GENETIC FACTORS INVOLVED IN AUTISM SPECTRUM DISORDERS

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S u m m a r y. The review of the literature shows the researchers agreed that the occurrence of autism in monozygotic twins is twice as frequent as in dizygotic twins, indicating a high level of inheritance (60% -90%), and suggesting a less significant involvement of common environmental factors. Clinical and genetic heterogeneity of the disease is an important obstacle to correlate the genotype with the autistic phenotype, which in combination with the polygenic background makes linking the specific presence of clinical features to a single gene or even a set of genes very difficult.

K e y w o r d s: genetic factors, autism spectrum disorders

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In the last 15 years, the knowledge about the genetic background of autism has grown considerably. In the earlier period, the most common research strategy was the use of a quite controversial hypothesis, i.e. common disease-common variant, assuming that the polymorphisms frequently occurring in the general population influence the phenotype (clinical symptoms) of common diseases [1]. Unfortunately, genomic-wide association studies (GWAS), which identify genetic risk factors of common diseases, found only a few common variants as more promising [1a]. Among other things, mutations of genes *SHANK3, NRXN1, NLGN3, NLGN4X* and *CNTNAP2* have been linked to autism [2, 3].

The opinions about the source of mutations responsible for the susceptibility to ASD are fluctuating. Some sources emphasize greater involvement of *de novo* mutation [27], others state that congenital factors, both rare and common gene variants deserve more attention [21]. So far, no com-

mon variants have been convincingly confirmed as predisposing to ASD, which may suggest that the rarest variants are responsible for the risk of developing this disease [22]. In particular, changes in the genes involved in synaptic conduction are most often cited in genetic and functional animal studies [58]. Some researchers present common genetic variants as more prominent in inheriting ASD [21].

Lack of significant success aside ambiguity of GWAS research is not limited to autism. Despite large financial funding for the methodology, the majority of genetic variations related to the risk of common diseases still remain unexplained. One of the more interesting discoveries regarding ASD was the identification of a common variant located between the 9th and 10th cadherin intergenic region, the discovery of particular importance in the context of the mechanism of neuronal conduction in which cadherin proteins play a particularly important role [4].

The co-existence of so-called monogenic diseases inherited according to Mendelian laws is observed in the course of ASD. It is recorded in about 10-15% of ASD cases. The most common monogenic diseases co-existing with ASD are fragile X syndrome (*FMR1* gene), Rett syndrome (*MECP2* gene), tuberous sclerosis (*TSC1* gene), neurofibromatosis type I (*NF1* gene), Cowden syndrome (*PTEN* gene), Joubert syndrome (*AHI1* gene), and Timothy syndrome (*CACNA1C* gene), as well as Smith, Lemli and Opitz syndromes (*DHCR7* gene) [5, 6].

Also interesting is the fact that both ASD and intellectual disability (ID) occur together in a significant number of cases. Both diseases exhibit a high degree of heterogeneity, and appear to be closely related in biochemical and molecular terms. Approximately 70% of patients with ASD show some level of intellectual disorders while the remaining 30% have other disorders, and about 10% of patients with intellectual disabilities have autistic symptoms [7].

In 1997, the studies on twins provided first serious scientific evidence on the important role of genetic etiology [8, 9, 10, 11, 12]. The review of the literature shows the researchers agreed that the occurrence of autism in monozygotic twins is twice as frequent as in dizygotic twins, indicating a high level of inheritance (60% -90%), and suggesting a less significant involvement of common environmental factors [12a, 13, 14, 15, 16].

Other publications cite a lower percentage of genetic impact but a greater environmental impact [17, 18, 19, 20]. Therefore, the autistic phenotype can be attributed to the significant impact of genetic conditions, and the indirect impact of environmental factors. A more general statement that autism is a complex disease consisting not only in the interaction of genes with each other, but also the interaction of genes with other non-genetic factors seems to adequately define the etiology of the disease.

Traditional approach of genetic testing to the clinical evaluation of psychiatric genotype and phenotype is based on a comparative analysis of disease-related cases with control group. Many of such studies have produced quite tangible results, combining congenital common polymorphisms and single *de novo* with the risk of developing ASD [21, 22].

