Annotated Bibliography
2001-2002

2001

Pozzi N, Gaetaniello L, Martire B, De Mattia D, Balestrieri B, Cosentini E, Schlossman SF, Duke-Cohan JS, Pignata C. Defective surface expression of attractin on T cells in patients with common variable immunodeficiency (CVID). *Clin Exp Immunol* (2001) 123: 99-104.

Although patients with Common Variable Immunodeficiency (CVID) exhibit an impaired differentiation of B lymphocytes into Ig-secreting plasma cells, this appears to be predominantly the result of defective T cell help rather than an intrinsic B cell defect. The molecular machinery for antigen presentation appears to be intact in CVID patients and except in a minor subset of patients, there is no real evidence of downstream signaling problems in T cells. This suggests that some aspect of the interaction of T cells and antigen-presenting cells during the formation of an immunoregulatory cluster may be aberrant. Since attractin may play a role in this interaction, its expression was examined in patients with CVID. Activation of CD3+ T cells from these patients does not result in an upregulation of attractin as normally observed, although other markers of T cell activation are upregulated including CD26 and IL2R.

The defective expression of attractin, however, may not be primary since the basal level of expression of CD26 is characteristic of a partially-activated T cell, allowing the possibility that attractin was expressed previously and the cell is now refractory to further expression of attractin, as is seen with normal T cells that have been activated in vitro, express attractin, lose it and in the presence of IL-2 and/or the initial stimulus are incapable of re-expressing attractin. Supporting the extracellular role for attractin, addition of exogenous attractin significantly increased the proliferative responses of CVID leukocytes to CD3-crosslinking in the presence of suboptimal amounts of IL-2.

Kuramoto T, Kitada K, Inui T, Sasaki Y, Ito K, Hase T, Kawagachi S, Ogawa Y, Nakao K, Barsh GS, Nagao M, Ushijima T, Serikawa T. Attractin/mahogany/zitter plays a critical role in myelination of the central nervous system. *Proc Natl Acad Sci U S A* (2001) 98: 559-64.

In a surprising discovery, positional cloning of the zitter mutation in rats reveals that the phenotype (consisting of tremors that develop at 3 weeks of age, flaccid paresis of the hind limb at around 6 months of age and progressive hypomyelination and vacuolation in the central nervous system) is due to aberrant attractin expression consequent apparently to an 8 bp deletion at the splice donor site of intron 12. Although it is a weak mutation, it was the only abnormality noted after sequencing the complete coding region and all exon-intron boundaries. At the molecular level, there is a severe depression in attractin mRNA. Equally surprising was the identity of a second species of mRNA of 4.5 kb, in addition to a 9 kb band, that appears to code for a secreted form of attractin by skipping

a splice donor site in intron 24. This is surprising because there is no real evidence of a secreted form in the mouse and that in the human appears to be the result of a primate-specific retrotransposon event that introduces a new exon coding for 5 amino acids. In fact, the putative rat product is remarkably similar to that of the human since the extended read into exon 24 only adds 5 amino acids before reaching a termination codon. Two different splicing mechanisms in rat and human generate remarkably similar products - convergent evolution, or chance?

The focus of the report then falls on the pathology associated with attractin deficiency that might account for the severe neurological phenotype. CNS histology in this report and previous reports suggests that initial axon projections and myelination are normal and that there is no inability to synthesize the components of myelin. Nevertheless, there is a progressive hypomyelination and vacuolation, with swollen astrocytic processes suggesting that attractin is involved in axon-accessory cell interactions involved in maintenance of neuronal architecture. Conclusive evidence for the involvement of attractin is provided by rendering the zitter rats transgenic for membrane and soluble attractin - the membrane form rescued normal neurological phenotype, not so in the instance of the soluble form. Furthermore, the *zi* mutation is on a WTC albino background so, after backcrossing the mutation on to an agouti ACI/N strain, consequences of the mutation upon pigmentation could be observed - the *zi* (Atrn) mutation exhibits a mahogany color that returns to wild-type agouti in membrane attractin transgenics but remains mahogany for soluble attractin transgenics. To sew up the association with neurological phenotype, mg-3j/mg-3j mice exhibit a very similar neurological phenotype to zitter with cerebral vacuolation and constant tremors in rear skeletal muscle. Given the possibility of expression of a soluble form, the zitter rat may prove to be a most valuable model for the human.

