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Of mothers and myelin: Aberrant myelination phenotypes in mouse model of Angelman Syndrome are dependent on maternal and dietary influences

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Abstract

Angelman Syndrome (AS) is a neurodevelopmental disorder characterized by a number of neurological problems, including developmental delay, movement disorders and epilepsy. AS results from the loss of UBE3A (an imprinted gene) expressed from the maternal chromosome in neurons. Given the ubiquitous expression of Ube3a and the devastating nature of AS, the role of environmental and maternal effects has been largely ignored. Severe ataxia, anxiety-like behaviors and learning deficits are well-documented in patients and AS mice. More recently, clinical imaging studies of AS patients suggest myelination may be delayed or reduced. Utilizing a mouse model of AS, we found disrupted expression of cortical myelin proteins, the magnitude of which is influenced by maternal status, in that the aberrant myelination in the AS pups of AS affected mothers were more pronounced than those seen in AS pups raised by unaffected (*Ube3a* (m+/p-)) Carrier mothers. Furthermore, feeding the breeding mothers a higher fat (11% vs 5%) diet normalizes these myelin defects. These effects are not limited to myelin proteins. Since AS mice have abnormal stress responses, including altered glucocorticoid receptor (GR) expression, we measured GR expression in pups from Carrier and affected AS mothers. AS pups had higher GR expression than their WT littermates. However, we also found an effect of maternal status, with reduced GR levels in pups from affected mothers compared to genotypically identical pups raised by unaffected Carrier mothers. Taken together, our findings suggest that the phenotypes observed in AS mice may be modulated by factors independent of Ube3a genotype.

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Conflict of Interest:

The authors have declared that no conflict of interest exists.

Keywords

Angelman Syndrome; Ube3a; E6AP; Myelin; Maternal Effects; Breeding

1. Introduction

Angelman syndrome (AS) is a neurodevelopmental disease characterized by developmental delay, speech impairment, movement disorders, sleep disorders and refractory epilepsy [1]. These symptoms have a devastating impact on the quality of life for individuals with AS and their caregivers. AS results from the loss of neuronal UBE3A, an E3 ubiquitin ligase derived from the *UBE3A* gene [2]. *UBE3A* is maternally imprinted in neurons, such that it is expressed nearly exclusively from the maternal chromosome while the paternal chromosome is epigenetically silenced [3;4].

A growing body of clinical evidence has suggested that myelin dysfunction may be a contributing factor to morbidity in a variety of neurodevelopmental syndromes, including Tuberous Sclerosis Complex and cryptogenic autism [5–7]. Several studies utilizing diffusion tensor imaging (DTI) to evaluate white matter tracts in AS have revealed alterations in DTI signals in several brain regions[8;9]. MRI studies have also revealed delayed myelination and thinned corpus callosa in Angelman patients[10]. Additionally, studies in AS mice revealed decreased mRNA expression for Myelin Associated Glycoprotein (MAG) in the cerebellum [11]. The extent to which myelin abnormalities contribute to the pathophysiology of Angelman Syndrome and the potential mechanistic link between Ube3a and myelin dysfunction remain unclear.

To determine the role of Ube3a on myelination, we utilized an existing AS mouse model [12] to characterize myelin protein expression in the mouse central nervous system and sciatic nerve. Myelin protein expression throughout the cortex in the AS mouse was consistently abnormal but widely variable between animals. Further subgroup analysis demonstrated that the observed myelin phenotype in the AS mouse model is exquisitely sensitive to maternal influences and diet. Here we evaluate the possibility that the phenotypes observed in AS are influenced by factors including, but not limited to, maternal status and diet.

