Herbert et al.[1] note that not all cross-reactive immunologic material–negative infantile Pompe patients developed neutralizing antibodies, while they all experienced poor clinical outcome, and that the role of neutralizing antibodies should not be overlooked. We agree that neutralizing antibodies are not the only explanation for a poor response to ERT. First, ERT does not compensate in all cases for α-glucosidase (GAA) deficiency to an activity level above the critical threshold. The reason for this could be that the dosage is too low or the therapy is inefficient due to the formation of anti-recombinant human GAA antibodies, which neutralize GAA activity and/or interfere with cellular uptake. Second, Pompe disease may have progressed too far and tissue damage has become beyond repair. Third, as yet unknown modifying factors may enhance or decrease the effect of ERT. Fourth, the lysosomal storage of glycogen in Pompe disease induces secondary cellular responses, such as a block of autophagic flux and mitochondrial dysfunction—processes bound to interfere with ERT. Evidently, antibodies are just one of several factors determining the outcome of ERT. This is also emphasized by the heterogeneous response to ERT in patients with no or low antibody titers in our study.

Herbert and colleagues[1] suggest that assay variability "appears to be associated with control reagents" rather than titering patient samples. It is unclear to us why the authors conclude this as this is misconstrued from our paper; we did use patient samples over the titer range to determine assay variability.

Herbert et al.[1] question whether the assay used in our study to measure neutralizing effects has been standardized and whether the cellular matrix could cause variability. The assay has been standardized and the same cellular matrix (fibroblasts from a classic infantile patient without any detectable GAA activity) was used in all experiments. We would like to emphasize that assessment of neutralizing effects is an important aspect to investigate the potential impact of antibodies on ERT, and we wish to promote its assessment as a standard assay whenever high antibody titers are found.

The authors also question the use of our clinical outcome measures as a readout for efficacy. We note that the outcome measures have been internationally recognized in consensus meetings and have been found suitable for the detection of changes in patient performance in response to ERT in multiple clinical studies. We recommend testing for the presence of neutralizing antibodies in the case of infusion-associated reactions and when clinical outcome declines.

Herbert et al.[1] state that "genotype alone is not responsible for immune response" and that "the observation of genotype association in LOPD should be stated with caution". We regret what appears to be a misunderstanding of our work. We did not state in our article that genotype alone is responsible for the immune response. We did, however, state that our results should be confirmed in a larger patient group.

In summary, we have conducted an in-depth study in which we measured antibody titers and their neutralizing effects at multiple time points over a period of 3 years. This showed that titers declined on a group level, a limited number of patients developed high antibody titers, and a subset of these patients showed high sustained titers, but in only one patient was a clear impact of antibodies on the effect of ERT likely.

DISCLOSURE

A.T.vdP. has provided consulting services for various industries in the field of Pompe disease under an agreement between these industries and Erasmus MC. The other authors declare no conflict of interest.

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Pitfalls of trio-based exome sequencing: imprinted genes and parental mosaicism—*MAGEL2* as an example

To the Editor: Family-based whole-exome sequencing has proven to be an effective diagnostic strategy for the identification of causative variants in individuals with intellectual disability (ID) and congenital malformations

(CM). De novo mutations play a major role in ID/CM and it is estimated that they are responsible for up to 40% of cases in non-consanguineous populations.[1] Most whole-exome sequencing filtering strategies applied in laboratories worldwide are focused on de novo, X-linked and recessive inheritance. However, in their article "Imprinting: The Achilles Heel of Trio-Based Exome Sequencing," Aten et al.[2] recently highlighted the importance of taking into account mutations in imprinted genes as a cause of ID. They described the difficulties they confronted in the identification of the causative variant in a large family with several affected members. The family was studied in parallel in two independent centers using different diagnostic approaches. A trio-based approach was used for one part of the family and single-exome sequencing for another member; both failed to identify the pathogenic mutation. Only when the analysis of the family pedigree showed that all affected individuals were linked through their fathers was the causative variant, a paternally inherited frameshift mutation in *MAGEL2*, identified. Truncating mutations in the paternally expressed allele of *MAGEL2*, located in the imprinted 15q11q13 Prader–Willi syndrome region, are responsible for Schaaf–Yang syndrome.[3] An additional pitfall when applying the common filtering strategies may be encountered if parental mosaicism is present. Recent unpublished data from the Deciphering Developmental Disorders study estimate that around 2% of pathogenic de novo mutations in children are mosaic in parental tissues.

