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LETTERS

ZNF277 microdeletions, specific language impairment and the meiotic mismatch methylation (3M) hypothesis

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We were intrigued by the pedigrees in the paper by Ceroni *et al*[1] in the October 2014 issue that explored the association between *ZNF277* microdeletions and specific language disorder (SLI). The authors make the case for *ZNF277* microdeletions contributing to the risk of SLI while acknowledging that there was little evidence of co-segregation of these microdeletions with SLI in their discovery pedigree plus eight other families. The authors only discuss this discrepancy in terms of reduced penetrance, that is, unaffected siblings that carry the microdeletion, but say little about the reverse discordance in two of their three multiplex families, that is, where one of the two affected siblings has inherited the maternal microdeletion and the other has not. This reverse discordance has been reported in multiplex families with autistic spectrum disorder (ASD) in which a putative risk copy number variation (CNV) is segregating.[2] This paper on ASD, and our finding of a maternal grandmother age effect in ASD prompted us to propose the meiosis mismatch methylation (3M) hypothesis.[3] This states that, in female early meiosis I, the pairing of a chromosome carrying a microdeletion with a wild-type homologue increases the chance of abnormal methylation due to chromosome looping through misaligned pairing, such as would occur with silencing of a transposon.[4] This in turn results in the functional silencing of the wild-type gene when transmitted to offspring. 3 M predicts that all offspring of a woman carrying a risk CNV are at risk of the condition even though only half inherit the CNV. Independently, around the time of formulating the 3 M hypothesis, an ASD family was reported in which one affected sibling had inherited a maternal deletion containing the oxytocin receptor gene (*OXTR*) and the other affected sibling had no deletion, but had epigenetic misregulation of this gene through aberrant gene silencing by DNA methylation.[5] We suggest that it would be useful to examine at least the DNA methylation status of the maternally inherited *ZNF277* allele in the siblings that have SLI but not the microdeletion.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Marcus Pembrey*[,1,2], Jean Golding[1] and Jessica Connelly[3]
[1]*Centre for Child and Adolescent Health, School of Social and Community Medicine, University of Bristol, Bristol, UK;*
[2]*Institute of Child Health, University College, London, UK;*
[3]*Department of Psychology, University of Virginia, Charlottesville, Virginia, USA*
E-mail: M.Pembrey@bristol.ac.uk

1 Ceroni F, Simpson NH, Francks C *et al*: Homozygous microdeletion of exon 5 in ZNF277 in a girl with specific language impairment. *Eur J Hum Genet* 2014; **22**: 1165–1171.
2 Bucan M, Abrahams BS, Wang K *et al*: Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. *PLoS Genet* 2009; **5**: e1000536.
3 Golding J, Steer C, Pembrey M: Parental and Grandparental Ages in the Autistic Spectrum Disorders: A Birth Cohort Study. *PLoS One* 2010; **5**: e9939.
4 Walsh CP, Chaillet JR, Bestor TH: Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. *Nat Genet* 1998; **20**: 116–117.
5 Gregory SG, Connelly JJ, Towers AJ *et al*: Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Med* 2009; **7**: 62.

Reply to Pembrey *et al*: '*ZNF277* microdeletions, specific language impairment and the meiotic mismatch methylation (3M) hypothesis'

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In a recent paper,[1] we described a homozygous exonic microdeletion in *ZNF277* in a girl with specific language impairment (SLI). This microdeletion was also identified in the heterozygous form in eight families of the SLI Consortium (SLIC) cohort and four families with ASD cases from the IMGSAC Cohort. We observed an increased allelic frequency of *ZNF277* microdeletions in SLI probands (1.1%) compared with both ASD family members (0.3%) and unrelated controls (0.4%), suggesting that these microdeletions might be a risk factor for SLI. However, as the *ZNF277* microdeletions showed incomplete segregation with the SLI phenotype, as they were also identified in unaffected family members and, in some cases, they were not inherited by the affected children (reverse discordance), we hypothesised that these CNVs might contribute to the SLI susceptibility in a complex manner, acting as a risk factor with a reduced penetrance.