He L, Gunn TM, Bouley DM, Lu XY, Watson SJ, Schlossman SF, Duke-Cohan JS, Barsh GS. A biochemical function for attractin in agouti-induced pigmentation and obesity. *Nat Genet*.(2001) 27: 40-7.

Consolidation of the results from a number of reports suggest that the antagonism of alpha-MSH binding to Mc1R by agouti in the mouse a) requires membrane attractin, and b) is dependent upon the C-terminal of agouti binding to the Mc1R. The C-terminal of agouti shares some structural similarities with Agouti-related protein (Agrp) thus providing a likely explanation for the pleiotropic effects of ectopic agouti in Ay mice where agouti is likely functioning beyond the Mc1R localized to the hair follicle and is interfering with the Agrp-Mc3R/Mc4R system in the brain. That Agrp might also require membrane attractin for its function seemed to be lent support by crosses between Atrn-mg3J/Atrn-mg3J mice and Ay that suppress completely the effects of Ay not only upon pigmentation but also upon obesity.

In a surprising result, however, in a cross between Atrn-mg3J/Atrn-mg3J mice and mice constitutively expressing Agrp under control of a beta-actin promoter, the mice carrying the Agrp transgene have an increased body weight by 8 weeks of age leading to a 50% increase over wild-

type over the next 12 weeks in an effect that is completely independent of Atrn-mg3J/Atrn-mg3J genotype! Agrp does not require attractin to function so the observed reduced weight of Atrn-mg3J/Atrn-mg3J mice is not due to a regulation of Agrp function. These results open up the possibility that agouti can interact with attractin but that such an interaction does not occur with Agrp. In testing this possibility using surface plasmon resonance to measure binding, the predicted result was confirmed and underpinned by showing that the N-terminal of agouti interacts with attractin but the C-terminal exhibits no binding, and that Agrp does not bind to attractin. The zitter rat, as mentioned above, has tremors and electromyograms revealed a similar phenomenon in Atrn-mg3J/Atrn-mg3J mice. The tremors in the rat are likely the result of the attractin-related neurodegeneration and the authors in this report confirm the presence of extensive spongiform vacuolation/neurodegeneration in Atrn-mg3J/Atrn-mg3J mice. And, as in the rat, expression of the attractin transgene under the control of a constitutively-expressed promoter can correct the neurodegeneration. Is it possible that the reduced weight of Atrn-mg3J/Atrn-mg3J is simply the consequence of neurodegeneration-induced inadequate food-intake or even of the tremors (unconscious exercise!)?

Other intriguing effects are revealed in this study: a) attractin transgene expression driven by a keratinocyte-specific promoter (Keratin14) does not rescue the pigmentation defect of Atrn-mg3J/Atrn-mg3J mice, while expression driven by the Dopachrome tautomerase (Dct) promoter (that operates in melanoblasts, melanocytes, pigmented retinal epithelium and parts of the brain) is able to restore the pigmentation functionality of agouti, thus the attractin defect in Atrn-mg3J/Atrn-mg3J mice is melanocyte autonomous. b) Other possibilities for the functions of attractin are raised by the observation that C. elegans and Drosophila have homologues of membrane attractin with remarkable domain conservation but the melanocortin receptors and their peptides are characteristically vertebrate - are there other as yet unidentified or uncharacterized ligand/receptor interactions modified by attractin? In any event, early development of the neural systems of both Atrn-mg3J/Atrn-mg3J mice and zitter rats does not seem to be affected from which we may infer that attractin is involved in maintenance of neural architecture rather than patterning or axon guidance.

Bronson RT, Donahue LR, Samples R, Kim JH, Naggert JK. Mice with mutations in the mahogany gene Atrn have cerebral spongiform changes. *J Neuropathol Exp Neurol* (2001) 60:724-30.

In this report, mutants arising on an agouti background in CAST/Ei (*Mus castaneus*) stock displayed a mahogany color and developed a tremor observable at around 10 days of age. Examination of the brains revealed a vacuolation. Crossing of the mice with Atrn-mg3J/Atrn-mg3J mice demonstrated that the mutation was an allele of Atrn and has been designated Atrn-mg6j. Prior to the characterization of the mutation as an Atrn allele, 3 other mutations with similar phenotype had been discovered (Atrn-mg2j, Atrn-mg4j and Atrn-mg5j) but were discarded. The mice have severe tremors and a sprawling gait and usually die at 3-4 weeks. After backcrossing onto a C3H/Snj background, the tremors are less severe and the sprawling is not apparent. In fact, the tremors are

