozygotic (MZ) twins is absolutely identical. Situations where this is not the case are extremely rare, but can arise, for example in mosaicism or chimaerism, which both involve admixture of cells with differing types of genomes (Pearson, 2002). Somatic mutations and mobile element transposition could also be expected to generate cell-specific genomic divergence between MZ twins, but phenotypic divergence arising as a consequence of such events is unlikely (see Gringras & Chen, 2001, for review). Epigenetic mechanisms such as methylation, however, may cause changes in gene function without alteration in DNA sequence (Russo et al., 1996), and are generally more frequent than mutations in primary sequence. Such epigenetic changes therefore represent a more salient process by which biologically-based phenotypic divergence between MZ twins could occur.

One important epigenetic modification, of specific relevance to female MZ twins (MZF), is X inactivation. X inactivation takes place in all females and across the majority of one of their two X chromosomes, with the exception of a small proportion of genes that escape inactivation (e.g. Brown et al., 1997). X inactivation compensates for the greater dosage of X-linked genes in females, who have two X chromosomes, than in males, who have only one. Whether the paternal or the maternal X is silenced is determined early in embryogenesis and is usually random for each cell lineage, but maintained throughout subsequent cell divisions. Consequently, females are generally mosaic for cells having an active paternal X and cells having an active maternal X, with roughly equal numbers of each cell type. Skewed patterns of inactivation, however, demonstrating a departure from the anticipated 50:50 ratio, may arise by chance, particularly if tissues develop from relatively few cells. Extremely skewed inactivation patterns can result from mutations affecting the X chromosome, such as deletions and translocations (see Heard et al., 1997). There are also reports of pedigrees in which skewed X chromosome inactivation segregates with mutations affecting the XIST locus, which has been implicated in initiating the X inactivation process (Plenge et al., 1997).

X inactivation is, therefore, an important process by which each member of a female MZ twin pair has the potential to express different proportions of maternal and paternal X chromosome alleles in corresponding tissues. Although the DNA sequence along the X chromosomes is the same for each member of the twin pair, whether it is the maternally- or paternally-received version of the gene that is predominantly active may differ for each. This has already been found to be the case in several MZ female twin pairs who are discordant for X-linked single gene

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disorders such as Duchenne muscular dystrophy (Abbadi et al., 1994; Zneimer et al., 1993) and Fragile X syndrome (Kruyer et al., 1994). In these cases, the affected twin has more cells in which the disease allele is on the active X and the normal allele is on the inactive X, while the unaffected twin has predominant inactivation of the chromosome carrying the disease allele, or random inactivation (with a 50:50 ratio) (see Tiberio, 1994). It follows that a similar phenomenon of non-identical X chromosome expression could decrease the phenotypic similarity between female MZ twins on complex behavioural traits that are affected by any of the 1000 or so X-linked genes.

Therefore, we explored the hypothesis that non-identical X chromosome expression, being the only sex-specific source of variation in gene expression within twin pairs, causes MZF twins to correlate less strongly than male MZ twins (MZM) on complex behavioural traits affected by X-linked loci. We investigated several complex traits in young twins, including behaviour problems and cognitive skills. Mean sex differences have been observed for most of these traits, which could indicate the involvement of X-linked loci, although there is as yet little empirical evidence to enable us to identify specific genes on the X chromosome that influence these behaviours. Nevertheless, there are indications that X-linked genes may be involved in certain complex behavioural traits, such as conduct, socialisation and cognitive ability (see Brunner et al., 1993; Skuse et al., 1997; Zechner et al., 2001). A comparison between MZ girl twins and MZ boy twins may provide additional evidence for the involvement of X-linked genetic factors in some of these complex phenotypes.

