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Re-Evaluation of Clinical Exome Can Identify Pathogenic Variants For Patients With Autism Spectrum Disorder

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Abstract

Approximately half of patients with rare genetic diseases remain undiagnosed after clinical exome sequencing (CES). We performed a genetic re-evaluation of undiagnosed patients more than 4 years after their initial negative CES report to determine the efficiency of reanalysis strategies. A total of 28 clinical exomes from patients with autism spectrum disorder (ASD) were genetically re-evaluated after several years from negative test results. The clinical phenotypes were categorized as ASD-Simplex (no dysmorphic features) and ASD-Complex (with dysmorphic features) by a specialist. No pathogenic or likely pathogenic mutations were identified in all patients through chromosomal microarray (CMA) and clinical exome sequencing panels at the initial diagnosis. We found that 14 % (4/28) of the patient's diagnosis could be resolved after re-evaluating clinical exome data. Demonstrating that reanalysis after several years is a necessary action to find causative gene disorder.

Keywords: Autism Spectrum Disorder; ASD; clinical exome sequencing; CES.

Introduction

Autism is a group of genetically distinct neurodevelopmental disorders characterized by impaired early social interaction and repetitive behaviors and interests **[1]**. Autism is four times more prevalent in males than in females, and it frequently co-occurs with epilepsy, melancholy, anxiety, attention deficit hyperactivity disorder, and challenging behaviors such as sleep and self-harm **[2]**. Autism can be classified as simplex or essential (when there are no physical abnormalities or microcephaly present), complicated (when there are dysmorphic traits and microcephaly present), or syndromic (when autism is part of a genetic condition that has already been described) **[3]**. Currently, several specific genetic variants are known to be associated with ASD. As of May 2023, the Simons Foundation Autism Research Initiative (SFARI) included a list of approximately 1195 risk genes and more than 20 recurrent copy number variant (CNV) loci that are relevant to both syndromic and non-syndromic autism (https://gene.sfari.org/). The American Academy of Pediatrics and the American College of Medical Genetics and Genomics both recommend chromosomal microarray (CMA), which is a technique that detects large duplications or deletions, as part of the first-tier evaluation for children with either a developmental disability or ASD **[4]**. Current guidelines recommend for ASD next-generation sequencing (NGS) panel testing if CMA-based evaluations yield a negative result.

On the other hand, the majority of these patients will have normal results on both tests. Recent research indicates that periodic negative Clinical Exome data reanalysis using improved bioinformatic tools and current gene–disease databases can identify additional candidate variants. Estimates suggest that the diagnostic yield by Clinical Exome could be increased by ~15 % when using up-to-date software, literature, and phenotypic information for reinterpretation **[5-10]**. Thus, there is a need to re-analyze negative Clinical Exome to give a better possibility for those who do not receive a molecular diagnosis in the first evaluation. In this study, using a clinical exome cohort enrolled in the Altamedica Center, we present a retrospective reanalysis of 28 negative clinical exome tests.

Methods

Patients included in this study were 28 patients with complex and simplex ASD, originally enrolled at Altamedica Medical Center. Written consent was obtained from the carers or guardians on behalf of the participating minors. Genomic DNA was extracted from peripheral blood using the DNeasy Blood & Tissue Kit and QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The array comparative genomic hybridization (aCGH) analysis was performed using the 44K platform (Agilent Technologies, Santa Clara, CA, USA) on DNA from blood to characterize the presence of DNA deletions or duplications, as previously reported. According to the manufacturer's instructions, clinical exome sequencing (CES) was carried out using the TruSight

One Sequencing Panel (Illumina, San Diego, CA, USA). The panel covers 4813 disease-associated genes. The targeted exonic regions underwent paired-end sequencing on an Illumina platform, using a NextSeq 550Dx sequencing system (Illumina, San Diego, CA, USA). The data analysis variants were carried out with the enGenome tool. The detected variants were annotated and filtered based on the information of the functional prediction tools [e.g., Polyphen2, SIFT, REVEL) and public disease variant databases (e.g., ClinVar, HGMD, OMIM, dbSNPs, and GWAS). The identified variants in the genes were verified using Sanger sequencing. The PCR was performed in a 50-µL reaction containing a final concentration of 1× PCR Buffer (Applied Biosystems, Foster City, CA, USA), 50 µmol/L each of dNTP, MgCl2 1.5 mM, 1.25 AmpliTaq Gold (Applied Biosystems), and 0.2 µmol/L each forward and reverse primers. The reaction mixture was subjected to 95◦C for 5 min, followed by 35 cycles of 95 ◦C for 15 s, 57 ◦C for 15 s, and 72 ◦C for 1 min, followed by 72 ◦C for 7 min. The cycle sequencing was performed using the BigDye version 3.1 terminator cycle-sequencing kit, according to the manufacturer's instructions (Applied Biosystems). The cycle-sequencing conditions were 95 ◦C for 30 s, followed by 35 cycles of 95 ◦C × 15 s, 50 ◦C × 15 s, and 60 ◦C × 4 min. The products were analyzed using a SeqStudio Genetic Analyzer (Applied Biosystems).

Results and Discussion

To emphasize the significance of CES reanalysis, we revisited a cohort of 28 patients with complex (n=9) and simplex ASD (n=19), originally enrolled at Altamedica Medical Center. We selected all negative Array Comparative Genomic Hybridization (Array-CGH) and negative clinical exome analyzed over more than 4 years to gain insight into the relative contribution of reanalysis strategies. Array CGH was performed to exclude microdeletion/microduplication copy number variants (CNV) as possible causes of ASD. We performed a systematic reanalysis, with an advanced clinical exome pipeline (i.e., including low-quality variants, copy number variant (CNV) analysis, and up-to-date disease-gene panels), of all patients in this cohort. Systematic follow-up of all patients without a conclusive diagnosis after diagnostic testing (n=28) revealed 4 new definitive genetic diagnoses in simplex ASD patients, resulting from updated bioinformatic information tools **(Table 1)**. In total, 14 % (4/28) of the patients in this study required reanalysis for conclusive diagnoses based on variants not identified in the initial analysis. For these 4 patients, the definitive genetic diagnosis was based on variants not prioritized in the initial CES analysis **(Table 1)**. Variants were detected after updated bioinformatic analyses and interpretation. Publications of the 4 disease-variants associations appeared after our initial research since they were not seen or prioritized during the initial CES analysis. This emphasizes the necessity of routinely reviewing CES data since novel genotype-phenotype relationships can be discovered at almost any moment.

Table 1. Novel genetic diagnoses after more than 4 years from the initial analysis.

Id Sample	Clinical Phenotype	Initial Analysis	Re-Analysis	Variant	Protein Change	Effect	Interpretation (Last Evaluated)
666	ASD	17/04/2017	AUTS2	c.1611C>A	p.His537Gln	Missense	Likely pathogenic (May 13, 2021)
59	ASD	19/06/2018	SHANK3	c.5209C>T	p.Gln1737Ter	Nonsense	Pathogenic (Dec 21, 2020)
245	ASD	11/03/2016	FOXP1	c.494del	p.Gly165fs	Frameshift	Pathogenic (Aug 23, 2022)
1518	ASD	01/09/2018	AUTS2	c.2218del	p.His740fs	Frameshift	Pathogenic (Oct 04, 2022)

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Informed Consent Statement: Informed consent was obtained from subjects involved in the study.

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Conflicts of Interest: The authors declare no conflict of interest.

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