Reviewer's report

Title: Twin discordance and disease: not just an environmental cause?

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Reviewer: Mario F.Fraga

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This manuscript reviews the role of epigenetics in twin discordance and discusses the relative contribution of a number of possible factors on epigenetic variation over time. Whilst the topic is interesting, the manuscript needs substantial improvement before being published:

The title does not reflect the content of the review because it does not contain the word epigenetics. Moreover, what does ‘twin discordance and disease’ mean? Disease discordance in twins? What do the authors want to suggest by "not just an environmental cause"? On reading the abstract it seems that the alternatives are "differences in the in utero environment, mosaicism and epigenetics". In my opinion, the alternatives to adult environmental conditions are in utero environmental conditions and stochastic molecular alterations (during embryo development and adult life) that depend, at the same time, on genetic and extrinsic factors. These stochastic and environmental factors can affect the epigenetic marks and generate mosaicisms. An alternative title could be "The value of twin studies in environmental epigenetics" or something similar.

The concept of non-shared environmental conditions should be clarified. The authors assume that during the embryos' development the environmental conditions are identical but this is not accurate as it is known that, for example, one of the siblings may receive more nutrients than the other.

There are many paragraphs that seem to be out of context and some sections that do not contain what is stated in their title. One example is "Environment and discordance" in which the authors mainly explain the biology of twinning.

The paragraph "Slight differences in eye or hair color as ...... from shared environmental exposures in twins." should be rewritten. These phenotypic differences can arise from stochastic and/or environmental-dependent molecular alterations during embryo development and/or adult life but the underlying molecular mechanisms are still largely unknown.

The paragraph "Certain cases of differential allelic expression (DAE), including random monoallelic ....... within between (sic) the co-twins, still leaving room for stochastic effects." should be rewritten as it contains many factual inaccuracies: Cheung et al found that "in 50% of genes expressed in lymphoblastoid B cells, the entire distribution of the allelic expression ratio is significantly shifted away from the expected mean of 0.5 (equal allelic expression)" and not, as the authors state, that "about 50% of heterozygous loci are subject to DAE within MZ twin pairs"; Baranzini’s study analyzed 3 pairs of twins (and not only a single pair),
The paragraph "The source as well as significance of epimutations in twin phenotypic variability ....that epimutation rates are lower in cells in vivo[25, 35, 80]." should be rewritten. The stochastic epimutations depend on genetic and environmental factors because, for example, the fidelity of the methyltransferases may depend on both genetic and environmental conditions. The authors should also endeavor to clarify the following concept: "there is a stochastic epigenetic variation during embryo development and adult life that depends on genetic and environmental factors but their relative contribution is not known. Taking it into account, the following sentence seems speculative and should be removed:" In contrast to ......showing similar concordance for personality and social attitudes in MZ twins raised apart to those raised together [82].". In general, I believe that it is difficult to compare the two studies in references 29 and 82 because the age range of the study populations is completely different. In addition, the following inaccuracies should be corrected: The study quoted in reference 29 does not report "a trend of steady accumulating changes to the epigenome with age"; it simply identifies more epigenetic differences in a group of older twins than in another group of younger twins. As the study does not involve a continuous range of ages, it is better not to talk about a tendency. The values in the sentence "Estimates based on 20 MZ and 20 DZ pairs indicated that methylation heritability was very low (0.014) in white blood cells, but rose to about 0.3 in buccal tissue (findings based on 19 MZ and 20 DZ pairs), and up to 0.7 when dichorionic twins only were considered." must be reviewed. They do not correspond with those published in the reference cited.

The next paragraph should also be reviewed. In reference 29, young twins present some epigenetic differences but, when compared with older twins these differences are statistically non-significant. The sentence "despite low heritability, the intraclass ...... epigenome-wide findings of ref 29" is not correct because in ref 29, epigenetic differences between twins within the range of ages analyzed in ref 84 (5 and 10-yo) are also stable. In general, the conclusions of the three studies cited in this paragraph are difficult to discuss together for two reasons: Firstly, the technologies used are not the same and this in itself could explain the identification of more epigenetic differences in young twins in one study than in others and, secondly, because the type of DNA sequences identified in the different studies are not the same (repeated DNA vs single copy genes).

In the section Methylation Studies and Human Disease, the authors should discuss the effect of the tissue-type analyzed. In many of the studies cited, the tissue analyzed is not the disease-targeted tissue. It must be stated that this strategy only allows the identification of systemic epimutations and that tissue-specific epimutations (which, in my opinion are more relevant) are not detected.

Other minor points are:

It should be mentioned that Mosaicisms can also be generated due to epigenetic alterations.
This sentence needs a reference: "There is evidence for transgenerational inheritance of epigenetic changes but the scope and mechanisms are under study."

A paragraph or section should be included which describes studies on environmental epigenetics in genetically identical animals.

There many typos and mistakes that must be corrected. Examples are: " were concordant within between the co-twins", " Rather, the term rather described the way", "the significance of age in studies DNA methylation twin discordance", italic format " which have been implicated by a previous study [96]." or the references Esteller et al., 2005, PNAS, Spielman et al., 2008, AJMH).

Figure 1 should be improved and clarified.

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests