"The discoverer of Canavan syndrome gene: how problems of diagnosis and treatment are being changed over time"

By:

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Introduction

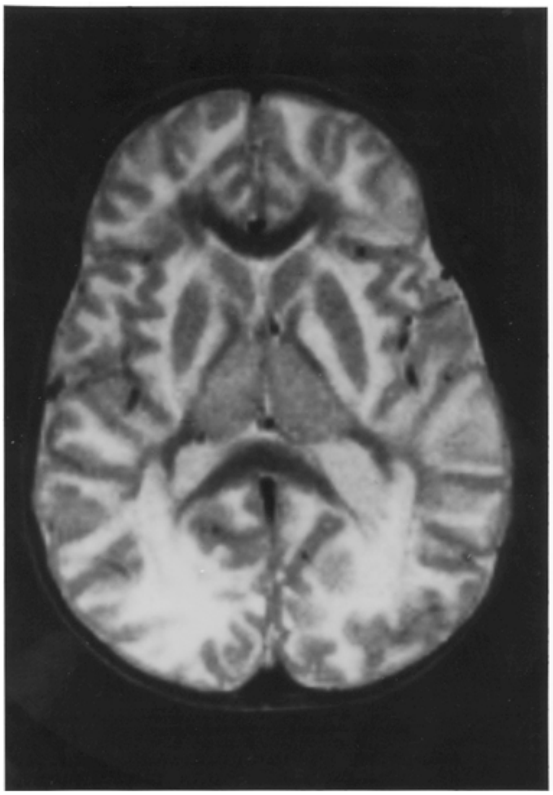
- Canavan Disease is an autosomal recessive neurodegenerative disorder
- The Basic Defect is Asparoacylase (ASPA) deficiency leading to accumulation of N-Acetylaspartic Acid (NAA)
- The disease is panethnic, but more prevalent among Ashkenazi Jews with carrier rate of 1/40 – 1/60.

Different Severities

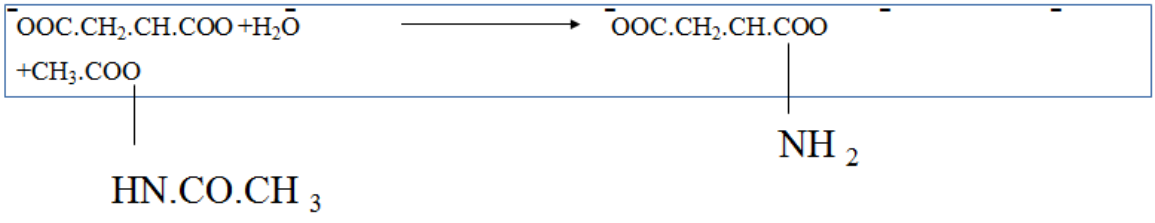
- **VARIANT FORMS OF CANAVAN DISEASE**
  - **CONGENITAL**
  - **INFANTILE**
  - **JUVENILE**

Atypical Canavan Disease Mutations

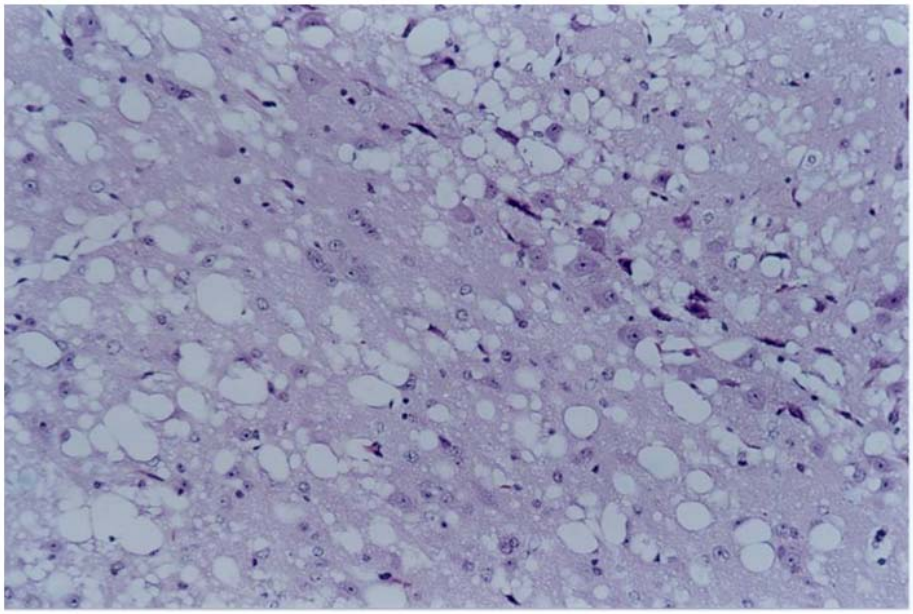
- **MUTATION ON CHROMOSOME 17**
- **E285A AND Y231X, ACCOUNT FOR > 98% IN ASHKENAZI JEWS**
- **MILD MUTATION**
  - **Y288C**
  - **R71H**
- **SEVERE MUTATIONS**
  - **A350E, MOST COMMON IN NON-JEWS**
- **NEW MUTATION**
  - **HOMOZYGOUS C432+1G>A MUTATION (UNALP A, ALTIOK E, URAN N, OZTÜRK A, YÜKSEL S., J TROP PEDIATR. 2007 NOV 12)**

