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Trainee Clinical Scientist Project
ACC/CMGS Spring Conference 2011

Implementation of a Molecular Genetic Diagnostic Test for Niemann-Pick Type C



Background

- Enhanced Genetic Services project
- Reduce neonatal mortality & morbidity in the Birmingham region
- Diagnostic testing for rare recessive diseases selected by Clinical Genetics
- Gaucher's, Pompe disease, citrin deficiency and

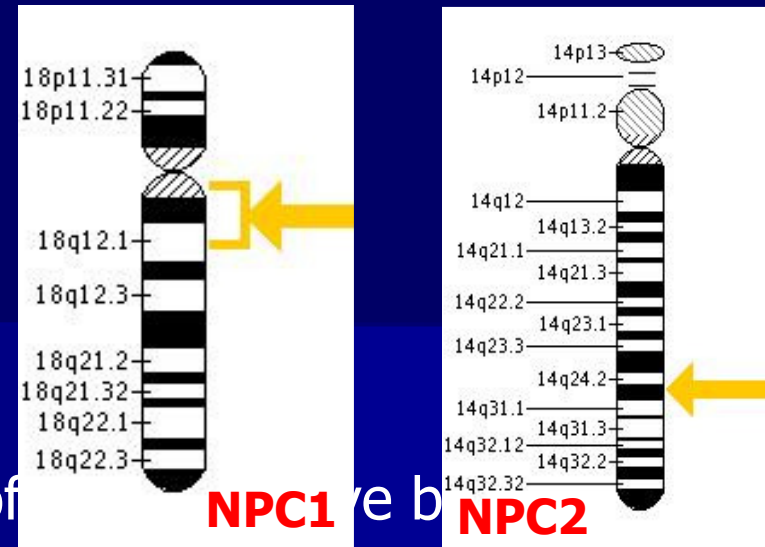
Niemann-Pick Type C

Niemann-Pick Type C (NPC)

- Neurovisceral lysosomal lipid storage disorder
- Heterogeneous presentation: symptoms can include vertical gaze palsy & liver problems
- Progressive neurological problems become severe & limiting
- Average age of death \approx 16yrs but half of patients die before 12.5yrs
- Rare cases of much longer survival
- Disease progression & life expectancy primarily dependent on the age at onset of neurological symptoms
- No cure - Miglustat

