Letter to the editor

Reply: **Genotype-phenotype correlation in *ATAD3A* deletions: not just of scientific relevance!**

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Sir,

We were greatly interested to read the letter from Peeters-Scholte *et al*. (2017), written in response to our recent article "*ATAD3* gene cluster deletions cause cerebellar dysfunction associated with altered mitochondrial DNA and cholesterol metabolism" (Desai *et al.*, 2017). Our study described six subjects from five families with *ATAD3* deletions or gene conversions, and linked *ATAD3* to cholesterol homeostasis and maintenance of the mitochondrial genome, providing functional insight into the disease pathology. These results were indeed not only of scientific interest, but had clinical implications that are further highlighted by Peeters-Scholte *et al.* (2017) reporting six additional cases involving *ATAD3*

deletions/mutations, potentially providing more information to enable prediction of genotype/phenotype correlations.

Peeters-Scholte *et al.* (2017) stated that there are now nine cases reported in the literature "with homozygous deletions of the *ATAD3A* gene". It is more accurate to say there are ten cases with biallelic deletions that extend into the *ATAD3A* gene. The first case, described by Harel *et al.* (2016) is discussed below. The other nine had biallelic *ATAD3B*/*ATAD3A* deletions, including five subjects (from four families) we described with homozygous or compound heterozygous 38 kb deletions (S1a-S4; Desai *et al.*, 2017), and four subjects Peeters-Scholte *et al.* (2017) now report. The clinical spectrum in these 9 cases was strikingly similar (Table 1), clearly indicating *ATAD3* deletions are a substantial cause of pontocerebellar hypoplasia (PCH). In all cases bar one, the subjects died in the first two weeks of life, and the child in our study who died at 7 mo (S4) was incapable of breathing without support, so the increased lifespan appears to have been due to the duration of intervention, rather than a milder phenotype. The nine cases with biallelic *ATAD3B/ATADA* deletions were of Iranian, Dutch, Indian, Chinese, Moroccan or Egyptian ancestries and we detected four different breakpoints giving rise to 38 kb deletions. These data imply that the deletions are recurrent mutations due to non-allelic homologous recombination and that biallelic *ATAD3B/ATADA* deletions cause similar severe presentations and early death across a range of ethnicities.

A complete *ATAD3* knockout is embryonic lethal in mice (Goller *et al.*, 2013), and results in early larval arrest in *C. elegans* and *Drosophila* (Hoffmann *et al.*, 2009; Gilquin *et al.*, 2010), indicating that ATAD3 plays a crucial role during development. Like most species, mice, worms and flies have only a single *ATAD3* gene, while hominids have a cluster of three genes: *ATAD3C*, *ATAD3B*, and *ATAD3A*. We do not know of any evidence suggesting that the *ATAD3C* gene is expressed and it seems likely that complete loss of *ATAD3A* and *ATAD3B* would not be compatible with livebirth. The biallelic *ATAD3B*/*ATAD3A* deletion subjects we reported lack the *ATAD3A* promoter and instead rely on the weaker *ATAD3B* promoter, which explains the greatly decreased expression of the ATAD3B/ATAD3A fusion protein that presumably is sufficient for foetal development, but cannot sustain life beyond the neonatal period or very early infancy.

Other recessive and dominant mutations in the *ATAD3* locus cause milder clinical presentations but an additional three cases were associated with brain malformations and

neonatal lethality, all of which seem likely to have had a null mutation on one allele. Harel *et al.* (2016) reported a case with a 38kb *ATAD3B/ATADA* deletion plus a larger 68kb deletion allele (II-1; family 7). The 68kb deletion is predicted to result in an *ATAD3C*/*ATAD3A* fusion gene, which seems unlikely to be expressed. Subjects 2a and 2b described by Peeters-Scholte *et al.* (2017) had compound heterozygous nonsense and missense mutations in *ATAD3A*. The simplest explanation for these data is that, like the biallelic *ATAD3B/ATAD3A* deletions, very low but significant expression of ATAD3 protein allows foetal development but causes early lethality. More detailed descriptions of the deletions reported by Peeters-Scholte *et al.* (2017) could strengthen this conclusion.

