Clinical and Molecular Spectrum of Patients with Infantile-Onset Pompe Disease in Malaysia

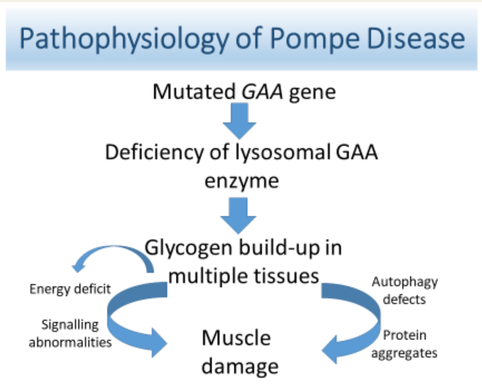
Chan Mei Yan[1], Julaina Abdul Jalil[2], Ernie Zuraida Ali[4], Mohd Khairul Nizam Mohd Khalid[2], Yusnita Yakob[2], Siti Aishah Abdul Wahab[2], Jeya Bawani Sivabalakrishnan[3]
Chew Hui Bein[1], Leong Huey Yin[1], Edward Eu[1], Ngu Lock Hock[1]

[1]*Department of Genetics, Hospital Kuala Lumpur*
[2]*Institute for Medical Research Kuala Lumpur*
[3]*Department of Pediatric Cardiology, Hospital Tunku Azizah Kuala Lumpur*
[4]*Unit of Inborn Error of Metabolism and Genetic, Nutrition, Metabolism and Cardiovascular Research Centre, Institute for Medical Research, National Institutes of Health, Selangor.*

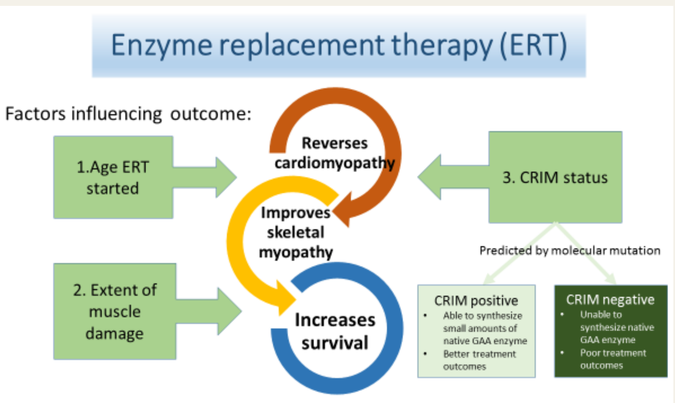
Introduction

Pompe disease is a rare autosomal recessive disorder caused by mutations in the *GAA* gene, leading to deficiency of the enzyme alpha-glucosidase (GAA).

This leads to glycogen deposition in multiple tissues, particularly cardiac and skeletal muscle, causing muscle damage.



Infantile-onset Pompe disease (IOPD) patients present within the first year of life with rapidly progressive cardiomyopathy and profound hypotonia. Untreated patients often die before the age of 2 years from cardiorespiratory failure.[1]



Treatment with enzyme replacement therapy (ERT) alglucosidase alpha ameliorates cardiomyopathy, improves motor function and increases survival.

•Early treatment with ERT prior to the onset of irreversible muscle damage leads to better patient outcomes.[2]

•However response to ERT is variable, with suboptimal outcomes in some patients even when started early.

•Cross-reactive immunological material (CRIM) negative patients have high titres of recombinant human GAA antibodies and respond poorly to treatment.

The *GAA* gene encodes for the lysosomal GAA enzyme. It is located at chromosome 17q25.3, and contains 20 exons. To date, 648 disease causing variants have been identified and are listed in the Pompe disease variant database.[3] The molecular spectrum of Malaysian IOPD patients has never been studied.

The aim of this study is to analyze the genotype, phenotype and long term treatment outcomes of IOPD patients in Malaysia.

Methods

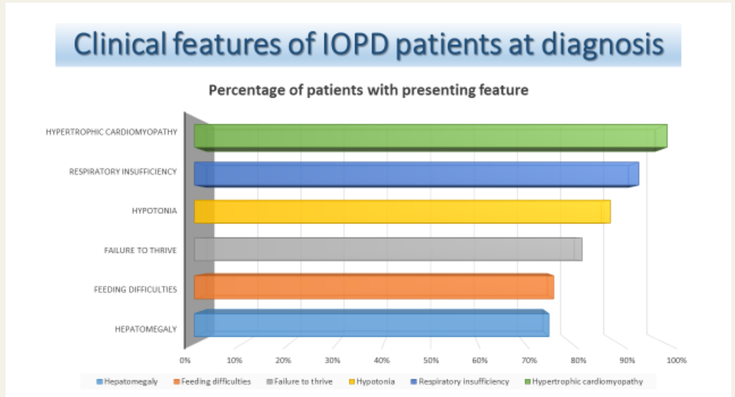
All patients diagnosed with IOPD between 2000 and 2020 were included in this national multicenter, retrospective study. This study was approved by the Malaysian Research and Ethics Committee.