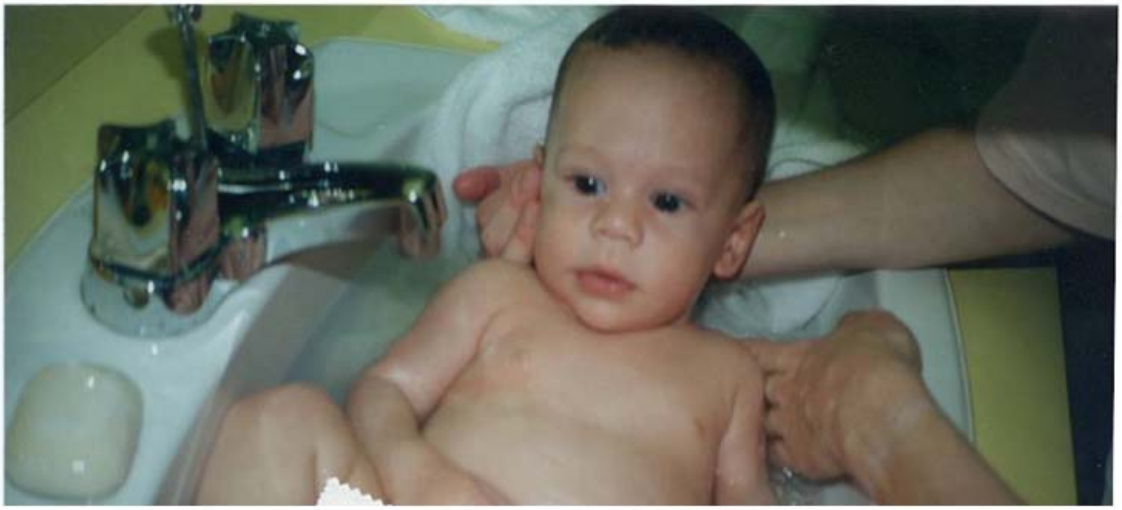


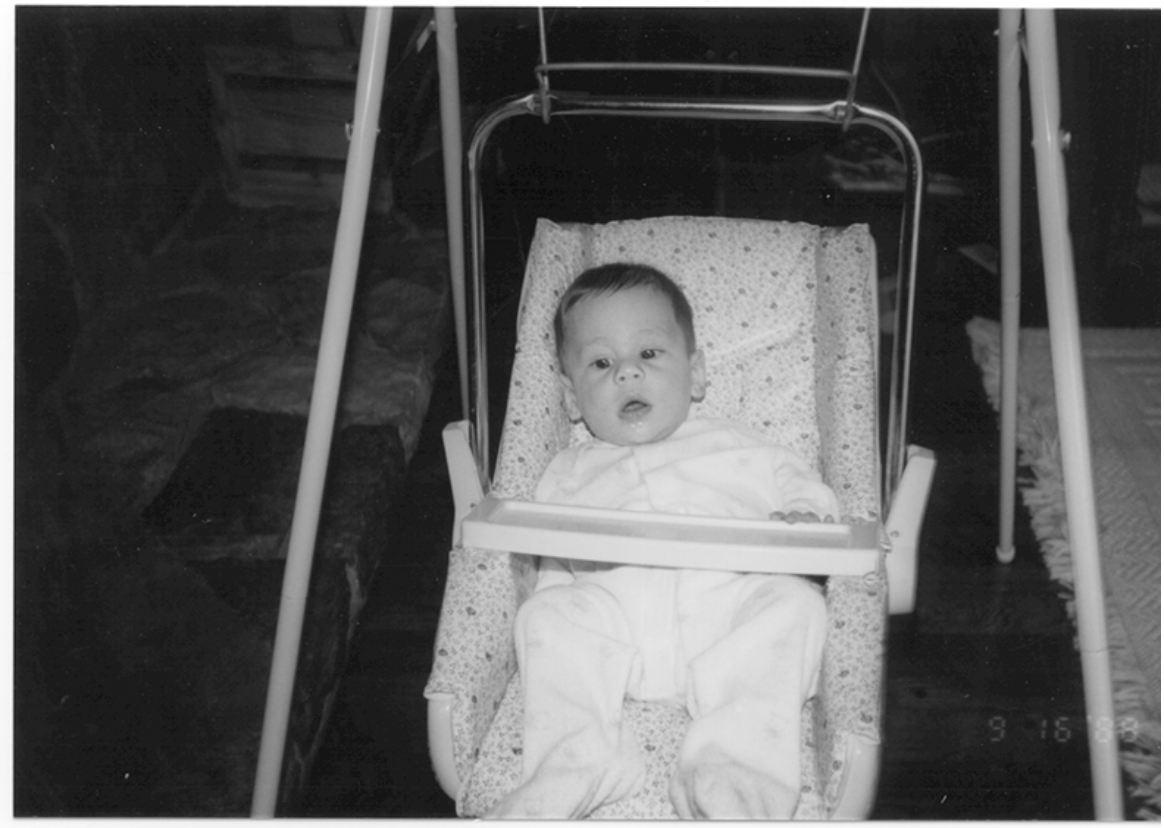
ASPA

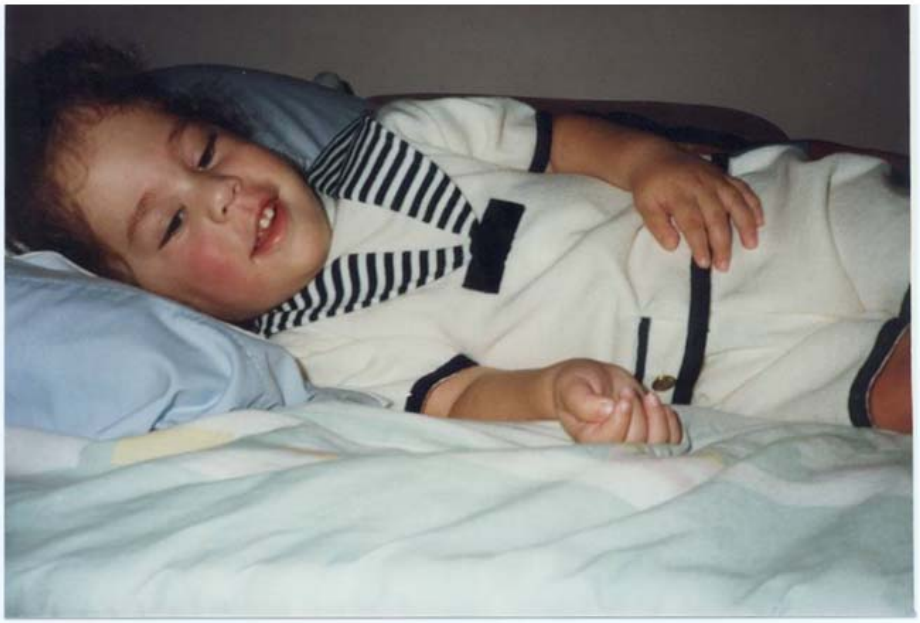


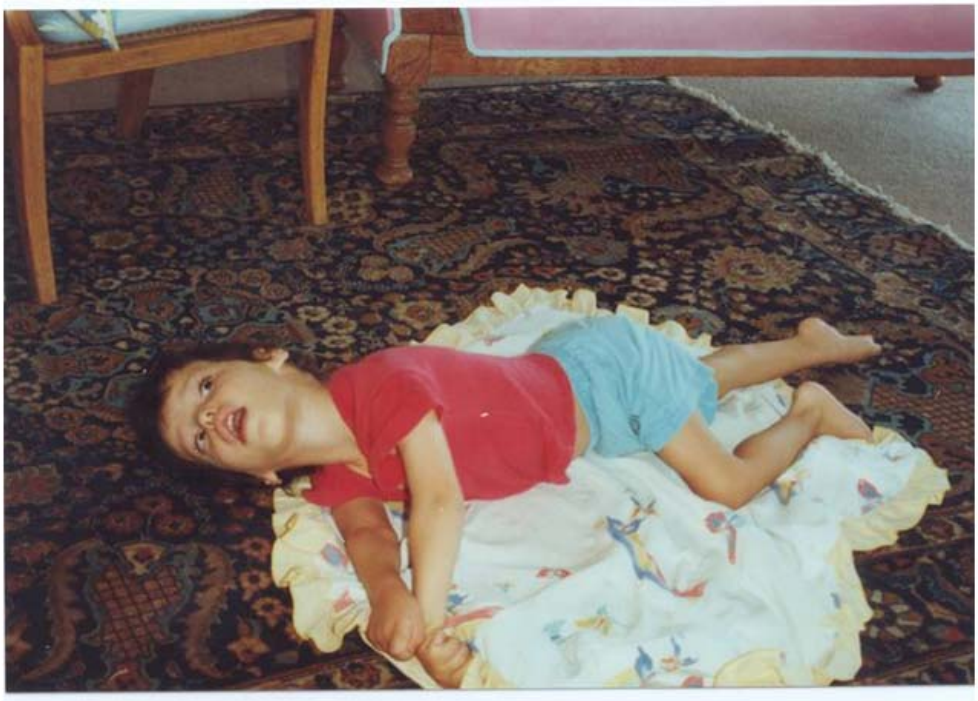
NAA **L-Aspartic acid** **Acetate**

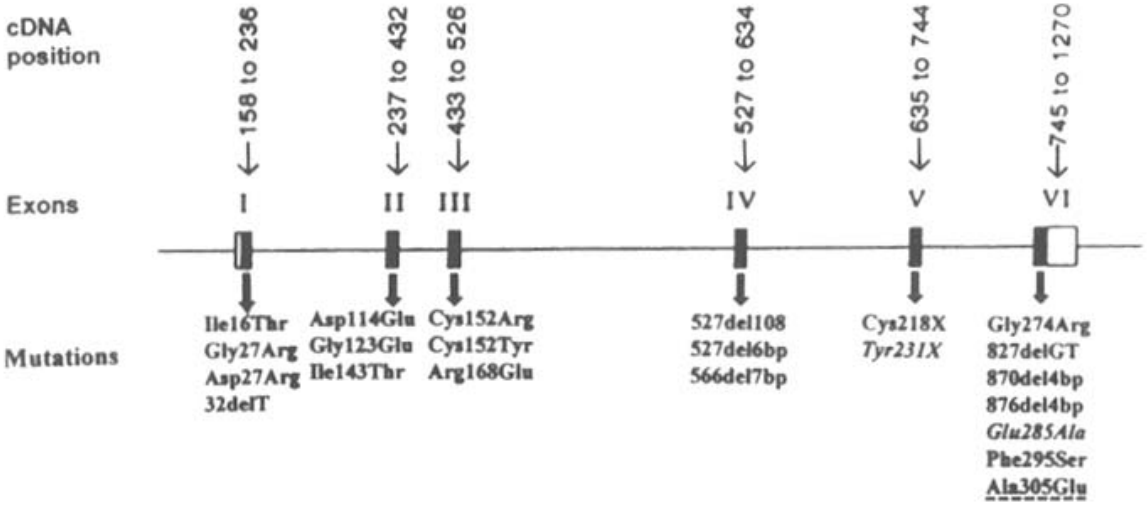








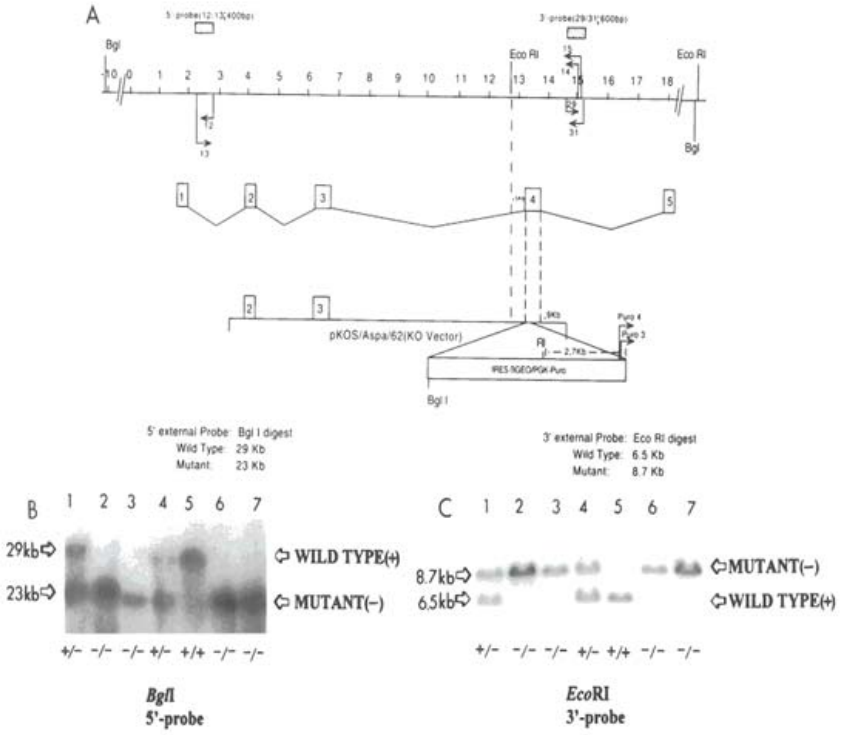



cDNA position
158 to 236
237 to 432
433 to 526
527 to 634
635 to 744
745 to 1270
Exons
I
II
III
IV
V
VI
Mutations
Ile16Thr
Gly27Arg
Asp27Arg
32delT
Asp114Glu
Gly123Glu
Ile143Thr
Cys152Arg
Cys152Tyr
Arg168Glu
527del108
527del6bp
566del7bp
Cys218X
Tyr231X
Gly274Arg
827delGT
870del4bp
876del4bp
Glu285Ala
Phe295Ser
Ala305Glu

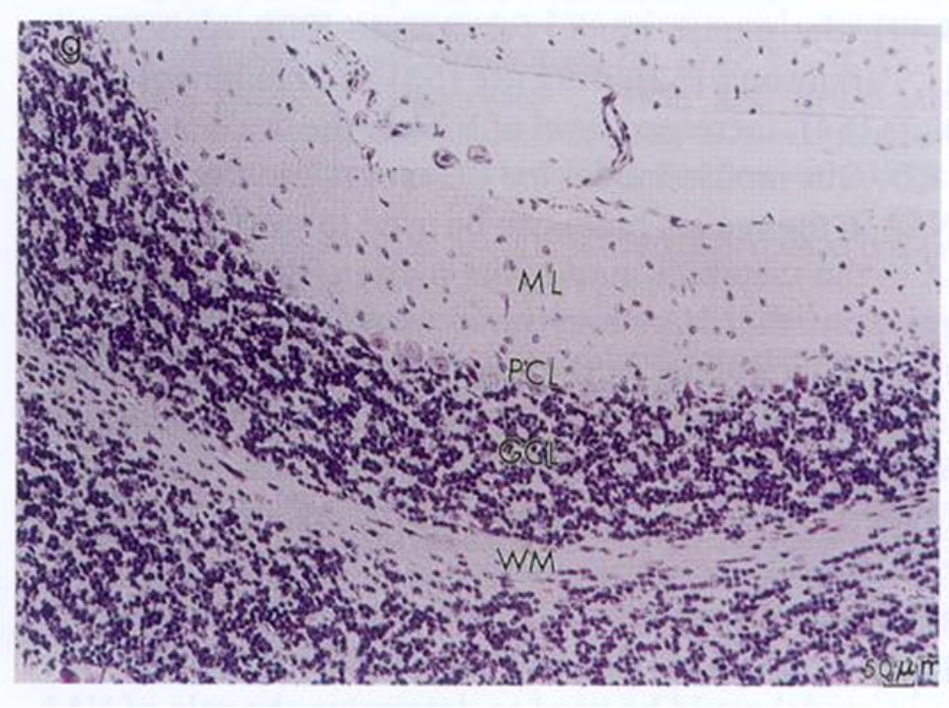
Knockout mouse

- Human and mouse aspartoacylase genes have been cloned and characterized.
- Ten base pairs were deleted from exon 4 of the mouse Aspartoacylase cDNA.
- Following homologouse recombination a Canavan mouse was produced.

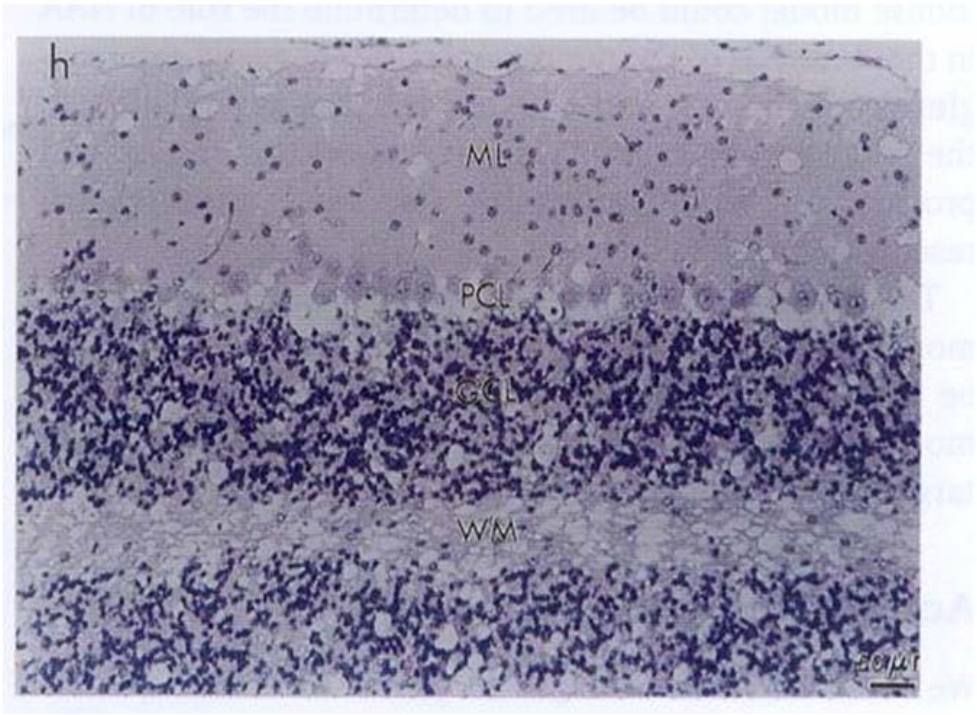
Targeted Disruption of the Murine Aspartoacylase Gene