2. Materials and Methods

2.1 Mouse Breeding and Genotyping

Ube3a deletion mice, derived by Jiang [12] and maintained on a C57/B6J background were used for all studies. WT males *Ube3a* (m+/p+) were crossed with AS females (*Ube3a* m-/p+), referred to as affected mothers, to produce WT-AM and AS-AM littermates. WT males *Ube3a* (m+/p+) were crossed with paternal deletion *Ube3a* (m+/p-) referred to as Carrier mothers to produce WT-Carrier (WT-CM) and AS-Carrier (AS-CM) littermates (Table 1). Paternal deficient mice were bred by crossing *Ube3a* (m+/p-) or (m-/p+) males with WT mothers. PCR analysis was performed on tail tissue to determine genotype with confirmatory testing of all experimental animals determined by western blot of Ube3a

protein expression in brain. PCR primers and protocol were modified from those described by The Jackson Laboratory. The primers used as followed: P1 (Common), 5'-CCAATGACTCATGATTGTCCTG-3'; P2 (WT reverse), 5'-TCAAACATTCCAAGTTCTCCC-3'; and P3 (mutant), 5'-TGCATCGCATTGTGTGAGTAGGTGTC-3'. PCR protocol: 94°C for 3 minutes, (94°C for 30 seconds, 58.3°C for 1 minute, 72°C for 1 minute)×30 cycles. Male and female mice were used in all experiments. Mice were fed a standard laboratory diet (Lab Diet 5L0D), with a 5% fat content or to determine the effect of alterations of fat in the diet, a subset of breeding animals were fed a breeder diet (Lab Diet 5LJ5) containing 11% fat with the resulting pups weaned onto the same breeder diet. All procedures were approved by the Vanderbilt Institutional Animal Care and Use Committee (IACUC).

2.2 Tissue Collection

Brain tissue was collected from mice aged 6 weeks to 3 months. Unless otherwise stated, experiments were performed on tissue from 6 to 7 week old mice. Mice were decapitated and the brains were removed quickly, chilled in ice cold artificial cerebrospinal fluid (aCSF) and dissected to isolate the cortex from all other structures. Additional tissues collected included the spinal cord and sciatic nerve. All tissue was flash frozen in a dry ice/ethanol slurry and stored at -80°C until being prepared for western blot.

2.3 Western Blot

Tissue was homogenized with a sonicator (QSonica) in a modified RIPA buffer containing (50 mM Tris (pH = 7.4), 150 mM NaCl, 1% NP-40, 0.2% sodium deoxycholate, 1 mM EDTA) with protease and phosphatase inhibitors (Sigma). Protein concentration was determined with the Bradford assay (Bio-Rad), and samples were diluted to a final concentration of 1 to 3 µg/µL in loading buffer containing β-mercaptoethanol. Samples were denatured at 60° C for 10 minutes before being loaded onto a 4–20% gradient SDS gel (Invitrogen). Proteins were transferred in a Tris-glycine transfer buffer onto Immobilon-FL PVDF membranes (Millipore). Membranes were then blocked in Odyssey blocking buffer (LI-COR) for 1 hour at room temperature. Membranes were incubated with primary antibody in 5% BSA, TBS with 0.1% Tween-20 (TBS-T) overnight at 4° C. Primary antibodies used included: Ube3a 1:2000 (Sigma E655), actin 1:10000 (Sigma A5441), and myelin basic protein (MBP) 1:1000 (Abcam ab7349), and MAG (Cell Signaling D4G3), CNPase (Cell Signaling D83E10) and Glucocorticoid Receptor (Cell Signaling D8H2) at 1:1000. Membranes were washed at least three times for 10 minutes in TBS-T and then incubated with fluorescent secondary antibodies at 1:10000 (LI-COR, IRDye800CW goat anti-mouse, IRDye680RD goat anti-rabbit) in TBS-T for 1 hour at room temperature. Membranes were then washed three times for at least 10 minutes with TBS-T. Imaging was performed on a LI-COR Odyssey fluorescence scanner and data was analyzed with Odyssey imaging software.

2.4 Statistics

Data are reported as mean ± SEM. All statistical analyses were performed using GraphPad Prism (ver 5.02, La Jolla CA) software. Unless otherwise specified, an unpaired t-test was used to compare two groups, while one-way ANOVA with Tukey's post hoc test was used

to determine if there were significant differences ($p < 0.05$) among different maternal conditions. A two-way ANOVA with Bonferroni's post-test was used for two-factor analysis of maternal status and pup genotype.