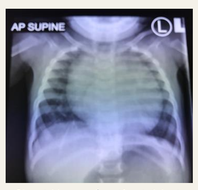
- Clinical and biochemical data were obtained from patients' medical records and analyzed.
- GAA enzyme levels were performed on dried blood spots with and without acarbose inhibition.[4]
- Molecular analysis of the *GAA* gene was performed by polymerase chain reaction and Sanger sequencing.
- CRIM status and severity of mutation was predicted according to the Erasmus Pompe database.[3]
- Structural modeling was performed to illustrate the effect of novel mutations on protein structure.[5]

Results

Clinical features:
17 patients were diagnosed with IOPD between 2000 and 2020, consisting of 11 females (65%) and 6 males (35%). 76% were of Chinese ethnicity.

- Median age of presentation: 3 months
- Median age of diagnosis: 6 months
- Median age of ERT initiation: 7 months





Chest X-ray of patient 14 at presentation showing a grossly enlarged, globular heart.

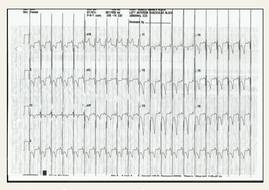
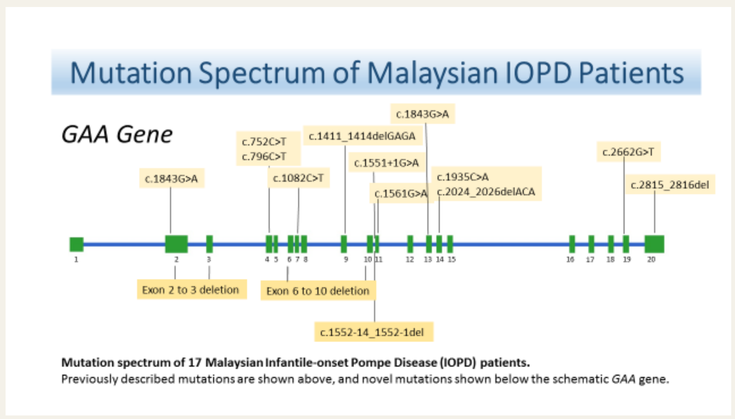

ECG of patient 14 showing giant QRS complexes and short PR interval


Photo of IOPD patient showing respiratory insufficiency

GAA enzyme activity:
All measured GAA enzyme activity levels were below the normal range

- Mean net GAA level: 1.03 nmol/punch/hour (Normal range >15nmol/punch/hour)

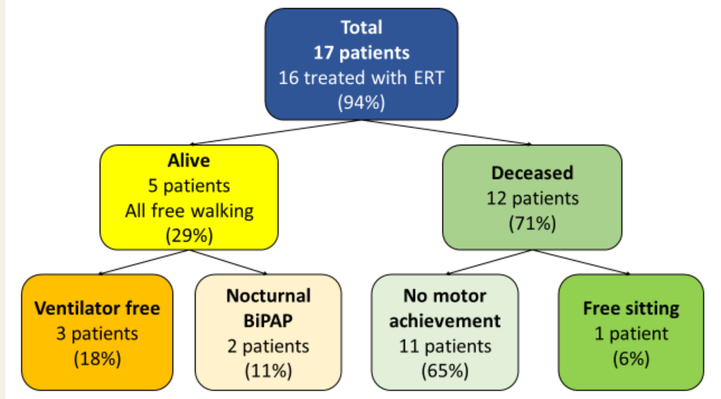
Molecular features:
The most common mutation identified was c.1935C>A, occuring in seven patients (10 alleles) with allele frequency of 33%.



Mutation spectrum of 17 Malaysian Infantile-onset Pompe Disease (IOPD) patients.
Previously described mutations are shown above, and novel mutations shown below the schematic *GAA* gene.

The mutations are well distributed across the *GAA* gene.

Long term treatment outcomes:



Pt	Ethnicity	*GAA* mutation		Predicted CRIM status	GAA enzyme level (nmol/punch/hour)	Age at presentation (months)	Age ERT started (months)	Best motor achievement	Current status	Current age / age of death
		Allele 1	Allele 2							
1	Chinese	c.1082C>T	c.2815_2816delGT	Positive	0	12	64	Walks unaided	Alive	20 years
2	Chinese	c.1082C>T	c.2815_2816delGT	Positive	0	10	20	Walks unaided	Alive	17 years
3	Chinese	c.796C>T	Exons 2-3 deletion	Positive	1.2%	3	7	None	Deceased	3.5 years
4	Indigenous	NA	NA	Unknown	0.05	5	11	None	Deceased	1 year
5	Chinese	c.2024_2026delACA	c.1935C>A	Positive	NA	4	7	None	Deceased	8 months
6	Chinese	c.1843G>A	c.1935C>A	Positive	NA	6	9	None	Deceased	10 months
7	Chinese	c.1411_1414delGAGA	c.1935C>A	Positive	<0.1	3	7	None	Deceased	2 years
8	Chinese	NA	NA	Unknown	0.47	3	4	Walked with support	Deceased	1 year 11 months
9	Chinese	c.1411_1414delGAGA	c.1935C>A	Positive	NA	3	7	None	Deceased	3 years
10	Chinese	c.1843G>A	c.2815_2816delGT	Positive	1.58	2	Declined ERT	None	Deceased	6 months
11	Indian	c.1551+1G>A	c.1561G>A	Positive	2.06	3	7	Turned supine to prone	Deceased	11 months
12	Chinese	c.2024_2026delACA	c.1935C>A	Positive	2.09	2	2	Walks unaided	Alive	5 years 10 months
13	Chinese	c.1935C>A	c.1935C>A	Positive	0.95	4	8	None	Deceased	2 years
14	Indian	c.1A>G	Exons 6-10 deletion	Unknown	1.29	6	7	Walks unaided	Alive	4 years 1 month
15	Chinese	c.2662G>T	c.1935C>A	Positive	0.07	4	6	None	Deceased	7 months
16	Chinese	c.1552-14_1552-11del	c.2662G>T	Unknown	0.24	1	3	Walks unaided	Alive	3 years 5 months
17	Malay	c.1935C>A	c.1935C>A	Positive	0.55	2	2.5	None	Deceased	6 months