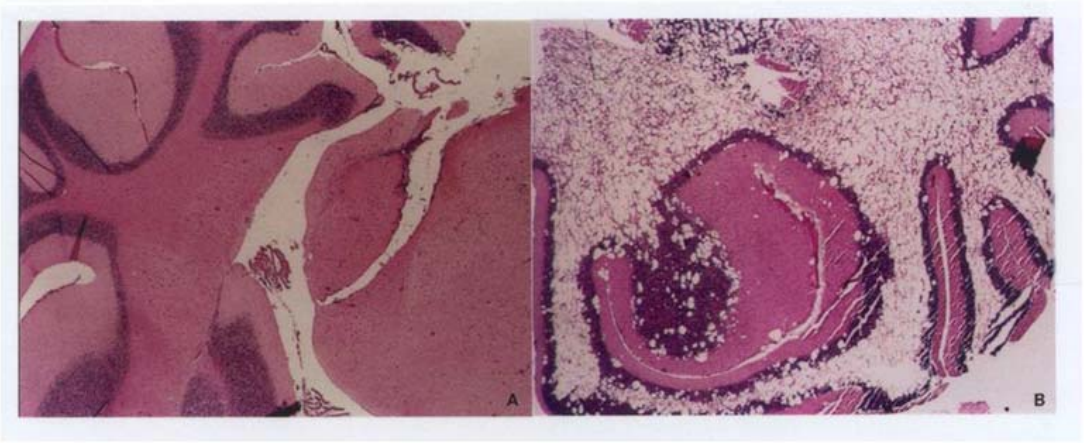
Normal Mouse Cerebellum



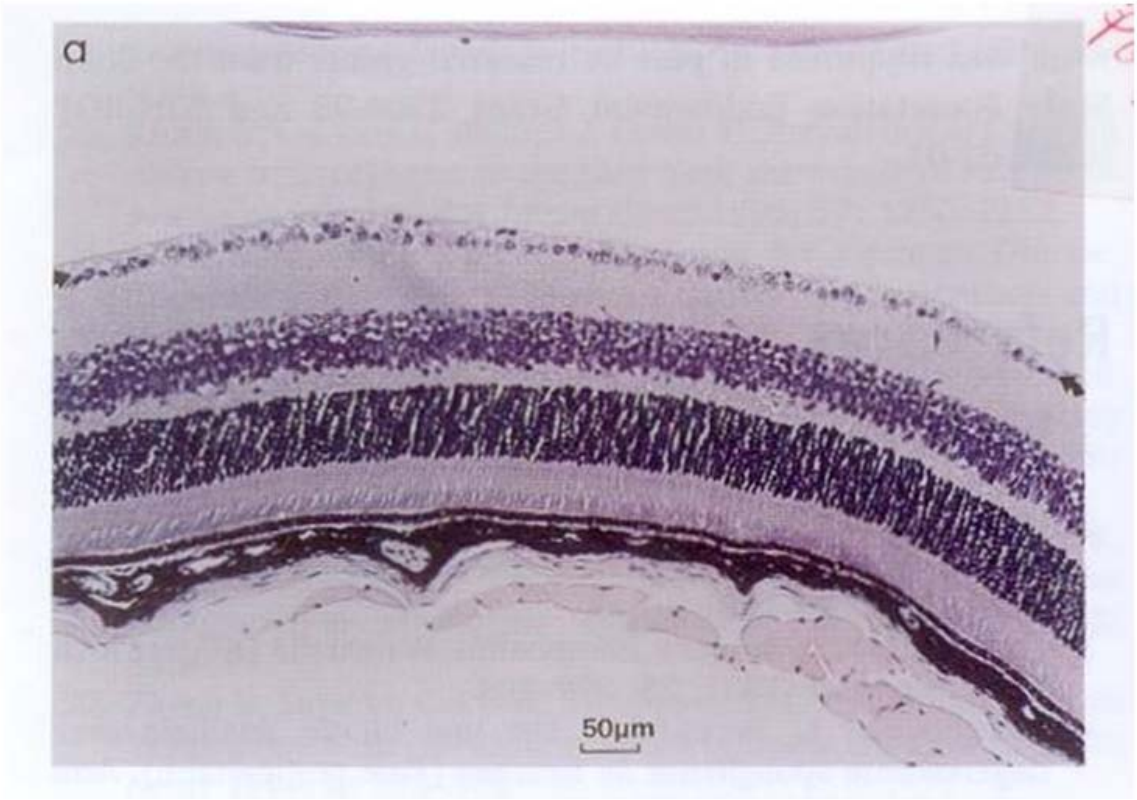
Knock Out Mouse Cerebellum



Cerebellum of the Knock Out Mouse

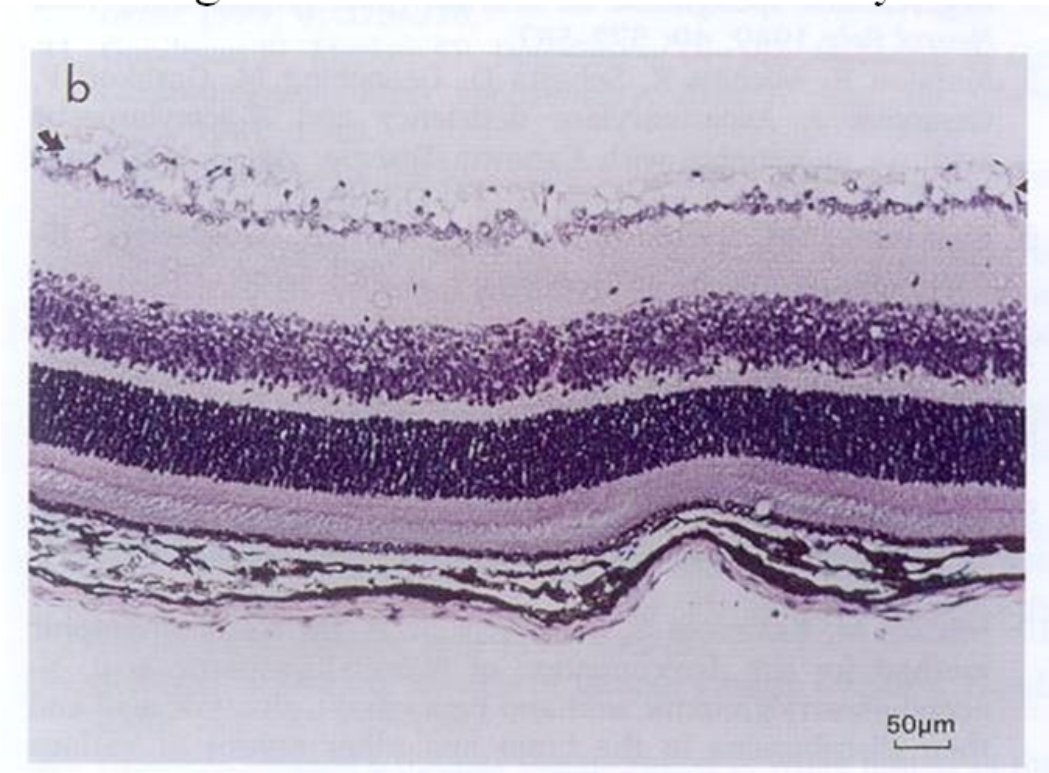


Normal Mouse Retina

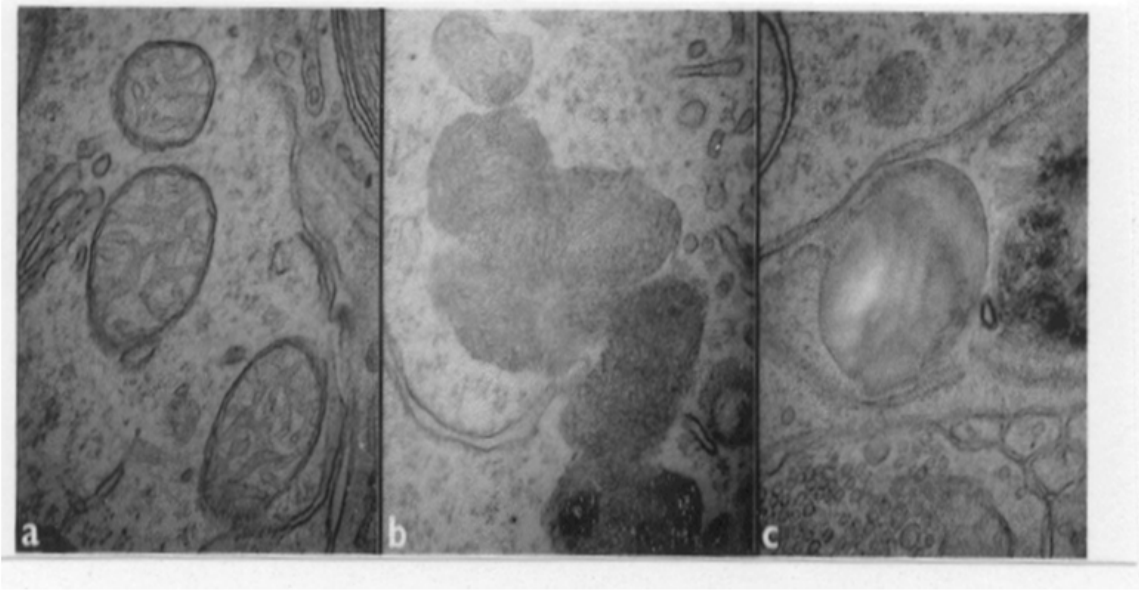


Knock Out Mouse Retina

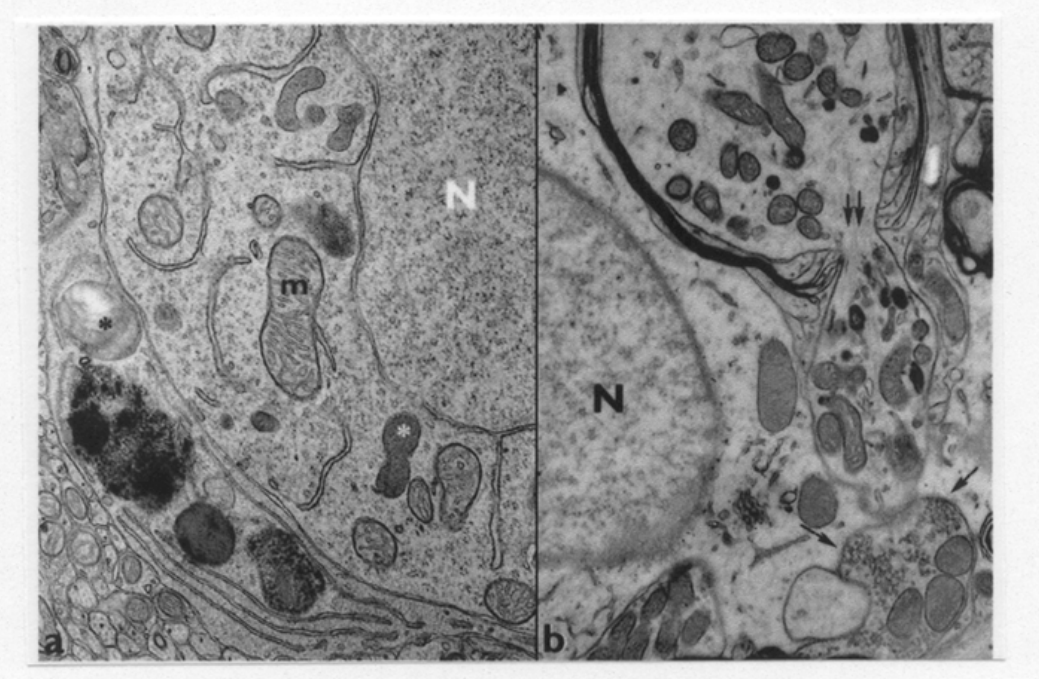
Ganglion cells and Nerve Fiber Layers



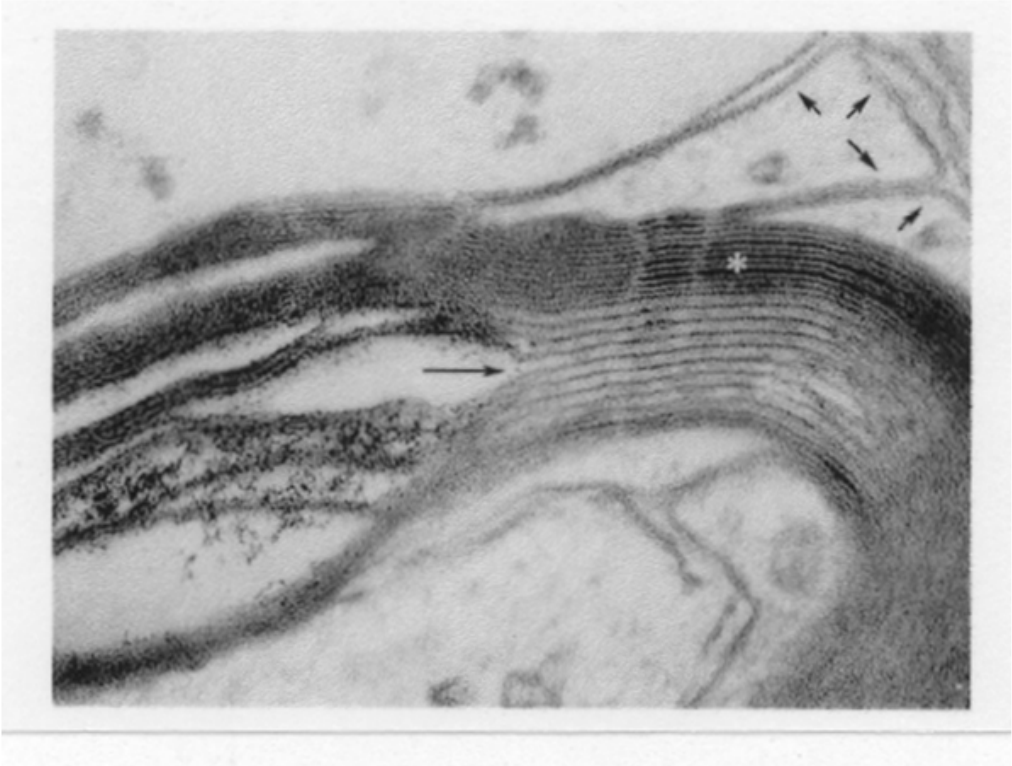
Stages in Degeneration of Mitochandria



a. Neuron Body b. Interrupted Myelin



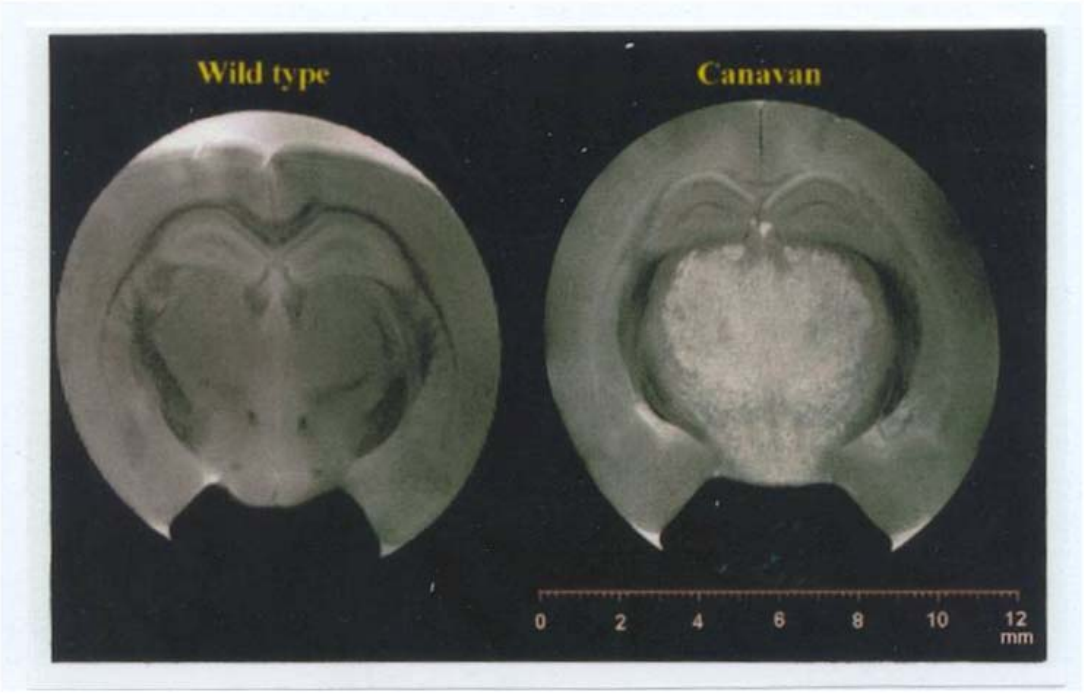
Split of Myelin Sheath

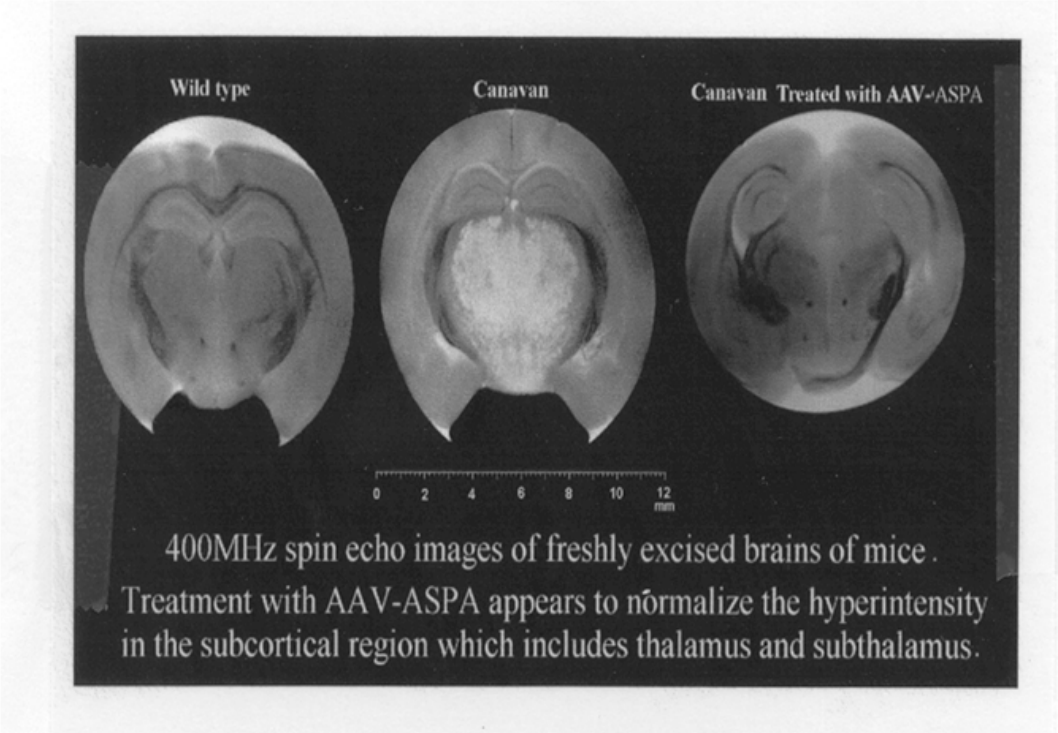


Molecular Studies

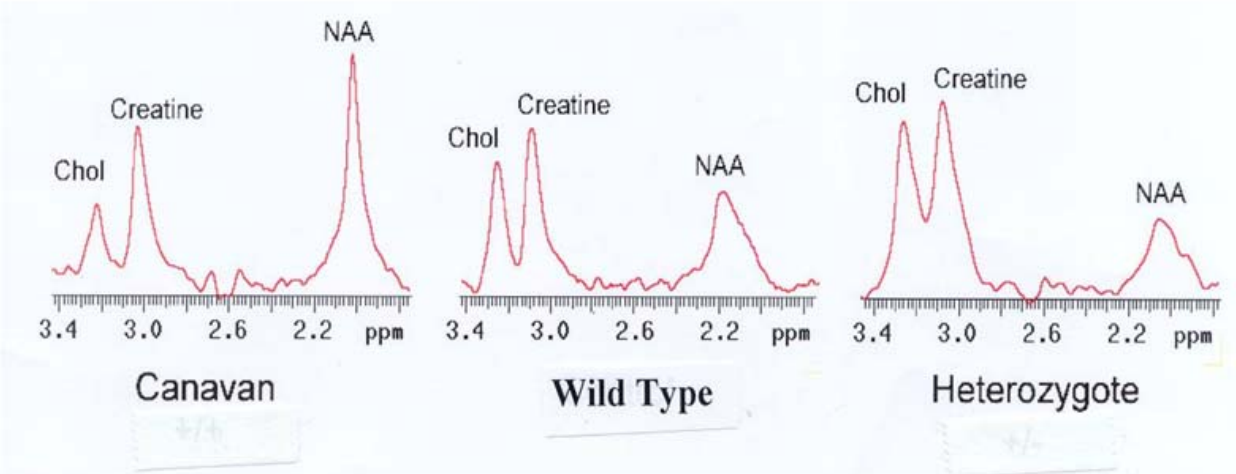
