

Genetic analysis of intellectual disability and autism

Pietro Chiurazzi^{1,2}, Aysha Karim Kiani³, Jan Miertus^{4,5}, Shila Barati³, Elena Manara³, Stefano Paolacci⁵, Liborio Stuppia⁶, Fiorella Gurrieri⁷, Matteo Bertelli^{3,5,8}

¹Istituto di Medicina Genomica, Università Cattolica del Sacro Cuore, Rome, Italy; ²Fondazione Policlinico Universitario “A. Gemelli” IRCCS, UOC Genetica Medica, Rome, Italy; ³MAGI EUREGIO, Bolzano, Italy; ⁴Génus n.o., Mestská Poliklinika, Starohájska 2, Trnava, Slovakia; ⁵MAGI'S LAB, Rovereto (TN), Italy; ⁶Dipartimento di Scienze Psicologiche della Salute e del Territorio, Università degli Studi “G. D’Annunzio”, Chieti, Italy; ⁷Genetica Medica, Università Campus Bio-Medico, Rome, Italy; ⁸EBTNA-LAB, Rovereto (TN), Italy

Abstract. *Background and aim:* Intellectual disability (ID) and autism spectrum disorders (ASD) are neurodevelopmental conditions that often co-exist and affect children from birth, impacting on their cognition and adaptive behaviour. Social interaction and communication ability are also severely impaired in ASD. Almost 1–3% of the population is affected and it has been estimated that approximately 30% of intellectual disability and autism is caused by genetic factors. The aim of this review is to summarize monogenic conditions characterized by intellectual disability and/or autism for which the causative genes have been identified. *Methods and Results:* We identified monogenic ID/ASD conditions through PubMed and other NCBI databases. Many such genes are located on the X chromosome (>150 out of 900 X-linked protein-coding genes), but at least 2000 human genes are estimated to be involved in ID/ASD. We selected 174 genes (64 X-linked and 110 autosomal) for an NGS panel in order to screen patients with ID and/or ASD, after fragile X syndrome and significant Copy Number Variants have been excluded. *Conclusions:* Accurate clinical and genetic diagnosis is required for precise treatment of these disorders, but due to their genetic heterogeneity, most cases remain undiagnosed. Next generation sequencing technologies have greatly enhanced the identification of new genes associated with intellectual disability and autism, ultimately leading to the development of better treatment options. (www.actabiomedica.it)

Key words: intellectual disability, autism spectrum disorders, next-generation sequencing, targeted gene panels

Intellectual Disability (ID) and Autism Spectrum Disorders (ASD) are neurodevelopmental disorders that derive from an altered function of our brain (1). Their clinical manifestations overlap just as much as their etiology. In fact, the development of a functional brain depends on a complex sequence of events that include neuronal and glial cell proliferation and migration, neuronal maturation and survival, efficient connectivity at both the axonal as well as the synaptic level. Thousands of genes encode the many proteins that are needed for an efficient brain function, therefore next-generation sequencing greatly facilitates the identification of genetic determinants of ID and ASD (2).

Intellectual Disability

Intellectual disability (ID), previously known as “mental retardation” (3), represents a major public health concern. It is characterized by a congenital deficit in intellectual function and adaptive behaviour, impacting on social interactions as well as on mental and practical abilities of affected individuals. ID may be “isolated” or “syndromic” when patients have a peculiar facies, specific physical signs and/or an abnormal growth pattern. Many genetic and environmental factors, may cause intellectual disability (1). Common (but potentially preventable) causes of ID are iodine defi-

ciency and malnutrition, affecting millions of people worldwide. Prenatal infections or maternal exposure to toxic substances (including alcohol, nicotine and several teratogenic drugs) as well as premature birth and perinatal asphyxia, may result in mild to severe ID in the child. The frequency of these “external” factors varies greatly among different countries and depends on (maternal) lifestyle and quality of health care, both indirectly influenced by socio-economic conditions. Genetic factors causing and/or contributing to ID (from chromosomal imbalances to monogenic syndromes) are less variable in frequency and increase with parental age (chromosomal non-disjunctions increase with maternal age while dominant *de novo* point mutations increase with paternal age). The reported prevalence of ID in children in the United States was 1.1-1.2% between 2014 and 2016 (4). In 2016, Chiurazzi and Pirozzi (1) listed 818 human genes associated with ID by searching the OMIM database (Table S1 - not present in the article, please see at <https://mattiolihealth.com/wp-content/uploads/2020/11/Supplementary-Tables-10684.zip>); now we retrieved 1356 human genes from the OMIM database whose entries contain one of the following keywords: “intellectual disability”, “mental retardation”, “cognitive impairment” or “developmental delay” (Table S2 - not present in the article, please see at <https://mattiolihealth.com/wp-content/uploads/2020/11/Supplementary-Tables-10684.zip>). Another valuable source of ID genes is provided by the SysID database (5), which also provides a useful distinction of the clinical phenotypes, based on disease severity and complexity (“sindromicity”): SysID presently lists 2588 human genes that can be easily accessed at <https://sysid.cmbi.umcn.nl/> (Table S3 - not present in the article, please see at <https://mattiolihealth.com/wp-content/uploads/2020/11/Supplementary-Tables-10684.zip>). Many of these genes have been found mutated in just one family and therefore their causative role should still be proven either by finding new unrelated patients with pathogenic variants or by functional studies (6). A note on the clinical phenotype should be made: broadly speaking ID is “isolated” or “pure” when the causative gene is expressed only in the brain while “ID syndromes” are either due to variants in a ubiquitously expressed gene that affect several tissues or to microdeletions/duplications (Copy Num-