Commonly genotyped single nucleotide polymorphisms (SNPs) can be considered causal in at least 20% ASD [23, 24, 25]. The *de novo* variants were found in 10-20% of cases, although cumulative *de novo* mutations account for less than 5% of the causes of ASD [26]. Some SNPs implicated in the risk of developing autism are also associated with the risk of schizophrenia [27], a fact that also correlates with the results of diagnostics assessing the effects of copy number variants (CNV) [28, 29]. CNV in the chromosomal regions 1q21 and 15q13 are often associated with mental diseases such as schizophrenia, autism, epilepsy, and delayed mental development.

Almost all gene mutations considered as risk factors for ASD are also found in healthy individuals. One example are patients who have the abovementioned genetic changes or a deletion in the 16p11.2 region, which is also considered one of the major risks for developing ASD. Healthy people, parents of an autistic child, have these mutations and do not meet other criteria for the diagnosis of ASD [30]. These observations indicate that chromosomal aberrations alone are neither necessary nor sufficient to cause autism, rather they indicate patient's considerable susceptibility to a wide range of psychiatric symptoms.

Besides, considerable differences in the quality of social interactions and the ability to communicate with others are observed among the healthy population [31]. Despite observed and defined phenotypic differences of mental disorders, the linking of genetic changes with specific neuropsychiatric diseases and associating them with characteristic differences in social behavior may be difficult.

Disregarding different theories on the etiology, genetic research and testing are still considered critical in the diagnosis of ASD, and numerous heterogeneous genetic changes are found throughout the entire genome in the patients affected by the disease [32].

ASD has also been associated with known genetic factors that accompany other diseases of the nervous system in about 10-15% of cases. One of the most frequently quoted aberrations are fragile X syndrome (about 3%), tuberous sclerosis (about 2%) as well as other cytogenetic changes such as duplication in the 15q1-q13 region (about 2%), and deletions with duplications in the region 16p11 (around 1%) [33]. None of these genetic changes is specific to ASD, but rather characteristic of a certain range of phenotypes, including intellectual development of various degree. In recent years, a project using sequencing of the whole exome has again confirmed that a single gene associated with a significant ASD risk cannot be indicated. Rare variants considered as more likely to be causative were found scattered among hundreds of different genes [35].

GENETIC DIAGNOSIS OF ASD BY THE ACGH

Considering classic cytogenetic studies in ASD, diagnostic efficiency of conventional karyotyping is only around 3% [34]. One of the key elements of precise diagnosis of genetic diseases is the detection of changes in the number of DNA copies in the patient's genome, and therefore molecular diagnostic methods providing the most satisfactory results are most useful. The diagnostic efficiency of molecular tests increases in patients who have autistic symptoms accompanied by other clinical features, e.g. dysmorphism and/or delayed intellectual development [1].

One of the more useful techniques is array Comparative Genomic Hybridization, (aCGH). It consists in a comparative analysis of the patient's DNA and the reference DNA of a healthy person (control). Equivalent amounts of DNA are hybridized to so-called matrix. The matrix is a glass or plastic plate on which arranged molecular probes are placed, to which the fragments of the standard and control DNA are hybridized. This comparative method allows for the detection of deletions or amplifications of DNA fragments. Microarrays allow a fairly precise detection of unbalanced genomic changes. However, the method does not allow for the diagnosis of gene balanced rearrangements and chromosomal rearrangements [1].

Comparative microarray genomic hybridization studies are widely used to identify unbalanced changes in the genome of patients with intellectual disabilities, autism, dysmorphic traits, congenital malformations, as well as in oncological and prenatal diagnostics [37].

Depending on the research profile, two different diagnostic approaches are applied. Targeted microarrays that serve to detect known, predetermined, clinically significant genetic changes, and whole-genome microarrays of the entire genome. The detection of *de novo* genetic changes is much easier when whole-genome microarrays are used. The clinical performance and resolution of microarrays depends on the number of DNA probes, counted from hundreds of thousands to over one million probes [38].

aCGH is recognized as a clinically relevant and comprehensive diagnostic tool for genomic testing, in particular for the detection of sub-microscopic deletions and group-defined duplications as copy number variability (CNV). High resolution is a very important aspect of this method as it allows to significantly increase the diagnostic precision, especially as the impact of rare CNV is widely recognized in the pathogenesis of ASD, and high resolution of diagnostic methodology is very important in that respect [35].