- Human and mouse aspartoacylase genes have been cloned and characterized.
- Ten base pairs were deleted from exon 4 of the mouse Aspartoacylase cDNA.
- Following homologous recombination a Canavan mouse was produced.

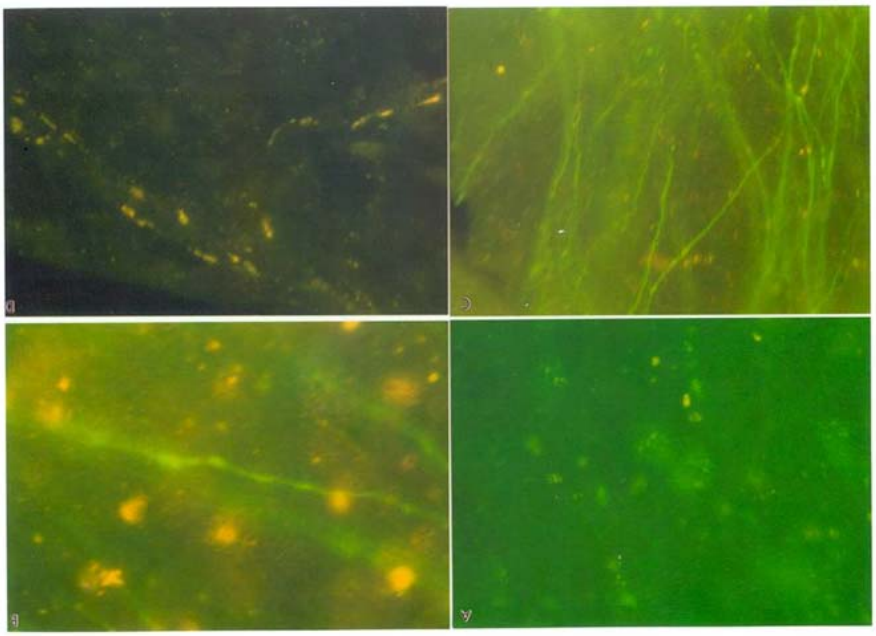
MRI of Mouse Brain

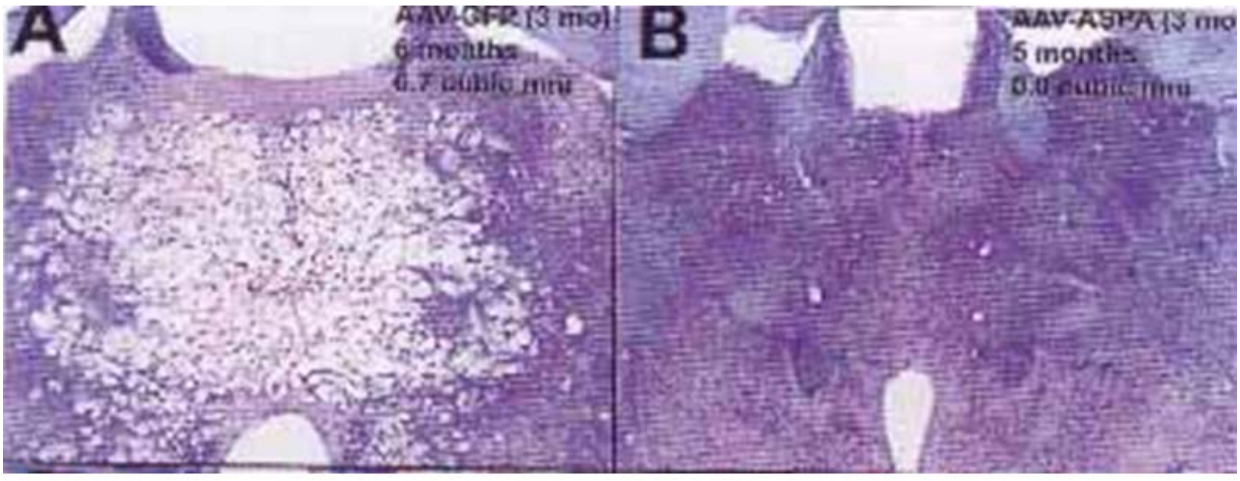


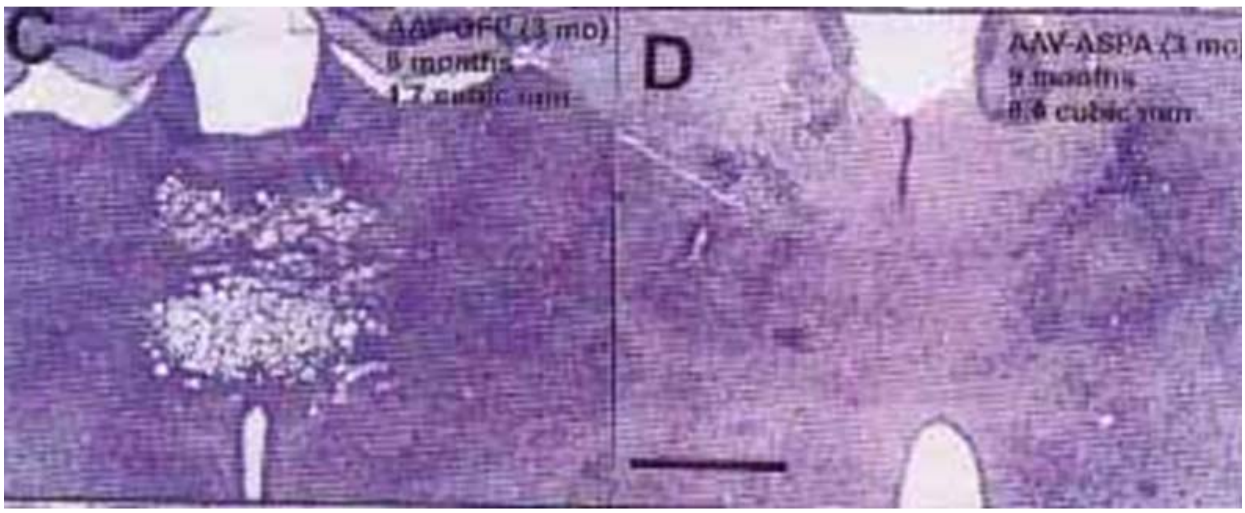


MRS of Mouse Brain






A
B



Gene Expression in CD Mouse Brain Downregulation

Genes	Expression ratio
Glutamate transporter-EAAT4	9.7↓
Gamma-aminobytyric acid A receptor, subunit	110.1↓
G-substrate mRNA	16.4↓
Mitochondrial ribsomal protein S12	10.7↓
Dao-ld mRNA for D-amino acid oxidase	16.0↓

