EXPRESSIVITY OF TRAITS AND ALTERNATIVE SPLICING : CASE STUDY OF *AXIN^{Fu}* MUTATION IN MICE

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INTRODUCTION

The majority of mutations demonstrate variable expressivity and incomplete penetrance and exact causes of these phenomena are usually unknown. The *Axin^{Fu}* mutation in mice shortens tail length (Ruvinsky *et al*., 1991). It results from insertion of an intracisternal-A particle (IAP) into intron 6 of the *Axin* gene (Vasicek *et al*., 1997). Produced mRNA is not only spliced alternatively, but can also undergo correct splicing. It was suggested that *Axin^{Fu}* causes fused phenotype by encoding a competing form of Axin protein (Hsu *et al*., 1999). The expressivity and penetrance of this mutant trait varies depending on genetic background and direction of the crosses. Using RNase protection assay (RPA) we measured the relative quantities of wild and alternatively spliced Axin mRNAs produced by different genotypes. This allows to study whether the mRNA profiles correlate with expressivity of the mutant trait.

MATERIAL AND METHODS

Mice. Strains 129/Rr *Axin^{Fu}*/+ and TF/Le (*tf/tf*) were obtained from the Jackson Laboratory (USA). *Axin^{Fu}*/+ mice were crossed to *tf/tf* mice. The following crosses were made: Cross 1. **m** *Axin^{Fu}*+/+*tf* X **f** +*tf*/+*tf*, Cross 2. **m** +*tf*/+*tf* X **f** *Axin^{Fu}*+/+*tf* , Cross 3. **m** *Axin^{Fu}*+/+*tf* X **f** *Axin^{Fu}*+/+.

PCR amplification. DNA for genotyping was isolated by phenol/chloroform extraction following overnight digestion with proteinase K. Conditions for multiplex PCR were described elsewhere (Vasicek, *et al*. 1997)

RNase Protection Assay. RNA was prepared from kidney samples stored at –20^{o}C in RNAlater (Ambion) using the RNaqueous RNA extraction system (Ambion). The probe used for RNase protection assay was generated using the Thermoscript RT-PCR system (Life Technologies) with primers described by Vasicek *et al*., 1997. cDNA derived from an *Axin^{Fu}* mouse was cloned into pBluescript II and the appropriate clone selected by direct screening of colony picks by PCR. Orientation of the insert relative to promoters was determined by sequencing. Antisense RNA probe was transcribed from the cDNA template cloned in pBluescript II using the Maxiscript transcription system (Ambion). Following transcription, reactions were electrophoreised on a 5% polyacrylamide gel. The position of the band corresponding to full-length probe was determined following exposure to Biomax MR autoradiography film (Kodak) and the band subsequently cut out and eluted overnight at 37^{o}C. RPAs were performed with the RPAIII system (Ambion). Assays were electrophoreised on a 5% polyacrylamide gel and exposed to Biomax MS autoradiography film (Kodak).

Gel Analysis. Autoradiographs from the RPAs were captured using UVP gel documentation system. Autoradiographs were placed in an UV/white light fluorescent darkroom (UVP) and captured under white light using a low level light camera (resolution 752x582 pixels) with a 8-

48mm zoom lens. Quantification of the bands was performed using Phoretix 1D gel analysis software incorporating the rubber band method of background subtraction.

RESULTS AND DISCUSSION

The level of penetrance between the crosses varied significantly: Cross 1 ~88%, Cross 2 ~24% and Cross 3 ~45%. In Cross 1 the majority of *Axin*^{Fu} + / + *tf* expressed typical tail deformities. On the contrary, heterozygotes from cross 2 generally expressed a milder tail phenotype. We aimed to determine if there is a connection between the level of wild and alternatively spliced RNAs with expressivity of the mutant trait. Tail length was used as a measure of expressivity of the mutant allele *Axin*^{Fu}. Strong differences were found between groups of mice with high and low levels of expressivity in crosses 1 and 3. To investigate the relative levels of wild type and alternative transcripts we employed RPA and observed 3 protected fragments; a standard (286) and two alternatively spliced (238 & 234), Figure. 1. These three bands, were found in all studied heterozygotes and homozygotes. Unexpectedly wild type mice also displayed two less intense mRNA fragments that seem to correspond to alternative transcripts.

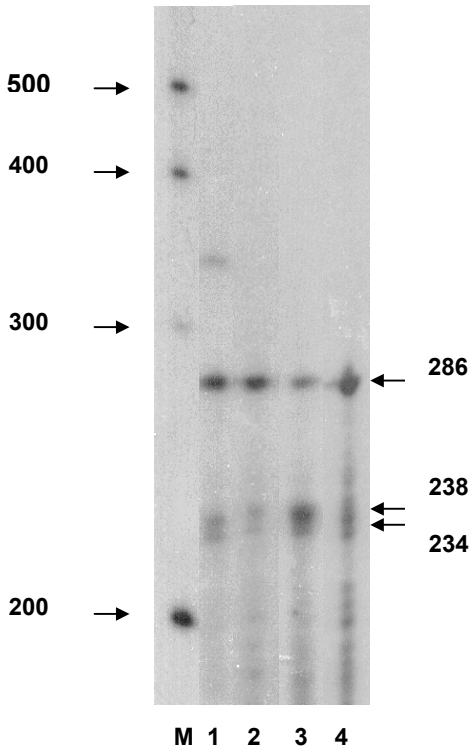

Figure 1. RNase Protection Assay Gel. Lane M, mol. weight markers. Lane 1, heterozygote from cross 1. Lane 2, heterozygote from cross 2. Lane 3, homozygote from cross 3. Lane 4, wild type

Correlation analysis reveals a connection between tail length and the relative intensity of the mRNA bands (Table 1). The majority of correlations are high and significant. A few conclusions can be drawn from the correlation analysis. Firstly, there is a significant positive correlation between tail length and the amount of standard *Axin* transcript. Secondly, there is a significant negative correlation between tail length and the amount of alternate transcript (238). This negative correlation likely indicates that there is competition between the standard and alternatively spliced products. Thus the data demonstrate that the shift in balance between the fractions of mRNA correlate with expressivity and possibly penetrance of the mutant trait.