not apparent in Atrn-mg/Atrn-mg mice, and Atrn-mg3J/Atrn-mg3J mice occasionally have late-onset seizures where they suddenly freeze and topple over. This suggests that there may be modifier genes that exacerbate or ameliorate the effects of attractin deficiency expressed at different levels in different strains. Vacuolation is observed for all allelic variant mutants, beginning at roughly eight weeks and getting progressively worse, beginning in the cerebellar cortex, deep layers of the cerebral cortex and within nucleii in the medulla, midbrain and thalamus. The white matter of brain and spinal cord was paler upon staining with luxol fast blue than that of wild type suggesting demyelination while myelination in the peripheral nervous system appeared unaffected. Importantly, and perhaps connected to the possibility of modifier genes, there was no connection between vacuolation and clinical severity despite the correction of the neuropathological phenotype by membrane attractin transgenes in mg mice and zitter rats.

Malik R, Mares V, Kleibl Z, Pohlreich P, Vlasicova K, Sedo A. Expression of attractin and its differential enzyme activity in glioma cells. *Biochem Biophys Res Commun* (2001) 284:289-94.

In a previous report, attractin was reported as having a DPPIV activity associated with a 570 kDa species that appeared to be a trimer of human secreted attractin. This DPPIV activity was completely resistant to trypsin digestion and, unlike CD26 (the canonical DPPIV) was inhibited by bestatin (a chymotrypsin-like serine protease inhibitor). In this more recent report, DPPIV activity was isolated from human glioblastoma-like cell lines U373 and U87 and 5% and 25% respectively of the DPPIV activity was found to be associated with a non-CD26 protease that migrated on gel filtration at 570 kDa, is completely resistant to trypsin digestion and is inhibited by bestatin as well as by more specific DPPIV inhibitors. This suggested that the origin of the high molecular weight DPPIV activity might be attractin.

Although the lower molecular weight (and dominant) activity has the size and enzymatic characteristics of CD26, it does not appear to react with anti-CD26 antibodies suggesting that this activity may be the result of other molecules with DPPIV activity recently identified. In at least the case of U87 glioma cells, the high molecular weight DPPIV activity was associated with anti-attractin reactivity by Western blotting, and fluorescence analysis revealed attractin expression on U373 cells. In general, mRNA levels detected by RT-PCR for 3'-proximal attractin exons did not correlate with enzyme activity leading the authors to contemplate alternative transcripts that may differ in, or be devoid of, enzyme activity. At least two forms incorporating 5' sequence differences in and around a potential chymotrypsin-like serine protease motif have been identified (AF034957 and AF106861). The authors also suggest that attractin may play a role in the development of gliomas since normal glial cells do not have attractin-related sequence detected by in situ hybridization

Thomas RE, Wolfgang WJ, Forte M, Cone RD. The Drosophila Mahogany/Attractin homologue, Distracted, affects neuronal connectivity. Society for Neuroscience Meeting, November 2001, San Diego, CA. (Abstract)

Report that stock of flies with a P-insertion element [EP(3)3400] 415 bp upstream of the transcriptional start of CG5634 have defects in retinal axon projection, a good general model for following neuronal connections. No information is provided in the abstract as to whether this P-element insertion directly affects the CG5634 locus or whether progeny with “imprecise excisions” have been generated. Since a yeast two-hybrid screen using the cytoplasmic tail identified at least one candidate involved in retinal axon projections, it is suggested that attractin may be involved in neuronal connectivity. This is more difficult to extend to vertebrate models of attractin deficiency since the neurological defects are juvenile in onset rather than embryonic and correlate developmentally more with the maturation and myelination of CNS axons rather than with the paths the axons take. The results may reflect compensatory mechanisms in higher organisms; in particular, there is a protein similar to attractin coded on chromosome 10 (KIAA0534) for which information is sparse but may reflect a degeneracy of function in mammals.

Gunn TM, Inui T, Kitada K, Ito S, Wakamatsu K, He L, Bouley DM, Serikawa T, Barsh GS. Molecular and phenotypic analysis of attractin mutant mice. *Genetics* (2001) 158: 1683-1695.