ber Variants) involving contiguous genes (1). For instance the *FMR1* gene is widely expressed in all tissues whereas the expression of *SYNGAP1* is restricted to the brain: in fact absence of the *FMR1* protein cause fragile X syndrome (7) while variants in *SYNGAP1* and *SHANK3*, encoding synaptic proteins, are associated with non-syndromic intellectual disability (8).

Autism Spectrum Disorders

Children with intellectual disability have a significantly higher risk of autistic behaviour, stereotyped movements, neuromuscular deficits and epilepsy that affect their daily life and well-being. Autism Spectrum Disorder (ASD), just as ID, is a broad clinical definition including neurodevelopmental conditions characterized by deficient social interactions, poor or absent communication, repetitive behaviours and apparently limited interests (9). ASD generally becomes apparent after the first year of life and it has been reported in an increasing number (2.2-2.7%) of children between 2014 and 2016 in the United States (4). ASD, but not ID, in a child has also an important effect on parental emotional and mental health (10). The Simons Foundation Autism Research Initiative aims at identifying the genetic determinants of ASD and its database (<https://gene.sfari.org/database/human-gene/>) now lists 960 different genes (Table S4 - not present in the article, please see at <https://mattiolihealth.com/wp-content/uploads/2020/11/Supplementary-Tables-10684.zip>), many of which are also responsible for ID. In fact, autism often coexists with intellectual disability, with 70% of ASD patients suffering also from ID whereas 40% of ID patients have ASD. The extended phenotypic overlap between ID and ASD is not surprising since they both derive from a more or less subtle alteration of brain functions and pathogenic variants in hundreds of genes expressed in neurons and/or glia cells underlie their pathogenesis, as proven in animal models (11).

Genetic determinants and molecular pathways of ID and ASD

For the accurate development as well as functioning of the brain, the coordinated and timely work of hundreds of genes is essential; 2000 to 3500 genes

critical for brain function and involved in neurodevelopmental disorders has been made (1). Given the large number of proteins that must be produced at the right time and right amount, it is not surprising that both ID and ASD are characterized by a great genetic heterogeneity (12). However, the first-tier genetic test that should be recommended is array-CGH (13), in order to exclude copy number variants (CNV), possibly integrated by karyotyping when a chromosome translocation is suspected. Another cheap and useful first-tier assay looks for the possible CGG expansion in the *FMR1* gene, since fragile X syndrome should be excluded before further testing, although recent reports argue that it should not be performed in the absence of family history and/or clinical features (14). Fragile X syndrome was the first monogenic ID condition whose gene was identified (in 1991), partly because of its frequency (relatively high, thanks to dynamic nature of its mutational mechanism) and partly because X-linked transmission is more easily recognized (7). In fact, X-linked conditions were identified first because of their typical inheritance pattern (affected males connected by carrier females) and, while 82 genes on the X chromosome had been associated with intellectual disability in 2008 (15), presently OMIM lists 167 X-linked ID genes (Table S2 - not present in the article, please see at <https://mattiolihealth.com/wp-content/uploads/2020/11/Supplementary-Tables-10684.zip>). Then, the improvement of sequencing technology facilitated the analysis of (even small) autosomal recessive families (16) and of sporadic patients with autosomal dominant *de novo* mutations (17): as indicated in Table S2, there are now 1170 genes in OMIM whose mutations result in ID. The corresponding proteins play very different roles in the various cells, as gene ontology analysis revealed (1), however their functions ultimately converge on key biological pathways (18). Also in ASD most genes work in converging networks that are enriched in neuronal signaling, synaptic function, chromatin remodeling and channel activity (19,20).

