Supplementary file: Ending a case of diagnostic odyssey

A clear example on how next generation sequencing could be used to extensively shorten the diagnostic phase is illustrated by the following case (individual P16, Table 1 main file) who was first presented to our department at the age of two months. He was the second child of healthy, non-consanguineous parents. The family history was unremarkable. The prenatal history only revealed bleeding in the eighth week of pregnancy. The patient had a history of frequent bronchopneumonia with stridor respiration and tricuspid valve regurgitation.

Dysmorphologies found including dolichocephaly, downslanted palpebral fissure, telecanthus, depressed nasal bridge, small and upturned nose, anteverted nostrils, cupid bow lips, philtrum ridge, high arched palate, broad thumbs, puffy hands, tapering fingers, puffy feet, broad toes, and disharmony of toe length (see Supplemental Figure 1, left side). There was also contracture on upper extremities, while genital examination was unremarkable. Upon the first evaluation and syndrome search, the differential diagnoses were Robinow syndrome, along with Rubinstein-Taybi syndrome and Pfeiffer syndrome.

Cytogenetic analysis of the patient revealed a normal 46,XY karyotypes. Sequencing was performed on dishevelled 1 segment polarity protein (*DVL1*), receptor tyrosine kinase-like orphan receptor 2 (*ROR2*), and wingless-type MMTV integration site family-member 5A (*WNT5A*) genes as the causative genes of Robinow syndrome. However, no pathogenic variants were found in the respective genes. An array analysis was also performed and showed abnormal male profile (arr(X,Y)×1,(1–22)×2).

Upon the next follow-up at 15-months of age, there were some physical changes, particularly regarding the shape of the head and structure of his face. Dysmorphic findings included brachycephaly, flat occiput, protruding eyes, downturned mouth, prominent ears, abnormal palmar creases, brachydactyly, scoliosis, and pectus excavatum-carinatum (see Supplemental Figure 1, right side). Tapering fingers and contracture on upper extremities were still evident. Meanwhile, from developmental history, the patient could tilt his body at six months of age and roll over at 12 months. He was already able to babble and respond to objects nearby at the time of follow-up. Based on this follow-up, the patient was assessed as probably Antley-Bixler syndrome (ABS).

Following the presumptive diagnosis, additional DNA sequencing was performed on fibroblast growth factor receptor (*FGFR2* and *FGFR3*) genes as the known causative genes for ABS without ambiguous genitalia

and impaired steroidogenesis (ABS2, OMIM #207410), as well as cytochrome p450 oxireductase (*POR*) gene for ABS with disordered steroidogenesis (ABS1, OMIM #201750). Sequencing results showed no pathogenic variants in *FGFR2*, *FGFR3*, and the *POR* genes.

Eventually, trio exome sequencing (ES) revealed a *de novo*, pathogenic hemizygous variant in the Filamin-A (*FLNA*) gene (NM_001456.3: c.3425A>T, see Table 1 main file). The variant leads to substitution of Aspartate to Valine, a highly conserved amino acid. It has previously been described as a gain-of-function pathogenic variant, which causes frontometaphyseal dysplasia type 1 (FMD1, OMIM #305620), a spectrum of otopalatodigital syndrome (*1*). The patient passed away at the age of 3 years 9 months.

Supplemental Figure 1. Frontal picture of P16 upon first evaluation (left) and on next follow-up (right). Note the dysmorphic features on first visit included dolichocephaly, downslanted palpebral fissure, telecanthus, depressed nasal bridge, small and upturned nose, anteverted nostrils, and cupid bow lips. On the follow-up, dysmorphic features found were brachycephaly, flat occiput, protruding eyes, downturned mouth, and prominent ears.

1. Moutton S, Fergelot P, Naudion S, *et al*. Otopalatodigital spectrum disorders: refinement of the phenotypic and mutational spectrum. J Hum Genet. 2016; 61:693-699.