This report attempts a multivariate analysis of relative presence of attractin transcript and protein to pigmentation, feeding, fat content, neurodegeneration and locomotor activity in the Atrn-mg, Atrn-mg-L and Atrn-mg3j mutant alleles. Realistically, the complexity of the parameters and the increasing likelihood of modifier gene activity renders difficult the drawing of hard-and-fast conclusions. Nevertheless, some important data are generated and can be summarized as follows:

The mutation in the Atrn-mg3j allele is a 5 bp deletion near the end of exon 16 causing a frameshift resulting in 914 codons being translated normally, S->L at codon 915 and a premature stop codon at 916. Northern blotting suggests a minute amount of normally-sized attractin but given the nature of the mutation the origin of this species is questionable. For all practical purposes, the Atrn-mg3j allele appears to be null for attractin. The mutation in the Atrn-mg allele results from insertion of an Intracisternal A Particle (IAP) element inserted in reverse orientation into intron 26. Northern blotting shows a heterogenous band of larger size than normal. A 5' probe detects a truncated 5.2 kb species not detected with downstream probes suggesting that transcription starts normally but that the IAP insertion results in aberrant splicing. The mutation in the Atrn-mg^{L} allele results from insertion of an IAP element in forward orientation into intron 27. Northern blotting reveals a large smear of transcripts larger and smaller than the normal transcript and the smear is absent using a 3' UTR probe, suggesting variable termination within the IAP due to defective splicing.

By Western blotting, no normal attractin is detected in the Atrn-mg allele but a smaller protein is detected. In the Atrn-mg^{L} allele, a normal and larger protein is detected. It is important to note that these are total cell lysates so there is no guarantee that any of the proteins are presented on the extracellular face of the plasma membrane and that some phenotypic effects may represent the activities of mutant protein being expressed in the wrong place at the wrong time and the wrong way intracellularly rather than loss of function. Hair phaeomelanin is significantly reduced in all the

mutant alleles and a subtle age-related loss of eumelanin is observed in 5-6 month Atrn-mg3j mice which then stabilizes - an effect independent of the agouti system. Atrn-mg^{L} mice did not differ to controls in body weight gain from birth to 6 months. In contrast, Atrn-mg and Atrn-mg3j allelic mice showed similar significantly decreased weight gain with the effect stronger in males than in females. There is a reduced adiposity for all three mutant alleles. Although not very significant, the Atrn-mg mutants may compensate with increased feeding. The increased feeding may, however, also be a response to high levels of nocturnal locomotor activity seen also to a lesser degree in Atrn-mg3j mutants but not observed in Atrn-mg^{L} mutant mice. There is no apparent vacuolation in Atrn-mg^{L} mutant mice but this is observed in Atrn-mg and Atrn-mg3j mice with the degree of vacuolation much greater in the latter.

Allele:	*Atrn mg-L*	*Atrn mg*	*Atrn mg3J*
Normal transcript	+/v	+/v	-
Abnormal transcripts	+++++	+++	-
Protein (normal)	+/v	-	ND
Protein (abnormal)	++ (large)	++ (small)	ND
Phaeomelanin	v	v	v
Body weight	Normal	v	v
Adiposity	v	v	v
Food intake	Normal	(v)	Normal
Locomotor activity	Normal	vv	v
Neurodegeneration	Normal	+	++

Maiti AK, Jorissen M, Bouvagnet P. Isolation, in silico characterization and chromosomal localization of a group of cDNAs from ciliated epithelial cells after in vitro ciliogenesis. *Genome Biology* (2001) 2: research0026.1-0026.9

Investigating gene expression associated with beating of cilia and sperm flagellae, the authors determined transcript expression in nasal epithelial cells after induction in vitro of ciliogenesis. A

transcript corresponding to a large part of exon 1 of attractin was expressed and was placed electronically on Chromosome 17, thus distinguishing it from attractin (Chromosome 20) and KIAA0534 (Chromosome 10). A role in cytoskeletal rearrangements during ciliogenesis is implied.

Sedo A, Malik R. Dipeptidyl peptidase IV-like molecules: homologous proteins or homologous activities? *Biochim Biophys Acta* (2001) 1550: 107-16

Compares the structure and relatedness of molecules related functionally or structurally to dipeptidyl peptidase IV leading to a grouping classified as "DPP-IV activity- and/or structure homologues" (DASH). Although the enzymatic properties of attractin remain speculative at present, genomic sequence similarity dendrograms place attractin as related to quiescent proline peptidase (QPP), proline carboxy peptidase (PCP) and thymus-specific serine protease (TSSP) that is related as a group to the NAALADase's with a weaker relationship of both these groupings to the DPPIV/FAP-alpha/DPP6/DPP8 family.