Peeters-Scholte *et al.* (2017) emphasized that routine SNP arrays failed to detect the biallelic deletions in their subjects 1a and 1b due to limited probe coverage. We encountered the same issue with our subject S3 and the initial SNP results for our subject S5 with a milder clinical presentation, surviving to adulthood, were also unclear (Desai *et al.*, 2017). In the latter case, a standard cytoSNP-12 (Illumina) array suggested a homozygous deletion affecting *ATAD3C*/*ATAD3B,* which was not compatible with subsequent RNA studies. Further investigation using a CoreExome-24 (Illumina) array was more informative, since it had 41 probes covering the *ATAD3* locus (including 13 in *ATAD3C*, 17 in *ATAD3B* and 10 in *ATAD3A*) compared to a total of 10 probes in the cytoSNP-12 array. Long-range PCR also suggested a 38 kb *ATAD3B/ATAD3A* deletion in subject S5 that was incompatible with RNA studies. While the *ATAD3* deletions/rearrangements in S5 are not completely resolved, the apparent deletions observed by the cytoSNP-12 array and long-range PCR appear to be explained by a deletion on one allele and gene conversion events on the other allele. Likewise, we have found that SNPs located in homologous stretches shared between *ATAD3B* and *ATAD3A* can result in incorrect mapping and sequence alignment. These examples demonstrate that whilst care needs to be taken to detect and correctly define *ATAD3* rearrangements they are ultimately tractable to current technologies available to most centres carrying out genetic diagnosis.

Given the complexity of the *ATAD3* genomic region, we require concordant predictions for the size and location of the deletion(s) in genomic DNA by two independent methods (e.g., SNP array, long-range PCR or exome sequencing), or one genomic method supported either by cDNA or protein studies. We recommend others apply a similar depth of analysis to ensure diagnoses are robust. Importantly, we see a strong correlation between the amount of

residual ATAD3 protein and clinical outcome in our subjects. In fibroblasts and tissues, all of the subjects with biallelic *ATAD3B*/*ATAD3A* deletions have striking reductions in ATAD3 protein levels, while subject S5 retained one *ATAD3A* allele and expressed appreciably more ATAD3A protein in fibroblasts correlating with their milder clinical presentation (Desai *et al.*, 2017). Thus measurement of ATAD3 protein levels by immunoblotting is an important tool for the diagnosis of ATAD3 deletions that can enable labs with limited genomic mapping expertise to confirm a genomic diagnosis.

Genotype/phenotype correlations in the *ATAD3* region are more difficult to establish for missense mutations given they can be associated with either recessive or dominant inheritance (Harel *et al.*, 2016; Cooper *et al.*, 2017). In the case of subjects 2a and 2b described by Peeters-Scholte *et al.* (2017), who had compound heterozygous nonsense and missense mutations in *ATAD3A*, follow-on protein and RNA studies would be important to determine whether the alleles are expressed, if they have any impact on RNA splicing or stability and how the mutations affect protein levels. No data on predicted pathogenicity were described by Peeters-Scholte *et al.* (2017) for the *ATAD3A* missense and nonsense mutations they reported. The missense mutation in their subjects (Chr1(GRCh37): g.1451416T>G (NM_018188.4): c.230T>G:p.(Leu77Arg)), is not present in the gnomAd database (Lek *et al.*, 2016), is highly conserved, and multiple *in silico* analyses predict it to be probably damaging (MutationTaster, Align GVGD, Polyphen-2). Given it is inherited in combination with a nonsense mutation in two affected siblings, and the parents are not reported to be affected, it seems very likely to be acting as a severely deleterious recessive variant. The nonsense mutation itself (Chr1(GRCh37):g.1454346C>T: NM_018188.3: c.634C>T:p.(Gln212*) has been reported in gnomAd at a very low frequency (dbSNP rs760826883; MAF 0.000004063).

Two siblings with a homozygous *ATAD3A* missense mutation (p.Thr53Ile) described by Harel *et al.* (2016) (II-1 and II-2; family 6) had a milder disease course including cerebellar atrophy and survival to adulthood, which may relate to the overall levels of functional protein, or the functional severity of the mutation on ATAD3. Investigations of ATAD3 protein levels were not performed in these patients and so there is no established link between protein levels and disease phenotype. However, several subjects reported with heterozygous dominant *ATAD3A* mutations all had much milder clinical presentations than the *ATAD3A* deletions (Harel *et al.*, 2016; Cooper *et al.*, 2017) and the ATAD3A mutant protein levels

appeared similar to controls, suggesting some impairment of ATAD3A function, rather than the major diminution in activity caused by the deletions.

