Author's response to reviews

Title: Genetic, environmental and stochastic factors in Monozygotic Twin discordance with a focus on epigenetic differences.

Authors:

Witold WC Czyz (witold@well.ox.ac.uk)
Julia JMM Morahan (julia.morahan@well.ox.ac.uk)
George GCE Ebers (George.Ebers@clneuro.ox.ac.uk)
Sreeram SVR Ramagopalan (s.ramagopalan@qmul.ac.uk)

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Author's response to reviews: see over
Discordance in monozygotic twins and disease.

Dear Dr. Lee,

Many thanks for reviewing our paper. We have updated our manuscript in response to the comments made by the reviewers and the Journal. We hope you will find our manuscript now acceptable for publication.

The authors’ contributions are as following:

- **Review design and concepts**: Witold Czyz, Dr. Sreeram V Ramagopalan, Professor George Ebers
- **Analysis and Data interpretation**: Witold Czyz,
- **Manuscript draft**: Witold Czyz
- **Critical revision of the manuscript**: Dr. Julia Morahan, Dr. Sreeram V Ramagopalan, Professor George Ebers
- **Study supervision**: Dr. Sreeram V Ramagopalan, Professor George Ebers

All the authors gave their final approval for the submission of the manuscript. The authors of the review can be contacted under the following email addresses:

- George.ebers@ndcn.ox.ac.uk
- s.ramagopalan@qmul.ac.uk
- witold@well.ox.ac.uk
- julia.morahan@well.ox.ac.uk

All tables and figures have been created specifically for the review by one of the authors, Witold Czyz. The section below contains the response to the points raised by the reviewers.

**Reviewer - Dr. Mario Fraga**

The authors have addressed most of the points raised by the referees and the manuscript has been substantially improved. I only have two new suggestions:

1. In the first longitudinal study in adult MZ twins (recently published in Aging Cell, Talens et al. 2012) it has been shown that epigenetic changes during adult life are highly dependent on unique environmental conditions. Authors should include this study in their review.
The Talens et al. 2012 study has now been included in the review and table 1 as well as outlined on page 11 in the following paragraph: The most recent epigenomic study investigated methylation levels in a cohort of 230 MZ twin pairs (although 219 pairs appear in the analysis), whose age ranged from 18 to 89, both globally as well as across a panel of 9 chosen loci which have been implicated in age-related diseases and epigenetic regulation[88]. The authors adopted a cross-sectional approach, although a subset of 38 twins were re-assayed longitudinally after a 10-year interval. Although small intra-pair discordance for global methylation was observed, older pairs were found to be two times more discordant than the younger twins [88]. This trend held true also for the disease-related loci with older twins displaying 1.4 to 2.7-fold greater discordance and variation increasing proportionately to age (with very weak effects of changing cellular heterogeneity)[88]. Both global and locus-specific temporal increase in methylation discordance was confirmed by a longitudinal follow-up. The overall absolute global methylation differences between the twins were nonetheless small (1.1% in younger pairs in contrast to 2.1% in the older). The results appear to confirm the early findings of greater epigenetic discordance among older twin pairs shown by Fraga et al. (2005) and suggest that discordance increases progressively with age[ 88]. The study points to the importance of unique individual environment behind the discordance, but explains the trend with both the influence of stochastic and environmental factors, acknowledging the difficulty in separating their effects from each other and stressing that non-shared environmental exposures may also drive stochastic epigenetic discordance[88].

2. The manuscript still contains some mistakes. For example, first author of ref 29 is not Esteller.

The manuscript has been read and revised to eliminate spelling errors. The reference has been revised. The names of the first authors were sourced from Web of Science and PubMed database through Endnote, however ref 29 and 71 have been now manually amended in accordance to Dr Fraga’s suggestions. Other changes include: “Longitudinal studies of CpG methylation in MZ twin cohorts, optimally sampled at birth first, are better means to estimate the levels of epigenetic discordance and make inferences upon its nature.” on Page 10 and “such” on page 14. Also it has been mentioned in both the text (point 1, above) and the table that the number of MZ twins used in Talens et al. 2012 stated in the abstract and discussion of the study (230 pairs) is different to the number mentioned in results and methods section (219 pairs).

Thank you very much for your consideration.

We look forward to hearing from you,

Sreeram Ramagopalan MA DPhil