Table : Clinical, enzymatic and molecular features of Malaysian IOPD patients with treatment outcomes
Surviving patients

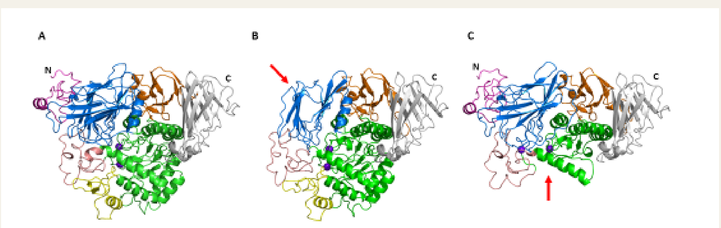
Discussion

Molecular features:
Common mutations:

- The c.1935C>A mutation has also been reported to be the most common mutation in Southern China (25%), Taiwan (36%) and Thailand (32.4%).

Novel mutations:

- c.1552-14_1552-1del: This nucleotide substitution is predicted to disrupt the consensus splice site and cause aberrant splicing and subsequent loss of function.
- Exons 2-3 deletion and Exons 6-10 deletion: The predicted effect of these large deletions by structural analysis are shown in the computational structural model below:

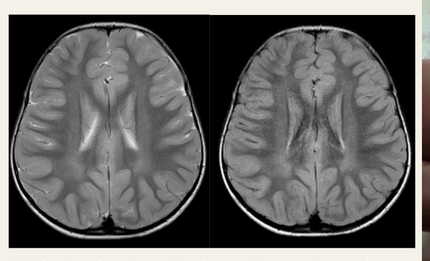


Structure of wild type and mutant models of GAA exon 2-3 and exon 6-10 deleted transcript variant with colored domains.
A) Structure of WT GAA. B) Structural model of ex2-3del GAA. Deletion of exon 2-3 results in loss of trefoil type-P domain and also missing some parts of the N-terminal β-sheet domain (indicated by a red arrow). **C) Structural model of ex6-10del GAA.** Deletion of exon 6-10 results in missing somes parts of the N-terminal β-sheet domain and the catalytic GH31 (β/α)8 barrel domain, including insert I and some parts of insert II (indicated by a red arrow). Cartoon representation of structure of GAA consists of the trefoil type-P domain (residues 81-136) (light magenta), the N-terminal β-sheet domain (residues 137-358) (marine), the catalytic GH31 (β/α)8 barrel domain (residues 359-720) (green) consisted of insert I (residues 444-492) (yellow) and insert II (residues 522-572) (salmon), the proximal (residues 721-820) (orange) and distal β-sheet (residues 821-952) (grey) domains. Catalytic residues (purple) is depicted as sphere. N, N terminus; C, C terminus.

Clinical features and long term treatment outcome:

- Our patients have a later median age of presentation (3 months) and diagnosis (6 months) compared to those in previous studies.
- This accounts for our survival rate of 29% which is lower than other cohorts (40% to 60%).
  - 71% of our patients were in established heart failure at presentation.
  - 82% of the deceased patients were started on ERT at the age of 6 months and above.

The long term morbidities experienced by the patients in our cohort are similar to those reported in previous studies, which include musculoskeletal, cardiac, respiratory, oropharyngeal, speech, hearing and neurocognitive dysfunction.


MRI brain of patient 14 taken at 3 years 8 months showing bilateral symmetrical T2/FLAIR hyperintense changes in periventricular white matter posteriorly.


IOPD patient treated with ERT on her first preschool day

Conclusion

- This is the first study that analyses the genotype and phenotype of IOPD patients in Malaysia, and adds to the IOPD data in the Asian region.
- This study establishes the c.1935C>A mutation as the most common mutation in Malaysian IOPD patients.
- The novel mutations identified in this study expands the mutation spectrum for IOPD.
- The survival of patients improves with early initiation of ERT.
- Early diagnosis via newborn screening for Pompe disease may achieve better survival and long term outcomes.

Acknowledgements

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