Gene Expression in CD Mouse Brain

Upregulation

Spi 2	29↑
Lzp-s mRNA for lysozyme p	25.4↑
Caspase-11 mRNA	4.4↑
Interleukin-1-beta converting enzyme	3.8↑

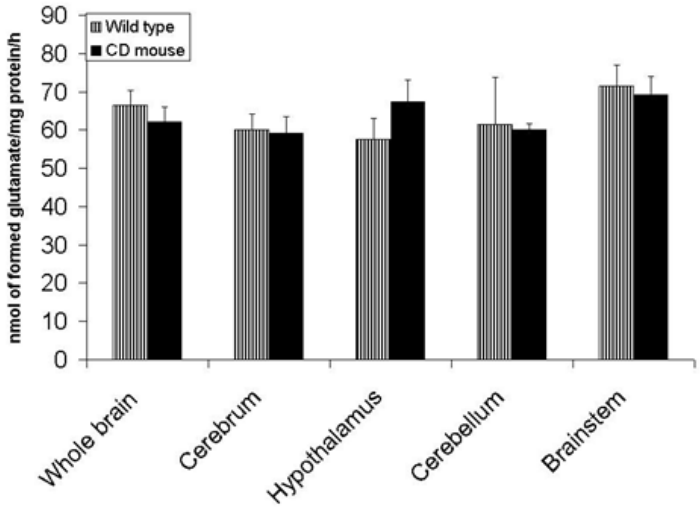
Levels of neurotransmitters in the CD mouse brain

Glutamate	2.5 times ↓
GABA	2.3 times 2.3↓

NAAG/ Cr ratio in the knock out mouse brain

Knockout	**0.032 ± 0.003**
Wild type	**0.028 ± 0.004**

NAALADase activity in the knockout mouse brain



Levels of succinic semialdehyde dehydrogenase and glutamate dehydrogenase in CD and WT mice.

	SSADH activity (mU/mg protein)	GDH activity (mU/mg protein)
Wild type:		3.81± 0.64
Cerebrum	0.27 ± 0.02	8.29 ± 1.04
Hypothalamus	0.64 ± 0.02	8.94 ± 0.74
Cerebellum	0.47 ± 0.05	6.9 ± 0.45
Brainstem	0.38 ± 0.02	
CD mouse:		2.7 ± 0.45
Cerebrum	0.27 ± 0.01	7.25 ± 1.67
Hypothalamus Cerebellum	0.76 ± 0.04	3.9 ± 0.42
Brainstem	0.36 ± 0.03	3.53 ± 0.31
	0.30 ± 0.03	

TREATMENT OF CANAVAN DISEASE

Therapeutic Attempts

1. Pharmacological agents
2. Gene therapy
3. Stem cell therapy

Pharmacological Agents

Trial with Diamox
Reduction of water in brain
Probably not sustained

Ketogenic Diet

- Acetoacetate increased in brain
- NO clinical improvement

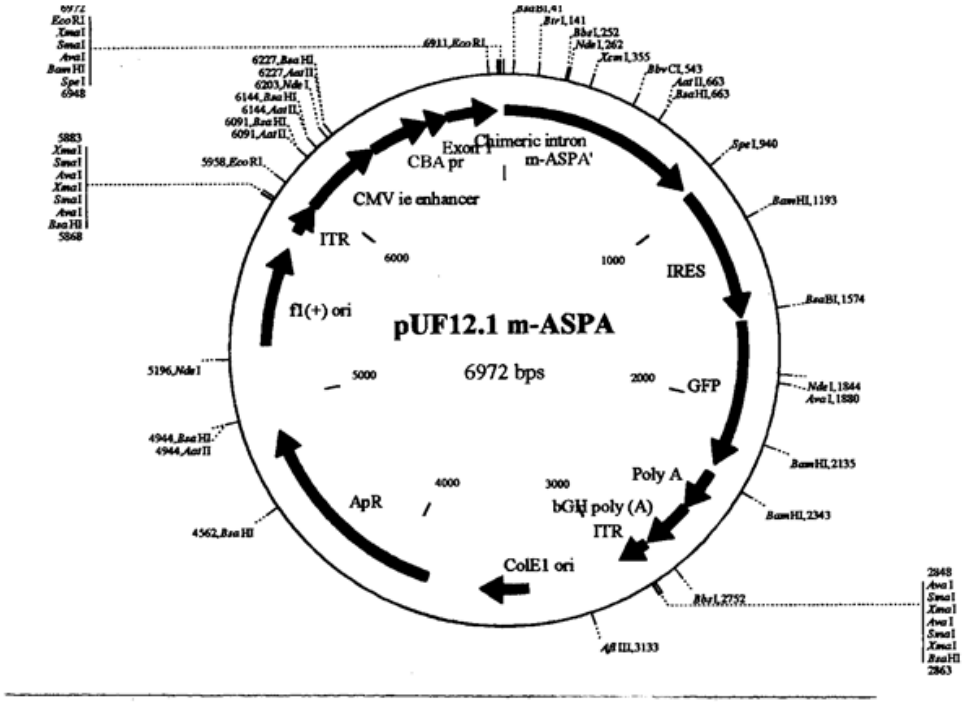
Gene Therapy

1. Currently being performed by Dr. P. Leone, these attempts have not been successful
2. A report from a few years ago on 2 patients

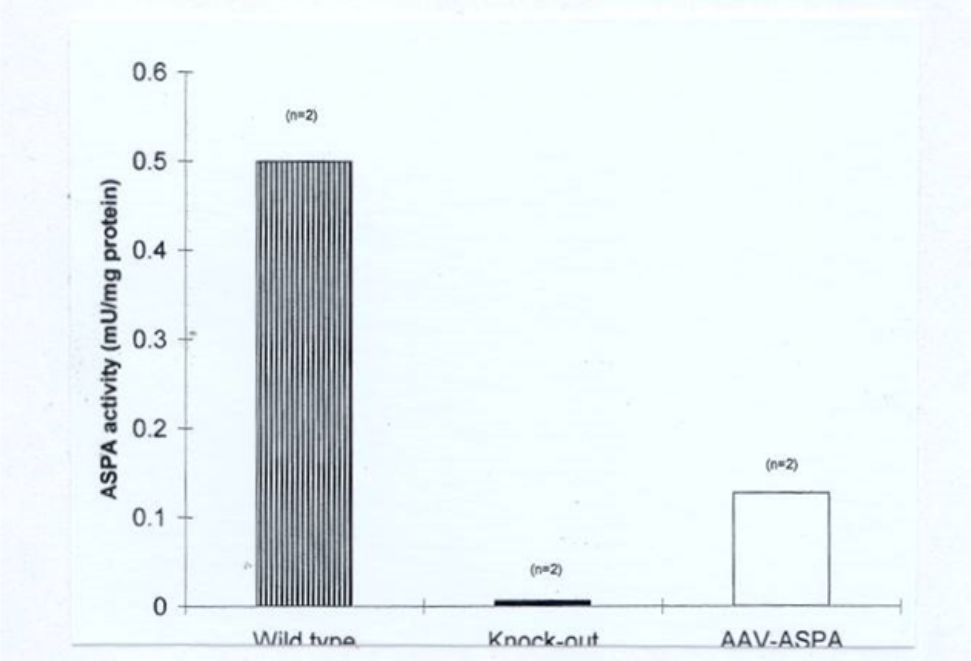
Adenoassociated virus, serotype 2, mediated gene therapy

rAAV-ASPA-GFP vector was injected into the striatum and thalamus of the CD mouse brain

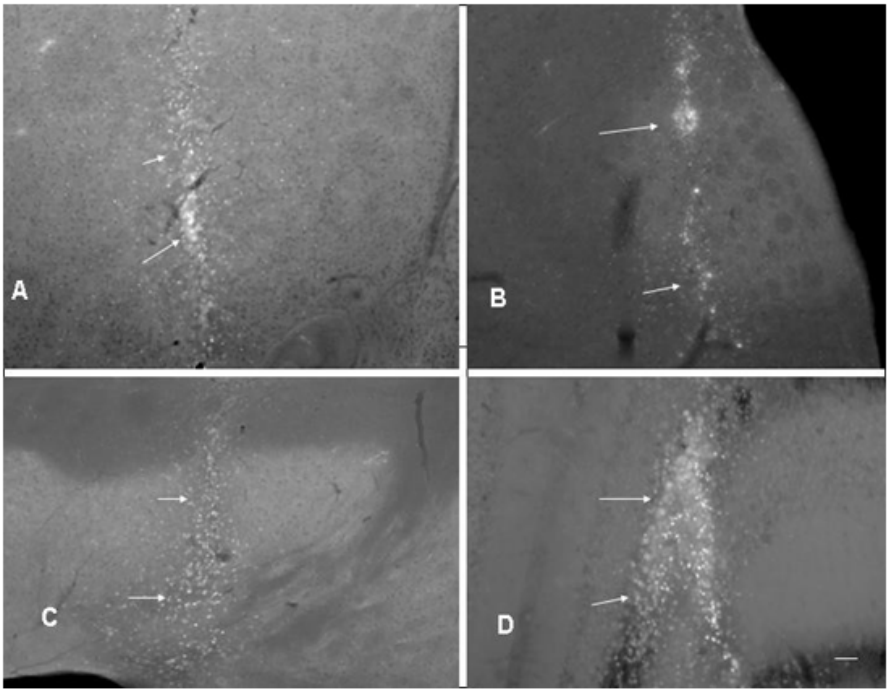
AAV Construct with Mouse ASPA cDNA



Aspartoacylase
10 Weeks after Treatment



WT forebrain CD forebrain



WT CD

Gene therapy on Knock out Mouse with Canavan Disease

Encouraging results

Better Vector dissemination is needed

Stem cell therapy

Neural stem cells were injected at a dose of 100,000 cells/ul

Striatum and cerebellum were injected

After one month of post transplantation period, animals were evaluated.

Stem Cell Therapy in the Knock Out Mouse for Canavan Disease

1. Trial with Genzyme Stem Cells

ASPA activity (mU/mg protein) 1 month after injection with Stem Cells

	WT	CD uninjected	CD injected
Juvenile	0.189	0	16% (4 weeks)
Adult	0.280	0	residual (5 weeks)