A possible difficulty for the proposed analysis arises from the fact that we do not have information on chorionicity for our dataset. Whether or not female MZ twins have similar X inactivation patterns is highly dependent on the timing of the twinning event, specifically whether inactivation was already established when the embryos split (Monteiro et al., 1998). Whilst dichorionic (DC) twins split early in development, monochorionic (MC) twins split later in development, after commitment to X inactivation has been made. Thus, monochorionic female MZ twins, who represent the majority of MZF twins, are less likely to differ as a result of differential X inactivation. However, early-splitting dichorionic twin pairs still account for around one third of MZ female twins, and the overall effect of differential inactivation in these pairs could still be large enough for detection here by way of female MZ twin correlations that are lower than male MZ twin correlations. The X chromosome represents around 5% of the human genome, and, assuming heritability of 50% and genetic effects that are evenly distributed across the chromosomes, genes on the X chromosome could be expected to account for at least 2.5% of the variance of a complex trait. This effect size corresponds to male and female MZ twin correlation differences of the order of 0.025. Our MZ twin correlations range from .50 to .94. Samples of 1000 MZM and 1000 MZF twin pairs provide 80% power (p = .05, two-tailed) to detect .025 correlational differences of .940 versus .915 and correlational differences as small as .94 versus .93 can be detected as significant (i.e., power of 50%).

We also calculated correlations on the same set of traits for male DZ (DZM) and female DZ (DZF) twin pairs. The effect that differential inactivation would have on the strength of correlations within DZF versus DZM pairs was expected to be different from its effect on MZF versus MZM pairs, with DZF correlations predicted to be stronger than DZM correlations. This effect would be seen because male siblings, always receiving their only X chromosome from their mother, have a 50% chance of receiving the mother's paternally-received X and a 50% chance of receiving her maternally-received X. If the mother is heterozygous for an influential X-linked quantitative trait locus (QTL), each son could therefore receive different alleles, and would be discordant to the full extent of the difference in the quantitative contribution to phenotype made by each allele. In contrast, females, though still liable to receive alternative X chromosomes from their mother, will of necessity have a second, paternally-received X chromosome, which is identical in sequence for them both and active in half of all cells (assuming no skewing of X inactivation and, for the sake of simplicity, ignoring the complication of genes which escape from inactivation, e.g., see Disteche, 1999). If the maternally-received X is indeed different for each female twin, its discordant influence will be moderated by the presence of the paternally-received identical X chromosome, thus reducing the phenotypic discordancy between sisters (Perez-Enciso et al., 2002). Hence, female non-identical siblings, including DZ twins, should be expected to be more similar than male siblings for traits affected by X-linked loci.

Materials and Methods

Participants

About 1000 pairs each of MZM, MZF, DZM and DZF twins were assessed close to their second, third and fourth birthdays (details of N at each age and for each trait are shown in Table 1). The twins were drawn from the Twins Early Development Study (TEDS), an ongoing longitudinal study in which all twins born in England and Wales between 1994 and 1996 were invited to take part (Trouton et al., 2002). The sample is reasonably representative of UK families with young children (Trouton et al., 2002). A parent-rated instrument was used to assign zygosity and has an accuracy of 95% as assessed against DNA

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markers (Price et al., 2000). The potential 5% inaccuracy in zygosity assignment is not likely to have an impact on the current results as twins with uncertain zygosity assignment were excluded from our analyses, although there is still a very small risk that zygosity assigned with certainty by parents may be inaccurate. Twin pairs were also excluded from the current analyses if at least one child in the pair had a specific medical syndrome such as Downs, or was an extreme outlier for birthweight, time spent in hospital (including special care at birth) or gestational age.

Measures

All measures were obtained by post from parents.

Anxiety, prosocial behaviour, hyperactivity, conduct problems and total behaviour problems were assessed using the Revised Rutter Parent Scales for Preschool Children (RRPSPC; Hogg et al., 1997) at ages 2 and 3, and the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997) at age 4. Peer problems at age 4 were also measured using the SDQ. The RRPSPC is based on the Preschool Behaviour Questionnaire (Behar, 1977; PBQ: Behar & Stringfield, 1974) which has been shown in previous studies to have good inter-rater reliabilities (teacher vs. classroom aide) ranging from .3 to .9. Test–retest reliabilities over 3–4 months for total scores rated by teachers averaged .87 (Behar et al., 1974). The clinical validity of the SDQ has been established in several studies (Goodman, 2001; Klasen et al., 2000) and the scales demonstrate reasonable reliability in the TEDS sample with alphas of .59 for anxiety, .67 for prosocial behaviour, .73 for hyperactivity and .51 for conduct problems. (For details of TEDS' behaviour problems measures and model-fitting genetic analyses, see Ronald et al., 2003).

