

Supplementary Material

Novel mutations in the asparagine synthetase gene (*ASNS*) associated with microcephaly

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Supplementary Figure 1. Conservation of amino acids in ASNS. Sequences were derived from the UniProt database (UniProtKB; Pundir et al., 2017; The UniProt consortium, 2017) and aligned using the Clustal Omega (1.2.4) multiple sequence alignment program implemented in UniProt. The following organisms were included: Human (*Homo sapiens*), Sumatran orangutan (*Pongo abelii*), Rhesus macaque (*Macaca mulatta*), Bovine (*Bos taurus*), Chicken (*Gallus gallus*), Mouse (*Mus musculus*), Rat (*Rattus norvegicus*), Chinese hamster (*Cricetulus griseus*), and Zebrafish (*Danio rerio*). Conservation: *(asterix) single fully conserved residue; :(colon) conservation between groups of strongly similar properties – scoring >0.5 in the Gonnet PAM 250 matrix; .(period) conservation between groups of weakly similar properties – scoring ≤0.5 in the Gonnet PAM 250 matrix (according to Uniprot; <http://www.uniprot.org>). Mutations described for ASNSD are given in blue, the mutations found in the family of this case report in green.

References

Pundir, S., Martin, M. J., & O'Donovan, C. (2017). UniProt Protein Knowledgebase. *Methods Mol Biol*, 1558, 41-55.

The UniProt consortium. (2017). UniProt: the universal protein knowledgebase. *Nucleic Acids Res*, 45, D158-D169.

UniProtKB ASNs	A6E	R49Q	
P08243	HUMAN	MCGIWAFLFGSDDDL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	160
Q5R6W9	ORANGUTAN	MCGIWAFLFGSDDDL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
F6SS13	MACAQUE	MCGIWAFLFGSDDDL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
Q1L2A3	BOVINE	MCGIWAFLFGSDDDL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
Q5ZJ3J	CHICKEN	MCGIWAFLFGSDECL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
Q61024	MOUSE	MCGIWAFLFGSDDDL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
P49088	RAT	MCGIWAFLFGSDDDL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
P19891	HAMSTER	MCGIWAFLFGSDDDL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
A3KQK2	ZEBRAFISH	MCGIWAFLFGSDECL SVQCL SAMKIAHRGPDADF RFENNVNGYTNCCFGFHR	60
Conservation	1	*****	60
glutamine amidotransferase domain			
K66Nfs*10 N75S			
P08243	HUMAN	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
Q5R6W9	ORANGUTAN	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
F6SS13	MACAQUE	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
Q1L2A3	BOVINE	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
Q5ZJ3J	CHICKEN	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
Q61024	MOUSE	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
P49088	RAT	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
P19891	HAMSTER	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
A3KQK2	ZEBRAFISH	PIRVKKYPYLWLCYNGEIIYNHKKMQHFEFEYQTKVDGEIILHL YDKGGIEQITCMLDGV	120
Conservation	61	*****	120
D138A L145S			
P08243	HUMAN	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
Q5R6W9	ORANGUTAN	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
F6SS13	MACAQUE	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
Q1L2A3	BOVINE	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
Q5ZJ3J	CHICKEN	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
Q61024	MOUSE	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
P49088	RAT	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
P19891	HAMSTER	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
A3KQK2	ZEBRAFISH	FAFVLLDTANKKVFVLRD TYGVRPLFKAMTEDGFLAVCSEAKGLVTLKHSTTFLPKVEPF	180
Conservation	121	*****	180
M201Wfs*28			
P08243	HUMAN	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
Q5R6W9	ORANGUTAN	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
F6SS13	MACAQUE	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
Q1L2A3	BOVINE	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
Q5ZJ3J	CHICKEN	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
Q61024	MOUSE	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
P49088	RAT	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
P19891	HAMSTER	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
A3KQK2	ZEBRAFISH	LPGHYEVLDPKNGKVASVEVKYVHHC RDVPLHALYDNVEKLPFGFEIETVKNNLRILFN	240
Conservation	181	*****	240
end asparagine synthetase domain			
V243A L247W G289A			
P08243	HUMAN	NAWKKRLMTDRRIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
Q5R6W9	ORANGUTAN	NAWKKRLMTDRRIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
F6SS13	MACAQUE	NAWKKRLMTDRRIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
Q1L2A3	BOVINE	NAWKKRLMTDRRIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
Q5ZJ3J	CHICKEN	NAWKKRLMAHRRIIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
Q61024	MOUSE	NAWKKRLMTDRRIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
P49088	RAT	NAWKKRLMTDRRIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
P19891	HAMSTER	NAWKKRLMTDRRIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
A3KQK2	ZEBRAFISH	NAWKKRLMAHRRIIGCLL SGGDLSSVLAATLLKQLKEAQVYQLQTFATIGMEDSPDLLAAR	300
Conservation	241	*****	300
T337I R340H			
P08243	HUMAN	KVADHIGSEHYEVL FNSEEGIQALDEVIFSL ETDYITVRASVGMYLISKYIRKNTDSVV	360
Q5R6W9	ORANGUTAN	KVADHIGSEHYEVL FNSEEGIQALDEVIFSL ETDYITVRASVGMYLISKYIRKNTDSVV	360
F6SS13	MACAQUE	KVADHIGSEHYEVL FNSEEGIQALDEVIFSL ETDYITVRASVGMYLISKYIRKNTDSVV	360
Q1L2A3	BOVINE	KVADHIGSEHYEVL FNSEEGIQALDEVIFSL ETDYITVRASVGMYLISKYIRKNTDSVV	360
Q5ZJ3J	CHICKEN	KVADHIGSEHYEVL FNSEEGIQALDEVIFSL ETDYITVRASVGMYLISKYIRKNTDSVV	360