3. Results

3.1 Cortical myelin constituent expression is abnormal in AS mice

A growing number of clinical studies in AS patients have suggested alterations in white matter pathways which may play a role in the pathophysiology of this disease [8–10]. To characterize expression of myelin proteins in AS mouse, cortical lysates (including the underlying corpus callosum) were assayed by western blot for expression of three independent myelin markers (Myelin-Associated Glycoprotein (MAG), 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) and Myelin Basic Protein (MBP)), each of which have differing levels of expression, localization and function in the myelin sheath [13]. We observed a 23% reduction in MAG, 32% reduction in CNPase and a 25% reduction in MBP in cortical lysates from AS mice collected at 6 weeks of age (Figure 1A).

To determine if the reductions in myelin protein expression were secondary to loss of neurons and/or their axons, we evaluated the expression of the neuronal marker PGP9.5, and saw no significant change (Figure 1B). To test if these changes were specific to oligodendrocyte derived proteins or a broader non-specific reduction in proteins we evaluated expression of the astrocytic marker, glial fibrillary acidic protein (GFAP). No difference in GFAP was observed (Figure 1). We also evaluated myelin protein expression at 2 and 3 months with results consistent with those seen at 6 weeks (data not shown). We also evaluated the visual appearance of the myelin structure using immunohistochemical staining for MBP as well as Sudan Black (a lipid stain), however the appearance of the myelin was similar between genotypes, with a slight reduction in the intensity of the staining (data not shown).

3.2 Normal myelin expression in the spinal cord and sciatic nerve

To evaluate if this defect in myelination is confined to the brain, we measured the same myelin related proteins in the spinal cord and the sciatic nerve as a representative peripheral nerve. In these tissues, we observed no alterations in expression of myelin proteins (Figure 2A, 2B). This was an unexpected finding as *Ube3a* is imprinted in these tissues [14].

3.3 Decreased litter size in AS mice mothers vs carrier mothers, which is abolished by higher fat diet

In the course of our studies, we noted that fewer pups were born to AS mothers than our experience with mice on a C57 background led us to expect. To elucidate the role of maternal status (genotype and diet) on reproductive ability, we first assessed our mouse colonies' reproductive ability as measured by litter size and latency between litters by breeding condition. All groups included WT males bred with either *Ube3a* (m-/p+) affected mothers (AS) or *Ube3a* (m+/p-) unaffected mothers (Carriers), thereby producing AS-AM or AS-CM pups, respectively. As *Ube3a* is expressed from the maternally inherited allele in neurons [4], each of these conditions produces genetically identical offspring (WT and

Ube3a (m-/p+)) with variable maternal influences (Table 1). We observed an average 7.60 ± 0.56 pups per litter born to unaffected mothers, 7.94 ± 0.46 pups per litter born to WT mothers, compared to the significantly reduced 5.13 ± 0.35 pups born to affected AS mothers (Table 2). This does not appear to be due to postnatal death as the fraction of pups reaching weaning age is equivalent between groups (data not shown).

We next ruled out a selective loss of mutant animals by analyzing the genotypes of pups born in each maternal condition and find found an equal distribution of genotypes in all 4 maternal conditions, consistent with published reports that viability is not altered in mutant animals [12] (Table 3). Furthermore, the average latency between litters was 35.89 ± 3.10 days for WT mothers, 29.36 ± 3.35 days for unaffected mothers and 50.94 ± 3.65 days for affected mothers (Table 2). This indicates AS mouse mothers have decreased fecundity. In an attempt to improve breeding, we provided a subset of affected mothers a higher fat (11%) breeder diet. Affected mothers on the higher fat diet had litter sizes equal to WT mothers and unaffected mothers. Latency between litters was comparable to WT and unaffected mothers (Table 2).

3.4 Myelin abnormalities are altered by maternal status and diet

In the context of decreased litter sizes and wide variability in our myelination data, we hypothesized that the breeding method and diet may be influencing the myelination defects we observed. We therefore separated our data by maternal status (that is, whether the mother is an AS mouse or a Carrier mouse), to assess if maternal influences were impacting our studies. When separated into subgroups, AS-CM pups from unaffected mothers, showed significant alterations in MAG, but no differences in CNPase and MBP (Figure 3A). We found that AS-AM pups, (representing an alternate breeding method that results in offspring with the same genotype and we presumed to be equivalent to AS-CM pups) had a more severe myelin protein alteration with a 24% reduction in MAG, 44% reduction in CNPase and 41% reduction in MBP (Figure 3B). Similar deficits in expression of myelin constituent proteins were seen in hippocampal and cerebellar lysates from AS-AM pups (Supplemental Figure 1). This indicates that expression of myelin constituents in AS mice are influenced by factors beyond *Ube3a* expression status including maternal influences.