Chromosomal disorders are classified as pathogenic if supported by convincing clinical evidence, as variants of undetermined clinical value if the evidence is less convincing, or as minor polymorphisms [36, 37, 38].

The diagnostic value of aCGH technique has been shown in various clinical scenarios, and it is recommended as the first-choice method for genetic diagnosis of ASD patients, offering high detection sensitivity of submicroscopic gene changes [39, 40, 41]. The frequency of diagnosis of clinically significant CNVs by means of aCGH in patients with ASD ranged within 7% - 9% [42, 43]. Tammimies et al. demonstrated that diagnostic performance is significantly higher in patients with a more complex phenotype [44]. In other studies, the total diagnostic value of aCGH for ASD patients ranged from 18.2% to 22% [45, 46, 47, 48].

Among patients with ASD, pathogenic CNVs are most often localized in chromosomes 1, 4, 6, 8, 21, 22 and X [49, 50]. Also, chromosome 15 has five break sites along the proximal long arm, referred to as BP1-BP5, which contain

a reduced copy number of repeats, facilitating homologous recombination and subsequent potential gene expression changes [51, 52]. One of the more frequently cited genetic changes considered important in the etiology of neurodevelopmental diseases are repetitive microdeletions and microduplications of chromosome region of 15q11.2 BP1-BP2. The CNVs found in this region include the genes *TUBGCP5, CYFIP1, NIPA2* and *NIPA1*, which are considered to play an important role in axonal development and neural connections. [53, 54, 55]. Most studies confirm that deletions in this chromosomal region correspond to delayed speech development, movement disorders, autism, epilepsy, and also dysmorphic changes [56]. Of course, not all patients with genetic changes in this region have clinical symptoms, which can also be attributed to the phenomenon of incomplete penetration and variable gene expression [57].

Despite some success in identifying genetic changes that potentially contribute to the development of autism, including CNVs, the etiology of autism is still unexplained in most cases [28].

CONCLUSIONS

Clinical and genetic heterogeneity of the disease is an important obstacle to correlate the genotype with the autistic phenotype, which in combination with the polygenic background makes linking the specific presence of clinical features to a single gene or even a set of genes very difficult [28].

REFERENCES

1. Cook EH Jr, Sherer MR. Copy-number variations associated with neuropsychiatric conditions. Nature 2008;455:919–923.

1a. Veenstra-Vanderweele J, Christian SL, Cook EH Jr. Autism as a paradigmatic complex genetic disorder. Annu Rev Genomics Hum Genet 2004;5:379–405.

2. C. Betancur, "Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting," Brain Research, vol. 1380, pp. 42–77, 2011.

3. D. Pinto, A. T. Pagnamenta, L. Klei et al., "Functional impact of global rare copy number variation in autism spectrum disorders, " Nature, vol. 466, pp. 368–372, 2010.

4. Wang K, Zhang H, Ma D, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 2009;459:528–533.

5. 11. Miles J.H.: Autism spectrum disorders-a genetics review.Genet. Med., 2011, 13(4), 278-294.

6. Devlin B., Scherer S.W.: Genetic architecture in autism spectrum disorder. Curr. Opin. Genet. Dev., 2012, 22(3), 229-237.

7. Folstein S, RutterM. Genetic influences and infantile autism. Nature. 1977;265(5596):726-728.

8. Folstein S, RutterM. Infantile autism: a genetic study of 21 twin pairs. J Child Psychol Psychiatry. 1977;18(4):297-321.

9. Ritvo ER, Freeman BJ, Mason-Brothers A,MoA, Ritvo AM. Concordance for the syndrome of autism in 40 pairs of afflicted twins. Am J Psychiatry. 1985;142(1):74-77.

10. Steffenburg S, Gillberg C, Hellgren L, et al. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J Child Psychol Psychiatry. 1989;30(3):405-416.

11. Bailey A, Le Couteur A, Gottesman I, et al. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med. 1995;25(1): 63-77.

12. Lichtenstein P, Carlström E, RåstamM, Gillberg C, Anckarsäter H. The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. Am J Psychiatry. 2010;167(11):1357-1363.

12a. Ronald A, Happé F, Plomin R. The genetic relationship between individual differences in social and nonsocial behaviours characteristic of autism. Dev Sci. 2005;8(5):444-458.