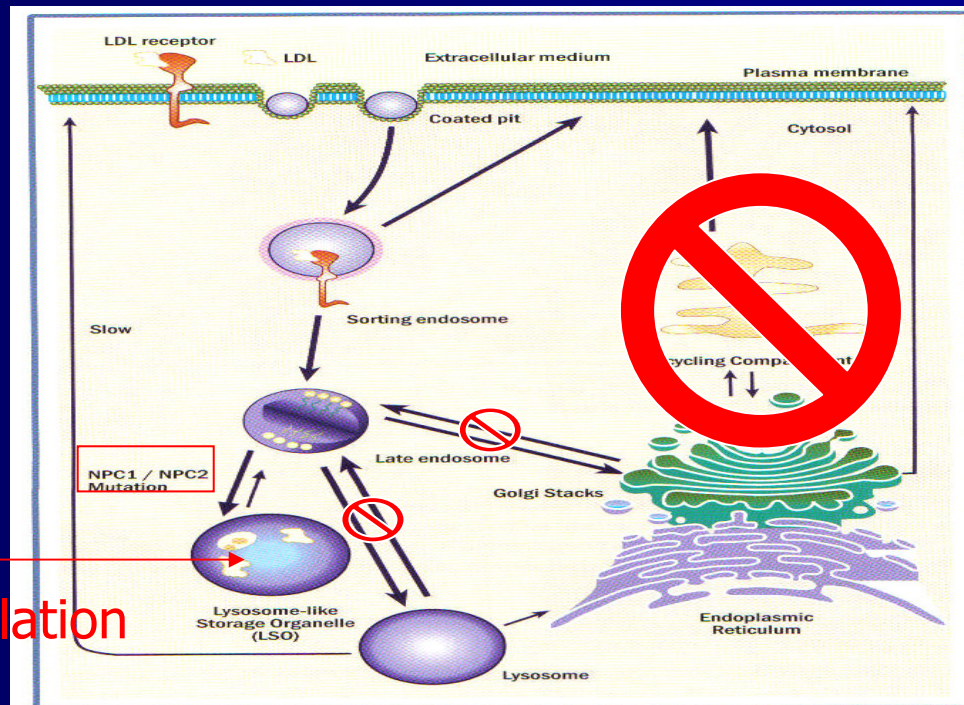
Etiology

- Panethnic
- Estimated minimal incidence of 1 in 1000
- \approx 95% of patients have mutations in NPC1 (18q11) which encodes a large membrane glycoprotein
- \approx 4% of patients have mutations in NPC2 (14q24.3) which encodes a small soluble lysosomal protein that binds cholesterol with high affinity
- Remainder of patients are biochemically proven cases for whom mutations have not been identified



Pathogenesis

- Cellular cholesterol & glycolipid trafficking
- **Non-function** Function sequentially in the same pathway
- Precise functions unknown



Lipid accumulation

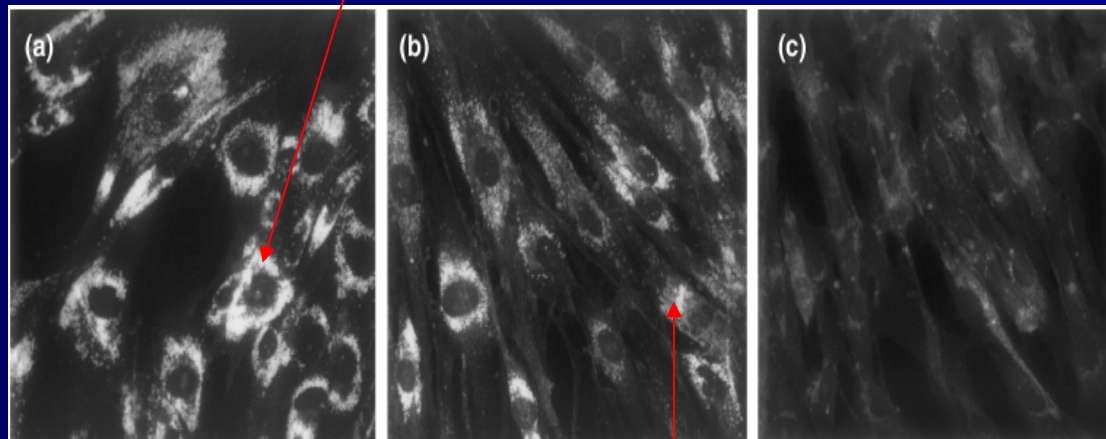
- Cholesterol processing disrupted
- Accumulation of lipids
- Structural & functional damage in cells & tissues

Diagnosis - Biochemical

- Impaired intracellular trafficking of lipids
- Primary diagnostic tests measure transport & storage of cholesterol by fibroblasts
- Impaired transport determined by measuring esterification
- Impaired storage determined through filipin staining of cultured fibroblasts

Diagnosis – Filipin Staining

- Filipin binds to LDL-cholesterol, sequestered in lysosomes around the cell nucleus, to form a fluorescent complex



(a) Classic NPC
Intense staining

(b) Variant NPC

(c) Normal

- 15% of patients have intermediate or 'variant' levels of cholesterol esterification (30-80% of normal) & a less distinctive staining pattern

Diagnosis - Genetic

- Confirmation of biochemical results, including 'variant' results
- Prenatal diagnosis: risk of error with biochemical tests
- Identification of carriers
- Treatment options based on genotype
- Early diagnosis before irreversible neurological lesions form could be essential as prospective therapies develop

Molecular Genetic Testing of *NPC1* & *NPC2*

- Existing testing for both mutations is essential for sequential testing and any subsequent cascade testing
- *NPC1* (78/138) of patients had a 2nd mutation 83% (115/138) of all patients
- 30% (23/78) patients with a 1st mutation have a 2nd mutation
- To identify both mutations of 13% of patients (23/138)