Glycceryl Triacetate

Acetate levels reduced (80%) in Canavan mouse

Lipid synthesis decreased

GTA is superior than Ca Acetate

What is Next

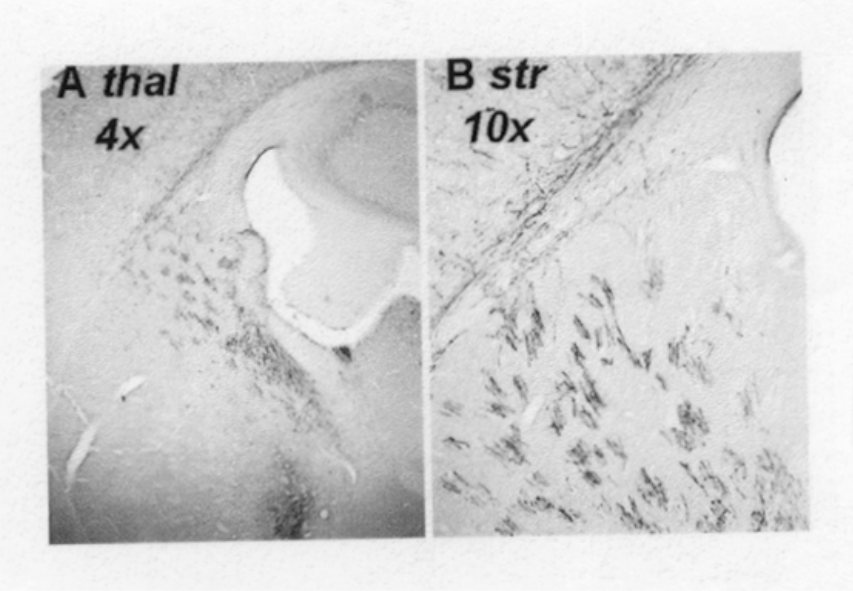
Trial with mice

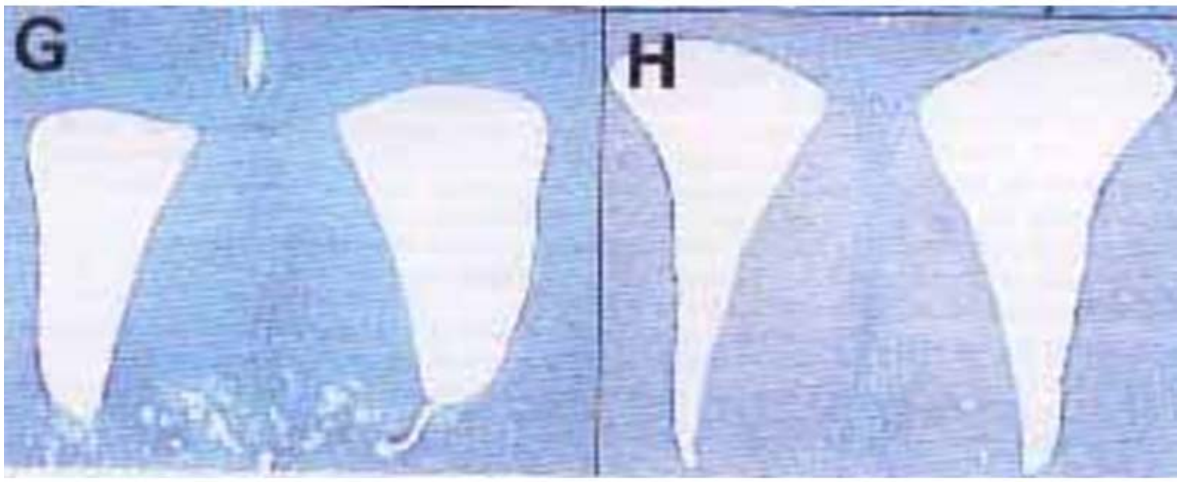
Trial with patients

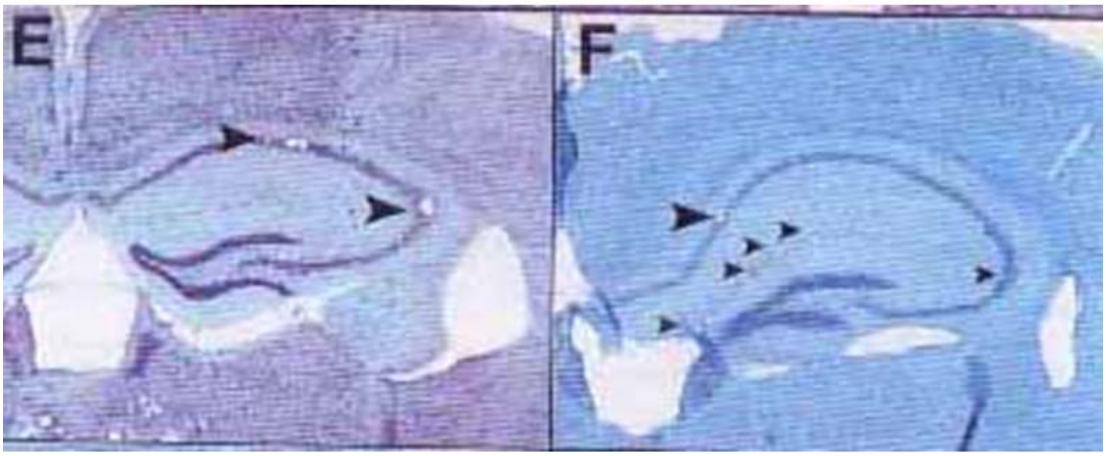
Who is likely to Respond

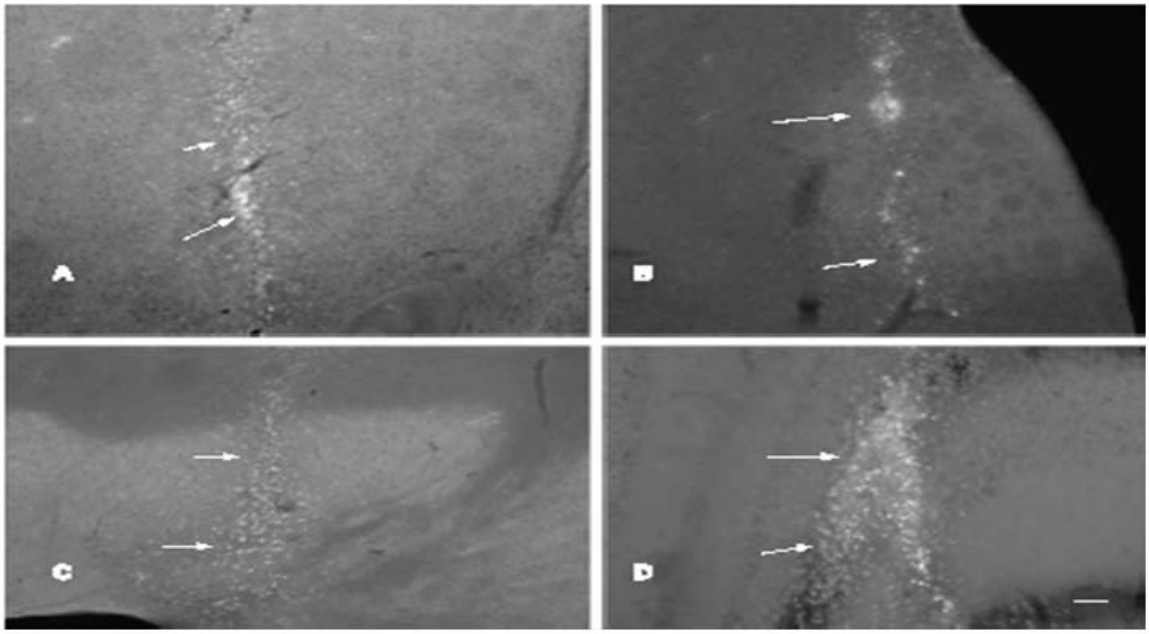
Mild forms of Canavan Disease

GFP Positive Fibers in a Canavan Mouse Treated with rAAV-ASPA

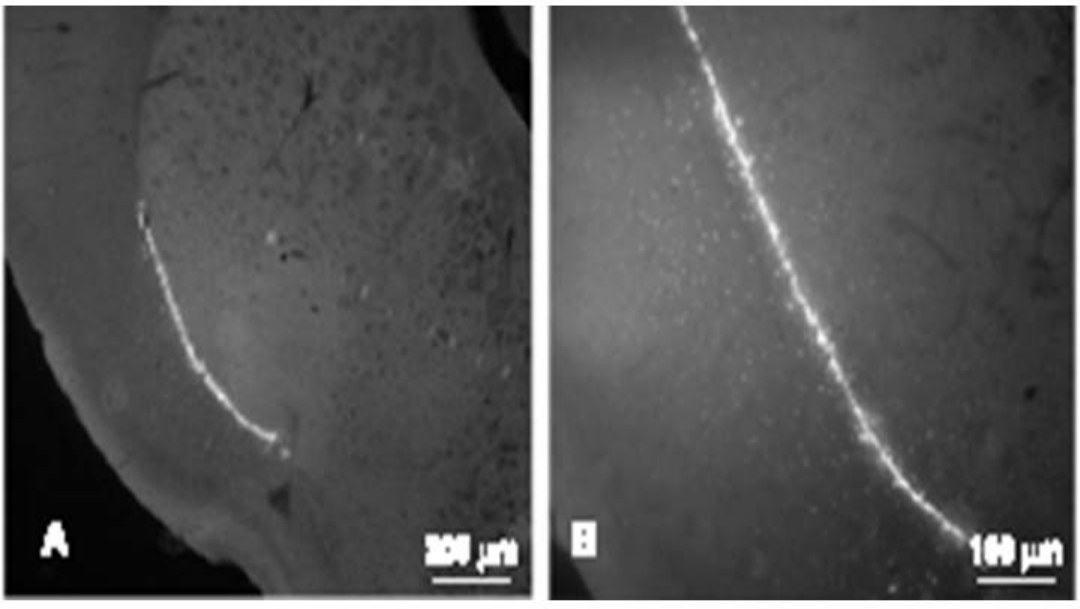



G
H

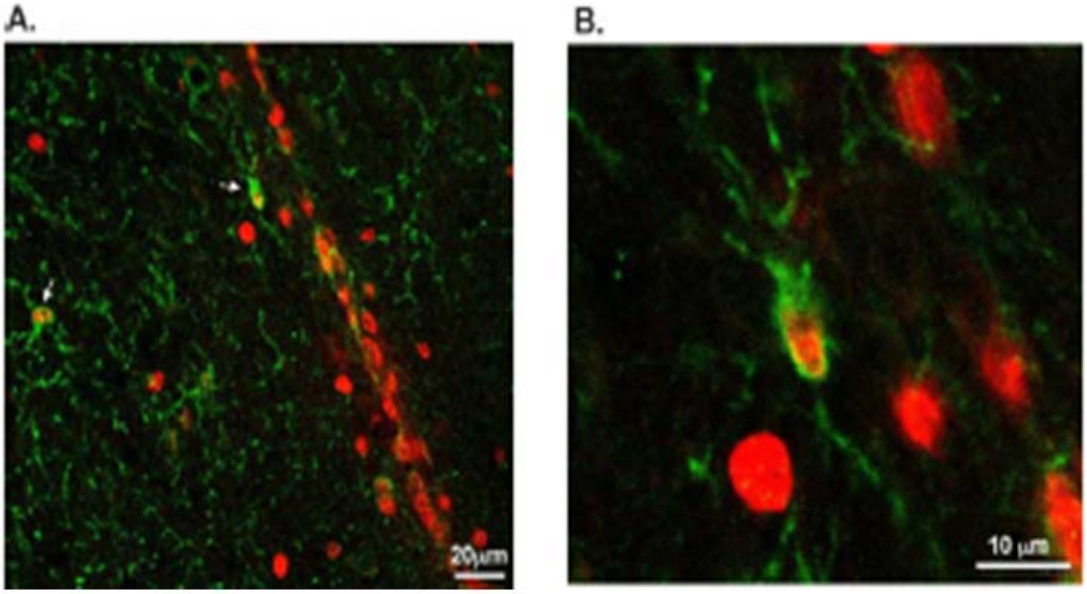

E
F



Neural progenitor cells transplantation to the brain of juvenile CD mouse BrdU positive neural progenitor cells (arrow) in the forebrain of (A) wild type and (B) CD mouse. Neural progenitor cells in the cerebellum of (C) wild type and (D) CD mouse. Interestingly, implanted cells migrated from the injected site in the CD mouse both in wild type and CD mice (bar=50μm).



Neural progenitor cells lining up towards the white matter of the CD mouse brain. (A) BrdU positive cells seemed to favor the white matter tracts. (B) The transplanted cells migrated from the white matter tracts into the neighboring cortical matter.



Transplanted neural progenitor cells differentiate into oligodendrocyte progenitor cells in the CD mouse brain. Neural progenitor cells stained with oligodendrocyte progenitor marker, NG2, showed positively stained cells. A and B show different magnifications.