We report a seven-year-old boy initially referred to our clinic at 3 years of age for clinical evaluation. He is the second child of a non-consanguineous healthy couple with no family history of note. His elder brother is healthy. Pregnancy was uneventful (normal fetal movements) and delivery was at term by C-section due to breech presentation. The birth weight was 2,830 g (15th centile). In the neonatal period, hypotonia, poor suck and scarce spontaneous movements were noted. On examination at 3 years of age, the patient showed marked growth delay (height: − 4 s.d., weight: − 3 s.d., occipitofrontal circumference: − 2.5 s.d.). He had dry skin and an abnormal hair growth pattern. Dysmorphic features consisted of dolicocephaly, low-set ears, a broad nasal root, a deep philtrum and widely spaced teeth. He had mild contractures of both knees, tapering digits with camptodactyly of fingers 2 to 5, and poorly developed palmar creases. He had male genitalia with a hypoplastic penis and scrotum. Bilateral cryptorchidism had been surgically corrected. His psychomotor development was markedly delayed: he was unable to walk independently, speech was almost absent, and he was just able to comprehend simple orders. He had suffered two seizures (normal magnetic resonance imaging and electroencephalogram) and was undergoing gastroenterology and endocrine follow-up due to chronic constipation and recurrent hypoglycemias of unknown origin. No clinical diagnosis could be established. Initial genetic testing included a karyotype, a custom-designed 60 K oligonucleotide array (KaryoArray v3.0) and the CytoSNP-850 K Beadchip (Illumina), all with normal results. Subsequent trio exome analysis using a de novo filtering approach revealed an apparently de novo heterozygous frameshift mutation in *MAGEL2* (NM_019066.4):c.1996dupC (p.Gln666fs) previously described in other patients with Schaaf–Yang syndrome. Sanger sequencing validation confirmed the variant in the proband and enabled us to identify the same frameshift variant in a mosaic state in his father. In fact, reanalysis of the parental data visualizing the BAM file detected this variant in two out of 50 reads. This finding was confirmed in the father using a custom next-generation sequencing clinical panel containing 1,253 genes involved in intellectual disability, autism spectrum disorders, and other common genetic disorders (Clinical panel V1.0). The variant was found again in 2 of 50 reads, and the mutant allele fraction was estimated to be around 4%. In this case, the low somatic mosaicism detected in the father allowed the identification of the variant in the proband when filtering by de novo variants because the genotyping tool used to obtain the variants (GATK HaplotypeCaller) establishes a cut-off of 10% of reads to call a de novo allele. At the time of diagnosis the father was expecting a baby with a different partner. The recurrence risk of 1 to 2% in the case of a de novo mutation due to the possibility of parental gonadal mosaicism substantially increased to 10 to 20% in view of *MAGEL2* being an imprinted gene and the presence of mosaicism in paternal tissues.

In conclusion, we report on another case of molecularly confirmed Schaaf–Yang syndrome, which—to the best of our knowledge—is the first report of a *MAGEL2* mutation inherited from a mosaic father. We also highlight the difficulties encountered in analyzing single patients when the causal variant is located in an imprinted gene or a parental mosaicism is present. Generally, trio-based analysis is a good approach in cases of unexplained ID/CM when a de novo variant is suspected; however, as illustrated by this case, a specific analysis pipeline for imprinted genes that does not include inheritance filtering should also be considered. To this extent de novo, inherited variants, and variants present in a mosaic state in the parents will be detected. In cases where the causative mutation is located in a non-imprinted gene, low parental mosaicism may be missed when applying a de novo filtering strategy and might be subsequently suspected in the direct visualization of the trio BAM file and/or during Sanger sequencing confirmation. Meanwhile, a pathogenic dominant mutation in a proband may be overlooked if parental mosaicism is present in a higher percentage and a common de novo filtering strategy is applied. Therefore, we also recommend a specific bioinformatic algorithm for imprinted genes, and raise awareness of parental mosaicism as a possible pitfall in routine de novo analysis and its implications in genetic counseling.

DISCLOSURE

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LETTERS TO THE EDITOR

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