When comparing AS-AM-HF animals to their WT-AM-HF littermates, we observed no differences in cortical myelin proteins (Figure 3C, 3D). This is not entirely unexpected as maternal higher fat diets are known to affect myelination in rodents [15]. However, this dietary effect on an apparent hypomyelination may not be generalizable to other models. We have tested the hypothesis that the higher fat breeding diet can restore myelination to normal levels other models of hypomyelination, (Supplemental Figure 2). In this model system, a higher fat breeder diet has no impact on myelin and associated proteins, indicating that the AS mouse model is more susceptible to dietary conditions than other mouse models.

3.5 Maternal status influences glucocorticoid receptor expression

Abnormal GR expression in AS mice has been previously described [16]. To explore the possibility that maternal status may influence other phenotypes, we assessed if GR expression was modified by maternal status. We found that both maternal status and pup

genotype are independent determinants of GR expression (Figure 4). In particular, AS pups express a modest, but consistently higher GR level than their WT littermates ($p < 0.01$, Two-way ANOVA). However, we also found that both WT and AS pups from affected mothers, express lower levels of GR relative to pups raised by Carrier mothers ($p < 0.001$, Two-way ANOVA).

3.6 Myelination in WT pups differs by maternal status

In light of the altered glucocorticoid receptor expression, we wanted to assess the impact of maternal influences on the myelin abnormalities we observed. We compared the baseline expression of myelin constituents in WT animals from each maternal phenotype and observed a marked increase in myelin associated proteins in pups reared by affected mothers (WT-AM), compared to WT mice reared by unaffected mothers (WT-CM) (Figure 5). *Ube3a* expression is unaltered, showing that the observed changes in myelin proteins are not due to bulk changes in protein expression. This suggests that the apparent amplification of myelin defects in the AS-AM vs AS-CM mice may stem from normalization to overexpressed proteins in WT-AM littermates .

4. Discussion

In this study we demonstrated that AS mice have alterations in cortical myelin that are susceptible to modulation by maternal and environmental influences. Expression of myelin proteins was also altered in subcortical brain regions, but remained normal in sciatic nerve and spinal cord, despite *Ube3a* imprinting in these tissues. Additionally, we show that the expression of GR and baseline myelin constituent expression were also influenced by maternal genotype. Lastly, we demonstrated the deleterious effect of the AS maternal status and beneficial effect of higher fat diet on litter size and frequency of litters. These findings highlight the importance of the maternal environment on the phenotypes observed in this model system.

To address the hypothesis that deficits in myelin protein expression may underlie the disrupted white matter pathways and structures found in AS patients, we set out to characterize myelin expression in AS mice. We observed marked decreases in multiple myelin markers in cortical, cerebellar, and hippocampal lysates. Expression of myelin proteins were not altered in either the spinal cord or in the sciatic nerve. This suggests that loss of *Ube3a* has differential effects on the oligodendrocyte progenitor cells (OPCs) originating in the medial ganglionic eminence that become cortical oligodendrocytes compared to OPCs originating in the ventral ventricular zone that become spinal cord oligodendrocytes [17]. Additionally, normal myelination in the sciatic nerve suggests that the neural crest derived Schwann cells respond differently to the loss of *Ube3a* than oligodendrocytes [18].