2002

Kuramoto T, Nomoto T, Fujiwara A, Mizutani M, Sugimura T, Ushijima T. Insertional mutation of the Attractin gene in the black tremor hamster. *Mammalian Genome* (2002) 13: 36-40.

The black tremor hamster has, as its name implies, a black coat with trunk and hindquarter tremors. The tremors are likely consequent to Central Nervous System-specific hypomyelination. The similarity of the pigmentation and neurological characteristics to the phenotype of *Atrn-zi* rat and the more severe *Atrn* mouse mutants led the authors to propose that the *bt* mutation may be a mutation at the hamster Atrn locus. Similar to the other rodent mutants, the black tremor hamsters exhibit CNS hypomyelination despite apparently normal levels of glial cells with consequent vacuolation throughout the brainstem, cerebral cortex, cerebellum and spinal cord.

In contrast to the 9 kb mRNA species observed in wild-type GN hamsters, *bt/bt* hamsters only expressed a smaller species of around 4.5 kb. Examination of this abnormal transcript revealed that in place of the normal splicing of 30 exons, the transcript was correct up to and including exon 23 followed by a portion of exon 24, 417 bp of unknown origin and a poly-A tail. Translation of this transcript would result in a secreted protein lacking transmembrane and cytoplasmic domains and corresponding to almost the entire ectodomain. Genomic analysis of *bt/bt* mutants identified that the unusual transcript arose from a 10 kb insertion with rodent LTR elements inserting 69 bp downstream of the intron 23-exon 24 junction. This mutation co-segregates with the mutant phenotype thus confirming that the CNS neurological aberrations and dark coat color are consequent to the mutation in the attractin locus. It further confirms results already hinted at in the

zitter rat study by the same group that the functionality of attractin resides in the cytoplasmic domain and that the soluble ectodomain, if it results in protein production in the *bt* mutant, cannot compensate for loss of the transmembrane splice variant.

Tang W, Duke-Cohan JS. Human secreted attractin disrupts neurite formation in differentiating cortical neural cells in vitro. *J Neuropathol Exp Neurol* (2002) 61: 767-777.

Although the alternatively-spliced secreted variant of human attractin circulates at high levels in the periphery, it is absent from CSF. The lack of secreted attractin in the CSF correlates with minimal mRNA levels detectable by Northern blot, and also with levels barely detectable by rt-PCR throughout discrete regions of the brain suggesting that transcription of the secreted form is downregulated throughout the CNS. In a model human cortical neuronal cell line that can be induced to take on a mature neuron phenotype with NGF and dibutyryl cAMP, the change from an undifferentiated state to mature neuron is accompanied by a specific downregulation of secreted form transcript. This suggests that the lack of secreted form in the CSF is due to transcriptional downregulation. Furthermore, it may be inferred that attractin does not normally cross the blood-brain barrier.

Several of the articles above address likely function of attractin and an emerging consensus is that attractin has the characteristics of an extracellular protein involved in cell-cell or cell-substrate communication. If this is so, is it possible that the transcriptional downregulation of the soluble form in the CNS and its prevention from transfer from the periphery is because the secreted form interferes with the interactions of the membrane form in the CNS? This report shows that soluble attractin does indeed interfere with neurite formation and this is mimicked by anti-attractin. Since the effects upon neurite extension are upon individual cells, the effects are cell autonomous and imply a cell-substrate role for attractin rather than a function in cell-cell interactions. The effect upon neurite extension is dramatic, the neurites no longer track out linearly from the cell body, but meander and tend to branch with subsidiary processes while the control extensions tend not to branch. This implies a role in the tracking of the neuron. Confirming that this effect is mediated by attractin, human serum that contains naturally high levels of the secreted form also affects neurite development, but if the serum is depleted of attractin, neurite extension reverts towards the normal condition. It is then proposed that the presence of secreted attractin, either by altered transcriptional control, release from CNS-localized inflammatory foci, or seepage across the blood-brain barrier interferes with membrane attractin function may lead to neurodegenerative processes similar to those observed in mutant attractin rodent models.

Kuwamura M, Maeda M, Kuramoto T, Kitada K, Kanehara T, Moriyama M, Nakane Y, Yamate J, Ushijima T, Kotani T, Serikawa T. The myelin vacuolation (mv) rat with a null mutation in the attractin gene. *Lab Invest* (2002) 82: 1279-1286.