13. Ronald A, Happé F, Price TS, Baron-Cohen S, Plomin R. Phenotypic and genetic overlap between autistic traits at the extremes of the general population. J AmAcad Child Adolesc Psychiatry. 2006;45(10):1206-1214.

14. Ronald A, Happé F, Plomin R. A twin study investigating the genetic and environmental aetiologies of parent, teacher and child ratings of autistic-like traits and their overlap. Eur Child Adolesc Psychiatry. 2008;17(8):473-483.

15. Skuse DH, Mandy WPL, Scourfield J. Measuring autistic traits: heritability, reliability and validity of the Social and Communication Disorders Checklist. Br J Psychiatry. 2005;187:568-572.

16. Hoekstra RA, Bartels M, Verweij CJ, Boomsma DI. Heritability of autistic traits in the general population. Arch Pediatr Adolesc Med. 2007;161(4): 372-377.

17. Hallmayer J, Cleveland S, Torres A, et al. Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry. 2011;68(11):1095-1102.

18. Frazier TW, Thompson L, Youngstrom EA, et al. A twin study of heritable and shared environmental contributions to autism. J Autism Dev Disord. 2014; 44(8):2013-2025.
19. Nordenbæk C, Jørgensen M, Kyvik KO, Bilenberg N. A Danish population-based twin study on autism spectrum disorders. Eur Child Adolesc Psychiatry. 2014;23(1):35-43.
20. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The familial risk of autism. JAMA. 2014;311(17):1770-1777.
21. Gaugler T, et al. Most genetic risk for autism resides with common variation. Nat Genet. 2014; 46:881–885.
22. Krumm N, et al. Excess of rare, inherited truncating mutations in autism. Nat Genet. 2015; 47:582–588.
23. Lee SH, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet. 2013; 45:984–994.
24. Bulik-Sullivan BK, et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat Genet. 2015; 47:291–295.
25. De Rubeis S, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature. 2014;515:209–215.
26. Iossifov I, et al. The contribution of de novo coding mutations to autism spectrum disorder. Nature. 2014;515:216–221.
27. Cross-Disorder Group of the Psychiatric Genomics, C. et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet. 2013; 45:984–994.
28. Stefansson H, et al. CNVs conferring risk of autism or schizophrenia affect cognition in controls. Nature. 2014; 505:361–366.
29. Talkowski ME, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell. 2012; 149:525–537.
30. Hanson E, et al. The cognitive and behavioral phenotype of the 16p11.2 deletion in a clinically ascertained population. Biol Psychiatry. 2015; 77:785–793.
31. Plomin R, Haworth CM, Davis OS. Common disorders are quantitative traits. Nat Rev Genet. 2009; 10:872–878.
32. Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L (2006) Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. Mol Psychiatry 11(1):18–28.
33. Kumar R, Christian S. Genetics of autism spectrum disorders. Curr Neurol Neurosci Rep 2009;9:188–197.
34. Reddy KS (2005) Cytogenetic abnormalities and fragile-X syndrome in autism spectrum disorder. BMC Med Genet 6:3.
35. Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R et al (2010) Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466(7304):368–372.
36. Riggs ER, Church DM, Hanson K, Horner VL, Kaminsky EB, Kuhn RM, et al. Towards an evidence-based process for the clinical interpretation of copy number variation. Clin Genet. 2012;81:403–12.
37. Rodriguez-Revenga L, Mila M, Rosenberg C, Lamb A, Lee C. Structural variation in the human genome: the impact of copy number variants on clinical diagnosis. Genet Med. 2007;9:600–6.
38. Hanemaaijer NM, Sikkema-Raddatz B, van der Vries G, Dijkhuizen T, Hordijk R, van Essen AJ, et al. Practical guidelines for interpreting copy number gains detected by high-resolution array in routine diagnostics. Eur J Hum Genet. 2012;20:161–5.
39. Roberts JL, Hovanes K, Dasouki M, Manzardo AM, Butler MG (2014) Chromosomal microarray analysis of consecutive individuals with autism spectrum disorders or learning disability presenting for genetic services. Gene 535(1):70–78.
40. Nicholl J, Waters W, Mulley JC, Suwalski S, Brown S, Hull Y et al (2014) Cognitive deficit and autism spectrum disorders: prospective diagnosis by array CGH. Pathology 46(1):41–45.
41. Battaglia A, Doccini V, Bernardini L, Novelli A, Loddo S, Capalbo A et al (2013) Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features. Eur J Paediatr Neurol 17(6):589–599.
42. McGrew SG, Peters BR, Crittendon JA, Veenstra-Vanderweele J (2012) Diagnostic yield of chromosomal microarray analysis in an autism primary care practice: which guidelines to implement? J Autism Dev Disord 42(8):1582–1591.
43. Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB et al (2010) Clinical genetic testing for patients with autism spectrum disorders. Pediatrics 125(4):e727–e735.