Genotype			
	Common Mutation/Common Mutation	Common Mutation/Other Mutation	Total
I1061T	8	47	55
P1007A	2	1	3
G992W	6	3	9
E20X	7	3	10
G992R	0	1	1
Total	23 = 30% (23/78)	55 = 70% (55/78)	78

NPC Screening Strategy at WMRGL

- **Sequence both *NPC1* and/or *NPC2* for small scale mutations**

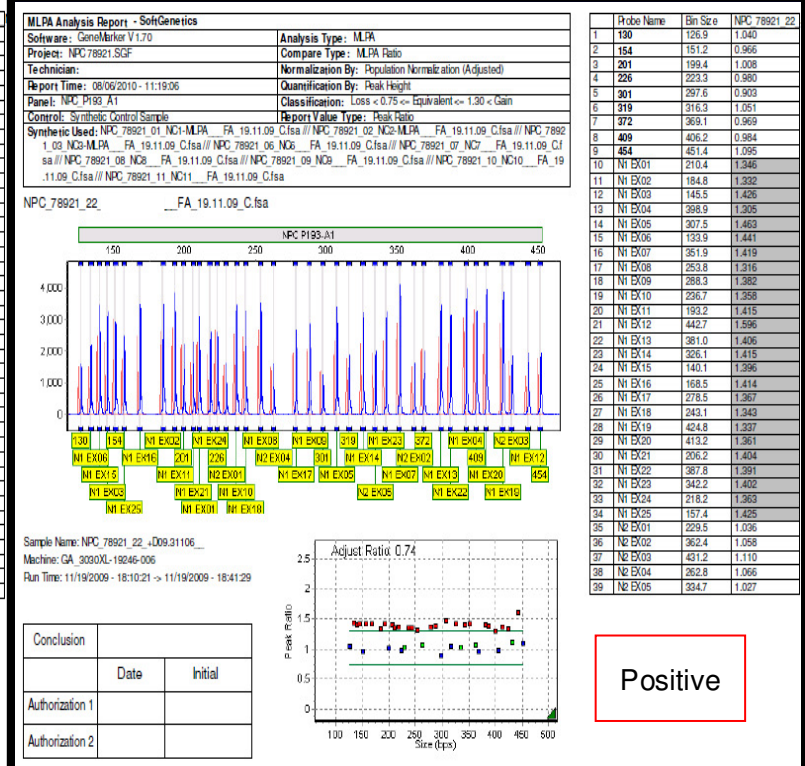
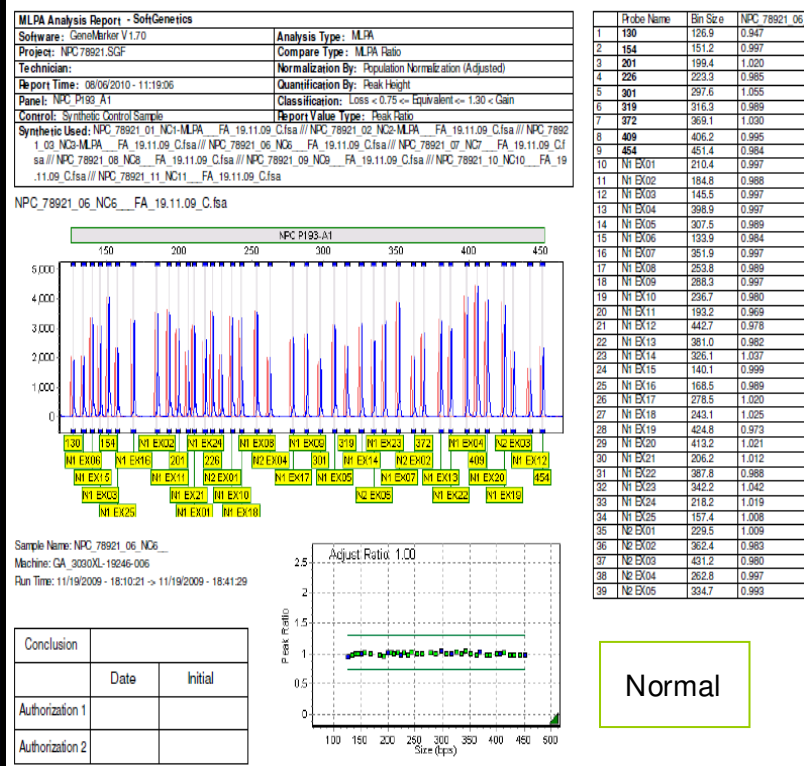
Plus

