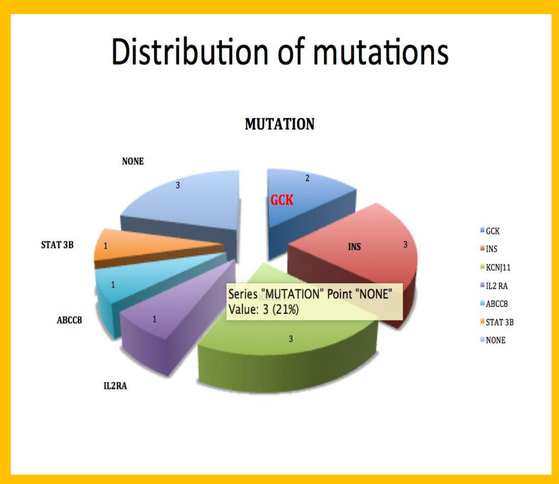
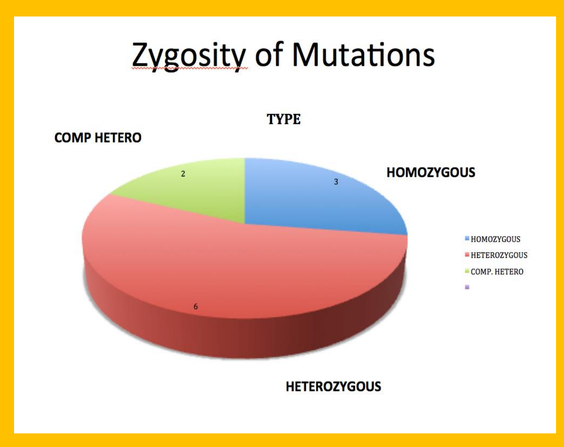
Clinical details, Molecular genetic analysis AND Clinical phenotype correlation of 14 patients with Neonatal diabetes from the South India – A Single Centre Experience

V. Sri Nagesh1, Andrew Hattersley2, Sian Ellard2, Elisa De Franco2, Sarah Flanagan2, Bipin Sethi[3], Altaf Naseem[4], Syed Tanveer Ahmed[4]
1. Consultant Endocrinologist, SriNagesh Clinic, Hyderabad 2. University of Exeter - Medical School 3. CARE Hospital, Hyderabad 4. Candy Children's Hospital, Hyderabad

Background

Neonatal diabetes typically presents within the first 6 months of life.
Often misdiagnosed as Type 1 Diabetes and on lifelong insulin therapy.
Doctors unaware of monogenic variants.
Recent studies report prevalence much higher at 1 in 90,000.
NDM prevalence is probably higher in India due to the high frequency of consanguineous marriages, especially in South India.
Few studies reported, mostly from South India.
No nationwide studies or genotype-phenotype co-relation



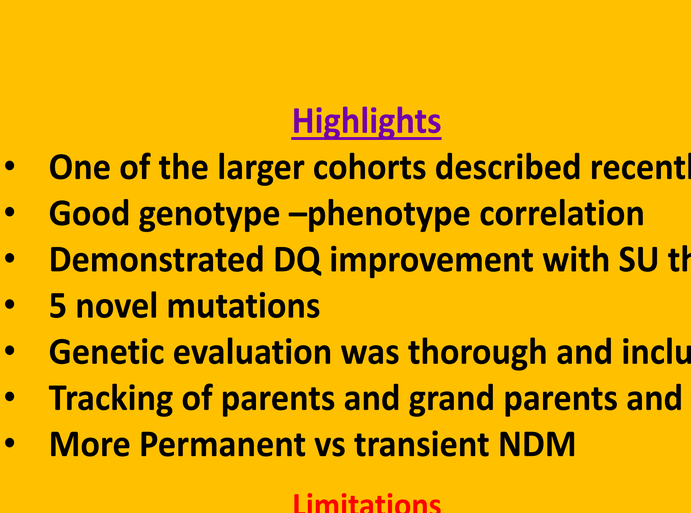


AIMS AND OBJECTIVES

- Describe the molecular genetics of a South Indian cohort of NDM patients referred to a single centre
- Correlate the clinical characteristics and follow-up picture to the genotype.
- Attempt transition to Sulphonylurea in children with ABCC8 and KCNJ11 mutations.

Materials & methods

- Patients referred with NDM between the period of Nov 2014 to April 2017 were included in the study.
- Retrospective analysis and case finding in patients who were assumed to have Type 1 diabetes mellitus and who were under follow-up, when the clinical phenotype was consistent with monogenic diabetes.
- Details of clinical presentation, birth and family history, clinical phenotype, biochemical data, imaging and management were collected using a standardised proforma.
- Study performed according to the principles of the Declaration of Helsinki with written informed consent given by the patients' parents for genetic analysis. Telephonic consent was also obtained from the parents prior to compiling information for this paper.



