Crowdfunding Effort Identifies the Causative Mutation in a Patient with Nystagmus, Microcephaly, Dystonia and Hypomyelination

Crowdfunding refers to the online gathering of finance via numerous small donations from individual supporters (the “crowd”) in order to fund a service, project or cause from various fields including business, arts, medicine and science (Sisler, 2012; Cameron et al., 2013; Weigmann, 2013; Wheat et al., 2013). Crowdfunding platforms facilitate the interaction between organizations soliciting funding for their projects and the people who wish to support them. As prices of molecular testing plummet, utilizing crowdfunding in order to support genetic research becomes increasingly feasible (Cameron et al., 2013). Whole Exome Sequencing (WES) has been used extensively for the purpose of identifying the genetic cause of rare mendelian diseases, uncovering novel mutations in previously implicated genes or identifying new disease-causing genes (Ng et al., 2009; Bamshad et al., 2011; Gilissen et al., 2011). The most common form of these studies involves sequencing a child suffering from a rare ailment and his parents (family trio) (Rivière et al., 2012; Veltman and Brunner, 2012). Today, WES costs about 1500$ per individual sequenced, allowing the cost of an entire trio WES project (about 4500$) to be completely funded through small donations made to a family. This type of funding is limited mainly by the extent of the family’s online social influence, in opposed to their financial capabilities, and therefore provides the means for economically disadvantaged patients to pursue a genetic diagnosis for their ailment.

Here we describe a crowdfunding effort to finance WES of a three-year-old girl with congenital nystagmus, progressive microcephaly, dystonia, developmental delay and brain hypomyelination, her parents and sibling. This project opened on Sep 1, 2013 and reached its financial goal of 5000$ after 50 days. Through analysis of the exome data, we identified a novel mutation in the patient’s Tubulin, β 4A Class IVa gene (TUBB4A) which was not found in any of the other family members. TUBB4A (also known as DYT4, TUBB4, TUBB5 or β-5) is a member of the highly conserved β-tubulin protein family. It is primarily expressed in the nervous system, with the highest expression in the prefrontal cortex, amygdala and cerebellum (Fig. S1) and is the major isotype in brain, where it represents 46% of all β-tubulins (Su et al., 2004). Other than the nervous system, moderate TUBB4A expression is restricted to the adrenal gland and the testis. This nearly exclusive expression of TUBB4A in the central nervous system (CNS) may explain the predominance of CNS-related phenotypes caused by TUBB4A mutations. TUBB4A has been implicated in the pathogenesis of two major neurologic disorders, depending on the mutation: whispering dysphonia (Dystonia type 4, DYT4) (Hersheson et al., 2013; Lohmann et al., 2013; Lohmann and Klein, 2014) and hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) (Ferreira et al., 2014; Miyatake et al., 2014; Pizzino et al., 2014). Common phenotypes in these two afflictions overlap the ones presented by our patient and include: hypomyelination, motor and speech delay, dysarthria, gait ataxia and dystonia (Blumkin et al., 2014). It was therefore concluded that the TUBB4A variant is the most probable genetic cause of the patient’s ailment. As funding for this study was collected through crowdfunding, we introduce it as an established, simple and efficient solution for families with rare genetic diseases lacking either private or outside funding sources.

The patient is a three-year-old girl who presented to the metabolic-neurogenetic clinic at the age of two months because of congenital nystagmus. Her parents are healthy non-consanguineous Ashkenazi and Libyan-Ashkenazi Jews. She has a healthy brother. There is no family history of neurologic disorders. Perinatal history was normal. She smiled at six weeks, laughed at three months and reached for toys at four months. On examination at seven months of age, her head circumference was 42 cm, on the 25th centile for age. Horizontal nystagmus was prominent and she had dystonic posturing of her hands. Brain MRI at that age demonstrated delayed myelination (Fig. 1A). At the age of one year, her head circumference declined to the 2nd percentile for age. The nystagmus and dystonic movements were prominent, her motor functions were delayed and she did not have any words. A repeat MRI demonstrated delayed myelination. MR spectroscopy revealed elevated choline.
Formal ophthalmologic examination revealed rotatory nystagmus in all directions of gaze, but normal optic nerves and retina. Hypomyelination was unchanged in an MRI performed at the age of 15 months (Fig. 1B). Examination at the age of 29 months showed that her head circumference was 44 cm (four standard deviations below the mean for her age) for her age and her weight was on the 10th percentile for age. She could not sit or stand unassisted and she walked with aids. She had normal eye movements with horizontal and rotatory nystagmus. She could follow an object but very slowly. She had generalized dystonia including the mouth, neck and limbs. Her muscle tone was decreased and tendon reflexes were normal. She understood simple commands and could reach and point at objects. At the age of 33 months, there was no significant progress. An extensive evaluation including Chromosomal Microarray Analysis (CMA) and Gap Junction Protein, γ 2 (GJC-2) sequencing was normal.