Three cognitive variables (verbal ability, nonverbal ability and "g") were investigated. Verbal ability was assessed using age-appropriate vocabulary and grammar scales from the MacArthur Communicative Development Inventory: UK Short From (MCDI: UKSF; Fenson et al., 1994). Nonverbal ability was assessed using age-appropriate versions of the Parent Report of Children's Abilities (PARCA; Oliver et al., 2002). Principal component analyses applied to the verbal and nonverbal abilities data indicated the appropriateness of a single component, "g", which would represent general cognitive ability. Standardised factor scores were combined into an average "g" score. These measures are described in greater detail by Spinath et al. (2003).

Analysis

Within-pair intraclass correlations were calculated for both MZ and DZ twins on each of the measures.

Z-transformations were performed to test for MZM vs MZF correlations and DZM vs. DZF correlations that were significantly different between the sexes using two-tailed tests (Cohen, 1988).

Results

Table 1 shows sample sizes, means, standard deviations and coefficients of variation for all the variables at all ages for MZM, MZF, DZM and DZF groups. With the large sample sizes, most mean and variance differences between the sexes are significant, and so coefficients of variation have been included to avoid the problem of scale effects. However, the only consistent differences across ages are mean sex differences, with boys showing higher mean hyperactivity scores, while girls have higher mean scores for all the cognitive measures as well as prosocial behaviour. Even these mean sex differences have small effect sizes, accounting for about 1% of the variance on average across the ages and measures for hyperactivity and prosocial behaviour, and for about 3% of the variance for general cognitive ability.

Table 2 shows MZM, MZF, DZM and DZF within-pair correlations on the nine behavioural measures, at ages 2, 3 and 4 years (with the exception of peer problems for which data are only available at age 4). Correlations for MZ twins are presented in the left hand side of the table and correlations that are stronger in males than females, as predicted by the X inactivation hypothesis, are shown in bold. Correlations for DZ twins are presented in the right hand side of the table and correlations that are stronger in females than males, as predicted by the X inactivation hypothesis, are shown in bold. Correlations that are significantly different between male and female twin pairs are marked with one asterisk ($p < .05$) or two ($p < .01$). Brackets around the asterisk indicate that the difference is significant but does not support the X inactivation hypothesis.

As shown in Table 2, there were four significant differences between MZM and MZF correlations, and three of these were in the direction of higher correlations for MZM than MZF twins, as expected by our hypothesis. The average correlation for MZM (.74) was only slightly greater than for MZF (.73), but in the direction supported by the hypothesis of an X inactivation effect. The effect size is small but in line with that anticipated, as mentioned earlier, especially given that we do not expect all of the phenotypes to be affected by loci on the X chromosome and so the full impact of skewed X inactivation may be obscured by the inclusion of all phenotypes when averaging.

In contrast, DZ twins tended to show the reverse pattern of sex differences, as predicted. That is, two DZM-DZF differences were significant and both correlated more strongly for DZF than DZM. The average correlation across the 25 variables for DZM was lower (.46) than for DZF (.49).

Discussion

Our results are generally consistent with our hypothesis that differential skewing of X inactivation renders

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Table 1

Sample Size (Pairs of Twins), Mean, Standard Deviation (SD) and Coefficient of Variation (CV) for Each Trait and Each Zygosity/Sex Group at Ages 2, 3 and 4 Years