In an attempt to understand the variability of our data, we assessed the breeding performance of our colony. We noted that AS females were generally poor breeders. As a result we spoke with the veterinary staff at our university and they recommended use of a higher fat breeder diet (11% fat) rather than a standard diet (5% fat) to provide additional nutritional support and possibly reduce physiological stress in the breeding mothers. This higher fat diet has

restored reproductive ability in at least one other rodent model of disease with small litters [19] and is suggested by veterinary staff at many research institutions for enhancing breeding performance. By employing this strategy, we were able to successfully normalize the litter sizes born to affected mothers as well as decrease the time between litters. This was a somewhat surprising finding since AS mice are prone to obesity [20–22] and obesity is known to decrease reproductive ability in mice. The rescue of litter size and reduction of time between litters would indicate that obesity is likely not the cause of the reproductive deficits described here as a higher fat diet would be expected to exacerbate the problem and result in smaller, even less frequent litters.

In our initial analysis of myelin expression, mice from all breeding conditions (including both AS and Carrier mothers and higher fat mice) were grouped together as we assumed that each condition was equivalent. When analyzed as a single group, this data was highly variable. The large differences in litter size and latency between litters in each breeding condition prompted us to assess the possibility that maternal influences may be modulating this model system. When analyzed in this manner, our data segregated by breeding cage type. We observed a modest hypomyelination phenotype in AS-CM pups (originating from unaffected mothers), a more severe hypomyelination in AS-AM pups (originating from affected mothers) and the unexpected finding of normal myelin protein expression in the AS-AM-HF mice. While it is tempting to speculate that a higher fat diet rescues the apparent defect in myelination in AS mice, an alternate hypothesis is that it acts to improve the maternal environment of the AS mothers and has no direct effect on expression of myelin proteins. It is unclear why MAG is resistant to differences in maternal conditions while CNPase and MBP are susceptible. One possible explanation is the documented defect in sialylation found in *Ube3a* KO mice [23], Sialylation is known to be important for normal myelination, playing a role in MAG interactions with neurons [24]. This may explain why differences in MAG are present in AS pups from both maternal conditions while CNPase and MBP are differentially affected, though future studies will be required to confirm this.

The AS genotype in pups is dependent upon acquiring a disrupted *Ube3a* allele from the mother, but not whether the mother is a Carrier or actually affected by AS herself. The devastating nature of AS precludes affected woman from raising children, therefore using Carrier mice more accurately models the human condition. However, only a limited number of publications clearly state the breeding scheme used. The use of Carrier mothers as breeders has only recently been explicitly reported [25–28]. There are a number of reasons why some laboratories, including our own, might instead use affected AS mothers. The primary reason being that obtaining Carrier mothers requires maintenance of a second breeding colony (crossing a *Ube3a* deletion heterozygote male with a WT mother), in which only 25% of the pups are female *Ube3a* (m+/p-) animals are used for breeding. In contrast, using AS mothers produces litters in which the mutant pups may be used for breeding or experiments, thereby minimizing the number of animals needed as directed by IACUC guidelines. Furthermore, given the global loss of *Ube3a* expression and wide array of neurological problems in AS mice, the possible effect of maternal status might be easily dismissed as unimportant.

Stress has been implicated in the development of phenotypes in several mouse models of disease including Angelman mouse models [29–31]. *Ube3a* maternal deficient mice have been shown to exhibit an altered stress response, elevated corticosterone and anxiety-like behaviors [16] that are not seen in *Ube3a* paternal deficient (Carrier) mice [32]. While our results with GR receptor appear at odds with previous findings of reduced glucocorticoid receptor expression in the hippocampus [16], further work from the same laboratory has shown a trend towards increased glucocorticoid receptor expression in cortical lysates [33], consistent with our results.

Due to the stark contrast in hypomyelination between AS-CM and AS-AM mice and the alteration in glucocorticoid receptor expression, we subsequently hypothesized there could be maternal influences on WT pups. By looking specifically at WT-AM pups we observed an increase in myelin associated proteins ranging between 25 and 50% in WT-AM pups compared to WT-CM pups, consistent with observations that prenatal stress is known to increase myelination in rodents [34]. This is also supported by the observation of a significant reduction in GR expression in the cortex of WT-AM mice compared to WT-CM. Previous studies have shown that GR expression is reduced in the prefrontal cortex and hippocampus of mice exposed to stress [35]. Taken together, this supports the notion that the myelin phenotypes we observed in the AS-CM mice could appear to be amplified by normalization to stress induced hypomyelination in WT-AM mice, rather than a pure hypomyelination in AS-AM mice.