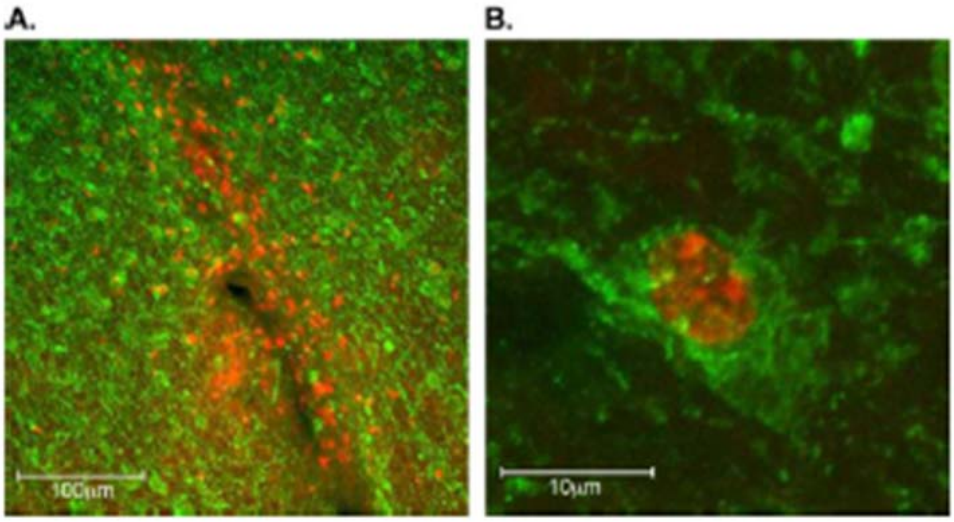
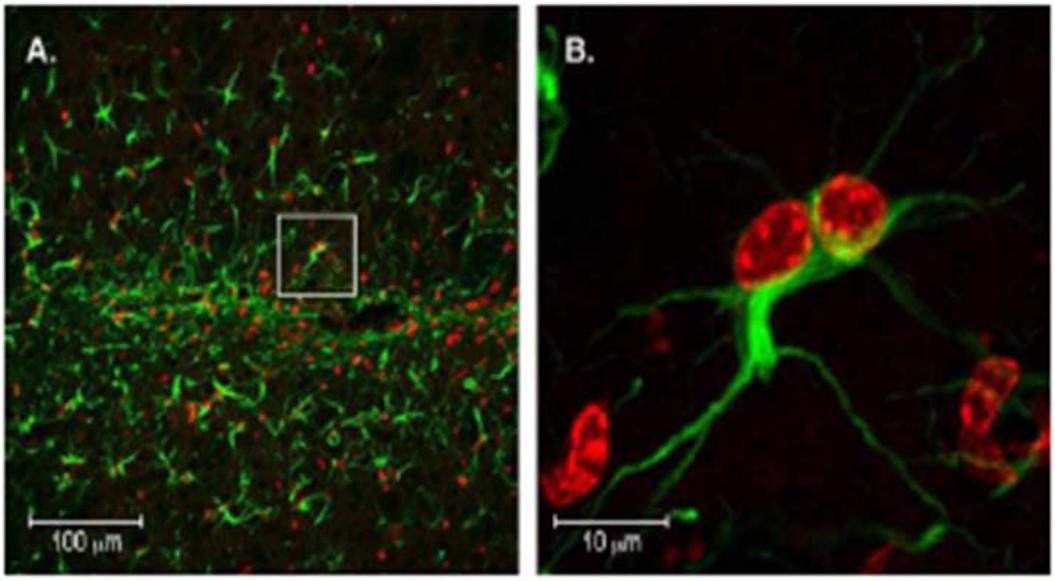
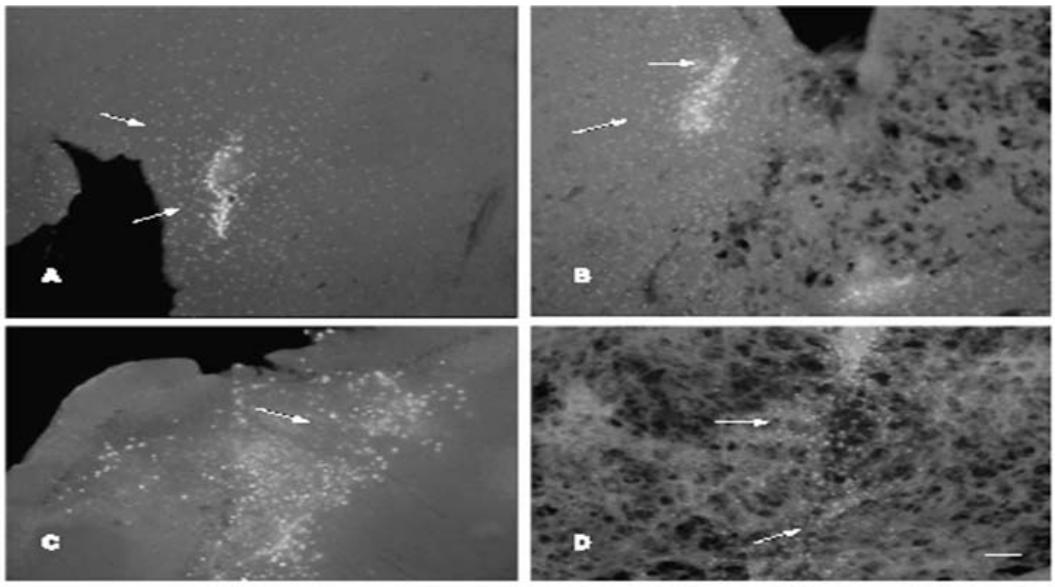


Image indicted CNPase (green) and BRDU (red) immunoreactive transplanted cells in the striatum of the CD mouse at different magnifications. Scale bars, (A) 100 μm; (B) 10 μm.



Implanted neural progenitor cells differentiate into astrocytes in the striaum of the CD mouse. (A) Staining with GFAP showed astrocyte positive neural progenitor cells. Image indicates GFAP (green) and BrdU (red) immunoreactive transplanted cells in the striatum. The rectangle enclosed area is shown at higher magnification in B.



Neural progenitor cell transplantation to the brain of adult CD mouse BrdU positive neural progenitor cells (arrow) in the forebrain of (A) wild type (B) CD mouse. Neural progenitor cells (arrow) in the cerebellum of (C) wild type and (D) CD mouse (bar=50 µm.

Conclusion I

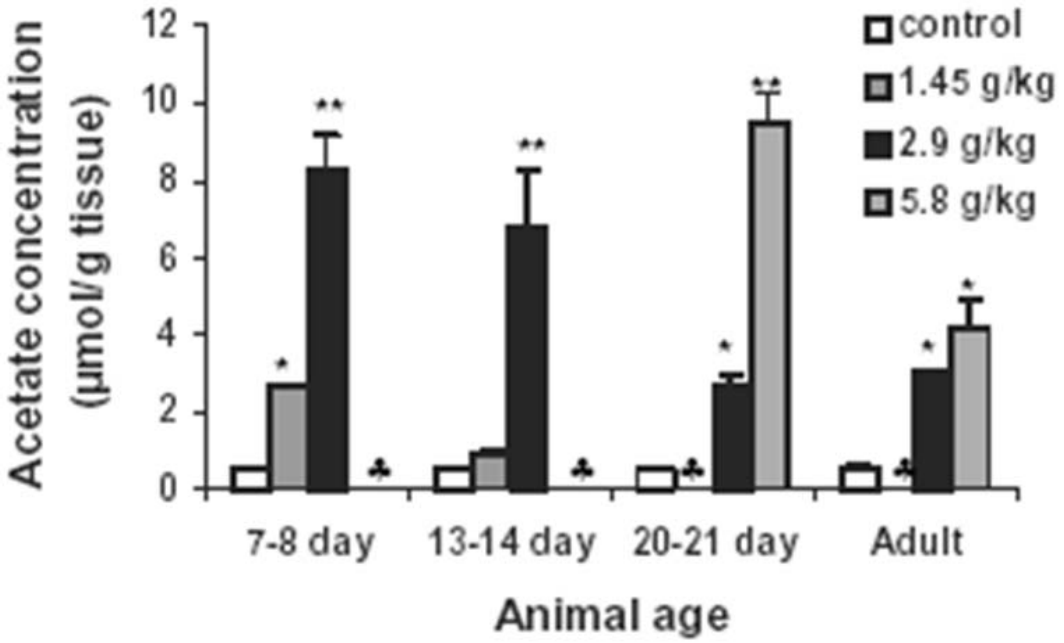
- **A knockout mouse for Canavan disease has been engineered.**
- **The mouse has a phenotype with neurological impairment.**
- **Biochemically and histologically has the characteristics of Human Canavan Disease.**
- **Elevated NAA seen in the mouse is not due to abnormal NAAG**
- **rAAV – ASPA treatment shows limited improvement in preventing or reversing spongy degeneration.**

Conclusion II

- **Enzyme expression is detected after 6th month of treatment.**
- **Type of cell induction that reverses the effect of the disease is yet to be determined.**
- **Dissemination of the vector to other parts of the brain need to be improved.**

Glycerol Triacetate

- Potential Beneficiaries
  - Non Canavan
  - N-acetylaspartic aciduria



Brain

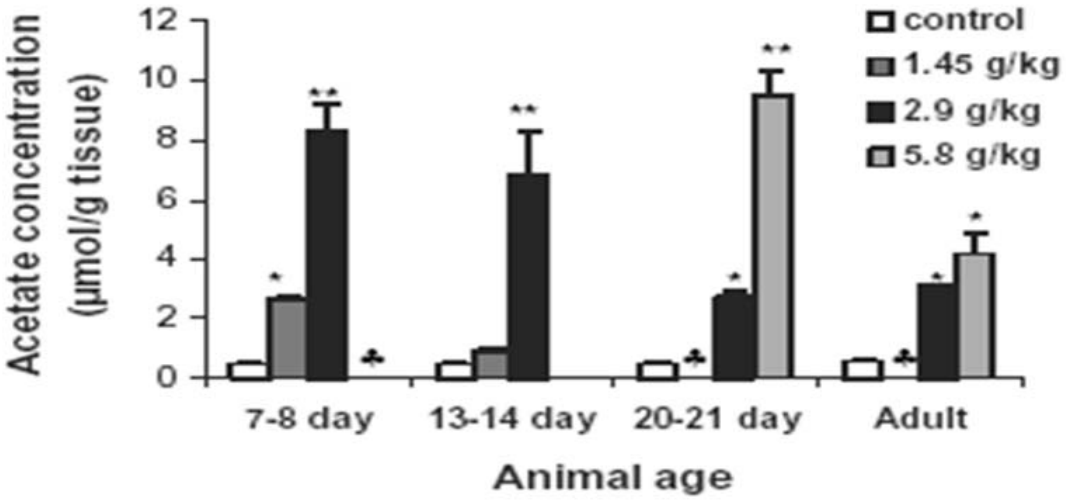
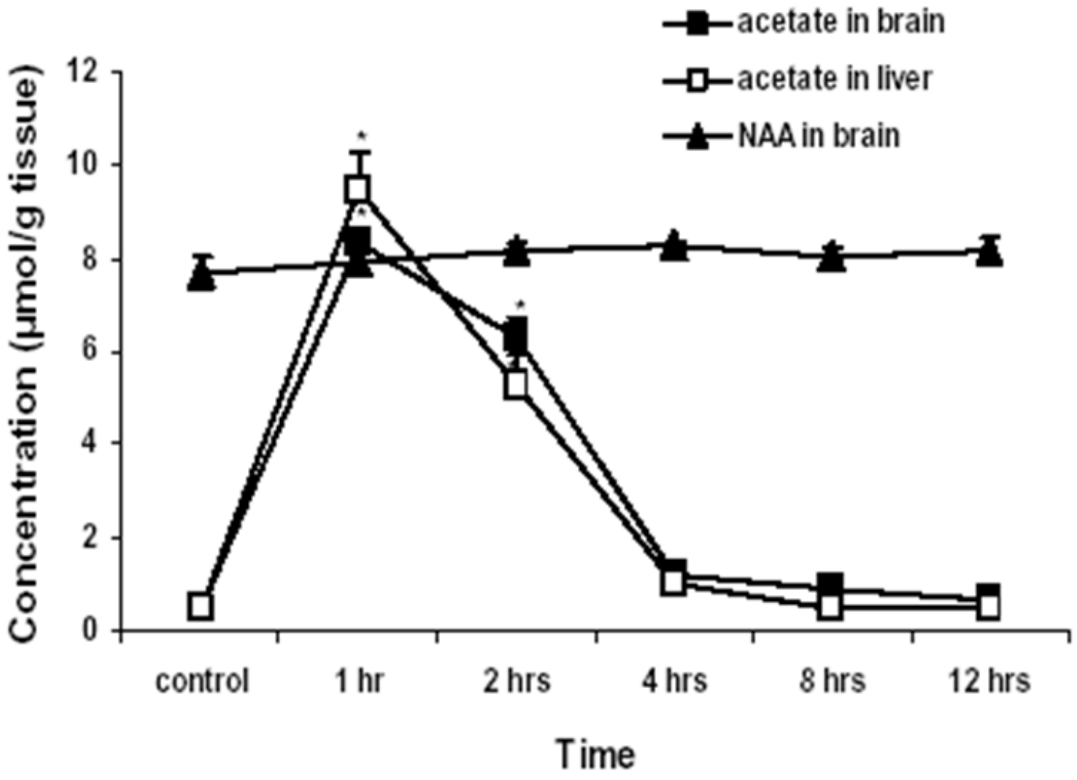


Figure 5

Liver



Mild form of Canavan Disease N-acetylaspartic aciduria

Mild Elevation of N-Acetylaspartic Acid and Macrocephaly: Diagnostic Problem, Sankar Surendran,Fiona J. Bamforth, Alicia Chan, Stephen K. Tyring, Stephen I. Goodman, Reuben Matalon **J Child Neurol 2003; 18:309-812**.