Pembrey and colleagues have suggested that an epigenetic mechanism may account for the reverse discordance observed for the maternal *ZNF277* microdeletions. According to their hypothesis, called meiotic mismatch methylation (3 M),[2] during the maternal meiosis I,

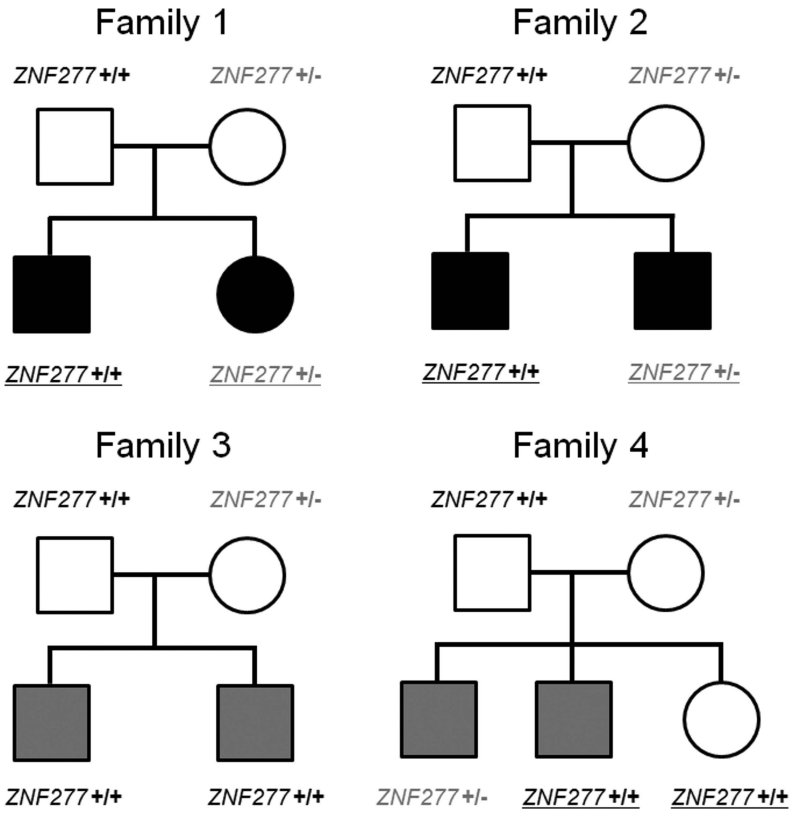

Figure 1 The figure shows the four ASD families carrying the *ZNF277* microdeletions. Black filling indicates a diagnosis of autism, grey filling indicates a diagnosis of pervasive developmental disorder (PDD). Lymphoblastoid cell lines from underlined children were available for the analysis.

a deletion on one chromosome might lead to abnormal methylation of the normal allele on the homologous chromosome, which would result in the silencing of the non-deleted allele. This hypothesis implies that all the children with a mother who carries a *ZNF277* microdeletion would be at risk, irrespective of the copy number of their inherited allele. In contrast, children in families where the father carries a *ZNF277* microdeletion would only be at risk if they directly inherit the event. Thus, one would expect to see an increased frequency of SLI in families in which the mother carries a *ZNF277* microdeletion.

In our discovery pedigree, we did observe reverse discordance: the proband inherited a deleted copy of *ZNF277* from both parents, whereas the affected sister and the brother, who had mild language impairment, did not carry the microdeletion. In the additional SLI families identified to carry a *ZNF277* microdeletion, we identified three maternal and three paternal *ZNF277* microdeletions. In both family types, we observe a similar level of affected children (four out of nine children are affected in maternal lines, while four out of eight children are affected in paternal lines). Moreover, in the maternal lines, we do observe unaffected children supporting the incomplete penetrance of this allele. Therefore, although the sample size is very small, in the SLI families we did not observe a clear maternal pattern that would support the 3 M hypothesis. In our study, however, the autism families did display aspects of maternal inheritance; in all four identified ASD families (of 252 screened), the *ZNF277* microdeletions were always carried by the mothers and a high number of offspring were affected (eight of nine offspring have ASD or PDD; Figure 1).

