Booked on	07/01/2017	**Patient ID**	011701070010	**Printed on**	23/02/2017
Name	Baby Coral Gupta	**Age**	2 Years	**Sex**	F
Ordering Physician					

Clinical Exome Analysis

Sample Information

Sample Type: Whole Blood

Clinical Indications

Baby *Coral*, born of a non-consanguineous marriage, presented with clinical indications of excessive drooling, overall growth retardation, inability to speak well, muscle weakness and elevated level of creatine phosphokinase. Baby *Coral* has been evaluated for pathogenic gene variations (Coverage of muscular dystrophy and congenital myopathy genes is given in appendix 1).

Results

Gene (Transcript)ǂ	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
POMT1 **(+)** (ENST00000372228)	Exon 11 Exon 19	c.1081C>C/T **(p.Gln361Ter)** c.2005G>G/A **(p.Ala669Thr)**	Heterozygous Heterozygous	Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A1, Congenital muscular dystrophy-dystroglycanopathy with mental retardation type B1, Limb-girdle muscular dystrophy-dystroglycanopathy type C1	Autosomal recessive	Likely Pathogenic

PATHOGENIC VARIANT CAUSATIVE OF THE REPORTED PHENOTYPE WAS IDENTIFIED.

Interpretation

Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A1 (OMIM#236670), congenital muscular dystrophy-dystroglycanopathy with mental retardation type B1 (OMIM#613155) and limb-girdle muscular dystrophy-dystroglycanopathy type C1 (OMIM#609308) are caused by homozygous or compound heterozygous mutations in the *POMT1* gene (OMIM*607423). Two heterozygous variations were detected in the *POMT1* gene (Table).

A heterozygous nonsense variation in exon 11 of the ***POMT1*** gene **(chr9:134387456; C>C/T; Depth: 111x)** that results in a stop codon and premature truncation of the protein at codon 361 **(p.Gln361Ter; ENST00000372228)** was detected (Table). The Gln361Ter variant lies in the MIR (protein mannosyl transferase, IP3R and RyR) domain of the protein [23], has not been reported in the 1000 genomes database and has a minor allele frequency of 0.0017% in the ExAC database. The *in silico* predictions# of the variant are damaging by LRT and MutationTaster2. The reference codon is conserved across species.

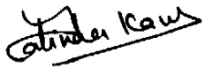
A heterozygous missense variation in exon 19 of the ***POMT1*** gene **(chr9:134397547; G>G/A; Depth: 161x)** that results in the amino acid substitution of Threonine for Alanine at codon 669 **(p.Ala669Thr; ENST00000372228)** was detected (Table). The Ala669Thr variant lies in the C-terminal four TMM region of protein-O-mannosyl transferase of the ***POMT1*** protein [23] and has not been reported in both the 1000 genomes and ExAC databases. The *in silico* predictions# of the variant are possibly damaging by PolyPhen-2 (HumVar and HumDiv) and damaging by SIFT, LRT and MutationTaster2. The reference codon is conserved across species.

Both of the observed variations have previously been reported, as compound heterozygous variants, in a patient affected with limb girdle muscular dystrophy with mental retardation and the patient had hypertrophy, low IQ, microcephaly and elevated level of creatine phosphokinase [24]. The Ala669Thr variant has also previously been reported, as one of the compound heterozygous variants, in a patient affected with motor disability, contractures in ankles, elbows and knees, microcephaly, severe mental retardation and elevated level of creatine phosphokinase [25].
Based on the above evidence, ***these POMT1 variations are classified as likely compound heterozygous likely pathogenic variants and have to be carefully correlated with the clinical symptoms***.

Recommendations

Validation of these variations by Sanger sequencing is recommended to rule out false positives.
Sequencing these variations in the parents and the other affected and unaffected members of the family is recommended to confirm their significance.
Genetic counselling is advised for interpretation on the consequences of these variations.