In conclusion, we agree with Peeters-Scholte *et al.* (2017) that determining a correlation between genotype and phenotype for *ATAD3* mutations is crucial to inform obstetric and neonatal care and for pregnancy planning. There is a clear correlation emerging, linking biallelic deletions extending into *ATAD3A* with severe clinical presentations, although the impact of missense mutations is not as well resolved. Identification of additional subjects with *ATAD3* point mutations coupled with detailed genetic and follow-on studies will provide important information towards resolving this. Not only will these investigations be of clinical importance to the specific cases of pathological *ATAD3* mutants, further scientific inquiry into the function of ATAD3, its roles in cholesterol and mitochondrial homeostasis, and its function in maintaining the mitochondrial genome can provide insights into a broad range of mitochondrial disorders and potentially the wider role of mitochondrial dysfunction in neurological and neurodegenerative diseases.

References

Cooper HM, Yang Y, Ylikallio E, Khairullin R, Woldegebriel R, Lin KL*, et al.* ATPase-deficient mitochondrial inner membrane protein ATAD3A disturbs mitochondrial dynamics in dominant hereditary spastic paraplegia. Hum. Mol. Genet. 2017; 26: 1432-1443.

Desai R, Frazier AE, Durigon R, Patel H, Jones AW, Dalla Rosa I*, et al.* ATAD3 gene cluster deletions cause cerebellar dysfunction associated with altered mitochondrial DNA and cholesterol metabolism. Brain 2017; 140: 1595-1610.

Gilquin B, Taillebourg E, Cherradi N, Hubstenberger A, Gay O, Merle N*, et al.* The AAA+ ATPase ATAD3A controls mitochondrial dynamics at the interface of the inner and outer membranes. Mol. Cell. Biol. 2010; 30: 1984-1996.

Goller T, Seibold UK, Kremmer E, Voos WKolanus W. Atad3 function is essential for early post-implantation development in the mouse. PLoS One 2013; 8: e54799.

Harel T, Yoon WH, Garone C, Gu S, Coban-Akdemir Z, Eldomery MK*, et al.* Recurrent De Novo and Biallelic Variation of ATAD3A, Encoding a Mitochondrial Membrane Protein, Results in Distinct Neurological Syndromes. Am. J. Hum. Genet. 2016; 99: 831-845.

Hoffmann M, Bellance N, Rossignol R, Koopman WJ, Willems PH, Mayatepek E*, et al. C. elegans* ATAD-3 is essential for mitochondrial activity and development. PLoS One 2009; 4: e7644.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T*, et al.* Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016; 536: 285-291.

Peeters-Scholte CMPCD, Adama van Scheltema PN, Klumper FJCM, Everwijn SMP, Koopmans M, Hoffer MJV*, et al.* Genotype-phenotype correlation in ATAD3A deletions: not just of scientific relevance! Brain 2017; submitted.

Table 1. Common features of *ATAD3* subjects with severe presentations

	Harel *et al.* (2016)	Desai *et al.* (2017)					Peeters-Scholte *et al.* (2017)					
	II-1, family 7	**S1a**	**S1b**	**S2**	**S3**	**S4**	**1a**	**1b**	**2a**	**2b**	**3**	**4**
Age at onset	32 weeks	38 weeks	33 weeks	33 weeks	34 weeks	37 weeks	28 weeks	29 weeks	unk[1]	30 weeks	unk	unk[2]
Age at death	Day 13	Day 5	Day 1	Day 5	Day 2	7 Months	Day 1	Day 1	Day 6	Day 3	Day 7	Day 5
Foetal Presentation												
Polyhydramnios		√		√	√		√	√		√		√
Reduced foetal movement	√	√	√	√		√	√	√				
Foetal distress/early delivery	√	√	√	√	√			√	√			
Neuroradiology and laboratory findings												
Pontocerebellar hypoplasia	√	√	ND	√	√	√	√	√		√	√	√
Simplified/delayed sulcal and gyral pattern	√	√	ND	√	√	√	√	√	√	√	√	
Elevated plasma or CSF lactate	ND	√		√	√	√			√	√	√	√
Elevated 3-methyglutaconate				√					√	√	√	√
EEG pattern: burst suppression; epileptiform discharges	√	√		√	√				√	√	√	√
Symptoms												
Respiratory insufficiency	√	√		√	√	√	√	√	unk	unk	unk	unk
Seizures	√				√						√	√
Contractures	√	√		√	√	√	√			√	√	
Corneal clouding and/or edema	√	√					√		√	√	√	√

ND, no data. unk, unknown. [1]Caesarean at 32 weeks. [2]Presentation in third trimester