44. Tammimies K, Marshall CR, Walker S, Kaur G, Thiruvahindrapuram B, Lionel AC et al (2015) Molecular diagnostic yield of chromosomal microarray analysis and whole-exome sequencing in children with autism spectrum disorder. JAMA 314(9):895–903.
45. Roberts JL, Hovanes K, Dasouki M, Manzardo AM, Butler MG. Chromosomal microarray analysis of consecutive individuals with autism spectrum disorders or learning disability presenting for genetic services. Gene. 2014;535:70–8.
46. Schaefer GB, Starr L, Pickering D, Skar G, Dehaai K, Sanger WG. Array comparative genomic hybridization findings in a cohort referred for an autism evaluation. J Child Neurol. 2010;25:1498–503.
47. El-Fishawy P, State MW. The genetics of autism: key issues, recent findings, and clinical implications. Psychiatr Clin North Am. 2010;33:83–105.
48. Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB, et al. Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration. Clinical genetic testing for patients with autism spectrum disorders. Pediatrics. 2010;125:e727–35.
49. Roberts JL, Hovanes K, Dasouki M, Manzardo AM, Butler MG. Chromosomal microarray analysis of consecutive individuals with autism spectrum disorders or learning disability presenting for genetic services. Gene. 2014;535:70–8
50. Schroer RJ, Phelan MC, Michaelis RC, Crawford EC, Skinner SA, Cuccaro M, et al. Autism and maternally derived aberrations of chromosome 15q. Am J Med Genet. 1998;76:327–36.
51. Locke, D.P.; Segraves, R.; Nicholls, R.D.; Schwartz, S.; Pinkel, D.; Alberston, D.G.; Eichler, E.E. BAC microarray analysis of 15q–q13 rearrangements and the impact of segmental duplications. J. Med. Genet. 2004, 41, 175–182.
52. Pujana, M.A.; Nadal, M.; Guitart, M.; Armengol, L.; Gratacos, M.; Estivill, X. Human Chromosome 15q11–q14 regions of rearrangements contain clusters of LCR15 duplicons. Eur. J. Hum. Genet. 2002, 10, 26–35.
53. Chen CP, Lin SP, Lee CL, Chern SR, Wu PS, Chen YN, Chen SW, Wang W. Familial transmission of recurrent 15q11.2 (BP1-BP2) microdeletion encompassing NIPA1, NIPA2, CYFIP1, and TUBGCP5 associated with phenotypic variability in developmental, speech, and motor delay. Taiwan J Obstet Gynecol. 2017 Feb;56(1):93-97
54. Vanlerberghe et al. 15q11.2 microdeletion (BP1-BP2) and developmental delay, behaviour issues, epilepsy and congenital heart disease: a series of 52 patients. Eur J Med Genet. 2015 Mar;58(3):140-7. doi: 10.1016/j.ejmg.2015.01.002.
55. Picinelli C, Lintas C, Piras IS, Gabriele S, Sacco R, Brogna C, Persico AM. Recurrent 15q11.2 BP1-BP2 microdeletions and microduplications in the etiology of neurodevelopmental disorders. Am J Med Genet B Neuropsychiatr Genet. 2016 Dec;171(8):1088-1098.
56. Jerkovich, A.M.; Butler, M.G. Further phenotypic expansion of 15q11.2 BP1–BP2 microdeletion (Burnside-Butler) syndrome. J. Pediatr. Genet. 2014, 3, 41–44
57. Burnside et al. Microdeletion/microduplication of proximal 15q11.2 between BP1 and BP2: A susceptibility region for neurological dysfunction including developmental and language delay. Hum. Genet. 2011, 130, 517–528.
58. Gai X, Xie HM, Perin JC, et al. Rare structural variation of synapse and neurotransmission genes in autism. *Molecular Psychiatry*. 2012;17(4):402-411.