Report Released by:



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Supplement Information Sheet

Comment

***Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:**

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).
Pathogenic	A disease causing variation in a gene which can explain the patients' symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed.
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Benign	A variant which is known not to be responsible for disease has been detected. Generally no further action is warranted on such variants when detected.
Likely Benign	A variant is not expected to have a major effect on disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non-disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

[ǂ]The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 75 gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.
Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.
[#]The *in silico* predictions are based on Variant Effect Predictor, Ensembl release 84 (SIFT version - 5.2.2; PolyPhen - 2.2.2); LRT version - November, 2009 release from dbNSFPv3.1 and Mutation Taster2 based on build NCBI 37 / Ensembl 69 [21].

Methodology

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions of the genome/genes is performed. Mutations identified in the exonic regions are generally actionable compared to variations that occur in non-coding regions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual.
DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80-100X coverage on Illumina sequencing platform. The sequences obtained are aligned to human reference genome (GRCh37/hg19) using BWA program [2, 3] and analyzed using Picard and GATK-Lite toolkit [4, 5] to identify variants relevant to the clinical indication. We follow the GATK best practices framework for identification of variants in the sample. Gene annotation of the variants is performed using VEP program [6] against the Ensembl release 84 human gene model [7]. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases – ClinVar, OMIM, GWAS, HGMD and SwissVar [8-15]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, ExAC, EVS, dbSNP141, 1000 Japanese Genome and our internal Indian population database [16-20]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, Mutation Taster2, Mutation Assessor and LRT. Only non-synonymous and splice site variants found in the clinical exome panel consisting of 6440 genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Total data generated	4.19 Gb
Total reads aligned (%)	99.87
Reads that passed alignment (%)	97.44
Data ≥ Q30 (%)	96.94

Disclaimer

- The classification of variants of unknown significance can change over time and MolQ cannot be held responsible for this. Please contact MolQ at a later date to inquire about any changes.
- Intronic variants are not assessed using this method.
- Large deletions of more than 10 bp or copy number variations /chromosomal rearrangements cannot be assessed using this method.
- Certain genes may not be covered completely and few mutations could be missed.

- The mutations have not been validated by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines [22] can also be given upon request.

References

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Appendix I: COVERAGE OF MUSCULAR DYSTROPHY AND CONGENITAL MYOPATHY GENES

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
ACTA1	100.00	*FKTN*	100.00	*POMGNT1*	100.00
ANO5	100.00	*FLNC*	100.00	*POMGNT2*	100.00
B3GALNT2	98.29	*GMPPB*	100.00	*POMK*	100.00
B3GNT1	100.00	*GNE*	100.00	*POMT1*	100.00
BAG3	100.00	*HNRNPA2B1*	100.00	*POMT2*	100.00
BIN1	100.00	*HNRNPDL*	100.00	*RYR1*	100.00
BVES	100.00	*ISCU*	100.00	*SEPN1*	93.71
CAPN3	100.00	*ISPD*	100.00	*SGCA*	100.00
CAV3	100.00	*ITGA7*	100.00	*SGCB*	100.00
CCDC78	100.00	*KBTBD13*	100.00	*SGCD*	100.00
CFL2	100.00	*KLHL40*	100.00	*SGCG*	100.00
CHKB	100.00	*KLHL41*	100.00	*SMCHD1*	100.00
CNTN1	100.00	*LAMA2*	100.00	*SPEG*	99.97
COL12A1	100.00	*LAMP2*	100.00	*SYNE1*	100.00
COL6A1	100.00	*LARGE*	100.00	*SYNE2*	100.00
COL6A2	100.00	*LDB3*	100.00	*TCAP*	100.00
COL6A3	100.00	*LMNA*	100.00	*TMEM43*	100.00
CRYAB	100.00	*LMOD3*	100.00	*TMEM5*	100.00
DAG1	100.00	*MEGF10*	100.00	*TNNT1*	100.00
DES	100.00	*MTM1*	100.00	*TNPO3*	100.00
DMD	100.00	*MYF6*	100.00	*TPM2*	100.00
DNAJB6	100.00	*MYH2*	100.00	*TPM3*	100.00
DNM2	100.00	*MYH7*	99.97	*TRAPPC11*	100.00
DYSF	100.00	*MYOT*	100.00	*TRIM32*	100.00
EMD	100.00	*NEB*	87.24	*TTN*	98.97
FHL1	100.00	*PABPN1*	100.00	*VCP*	100.00
FKRP	100.00	*PLEC*	100.00		