Exome sequencing generated 3,571,164 variants passing initial filters, 168,977 of which were rare variants (allele frequency <1% in all databases) and 1094 were found inside exons and resulted in an amino acid change. When searching for de novo variants found in the patient and in neither of the parents, only 13 variants remained. Removing variants also found in the sibling resulted in nine remaining variants (Table S1). Prioritization of these variants by combining variant and gene information revealed a novel heterozygous non-synonymous mutation in TUBB4A (exon 4, c.G535C, p.V179L) to be the top candidate variant. The variant was predicted to be deleterious by the highest number of prediction tools utilized (8 out of the 10). It is completely novel meaning that it is neither present in dbSNP138, the 1000 genomes project and the NHLBI exome sequencing project, nor is it found in our personal database of >250 Israeli exomes. Sanger sequencing confirmed the variant is present only in the patient and not in any other family member (Fig. S2).

Variant filtration and prioritization resulted in nine candidate variants in eight genes. We reviewed each of these variants manually in order to pinpoint the most likely candidate. The top scoring variant was the TUBB4A p.V179L mutation (Fig. 1C), supported by both variant and gene information. The variant was predicted by the majority of our employed tools to be deleterious, it is found in a conserved region of the gene and alternate allele coverage was >30×. Manually reviewing the eight other de novo variants (TRANK1 p.D2058G, ZFHX3 p.G3156S, BAX p.A177P, ALK p.A528T, FUCA2 p.22L and p.23L, KIAA1328 p.S8P and CYP251 p.G25W), we could not find additional viable candidates: TRANK1, ZFHX3, BAX, FUCA2, KIAA1328 and CYP251 variants had low alternate allele coverage (<10×), ZFHX3, BAX and FUCA2 variants were found inside low complexity regions, and ALK, FUCA2 and CYP251 variants were not predicted to be deleterious by any of the tools we employed. Phenotype over representation analysis was performed on all the affected genes (Table S2). Out of the eight genes, TUBB4A was the only gene with significant over-representation of relevant phenotypes (P = 0.0000305, 3 overlapping phenotypes out of 33, cerebral hypomyelination (HP:0006808), intellectual disability (HP:0001249) and dystonia (HP:0001332)). Through additional manual review of the associated phenotypes for the other genes, we could not find any phenotypes overlapping with the clinical presentation in our patient (Table S1). Genetic and phenotypic evidence combined with the fact that TUBB4A is expressed primarily in the brain suggests that the variant affecting TUBB4A is the causative variant in the affected patient.
Here we described how an online donation effort, crowdfunding, aided in the complete funding of WES for an entire family of four. The purpose of this project was to identify the genetic cause of congenital nystagmus, progressive microcephaly, dystonia, developmental delay and hypomyelination in one of the family members. Through computational analysis of the sequencing data, we managed to identify a single novel variant in the TUBB4A gene found only in the patient and in none of the other family members. Although WES is a valuable method for the study of genetic diseases, with numerous successful identifications of variant, it is still not offered as a routine test for families with rare genetic diseases and it is currently not funded in most countries. We demonstrate how crowdfunding represents a feasible option for WES projects funding for families with rare genetic diseases that otherwise cannot afford them.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgg.2014.12.004.

REFERENCES


Weigmann, K., 2013. Tapping the crowds for research funding. Crowdfunding, a common practice to support projects in the arts, music or gaming, has also attracted the attention of scientists. EMBO Rep. 14, 1043–1046.