These data indicate that AS model mice possess phenotypes that are susceptible to modulation by environmental factors, including, but not limited to maternal status and diet. We show that there is a deficit in cortical MAG expression in the Carrier mother breeding scheme. This hypomyelination phenotype is exacerbated when utilizing an AS mother breeding scheme, with deficits in CNPase and MBP in addition to MAG. The hypomyelination phenotype in the offspring of AS mothers is abolished when the mothers are fed a higher fat (11%) breeder diet. Our findings indicate that even in models of severe neurological disease, the importance of maternal status on their offspring cannot be underestimated or ignored. The importance of maternal genotype on pup phenotypes is only recently being appreciated. Several other mouse models have also shown that maternal genotype plays a role in pup phenotypes. These models include a serotonin mouse model of anxiety [36], a triple transgenic model for Alzheimer's disease [37] and a heterozygous Tuberous Sclerosis Complex mouse [38] all of which show a maternal genotype contribution to behavior phenotypes observed in the offspring of mutant mothers. These findings highlight the importance of considering the breeding scheme used in the generation of transgenic mice in even the most severe models of neurodevelopment disorders.

While our present data does not indicate whether the maternal influence is biological or behavioral, the combination of poor breeding and altered glucocorticoid receptor expression suggest that maternal behavioral stress is a prime candidate for these changes. Future studies, including cross fostering, will be required to determine the exact mechanism of this differential hypomyelination phenotype. Additional studies that should be undertaken include a formal evaluation of maternal care by mothers of both genotypes. Behaviors of interest include nesting behavior, pup retrieval tests and maternal aggression testing.

Additionally, increased ultrasonic vocalizations (USVs) have been reported in AS mice and are postulated to represent abnormal communication between the pups and dams. It would be very interesting to evaluate if the alterations in USVs are more severe in pups reared by AS mothers.

5. Conclusions

The findings presented here highlight the previously unrecognized importance of environmental variables and maternal influence on the biochemical phenotypes observed in AS mice. Additionally, they raise the exciting possibility that environmental or nutritional manipulations may help improve outcomes in children with this devastating disorder. Our findings indicate that breeding, Carrier mothers (*Ube3a* (m+/p-) crossed to a WT male is the most physiologically appropriate breeding scheme for this model system and is critical for the discovery of translationally relevant findings. This is the closest way to simulate the inheritance of AS as in humans the mutant allele is acquired from a mothers harboring a paternal deletion or the mutation occurs *de novo* [39]. While the use of this breeding scheme may be obvious to some, it has not been explicitly stated that this should be the preferred method for generating AS mice. Further studies will be required to assess the impact of maternal genotype/phenotype and environmental variables, including diet, on the behavioral and biochemical outcomes observed in AS mice. Whether these findings are generalizable to other genetic mouse models remains to be determined, though we would encourage the use of *Ube3a* paternal deficient females for breeding AS mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- AS mice have disrupted cortical myelin protein expression
- Maternal status influences myelin expression dramatically in this model
- A higher fat (11%) breeder diet normalizes myelin protein expression
- Use of an appropriate breeding scheme is critical for AS research

Figure 1. AS Mice have an apparent decrease in cortical myelin protein expression and imprinted Ube3a

(A) Expression of the myelin constituent proteins MAG, CNPase and MBP were significantly reduced in cortical protein lysates from AS mice compared to littermate controls. Ube3a expression is nearly abolished, consistent with imprinted expression (n=22–29 per group). (B) No significant differences in expression of the astrocyte or neuronal proteins GFAP or PGP9.5, respectively, were seen between AS and littermate control cortex (n=10–13 per group). (C) Representative immunoblots of littermate control (WT) versus AS cortical extracts. *** P<0.001 vs littermate controls.

Figure 2. AS Mice have normal expression of myelin markers in the spinal cord and sciatic nerve (A) No differences in expression of the myelin constituent proteins in the spinal cord (n=16–19 for MAG/CNPase and n=5–6 for MBP) or (B) in the sciatic nerve (n=18–19 for MAG/CNPase and n=6 for MBP) were seen for AS mice in comparison to littermate controls. (C) Representative immunoblots of control versus AS spinal cord lysates and (D) sciatic nerve lysates.