Latest estimates indicate that about 35% of studied mammalian genes may show an alternative splicing (Brett et al., 2000). If so, one may expect many examples similar to described above. For instance, it has been shown that among cystic fibrosis sufferers with a particular genotype, the severity of the disease depends upon the levels of correctly spliced mRNA transcript and

an alternatively spliced transcript (Chiba-Falek *et al*., 1998). In another example, low penetrance of the retinoblastoma-predisposition gene (RB1) may be caused by alternative splicing that leads to the production of a less severely disrupted protein (Onadim *et al*., 1992). It has also been suggested that clinical variability in neurofibromatosis type 1 (NF1) arises through differences in the relative ratios of alternatively spliced variants (Skuse and Cappione, 1997). Finally, extreme variation of phenotype in sufferers of familial adenomatous polyposis (FAP) has been attributed to alternative splicing, specifically within the adenomatous polyposis coli (APC) gene.

Table 1. **Correlations between tail length and the relative intensity of wild type and alternatively spliced mRNA bands produced by *Axin^{Fu}* hetero- and homozygotes**

Cross[A]	*Genotype*	No. of mice[B]	Correlations between tail length and relative intensity of mRNA bands[C]			
			Band 1 (286) Standard mRNA	Band 2 (238) Alternatively spliced mRNA	Band 3 (234) Alternatively spliced mRNA	Difference between Bands 1 and 2
1.	*Axin^{Fu}*+ /+*tf*	19	0.62 (P<0.01)	-0.54 (P<0.05)	-0.33	0.59 (P<0.01)
2	*Axin^{Fu}*+ /+*tf*	19	-0.01	-0.09	0.20	0.03
3	*Axin^{Fu}* /*Axin^{Fu}*	14	0.55 (P<0.05)	-0.41	-0.4	0.49
1. + 3.		33	0.58 (P<0.01)	-0.51 (P<0.01)	-0.41 (P<0.05)	0.56 (P<0.01)
Total[D]		55	0.52 (P<0.01)	-0.51 (P<0.01)	-0.29 (P<0.05)	0.52 (P<0.01)

[A] Crosses:
1. male *Axin^{Fu}*+/+*tf* x female +*tf*/+*tf*
2. male +*tf*/+*tf* x female *Axin^{Fu}* +/+*tf*
3. male *Axin^{Fu}* +/+*tf* x female *Axin^{Fu}* +/+*tf*

[B] The proportion of males and females was nearly equal

[C] Each band was adjusted to the total intensity of all 3 bands in each sample

[D] Including 3 mice of wild genotype

It is known that the major morphogenic events responsible for vertebral and tail development in mice occur in the developing embryo between 9-10 days. Though the Axin protein found around the same stage of development (Zeng *et al*., 1997), differences between embryos with normal and abnormal tail develop only 12-13 days after fertilization or later. For this reason and the fact that the *Axin* gene is expressed ubiquitously, adult mice were selected as a model for this study. It is still unclear what kind of relevant Axin mRNAs may appear during the crucial stage of tail development and this would be an interesting subject for future investigation. Our data allow suggesting that once the amount of alternatively spliced

transcript exceeds a particular level, a morphogenetic barrier is overcome and tail development deviates from the normal pathway. The ratio of competing mRNAs would establish the threshold for development of the mutant character, thus affecting expressivity and penetrance. If this is correct, one may propose that occasionally, a mouse may appear which does not have the mutant allele but develops non-inheritable tail abnormalities, which resemble the mutant trait. This study demonstrated that while the mRNA profile in heterozygotes obtained from reciprocal crosses was similar, there was a significant difference in correlation between tail length and studied mRNA fractions. This may be explained by suggesting that embryos obtaining the maternal $Axin^{Fu}$ allele may have a different ratio of normal and alternatively spliced mRNAs at the time of tail development. At a later stage of morphogesis, the ratio may normalise at a level similar to heterozygotes with paternally derived $Axin^{Fu}$. Developmental stage-specific expression of particular alternative transcripts has previously been suggested to interpret phenotypic variation in neurofibromatosis type 1 (Skuse and Cappione, 1997). Another explanation of the reciprocal effects suggests that Axin mRNA or protein may interact with a downstream component which could display an imprinting–like behaviour and thus interact quite differently in heterozygotes from reciprocal crosses. Accordingly one may expect that such a gene would reside in the vicinity of *Axin* on chromosome 17, otherwise recombination and segregation events would significantly reduce the chance of concomitant transmission of both alleles through the same gamete. Regardless, the passage of the $Axin^{Fu}$ allele or an unidentified gene (affecter) through the oocyte leads to a significant normalisation of the $Axin^{Fu}$ phenotype. Whether this is the result of occasional normalisation of the phenotype or is a long-term evolutionary adaptation remains unknown.

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