- **MLPA to detect large scale duplications/deletions**
- Difficult to estimate their frequency
- Small no. but worth considering in biochemically proven NPC patients with 0 or only 1 mutation identified
- Homozygous cases

Sequencing

- Primers for all exons amplify products within the optimal range ($\approx 250-750\text{bp}$) for sequencing
- Touchdown PCR
- Good quality sequencing for all exons
- Validation
 - 6 normal controls sequenced
 - Concordance with results achieved previously in a research setting

MLPA (P193-A1)



Normal control probe ratios are ≈ 1 . Positive control (trisomy chromosome 18) probe ratios for *NPC1* exons 1-25 are >1.3 = heterozygous duplication. A deletion = probe ratios of <0.7 .

Results

- 66 referrals
- 1 prenatal, 10 carrier tests , 2 confirmation & 34 for diagnosis
- 28/34 had 0 mutations
- 5/34 had 1 or more pathogenic mutations
- 1/34 had a homozygous UV
- \approx 15% pick up rate for NPC1/2 mutations
- 0 large scale duplications or deletions

NPC Mutations Identified

- Patient 1 = 3 pathogenic NPC1 mutations (case 1)
- Patient 2 = 1 pathogenic mutation & 1 UV in NPC1
- Patient 3 = 1 homozygous NPC2 UV (RNA studies in progress)
- Patients 4-6 = 1 pathogenic NPC2 mutation (case 2)

Patient	Mutation / Variant Identified						Comment
	Gene	Exon/Intron	NT Change	Protein Change	Type	State	
1	NPC1	Exon 18	c.2621A>T	p.Asp874Val	M	Het	1 of 3 pathogenic mutations identified
	NPC1	Exon 19	c.2882A>G	p.Asn961Ser	M	Het	
	NPC1	Exon24	c.3746-3749delGTTA	p.Ser1249ThrfsX2	F	Het	
2	NPC1	Intron 23	c.3591+4delA	N/A	S	Het	1 Pathogenic & 1 UV identified
	NPC1	Exon 24	c.3620T>C	Phe1207Ser	UV	Het	
3	NPC2	Intron 4	c.442-4A>C	N/A	UV	Hom	RNA Studies
4	NPC2	Intron 3	c.441+1G>A	N/A	S	Het	Only 1 mutation identified
5	NPC2	Intron 3	c.441+1G>A	N/A	S	Het	Only 1 mutation identified
6	NPC2	Intron 3	c.441+1G>A	N/A	S	Het	Only 1 mutation identified

Interesting Case 1

- <1yr old with jaundice & hepatosplenomegaly
- 3 heterozygous NPC1 mutations identified

Gene	Exon Intron	NT Change	Protein Change	Type	State
NPC1	18	c.2621A>T	p.Asp874Val	M	Het
	19	c.2882A>G	p.Asn961Ser	M	Het
	24	c.3746-3749delGTTA	p.Ser1249ThrfsX2	F	Het

- Missense mutations listed on the NPC-db & in the literature as pathogenic
- The frameshift mutation considered pathogenic

Interesting Case 1

- Diagnosis of NPC confirmed
- Mother confirmed as carrier of exon 18 mutation
- Father heterozygous for mutations in exons 19 & 24

Gene	Exon Intron	NT Change	Protein Change	Type	State
NPC1	18	c.2621A>T	p.Asp874Val	M	Het
	19	c.2882A>G	p.Asn961Ser	M	Het
	24	c.3746-3749delGTTA	p.Ser1249ThrfsX2	F	Het

- Since frameshift mutations considered severe could suggest mutation in exon 19 is moderate or non-pathogenic

Interesting Case 1

- **Classification**

- *1) all truncating mutations are severe*
- *2) severe phenotype = both alleles have severe mutations*
- *3) only 1 moderate mutation needed to confer a moderate phenotype*
- *4) mild phenotype in association with 1 moderate mutation means that 2nd mutation also moderate*

