Supplementary material

Dystrophin genotype and risk of neuropsychiatric disorders in dystrophinopathies: A systematic review and meta-analysis

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Supplementary table 1. Excluded studies with reasons.

Reference	Reason for exclusion
Banihani R et al (2015) [1]	Not outcomes of interest
Conway KC et al (2015) [2]	Not outcomes of interest
Gosar D et al (2021) [3]	Not outcomes of interest
Hendriksen JGM et al (2008) [4]	No genotype available
Hendriksen RGF et al (2018) [5]	Not outcomes of interest
Hinton VJ et al (2009) [6]	Not outcomes of interest
Latimer R et al (2017) [7]	No genotype available
Ozer U et al (2019) [8]	No genotype available
Pangalila RF et al (2015) [9]	No genotype available
Wu JY et al (2005) [10]	No genotype available

Supplementary references

[1]- Banihani R, Smile S, Yoon G, Dupuis A, Mosleh M, Snider A, et al. Cognitive and Neurobehavioral Profile in Boys With Duchenne Muscular Dystrophy. J Child Neurol. 2015 Oct;30(11):1472–82.

[2]- Conway KC, Mathews KD, Paramsothy P, Oleszek J, Trout C, Zhang Y, et al. Neurobehavioral Concerns Among Males with Dystrophinopathy Using Population-Based Surveillance Data from the Muscular Dystrophy Surveillance, Tracking, and Research Network. J Dev Behav Pediatr. 2015;36(6):455–63.

[3]- Gosar D, Košmrlj L, Musek PL, Meško T, Stropnik S, Krkoč V, et al. Adaptive skills and mental health in children and adolescents with neuromuscular diseases. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2021 Jan;30:134–43.

[4]- Hendriksen JGM, Vles JSH. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive--compulsive disorder. J Child Neurol. 2008 May;23(5):477–81.

[5]- Hendriksen RGF, Vles JSH, Aalbers MW, Chin RFM, Hendriksen JGM. Brain-related comorbidities in boys and men with Duchenne Muscular Dystrophy: A descriptive study. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2018 May;22(3):488–97.

[6]- Hinton VJ, Cyrulnik SE, Fee RJ, Batchelder A, Kiefel JM, Goldstein EM, et al. Association of autistic spectrum disorders with dystrophinopathies. Pediatr Neurol. 2009 Nov;41(5):339–46.

[7]- Latimer R, Street N, Conway KC, James K, Cunniff C, Oleszek J, et al. Secondary Conditions Among Males With Duchenne or Becker Muscular Dystrophy. J Child Neurol. 2017 Jun;32(7):663–70.

[8]- Ozer U, Tufan AE. Psychiatric comorbidities in cases with Duchenne muscular dystrophy: A case series. Dusunen Adam [Internet]. 2019;32(4):359–64. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85077876314&doi=10.14744%2FDAJPNS.2019.00052&partnerID=40&md5=28889347fdf2dded8fb8fcebd91e9b5e

[9]- Pangalila RF, van den Bos GA, Bartels B, Bergen M, Stam HJ, Roebroeck ME. Prevalence of fatigue, pain, and affective disorders in adults with duchenne muscular dystrophy and their associations with quality of life. Arch Phys Med Rehabil. 2015 Jul;96(7):1242–7.

[10]- Wu JY, Kuban KCK, Allred E, Shapiro F, Darras BT. Association of Duchenne muscular dystrophy with autism spectrum disorder. J Child Neurol. 2005 Oct;20(10):790–5.

Supplementary table 2. Diagnostic criteria for dystrophinopathies.

A. Advantages and disadvantages of the diagnostic methods of dystrophinopathies used by the authors of the included studies.

Diagnostic method	Advantage	Disadvantages
A. Genetic testing		
Multiplex Polymerase Chain Reaction (PCR)	Widely available and inexpensive	A priori only detects deletions Does not cover the entire gene
Multiplex ligation-dependent probe amplification (MLPA)	It detects more mutations than multiplex PCR It detects deletions and duplications It covers all exons It allows carrier study	Laborious and expensive design, although there are methods that make it affordable
Southern blotting	It detects deletions and duplications It determines the deletion and duplication endpoints, determining the effect on the reading frame	It requires isotopes and high molecular weight DNA It is tedious, and more time is required
Direct sequencing	In addition to detecting other mutations, it is very useful in point mutations	It is impractical and expensive
B. Muscle biopsy		
Immunohistochemistry	Very profitable High reliability in DMD and BMD: In DMD no fibers are detected, only reverted fibers (50% of patients); in BMD, reduction and/or irregularity in the staining of muscle fibers is observed	More sensitive to degradation or decomposition than Western Blot
Western blot	Very sensible It differentiates DMD from BMD better than immunohistochemistry Although it is rarely used, it has prognostic value: small amounts of functional dystrophin synthesized in some patients with DMD can be quantified, being associated with milder phenotypes	Different muscles may have different expression of dystrophin It is laborious and expensive