Age (yrs)	Trait	MZ Males				MZ Females				DZ Males				DZ Females			
		N	Mean	*SD*	CV	*N*	Mean	*SD*	CV	*N*	Mean	*SD*	CV	*N*	Mean	*SD*	CV
2	Anxiety	886	0.27	0.15	0.56	1035	0.28	0.16	0.57	982	0.31	0.17	0.55	903	0.31	0.17	0.55
	Prosocial Behaviour	881	0.58	0.19	0.33	1015	0.62	0.19	0.31	973	0.59	0.18	0.31	898	0.64	0.18	0.28
	Hyperactivity	891	0.39	0.23	0.59	1034	0.35	0.23	0.66	980	0.39	0.25	0.64	903	0.36	0.25	0.69
	Conduct Problems	884	0.28	0.17	0.61	1029	0.26	0.16	0.62	975	0.28	0.17	0.61	903	0.26	0.17	0.65
	Total Behaviour Problems	886	0.26	0.11	0.42	1033	0.25	0.11	0.44	981	0.27	0.12	0.44	903	0.26	0.11	0.42
	Verbal [1]	835	–0.3	0.89	—	971	0.14	0.91	—	925	–0.16	0.9	—	863	0.24	0.89	—
	Nonverbal [1]	878	–0.1	0.67	—	1024	0.05	0.68	—	968	–0.06	0.64	—	896	0.1	0.68	—
	g [1]	815	–0.31	0.98	—	956	0.14	1.01	—	904	–0.17	0.97	—	849	0.27	0.97	—
3	Anxiety	887	0.28	0.17	0.61	1010	0.3	0.18	0.60	937	0.31	0.18	0.58	913	0.33	0.19	0.58
	Prosocial Behaviour	888	0.67	0.16	0.24	1008	0.7	0.16	0.23	938	0.67	0.16	0.24	907	0.72	0.16	0.22
	Hyperactivity	888	0.38	0.24	0.63	1011	0.33	0.23	0.70	938	0.38	0.26	0.68	913	0.35	0.25	0.71
	Conduct Problems	889	0.3	0.17	0.57	1009	0.25	0.16	0.64	938	0.29	0.18	0.62	907	0.26	0.17	0.65
	Total Behaviour Problems	890	0.26	0.12	0.46	1011	0.24	0.12	0.50	937	0.27	0.12	0.44	913	0.26	0.12	0.46
	Verbal [1]	743	–0.2	0.89	—	866	0.09	0.85	—	800	–0.1	0.83	—	794	0.2	0.81	—
	Nonverbal [1]	882	–0.12	0.74	—	1009	0.1	0.72	—	926	–0.15	0.75	—	901	0.16	0.71	—
	g [1]	736	–0.25	1.04	—	853	0.12	0.99	—	793	–0.2	0.98	—	787	0.25	0.97	—
4	Anxiety	1030	0.16	0.17	1.06	1198	0.17	0.18	1.06	1077	0.18	0.19	1.06	1116	0.19	0.19	1.00
	Prosocial Behaviour	1030	0.71	0.18	0.25	1198	0.76	0.17	0.22	1077	0.72	0.19	0.26	1116	0.76	0.18	0.24
	Hyperactivity	1030	0.44	0.22	0.50	1197	0.38	0.21	0.55	1077	0.43	0.25	0.58	1114	0.37	0.24	0.65
	Conduct Problems	1030	0.23	0.16	0.70	1197	0.19	0.14	0.74	1077	0.23	0.16	0.70	1116	0.2	0.16	0.80
	Peer Problems	1030	0.14	0.14	1.00	1196	0.12	0.13	1.08	1076	0.17	0.16	0.94	1115	0.14	0.15	1.07
	Total Behaviour Problems	1030	0.25	0.12	0.48	1198	0.22	0.11	0.50	1077	0.26	0.13	0.50	1116	0.23	0.12	0.52
	Verbal [1]	956	–0.1	0.89	—	1118	–0.01	0.83	—	1021	–0.06	0.88	—	1063	0.1	0.78	—
	Nonverbal [1]	1038	–0.1	0.72	—	1207	–0.01	0.68	—	1088	–0.05	0.73	—	1128	0.04	0.68	—
	g[1]	946	–0.17	1.06	—	1110	–0.02	0.98	—	1008	–0.08	1.05	—	1058	0.09	0.94	—

Note: [1]CV is not presented for the cognitive variables because these variables were standardized

Table 2

Intraclass Correlations for MZM, MZF, DZM and DZF Twins at 2, 3 and 4 Years of Age