- Accordingly, this mutation, in conjunction with c.1997G>A (S666N), was reported in association with adult onset NPC **without** neurological symptoms
- Indicating that c.2882A>G & c.1997G>A are mild mutations

Interesting Case 2

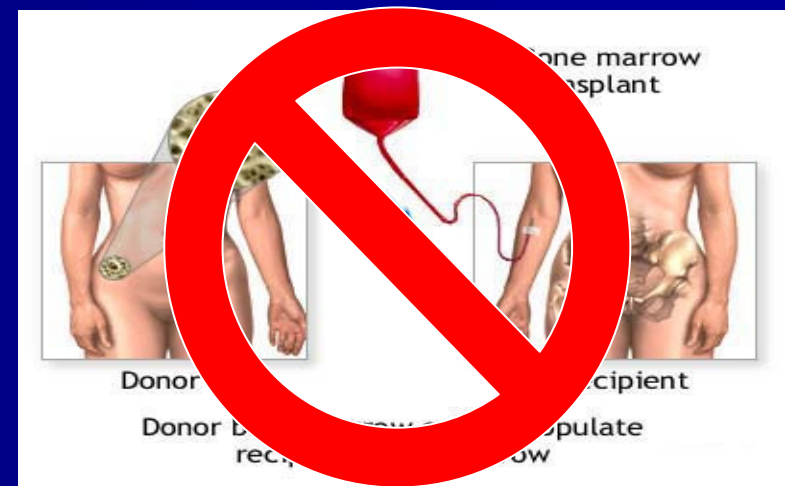
- <2yr old with clinical features of NPC (liver problems)
- 1 heterozygous splicing mutation identified

Gene	Exon Intron	NT Change	Protein Change	Type	State
NPC2	Intron 3	c.441+1G>A	N/A	S	Het

- Not listed on database or in the literature
- +1 is a highly conserved residue needed for correct splicing
- Considered to be pathogenic
- Pathogenicity confirmed by RNA studies

Interesting Case 2

- Clinical diagnosis not confirmed but more likely
- Bone marrow transplant (BMT)
- Used to treat other storage diseases e.g. Gaucher's
- No evidence that NPC1 is transducible between cells
- NPC2 is secreted & recaptured
- Engrafted donor blood cells secrete deficient protein which can be recaptured by protein deficient cells



Interesting Case 2



- Encouraging results in mice & humans
- Blood brain barrier could prevent positive neurological result
- Microglia cells (5% of brain cells) may be derived from stem cells
- Early BMT could be more neurologically beneficial

Incomplete Genotype

- Mutations present in regions not sequenced

L.Rodríguez-Pascau, M.J.Coll *et al* (2009) *Human Mutation* 30:E993-E1001

- novel mutation in intron 9 of NPC1 (c.1554-1009G>A)
 - creates a cryptic donor splice site
 - 194bp of intron 9 incorporated premature termination codon & NMD
 - Next generation sequencing
- Dominant negative effect
 - An unidentified NPC loci may exist
 - Diagnosis incorrect & identification of mutation in NPC1 or 2 a rare coincidence

Conclusions/Future



- Next generation sequencing – Roche Junior
- Actelion (Miglustat) funded project
 - Genotype/phenotype correlations
 - avoid unnecessary treatments through rapid diagnosis
- NPC on the BrumChip

Design and validation of a metabolic disorder resequencing microarray (BRUM1). Hum Mutat 31:1–8, 2010

- Microarray sequencing chip
- Dept of Inherited Metabolic Disorders
- Large no. of metabolic genes screened simultaneously
- Quicker diagnosis



Acknowledgements

- WMRGL Laboratory
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- Birmingham Children's Hospital
Paul Gissen & other members of the Department of Inherited Metabolic Disorders

Acknowledgements



Welcome to The Niemann-Pick Disease Group (UK)

Making a positive difference to the lives of those affected by Niemann-Pick Diseases



- <http://www.niemannpick.org.uk>

Leah's story was featured on ITV1 on 1st March. It can be viewed using ITV i-player