Figure 3. Apparent cortical myelin deficiency in AS mice is influenced by maternal status
 (A) Expression of MAG, but not CNPase or MBP, is reduced in AS-CM, when compared to WT littermates. (n=5–9 per group) (B) Expression of myelin constituents MAG, CNPase and MBP are significantly reduced in AS-AM mice when compared to WT littermates. (n=14–27 per group) (C) Expression of myelin constituent proteins is not different between WT-HF and AS-HF animals (n=8–11 per group). (D) Representative immunoblot of control (WT-HF) vs AS-HF cortex extracts. ^{^^^} P<0.001 vs WT-AM. ^{##} P<0.01 vs WT-CM controls.

Figure 4. Glucocorticoid receptor expression is modulated by both pup genotype and maternal genotype

(A) Expression of glucocorticoid receptor is reduced in WT-AM mice compared to WT-CM mice. ^{##} $P < 0.01$, $n=4-6$ per group. (B) WT-AM pups have reduced glucocorticoid receptor expression compared to WT-CM and AS-AM pups have slightly elevated glucocorticoid receptor expression compared to WT-AM littermates [^] $P < 0.05$ WT-AM vs WT-CM, [#] $P < 0.05$ WT-AM vs AS-AM. A secondary analysis of all four groups using a two-way ANOVA showed a modest, but consistent reduction of GR in pups from AS mothers compared to Carriers ($p < 0.001$) and increased GR in mutant AS pups compared to littermate controls ($p < 0.01$) (C),(D) Representative immunoblots of lysates from WT-CM vs WT-AM and WT-CM, AS-CM, WT-AM and AS-AM mice.

Figure 5. Baseline expression of myelin proteins is increased in WT pups from affected mother breeders

(A) Expression of myelin constituents is significantly increased in WT-AM pups when compared to WT-CM pups. Ube3a expression is unchanged. $n=7-8$ per group (B)

Representative immunoblots of control (WT-CM) vs WT-AM cortical extracts. [#] $P<0.05$, ^{##} $P<0.01$ vs WT-CM controls.

Table 1

Breeding Schemes and Nomenclature for Offspring

Breeding Scheme	Maternal Genotype	Dietary Status	Pup Nomenclature
Affected Mother	(m-/p+) AS mouse	Standard Lab Diet	Ube3a (m+/p+) WT-AM Ube3a (m-/p+) AS-AM
Carrier Mother	(m+/p-) (Carrier) mouse	Standard Lab Diet	Ube3a (m+/p+) WT-CM Ube3a (m-/p+) AS-CM
Affected Mother Higher Fat	(m-/p+) AS mouse	5LJ5 Higher Fat Breeder Diet	Ube3a (m+/p+) WT-AM-HF Ube3a (m-/p+) AS-AM-HF

Table 2

Litter size and latency between litters is altered in AS breeders.

Maternal Genotype	Average Litter Size	Latency Between Litters
WT	7.94 ± 0.46	35.89 ± 3.10
AS	5.13 ± 0.35 *\$#	50.94 ± 3.65 *\$#
Carrier	7.60 ± 0.56	29.36 ± 3.35
AS Higher Fat	7.17 ± 0.37	29.18 ± 2.34

Litter size is decreased in AS breeding mothers, but not in unaffected Carrier mothers or AS breeding mothers fed a higher fat diet. (n=16-36 litters per group, at least 5 breeding pairs per group). Latency between litters for AS breeding mothers is prolonged when compared to WT, Carrier, and AS mothers fed a high-fat diet. (n=9-22 inter-litter intervals per group, 3–5 breeding pairs per group).

* P<0.01 WT vs AS,

\$ P<0.01 AS vs ASHF,

% P<0.01 Carrier vs AS.

Table 3

Distribution of Pups in Each Breeding Condition

Maternal Genotype	WT Pups	Mutant Pups
WT	33 (48%)	35 (52%)
AS	99 (48%)	87 (52%)
Carrier	26 (48%)	28 (52%)
AS Higher Fat	101 (48%)	114 (53%)

Distribution of pup genotypes at weaning is unaltered by maternal status.