Age (yrs)	MZM	MZF	DZM	DZF
Anxiety				
2	.53	.57	.18	**.22**
3	.52	**.51**	.17	**.19**
4	.53	.59(*)	.31	.29
Prosocial behaviour				
2	.82	**.77****	.60	**.62**
3	.70	**.69**	.43	**.52***
4	.59	**.58**	.30	**.32**
Hyperactivity				
2	.67	**.65**	.15	.15
3	.65	**.61**	.07	.03
4	.51	**.50**	–.10	–.06
Conduct problems				
2	.72	**.68**	.43	**.45**
3	.71	.71	.46	**.51**
4	.65	**.62**	.29	**.35**
Peer problems				
4	.68	**.61****	.30	**.37**
Total behaviour problems				
2	.77	**.74**	.46	**.51**
3	.74	**.73**	.44	**.50**
4	.70	.71	.34	**.41**
Verbal				
2	.93	.94	.79	.79
3	.92	**.90***	.73	**.78***
4	.87	.87	.68	**.71**
Nonverbal				
2	.86	**.85**	.70	**.74**
3	.85	.85	.68	**.71**
4	.85	**.84**	.75	.71
"g"				
2	.92	.93	.79	**.80**
3	.91	.91	.74	**.78**
4	.90	**.89**	.73	**.74**
Average	.74	**.73**	.46	**.49**

Note: MZF correlations lower than MZM correlations and DZF correlations higher than DZM correlations (which are thereby consistent with the X inactivation hypothesis) are highlighted in bold.
* $p < .05$, ** $p < .01$
(*) indicates that the significant result is not consistent with the X inactivation hypothesis

X chromosome gene expression non-identical in a proportion of MZ females and thereby causes them to be less similar than MZ males, who have only one identical X chromosome to be expressed. The difference in average correlations is very small (rMZM = .74, rMZF = .73), but this is to be expected for genes of small effect, and it is probable that the inclusion of phenotypes that may not be affected by X-linked QTLs when averaging obscures a stronger effect for some phenotypes. Furthermore, three individual variables were significantly more strongly correlated in MZ male than MZ female twin pairs. Specifically these variables were prosocial behaviour at 2 years, verbal ability at 3 years and peer problems at 4 years. Importantly, each of these traits has previously been implicated in the literature as being influenced by QTLs on the X chromosome. For example, deficits in social functioning are characteristic of several X-linked disorders, such as Fragile X syndrome (Lesniak-Karpiak et al., 2003) and it has been argued that the X chromosome harbours more than its expected share of genes involved in cognitive ability (Zechner et al., 2001). Only anxiety was found to correlate more strongly in MZ females than MZ

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males at a significant level. This may be indicative of an absence of X-linked loci affecting the trait, and may also reflect a generally more consistent social expectation for girls to be anxious and shy, causing them to be more alike (Simpson & Stevenson-Hinde, 1985). The pattern of male-female differences in anxiety correlations was particularly inconsistent across ages and across zygosity in any case, suggesting that there is no consistent biological reason for this unexpected significant result.

As explained above, the hypothesis of the existence of X-linked genes affecting these phenotypes also predicts the reverse pattern of results for DZ twins. The present results for DZ twins, then, support our hypothesis since DZ female twin pairs were, on average, more similar than DZ male twin pairs, and they yielded significantly greater correlations for two variables (prosocial behaviour and verbal ability), both of which were also significant for MZ twins. The data for peer problems in DZ twins, which showed a significant difference for MZ twins, were nearly significant for DZ twins ($p < .1$). The fact that female twin similarity is greater than male twin similarity for dizygotic twins also serves to render less likely the possibility that MZM twins are more similar than MZF twins due to some social or environmental factor, unrelated to X-linked QTLs and differential X inactivation, since, if this were the case, the pattern would be expected to be consistent across MZ and DZ twins. It is theoretically possible that environmental factors do act differently on MZs and DZs, so that something about the monozygotic developmental environment means that boys are more similar, whilst in the dizygotic developmental environment, girls are more similar. Perhaps, for instance, mothers of MZ boy twins tend to treat their children more similarly than mothers of MZ girls treat their twins, but mothers of DZ girls treat their children more similarly than mothers of DZ boys. We have found no particular support for this in the literature, however, and can propose no intuitive reason why this should be the case.