Next-generation sequencing technologies in ID/ASD diagnostics

An accurate molecular diagnosis of neurodevelopmental disorders is important for their eventual

treatment. However, differentiating between these clinically overlapping conditions with huge genetic heterogeneity is very difficult and up to 50% patients suffering from ID and/or ASD remain molecularly undiagnosed. Next-generation sequencing (NGS) technologies have greatly improved the chance of identifying known as well as novel responsible genes, hence allowing clinicians to establish a molecular diagnosis in a time- as well as cost-effective manner (21-23). The most powerful techniques involve NGS sequencing of known coding exons ("whole exome" sequencing i.e. WES) or possibly the "whole genome" (WGS) but these approaches require large investment on data storage and bioinformatic analysis as well as the availability of DNA from at least the patient's parents (trio analysis) in order to sift through the huge amount of genetic variants that will be identified. Harripaul et al. (21) provide an excellent review of the evolution of NGS technologies with their potentials and pitfalls, while Han and Lee (22) describe the possible strategies and stepwise approach to diagnosing children with ID and/or developmental delay. Although WES and WGS may seem the best option to tackle the problem, increasing the amount of data comes with its limitations (e.g. insufficient coverage of part of some genes) and risks (e.g. incidental findings), even if increased costs were not a problem. Therefore, the choice of targeted gene panels as second-tier approach after chromosome studies, *FMR1* screening and array-CGH (22) is probably wise, given their higher coverage of the selected genes and the possibility of analyzing only the proband without his parents.

Gene panel sequencing

Targeted gene panels are indeed useful tools for parallel analysis of clinically relevant genes that should be "deep" sequenced with very high coverage in order to reliably exclude not only the presence of variants in phenotype-related genes, but also intragenic deletions/duplications (24). Hundreds of genes linked with intellectual disability are presently included in several academic as well as commercial panels that also allow some extent of customization related to targeted genes number and identity. Multiple factors regulate gene panel size, like the incidence of mutations in a spe-

cific gene among patients, clinical heterogeneity of the tested population, available infrastructure for sequencing and bioinformatic analysis as well as the clinical and analytical capabilities of the involved institute or center (see the Genetic Testing Registry at <https://www.ncbi.nlm.nih.gov/gtr/>). Redin et al. (25) reached a diagnostic yield of 25% when 106 selected patients with ID (but without congenital malformations, fragile X syndrome or CNV detectable by array-CGH), using a targeted gene panel with 99 X-linked and 118 autosomal genes. One year later, in 2015, Grozeva et al. (26) reported on their less selected population of 986 patients with moderate to severe ID screened with a larger panel of 565 genes and found likely pathogenic variants in 11% of them. More recently Yan et al. (27) used a panel with 454 genes to screen 112 Chinese patients, reaching a definite diagnosis in 9 of them (8% yield). Finally, Aspromonte et al. (28) designed a smaller panel including just 74 genes belonging to molecular pathways involved in the pathogenesis of both ID and ASD: given their careful selection of both genes and patients (negative for CNV, *FMR1* expansion, deletions/imprinting defects in 15q11q13, as well as variants in *MECP2*, *CDKL5* and *UBE3A*), they reached a 27% total diagnostic yield (41/150 patients). Considering all these previous experiences, we designed a new NGS panel targeting 174 genes (Table S5 - not present in the article, please see at <https://mattiolihealth.com/wp-content/uploads/2020/11/Supplementary-Tables-10684.zip>) that is being used to screen patients with ID and/or ASD preselected with array-CGH and *FMR1* CGG testing. We expect this approach to deliver a rapid, cost-efficient and sensitive analysis. Although gene panels are mostly useful for rare diseases that have well defined molecular origins, such an approach may serve well as second-tier screening test for individual patients. In case of a negative result, if DNA from relevant relatives is available, genome-wide techniques such as WES or WGS will be considered. However, given the rapid pace at which genetic knowledge accumulates, we anticipate that the panel structure will require a regular update to include newly identified genes and exclude those that are much less frequently mutated.

Conclusion

Intellectual disability and autism spectrum disorders are neurodevelopmental conditions characterized by cognitive impairment, defective adaptive behaviour and limited social interactions. ID and ASD have several environmental and genetic causes and 1400 human genes are described in OMIM associated either of the two or both. An accurate clinical as well as molecular diagnosis is essential for a deeper understanding of the pathogenesis of these conditions and for devising effective treatments. In fact, though some preclinical trials are ongoing, current therapeutic strategies are mostly symptomatic and aimed at controlling hyperactivity, anxiety, depression or epilepsy; sometimes patients develop undesired side effects especially when too many medications are co-administered. Therefore, NGS technology will hopefully facilitate an accurate diagnosis of the molecular basis of each individual condition so that every patient may receive a tailored treatment, fulfilling the promise of Precision Medicine (29).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Correspondence:
Pietro Chiurazzi, MD, PhD
Postal address: Istituto di Medicina Genomica,
Università Cattolica del Sacro Cuore,
Largo Francesco Vito 1, 00168 Rome, Italy
E-mail: pietro.chiurazzi@unicatt.it