This report, originating from the same groups that previously identified the Atrn-zi mutation in zitter rats and the Atrn-bt mutation in black tremor hamsters, now identifies that a spontaneously-arising tremor in a colony of Sprague-Dawley rats is due to a null mutation affecting the Atrn locus. At the histological level, the myelin vacuolation (mv) rats have strong vacuolization in the midbrain and spinal cord and to a lesser extent throughout the cerebellum and cerebral cortex. The myelin lamellae were often split, were thin, and axons appeared swollen, although the clinical phenotype does not appear to be as strong as in zitter rats or the Atrn-mg6j mice. This is surprising since the genetic defect appears to include a complete deletion of the proximal promoter region and exon 1 leading to the null phenotype, and one might expect the clinical consequences to be as severe as in other heavily-mutated Atrn mutants.

Furthermore, there are significant differences in the comparative brain regional vacuolization of myelin vacuolization and zitter rats. This strongly supports the proposal of modifier genes first put forward by Bronson *et al* (2001) above. Interestingly, in mouse Atrn mutants, pigmentation is the most sensitive indicator of attractin mutation (see Gunn *et al,* 2001, above) and there appears to be a complete suppression of agouti-induced phaeomelanin production. Within the limits of photo-reproduction in the article, however, the mv/mv rat is noticeably lighter than a non-agouti control (although significantly darker than the agouti-positive Atrn +/+ control) suggesting that there is some agouti-induced phaeomelanin synthesis independent of attractin.

As an aside, the loss of the promoter and exon 1 leads to a loss of all related attractin transcripts. This leads one to critically evaluate the large number of predicted human attractin products that have been virtually identified by GeneFinder and deposited in GenBank as reference sequences and have predicted start codons from exons downstream of exon 1.

Phan LK, Lin F, LeDuc CA, Chung WK, Leibel RL. The mouse mahoganoid coat color mutation disrupts a novel C3HC4 RING domain protein. J. Clin. Invest. (2002) 110: 1449-59.

The mouse mahoganoid mutation displays a pigmentation phenotype remarkably similar to attractin and both mahoganoid (*md*) and attractin (*Atrn*) map to the same genetic epistasis group. As do *Atrn* mutations, md mutations suppress both yellow pigmentation and the obese phenotypes induced by ectopic expression of high levels of agouti. The Atrn locus on chromosome 2 is distant from the md locus on chromosome 16. The likely functional pathway that would generate similar phenotypes for mutations in *Atrn* and *md* has remained speculative, with possibilities ranging from md coding for an attractin-interacting receptor on neighboring cells to both moieties having similar direct intracellular interactions modifying melanocortin receptor signaling. Identification of the product of the md gene would provide structural clues as to cellular location and function that may in turn allow conclusions to be drawn about attractin function.

In this report, the mahoganoid gene is identified by positional cloning as a 17 exon transcriptional unit that encodes a 494 amino acid protein containing a C3HC4 RING finger domain which is strongly associated with E3 ubiquitin ligase activity. The predominant mRNA species is about 3.9 kb with widespread tissue representation at variable levels – brain and kidney appear to have the highest representation. There are also less well represented species of 1.9kb and 5.2kb with differential tissue distribution. These normal transcripts were reduced by at least one order of magnitude in md/md and md^{2J}/md^{2J} mice while a significant number of abnormal transcripts were observed. The genomic basis for several mahoganoid alleles was determined. The md^{2J} results from an 8kb retroviral insertion within exon 12 and md5J results from a 5kb retroviral insertion in intron 2 just 3' of exon 2; md results from a 5kb retroviral insertion in intron 11 between exons 11 and 12. In each instance, the retroviral sequence was characteristic of an intracisternal type A particle.

The corresponding human transcript was identified as kiaa0544 and its regional tissue expression in the human is similar to that of *md* in the mouse. Although *md* suppresses the obesity of A^y mice, its effect when not co-expressed with an obesity-associated mutation is less clear and this report demonstrates that mahoganoid has no direct effect upon high-fat diet-induced obesity. Furthermore, the *md/md* animals demonstrate none of the hypomyelination and spongiform vacuolation observed in attractin-deficient mahogany mice.

The authors suggest several mechanisms that might account for mahoganoid regulation of Mc1R-regulated pigmentation and Mc3R/Mc4R-mediated regulation of hypothalamic control of feeding, but as they rightly surmise, the real clue will come from a) identifying whether the mahoganoid product has E3-ubiquitin ligase activity; b) if it does have such activity, what are the natural substrates for ubiquitination; and c) how do such substrates relate to attractin and its functionality.