It is known that DNA methylation profiles are a combination of inherited epigenetic marks and *de novo* modifications that can be spatio-temporally heterogeneous and dynamically regulated throughout development (as reviewed by Zhou[3]). The 3M hypothesis assumes a constitutive epigenetic effect on the expression of the risk gene, established at the very early stage of the life span (maternal meiosis). DNA methylation of *ZNF277* has not been directly examined in any of the SLI or ASD families included in our original article. However, as part of our previous study, we did examine *ZNF277* transcript levels in lymphoblastoid cell lines derived from the four ASD families (Figure 1). When we analysed all the available individuals carrying the *ZNF277* microdeletion, we observed a reduced expression compared with that of the housekeeping gene *GUSB*, which was chosen to have comparable expression levels to *ZNF277* (as shown in Ceroni *et al*[1]). In contrast, when we analysed all the children of mothers carrying the microdeletion, the *ZNF277* gene did not display altered expression (normalised average expression level of 1.01 across six samples), nor did we observe reduced expression when we restricted the analysis only to the children who did not inherit the *ZNF277* maternal deletion (normalised average expression level of 1.07 across four samples with lymphoblastoid samples available). Together, these data suggest that there is no constitutive silencing of the maternal copy of *ZNF277* by methylation. We recognise, however, that the number of available individuals is too small to conclusively rule out the possibility of an epigenetic regulation. Larger sample sizes and more extensive investigations, involving methylation sequencing in male and female lines, would be required to fully inform the 3 M hypothesis.

CONFLICT OF INTEREST

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Fabiola Ceroni[1], Nuala H Simpson[1], Clyde Francks[2,3], Gillian Baird[4], Gina Conti-Ramsden[5], Ann Clark[6], Patrick F Bolton[7], Elizabeth R Hennessy[8], Peter Donnelly[1], David R Bentley[9], Hilary Martin[1], IMGSAC[10], SLI Consortium[1], WGS500 Consortium[1], Jeremy Parr[10], Alistair T Pagnamenta[1,11], Elena Maestrini[12], Elena Bacchelli[12], Simon E Fisher[2,3] and Dianne F Newbury*[,1]

[1]*Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK;*
[2]*Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands;*
[3]*Donders Institute for Brain, Cognition & Behaviour, Nijmegen, The Netherlands;*
[4]*Guy's & St Thomas NHS Foundation Trust, Newcomen Children's Neurosciences Centre, St Thomas' Hospital, London, UK;*
[5]*School of Psychological Sciences, The University of Manchester, Manchester, UK;*
[6]*Speech and Hearing Sciences, Queen Margaret University, Edinburgh, UK;*
[7]*Departments of Child & Adolescent Psychiatry & Social Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, Kings College London, London, UK;*
[8]*University Child Health and DMDE, University of Aberdeen, Aberdeen, UK;*
[9]*Illumina Cambridge Ltd, Chesterford Research Park, Little Chesterford, Essex, UK;*
[10]*Institute of Neuroscience and Health and Society, Newcastle University, Newcastle upon Tyne, UK;*
[11]*NIHR Biomedical Research Centre, Oxford and Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK;*
[12]*Dipartimento di Farmacia e Biotecnologie, University of Bologna, Bologna, Italy*
E-mail: dianne@well.ox.ac.uk

1 Ceroni F, Simpson NH, Francks C *et al*: Homozygous microdeletion of exon 5 in ZNF277 in a girl with specific language impairment. *Eur J Hum Genet* 2014; **22**: 1165–1171.
2 Golding J, Steer C, Pembrey M: Parental and grandparental ages in the autistic spectrum disorders: a birth cohort study. *PLoS One* 2010; **5**: e9939.
3 Zhou FC: DNA methylation program during development. *Front Biol* 2012; **7**: 485–494.