AGE	RBS AT PRESENTA-TION	BIRTH WT	CONSAN-GUINITY	MUTATION	TRAN-SMISSION	PARENTS/KINDRED WITH MUTATION
94	558	1750	3^0	GCK N	HM	BOTH
5	446	1400	3^0	GCK N	HM	MOTHER
72	380	2500	--	INS	HT	FATHER
2	548	1700	--	INS N	CH	BOTH
240	360	1800	3^0	INS.	HT	NONE

S.NO	AGE	RBS AT PRESENTA-TION	BIRTH WT	CONSAN-GUINITY	MUTATION	TRAN-SMISSION	DNA (protein) description	KIND	PARENTS/KINDRED WITH MUTATION	PATHO-GENE-CITY
1.	94	558	1750	3^0	GCK	HM	c.854G>T	MISSENSE	BOTH	Y
2	240	360	1800	3^0	INS.	HT	c.265C>T	MISSENSE	NONE	Y
3	58	288	2500	--	KCNJ11	HT	c.685G>A	MISSENSE	F	Y
4	2	548	1700	--	INS	CH	c.-331del/c.-331C>A	PROMOTER VARIANT	BOTH	Y
5	18	396	3100	--	IL2 RA-	HM	c.65-?_819+?del	PARTIAL DELETION	BOTH HETERO	Y
6	82	600	2500	3^0	KCNJ	HT	c.601C>T	MISSENSE	NONE	Y
7	72	380	2500	--	INS	HT	c.188-40C>A	ABERRANT /NONSENSE	FATHER	Y
8	123	594	3000	3^0	*STAT3*	HT	c.592G>V	MISSENSE	FATHER	?
9	240	360	2300	--	NO	--	--			
10	215	553	2700	--	NO	--	--			
11	248	396	2500	3^0	NO	--	--			
12	5	446	1400	3^0	GCK	HM	c.820G>A	MISSENSE	MOTHER	Y
13	180	522	3800	3^0	ABCC8	CH	c.695G>A/760C>T	N/M	MOTHER	Y
14	4	594	2200	3^0	KCNJ11	HT	c.86G>A	MISSENSE	MOTHER, PAT GM	?

AGE	RBS AT PRESENTA-TION	BIRTH WT	CONSAN-GUINITY	MUTATION	TRAN-SMISSION	PARENTS/KINDRED WITH MUTATION	ASSOCIATED FEATURES	INSULIN DOSE
58	288	2500	--	KCNJ11	HT	F	TNDM	0.1 U/KG
82	600	2500	3^0	KCNJ 11	HT	NONE	Devp delay. Improved with SU. Partial transition	
4	594	2200	3^0	KCNJ11	HT	MOTHER, PAT GM	Mother n grandmother had mutation	
180	522	3800	3^0	ABCC8	CH	MOTHER		
18	396	3100	--	IL2 RA- N	HM	BOTH HETERO	HYPOTHYROIDISM ,IMMUNO-DEFICIENCY	
123	594	3000	3^0	*STAT3* N	HT	FATHER	? PATHOGENECITY	
240	360	2300	--	NO	--			
215	553	2700	--	NO	--			
248	396	2500	3^0	NO	--			

CRITERIA

Inclusion Criteria
- Age at onset <9 months
- Hyperglycemia sustained for ≥ 2 weeks
- Insulin dependence

Exclusion Criteria
- Exclusion of Hyperglycemia caused by stress and infection and drug therapies.

Highlights

- One of the larger cohorts described recently.
- Good genotype –phenotype correlation
- Demonstrated DQ improvement with SU therapy
- 5 novel mutations
- Genetic evaluation was thorough and included a 29 gene panel.
- Tracking of parents and grand parents and screening
- More Permanent vs transient NDM

Limitations

- Antibody testing to rule out T1DM - not financially feasible.
- Could not measure c-peptide prior to and during transition to SU
- Parents of a few children could not be tested due to various reasons like distance, death, diaspora and divorce.

Genetic Analysis

EDTA blood samples of infants and both parents (wherever possible) were sent for molecular genetic analysis.
Genomic DNA was extracted, and the coding regions and intron/exon boundaries of the ABCC8, KCNJ11, INS and EIF2AK3 genes amplified by PCR. Amplicons were sequenced using the Big Dye Terminator Cycler Sequencing Kit v3.1 (Applied Biosystems), and reactions were analysed on an ABI 3730 Capillary sequencer (Applied Biosystems)

- Sanger sequencing was used to validate the screened mutations and in parents for inherited or de novo mutations.
- Confirmed mutations were then searched in the human gene mutation database (HGMD), dbSNPl38, thousand genomes, and recent reviews. For all mutations, software Polyphen-2 was used to predict the pathogenicity.
- Statistical analysis was performed using IBM SPSS 22.0 for Windows
- statistical software. Wherever feasible, data was expressed as
- mean ± S.D.

TABLE 1 : CLINICAL CHARACTERISTICS

S.NO	VARIABLE	MEAN ± SD
1	MEAN AGE	114 ± 91
2	MEAN BW	2410 ± 613
3	MEAN GESTATION	37.28 ± 1.22
4	MEAN RBS	471 ± 102
5	MEAN INSULIN DOSE /KG BODY WEIGHT	0.73 ± 0.4

CONCLUSIONS

- Mutations in GCK, KCNJ11 AND INS were the commonest causes of NDM in our cohort.
- Underlying mutations established in 75%.
- More non-KATP channel mutations are likely to reflect the increased rate of consanguinity.
- In countries with more consanguineous marriages, focused searching for rarer causes of NDM and creation of database needs to be done, so that targeted high yield genetic sequencing can be performed

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