16 Year-old Caucasian male patient from western Canada had mild developmental delay

1. The white matter appeared to be normal
2. Magnetic resonance imaging of the brain showed increased signal intensity in the basal ganglia bilaterally

16 Year old Boy

Neonatal period unremarkable

Congenital vertical slow nystagmus 18 months

Possible concerns with development

Diagnosed with severe retinitis pigmentosa age11 years

16 Year old Boy (cont'd)

MRI of head : striking symmetric increased signal intensity within the region of the head of the caudate and anterior portion of the lentiform nucleus

Mutational analysis of NARP and MELAS negative

The white matter – normal

NAA mildly elevated

The Mutation Analysis

- Deletion -2A and -3C at the acceptor side of exon 3
- Y288c (863 a→G) in exon 6 considered polymorphic

Two Sisters, 2 and 4 ½ Years old

- Very mild delays
- Mild elevation of NAA in urine
- 957 mg/g creatinine in younger child
  - No macrocephaly
  - 471 mg/g creatinin in older child
  - No macrocephaly
- Suggested referral to Dr. Kolodny
- Asked for MRI & MRS


Miami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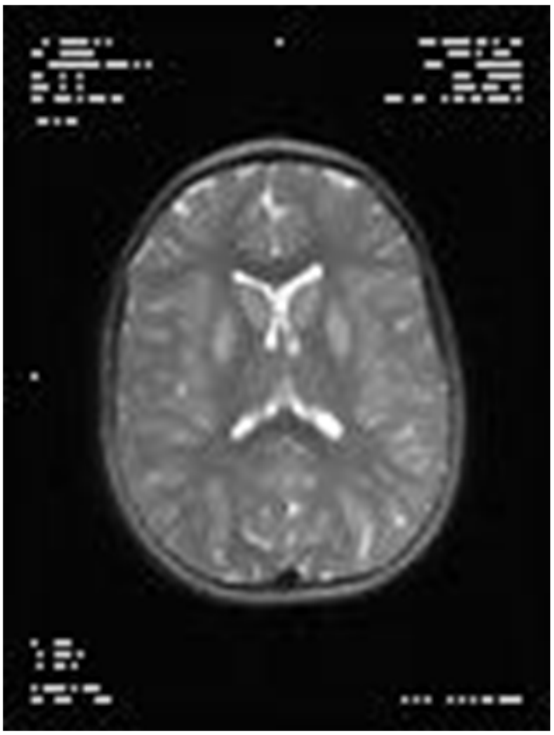

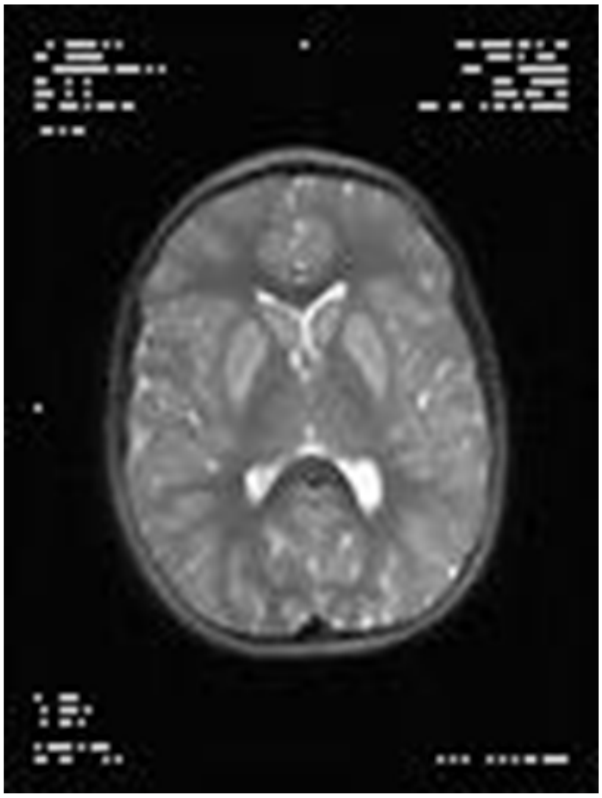

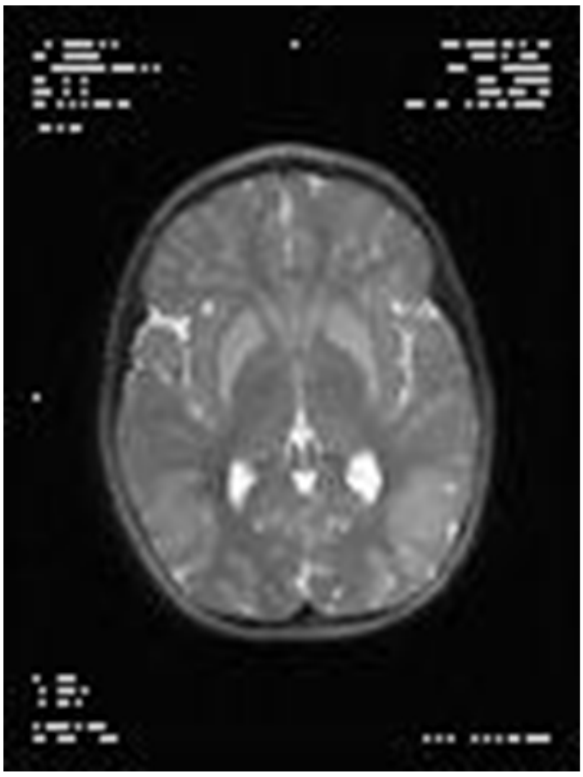

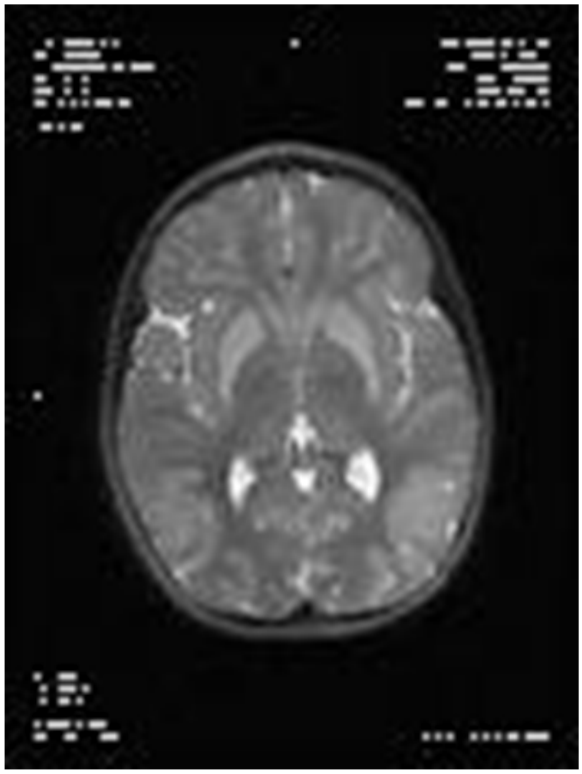

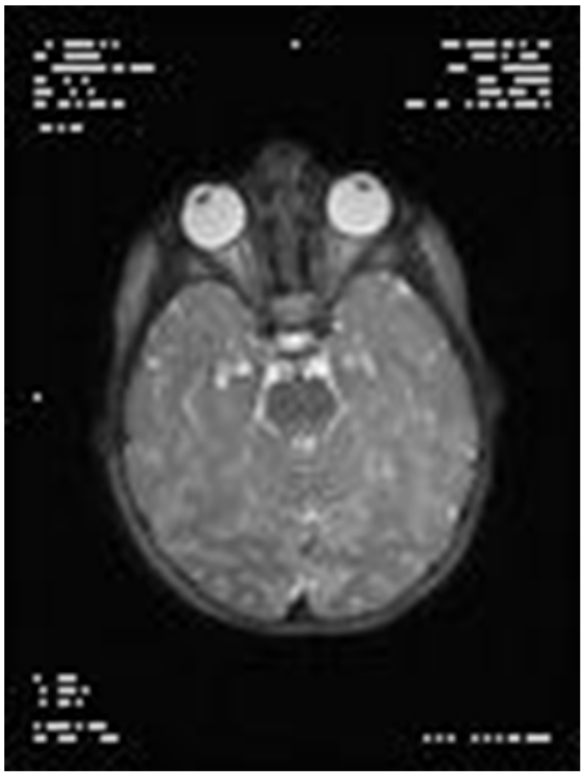

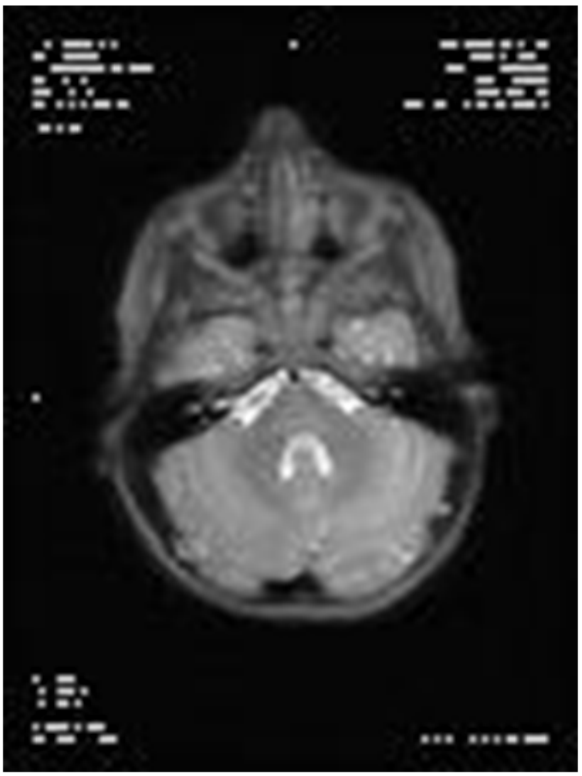

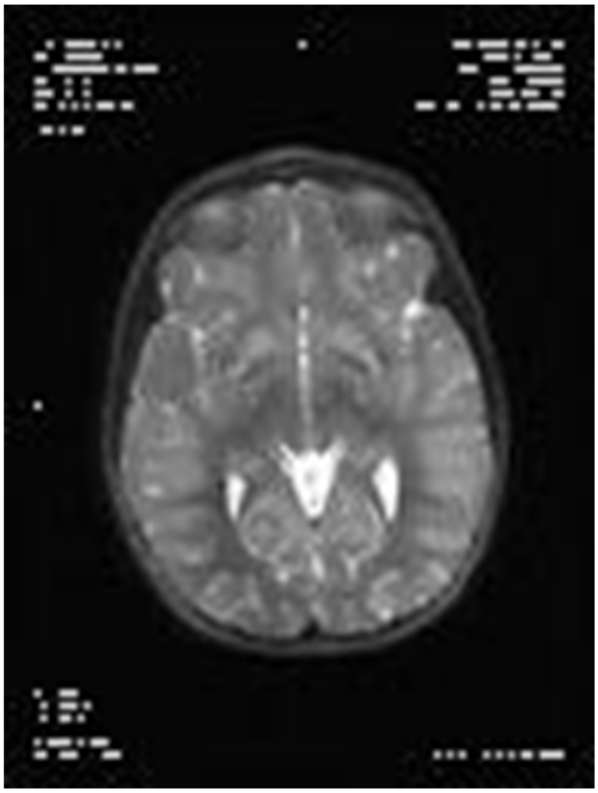

CONCLUSIONS

- There is a non Canavan n acelylaspartic aciduria
- Such defect may benefit from less drastic measures (e.g. gene therapy or stem cell therapy
- The possibility of combining gene or stem cell therapy and a form of acetate therapy may be an option for Canavan Disease
- Enzyme therapy may be also an option when blood brain barrier is overcome

Conclusion I

- **A knockout mouse for Canavan disease has been engineered.**
- **The mouse has a phenotype with neurological impairment.**
- **Biochemically and histologically has the characteristics of Human Canavan Disease.**
- **Elevated NAA seen in the mouse is not due to abnormal NAAG**
- **rAAV – ASPA treatment shows limited improvement in preventing or reversing spongy degeneration.**

Conclusion II

- **Enzyme expression is detected after 6th month of treatment.**
- **Type of cell induction that reverses the effect of the disease is yet to be determined.**
- **Dissemination of the vector to other parts of the brain need to be improved.**
- **New vectors have been developed, AAV8, 9, 10 that cross the blood brain barrier (BBB) when injected IV.**
- **These vectors disseminate readily in brain tissue and can restore the activity of ASPA.**
- **Experiments are now being conducted with these vectors.**

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