B. Diagnostic methods of dystrophinopathies used in each study.

Reference	Diagnostic method
Lambert JT et al (2020)	Clinical manifestation of BMD (exercise intolerance, elevated CPK, etc.), male gender, and diagnosis by genetic testing and/or biopsy
Mori-Yoshimura M et al (2018)	Remudy Database: Japanese Population Registry with Genetically Confirmed DMD/BMD. If it could not be confirmed with MLPA, the gene was sequenced. Clinically, BMD was considered when the person with confirmed dystrophinopathy was ambulant at 17 years of age
Mori-Yoshimura M et al (2019)	Participants with genetically or immunohistochemically confirmed BMD. Clinically, BMD was considered when the person with confirmed dystrophinopathy was ambulant at 17 years of age
Colombo P et al (2017)	Participants with DMD confirmed genetically or by muscle biopsy
Darmahkasih AJ et al (2019)	Clinical manifestation of DMD, and genetic confirmation
Pane M et al (2012)	Participants with genetic confirmation of DMD by MLPA or PCR and direct sequencing
Ricotti V et al (2015)	Participants with genetic confirmation of DMD
Saito Y et al (2022)	Participants with genetic confirmation of DMD
Thangarajh M et al (2019)	Participants with genetic confirmation of DMD
Fujino H et al (2018)	MLPA, multiplex PCR, Southern blotting, direct sequencing

Supplementary references

[1]. Dent KM, Dunn DM, Von Niederhausern AC, Aoyagi AT, Kerr L, Bromberg MB, et al. Improved molecular diagnosis of dystrophinopathies in an unselected clinical cohort. Am J Med Genet [Internet]. 2005;134 A(3):295–8.

[2]. Dinh LT, Tran VK, Luong LH, Le PT, Nguyen AD, Thi Nguyen BS, et al. Assessment of 6 STR loci for prenatal diagnosis of Duchenne Muscular Dystrophy. Taiwan J Obstet Gynecol. 2019 Sep;58(5):645–9.

[3]. Flanigan KM, von Niederhausern A, Dunn DM, Alder J, Mendell JR, Weiss RB. Rapid direct sequence analysis of the dystrophin gene. Am J Hum Genet. 2003 Apr;72(4):931–9.
[4]. García-Acero M, Pineda T, Guerra-Torres M, García-Robles R, Ayala-Ramírez P, Buitrago T, et al. Análisis del espectro mutacional de la distrofia muscular de Duchenne en un grupo de pacientes colombianos. Neurol Argentina [Internet]. :137–46. Available from: https://www.elsevier.es/es-revista-neurologia-argentina-301-articulo-analisis-del-espectro-mutacional-distrofia-S1853002818300417
[5]. Lalic T, Vossen RHAM, Coffa J, Schouten JP, Guc-Scekic M, Radivojevic D, et al. Deletion and duplication screening in the DMD gene using MLPA. Eur J Hum Genet. 2005 Nov;13(11):1231–4.
[6]. Nicholson L V, Johnson MA, Bushby KM, Gardner-Medwin D, Curtis A, Ginjaar IB, et al. Integrated study of 100 patients with Xp21 linked muscular dystrophy using clinical, genetic, immunochemical, and histopathological data. Part 3. Differential diagnosis and prognosis. J Med Genet. 1993 Sep;30(9):745–51.
[7]. Prior TW, Bridgeman SJ. Experience and strategy for the molecular testing of Duchenne muscular dystrophy. J Mol Diagn. 2005 Aug;7(3):317–26.
[8]. Salas AC. Distrofia muscular de Duchenne. An Pediatría Contin [Internet]. 2014;12(2):47–54. Available from: https://www.elsevier.es/es-revista-anales-pediatria-continuada-51-articulo-distrofia-muscular-duchenne-S1696281814701684
[9]. Stockley TL, Akber S, Bulgin N, Ray PN. Strategy for comprehensive molecular testing for Duchenne and Becker muscular dystrophies. Genet Test. 2006;10(4):229–43.

Supplementary table 3. Diagnostic criteria for main outcomes.

Reference	ASD	ADHD	Depression	Anxiety	OCD
Colombo P et al (2017)	ADOS	-	-	-	-
Darmahkasih AJ et al (2019)	Medical history	Medical history	Medical history	Medical history	Medical history
Fujino H et al (2018)	PARS	-	-	-	-
Lambert JT et al (2020)	Medical history	Medical history	Medical history	Medical history	Medical history
Pane M et al (2012)	-	DSM IV, CPRS-R:L, CTRS-R:L	-	-	-
Ricotti V et al (2015)	3Di-sv	-	-	-	-
Saito Y et al (2022)	DSM-V	DSM-V	-	-	-
Thangarajh M et al (2019)	-	Conners	-	-	-

3Di-sv: Developmental, Diagnostic and Dimensional Interview – short version; ADOS: Autism Diagnostic Observation Schedule; CPRSR: Conners' Parent Rating Scale-Revised; CTRS-R:L: Conners Teachers Rating Scales-Revised; DSM: Diagnostic and Statistical Manual of Mental Disorders; PARS: Pervasive Developmental Disorders/Autism Spectrum Disorders Rating Scale

Supplementary table 4. Developmental disorders included in Becker muscular dystrophy and affected participants.