It is a widely recognised, though infrequently discussed, phenomenon that males have greater variance than females on many traits in the general population (Hedges & Nowell, 1995). This could in turn lead to difficulties of scale effects between males and females, and for this reason the coefficient of variation has been presented alongside the standard deviation in Table 1. Scale effects ought to be consistent in both MZ and DZ twins, however, and might in fact be expected to cause males to yield lower correlations than females because of their greater variance; the fact that, in spite of this statistical effect, the opposite trend is seen in MZs, only serves to strengthen support for an X inactivation effect. In fact, the variances for males and females in our sample are quite similar and the impact of sex differences in variance is therefore likely to be minimal.

In traditional quantitative genetic analysis, the pattern of results that we have found (rMZM > rMZF and rDZM < rDZF) would yield lower heritability and higher shared environment for girls than boys. The usual interpretation, then, would be that genes seem to play a stronger role in affecting the relevant traits in boys than they do in affecting the same traits in girls. Why this should be true is unclear — one hypothesis might be that the sexually dimorphic influence of steroidal hormones causes girls to be more reactive, or susceptible, to environmental factors, and less strongly influenced by their genetic makeup, than boys. We propose, however, that such hypotheses would be post hoc, and somewhat convoluted, attempts to explain our findings; our hypothesis, on the other hand, predicts a priori the pattern of results that we obtained, and is perhaps the most cohesive and comprehensive explanation available. Thus, we suggest that phenotypes with higher phenotypic correlations in MZ males than MZ females but higher correlations in DZ females than DZ males may not, in at least some cases, be indicative of lower heritability in girls, but rather imply the presence of X-linked QTLs for the relevant traits.

DZF twins, as well as MZF twins, are susceptible to differential skewing of inactivation patterns, and this might be expected to reduce the moderating impact of the shared paternal X chromosome. Simulations that we have carried out to investigate varying levels of skewing suggest that an average skewing pattern of > 70:30 would have to be encountered in DZF pairs before the effects of the moderating, shared paternal X chromosome would be negated. According to Goodship et al. (1996), however, extreme skewing of X inactivation patterns tends to occur less frequently in DZ twins than MZ twins.

We suggest, therefore, that the observation of this pattern of correlations (rMZM > rMZF and rDZM < rDZF) in complex traits could be used as an indication that some of the variance caused by genetic factors is attributable to X-linked loci in particular. This could in turn help to narrow the focus for molecular genetic searches for behavioural QTLs that influence phenotypic traits such as those investigated here: prosocial behaviour, peer problems, and verbal ability.

Interestingly, several studies of chorionicity and within-pair MZ twin similiarities have reported stronger correlations within MC than DC MZ twin pairs on measures of cognitive ability, personality and risk for psychiatric disorder (see Prescott et al., 1999, for review). As already mentioned, differential X inactivation is more likely to occur for DC MZF twins than for MC (i.e., late-splitting) MZF twins. Thus, these findings could in part reflect the influence of skewed inactivation patterns in the female twin pairs within the MC samples used in the studies. Difficulties in obtaining chorionicity data for very large samples, and the lack of information about sex differences in the samples of

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known chorionicity that have already been reported, however, make it impossible for us to test this hypothesis that the X inactivation effect should be stronger for DC than MC MZ twins.

DNA from the TEDs twins is available, and we plan to conduct molecular genetic analyses to assess whether MZF twins with relatively large differences on relevant phenotypic traits do in fact have different X chromosome expression patterns, which will provide a direct test of the X inactivation hypothesis. Although there are fundamental practical difficulties in studying tissue-specific methylation patterns when the tissue of interest is the brain, simply establishing that expression profiles do differ within twin pairs in tissues that *are* available will indicate that this could also be true of cells in the brain. If our hypothesis is supported at the molecular level, then, far from being a hindrance to progress in the hunt for specific loci involved in behaviour, epigenetic mechanisms could in this case provide important clues as to where we ought to direct future molecular genetic searches for such loci.

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