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LETTERS

Does heritability hide in epistasis between linked SNPs?

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Much recent discussion addresses the question of 'missing heritability' in genome-wide association studies (GWAS). The problem can be illustrated using the example of human height. Classical pedigree studies show a high heritability of human height, in the order of 80%. This is part of our everyday experience: tall parents tend to have tall children. GWAS has identified more than 40 loci associated with height, but these variants together explain only a small part of phenotypic variation.[1] A number of hypotheses have been advanced to identify the source of the missing heritability, including large effects of rare variants and effects of copy-number variation.[2]

A conceptual difference between pedigree studies and GWAS does not appear to have been considered: pedigree-based heritability measures the phenotypic effects of much larger chunks of chromosome than GWAS-based heritability. This distinction can be illustrated with a simple example that elides complexities arising from diploidy. Consider two SNPS (A/T and G/C) in linkage equilibrium that are located 0.1 cm apart. The SNPs could, for example, encode two amino acid substitutions within a single protein. From the perspective of pedigree-based measures of heritability, the four haplotypes (AG, AC, TG and TC) are inherited as four alleles at a single locus, but from the perspective of GWAS these are biallelic polymorphisms at distinct loci. Suppose that the combinations AG and TC add a little bit extra to height but AC and TG subtract a little bit. Then, neither SNP will be correlated with height in GWAS, but the haplotypes, which are correlated with height, will be reliably transmitted from parents to offspring and will contribute to estimates of pedigree-based heritability. Put another way, the genetic effect on phenotype appears as part of the additive genetic variance in pedigree studies but as an unmeasured gene×gene interaction in GWAS.

The major constraint on measuring interactions in GWAS has been the very large number of possible interactions. If there are 10^6 SNPs on an array, then there are 5×10^{11} pairs of SNPs. However, the number of pairs is a much more manageable 10^6 if analysis is restricted to neighboring SNPs.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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Does the *HSD17B10* gene escape from X-inactivation?

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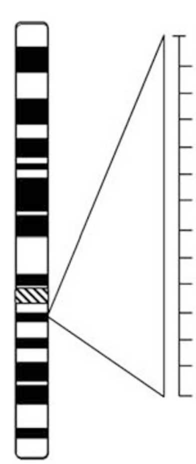
We read with great interest the recent report by Garcia-Villoria *et al*[1] regarding the expression of the *HSD17B10* gene from the inactive X chromosome that was published in the *European Journal of Human Genetics* (Advance online publication, 28 July 2010; doi:10.1038/ejhg.2010.118).

It had been reported previously that a cluster of six genes, including the *HSD17B10* (formerly *HADH2)* gene in Xpl 1.2, escapes X-inactivation.[2] Subsequently, Carrel and Willard, in a more detailed study,[3] showed that the escape of the *HSD17B10* gene from X-inactivation is not complete. The expression of the *HSD17B10* gene and the surrounding genes from the inactive X chromosome (Xi) is summarized in Figure 1 (adapted from Ref. Yang *et al*[4]).

Two female patients heterozygous for HSD10 deficiency were the subjects of this present study[1] in which skin fibroblast cultures were examined to determine the inactivation ratio of the normal and mutated X chromosomes. It appears that these studies were performed on cultures originating from a single biopsy from each patient. Mosaicism due to lyonization results in relatively large patches of skin with the same inactivated X chromosome, commonly illustrated by the coloration of calico cats. Thus, an analysis of cells from a single biopsy is probably not adequate to determine the X inactivation ratio. Analysis of a blood sample might be more informative.

In addition, the standard deviation (SD) appears to be relatively large, that is, >15% of the mean value in most cases. This limitation



	X_a	X_i	X_i expression in hybrids tested
TRO	+	–	0/5
MAGED2	+	–	0/9
FGD1*	+	+	1/9
TSR2	+	+	1/9
PHF8*	+	–	0/9
HUWE1*	+	+	1/9
HSD17B10*	+	+	1/9
RIBC1	+	+	7/9
SMC1L1*	+	+	7/9
KIAA0522	+	+	9/9
JARID1C*	+	+	9/9
TSPYL2	+	–	0/5
DXS6672E	+	–	0/5
TMEM29	+	–	0/5

Figure 1 Expression of transcripts of the *HSD17B10* and surrounding genes from inactive X (Xi) hybrids. Samples scored as positive are expressed at least >10% of the Xa levels, and their number is shown as the numerator. The total number of hybrids tested is shown as the denominator. Genes with mutation(s)[5] or copy number variation (CNV)[6] causing mental retardation are marked with asterisk.

makes it unlikely that partial (12%, Carrel and Willard[3]) escape of the *HSD17B10* gene from X-inactivation in the first female patient would be detectable. Moreover, although monoallelic expression in one of the cell lines indicates that this gene is subject to inactivation, lack of data from other tissue samples makes the inference of widespread monoallelic expression of the *HSD17B10* gene in the second female patient less than convincing. The statement that 'as the girl was severely affected, a similar unfavorable X-inactivation in other tissues could be expected'[1] does not suffice for correcting the defect in data. The conclusion that 'the *HSD17B10* gene does not escape X-inactivation as has been reported previously' is not adequately supported by the data included in this publication.

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Reply to He *et al*

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We appreciate the comments of He *et al.*[1] Our response is outlined below.

In fact, it had been reported that *HSD17B10* is a part of a multigene domain in Xp11.21–p11.22 that escapes X-inactivation.[2] Later results by Carrel *et al*[3] showed that this gene is probably subjected to X-inactivation as only one of nine hybrids escapes from it. This observation can not be inferred from Figure 2 of Yang *et al*,[4] while the results are more clarifying in the adapted figure of the letter of He *et al.*[1]

To elucidate whether *HSD17B10* cDNA doses differed between both sexes, we performed relative quantification (RQ) of wild-type *HSD17B10* cDNA alleles in four female and four male controls. The results did not show any significant difference between the doses in both sexes. Therefore, these results are in favour of an X-linked disease that does not escape X-inactivation and are in agreement with the observations of Carrel *et al.*[3]

Fibroblasts were obtained from a single biopsy, as it would not have been ethical to perform additional biopsies with the only purpose of performing these studies. In fibroblasts we not only performed genetic studies but also determined enzymatic activities with good correlation between both, which gives more strength to the results.

Relatively large deviations are often observed in real-time PCR quantification, owing to the low specificity of the probes and variability of the endogenous controls. However, despite these difficulties, the same expression levels in the first female patient and her brother were observed, which is in agreement with the sequencing results, the low enzymatic activity, the severe clinical presentation and the skewed X-inactivation pattern. The second female showed expression of both mutant and wild-type alleles, which is also in agreement with sequencing results, normal enzymatic activity, slight clinical presentation and random X-inactivation pattern.

In conclusion, our results are adequately supported by the studies in controls and are confirmed by the studies in patients.

We thank He *et al* for giving us the opportunity to clarify some issues, although we think that they do not change the conclusions of our study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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