Reference	Diagnostic criteria	Developmental/Neurodevelopmental disorders (DSM-V and ICD10 nomenclature)	Affected Participants
Mori-Yoshimura M et al (2018)	Data collection was carried out by self-reported questionnaire from the participants. Disorders were categorized according to DSM-IV, which corresponds to "neurodevelopmental disorders" in DSM-V	• Intellectual Disability (ICD10: F70/F71/F72/F73) • Global developmental delay (ICD10: F88) • Unspecified intellectual disability (ICD10: F79) • Language disorder (ICD10: F80.9) • Speech sound disorder (ICD10: F80.0) • Childhood-Onset Fluency Disorder (ICD10: F80.81) • Social communication disorder (ICD10: F80.89) • Unspecified Communication Disorder (ICD10: F80.9) • Autism Spectrum Disorder (ICD10: F84.0) • Attention-Deficit/Hyperactivity Disorder (ICD10: F90.0/F90.1/F90.2) • Other Specified Attention-Deficit/Hyperactivity Disorder (ICD10: F90.8) • Unspecified Attention-Deficit/Hyperactivity Disorder (ICD10: F90.9) • Specific Learning Disorder (ICD10: F81.0/F81.2/F81.81) • Developmental Coordination Disorder (ICD10: F82) • Stereotypic Movement Disorder (ICD10: F98.4) • Tourette's Disorder (ICD10: F95.2) • Persistent Motor or Vocal Tic Disorder (ICD10: F95.1) • Provisional Tic Disorder (ICD10: F95.0) • Other Specified Tic Disorder (ICD10: F95.8) • Unspecified Tic Disorder (ICD10: F95.9) • Other Specified Neurodevelopmental Disorder (ICD10: F88) • Unspecified Neurodevelopmental Disorder (ICD10: F89)	• Autism (integrated as autism spectrum disorder in DSM-V): 1 • Language disorders: 1 • Mental retardation (Intellectual Disability in DSM-V): 7
Mori-Yoshimura M et al (2019)	Data collection was performed by interviewing the participants by a neurologist. The categorization of the disorders was probably performed as in Mori-Yoshimura M et al (2018)	• Intellectual Disability (ICD10: F70/F71/F72/F73) • Global developmental delay (ICD10: F88) • Unspecified intellectual disability (ICD10: F79) • Language disorder (ICD10: F80.9) • Speech sound disorder (ICD10: F80.0) • Childhood-Onset Fluency Disorder (ICD10: F80.81) • Social communication disorder (ICD10: F80.89) • Unspecified Communication Disorder (ICD10: F80.9) • Autism Spectrum Disorder (ICD10: F84.0) • Attention-Deficit/Hyperactivity Disorder (ICD10: F90.0/F90.1/F90.2) • Other Specified Attention-Deficit/Hyperactivity Disorder (ICD10: F90.8) • Unspecified Attention-Deficit/Hyperactivity Disorder (ICD10: F90.9) • Specific Learning Disorder (ICD10: F81.0/F81.2/F81.81) • Developmental Coordination Disorder (ICD10: F82) • Stereotypic Movement Disorder (ICD10: F98.4) • Tourette's Disorder (ICD10: F95.2) • Persistent Motor or Vocal Tic Disorder (ICD10: F95.1) • Provisional Tic Disorder (ICD10: F95.0) • Other Specified Tic Disorder (ICD10: F95.8) • Unspecified Tic Disorder (ICD10: F95.9) • Other Specified Neurodevelopmental Disorder (ICD10: F88) • Unspecified Neurodevelopmental Disorder (ICD10: F89)	• Intellectual disorder (used as a synonym for intellectual disability by the authors): 5 • Pervasive developmental disorders (integrated as autism spectrum disorder in DSM-V): 1

Supplementary table 5. Risk of bias assessment.

Reference	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Colombo P et al (2017)															
Darmahkasih AJ et al (2019)															
Fujino H et al (2018)															
Lambert JT et al (2020)															
Mori-Yoshimura M et al (2018)															
Mori-Yoshimura M et al (2019)															
Pane M et al (2012)															
Ricotti V et al (2015)															
Saito Y et al (2022)															
Thangarajh M et al (2019)															

Green: good/low risk; Red: poor/high risk; Yellow: fair/some concerns/not applicable

Items for Study Quality Assessment Tools:

1. Was the research question or objective in this paper clearly stated?

2. Was the study population clearly specified and defined?

3. Was the participation rate of eligible persons at least 50%?

4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?

5. Was a sample size justification, power description, or variance and effect estimates provided?

6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?

7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?

8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?

9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?

10. Was the exposure(s) assessed more than once over time?

11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?

12. Were the outcome assessors blinded to the exposure status of participants?

13. Was loss to follow-up after baseline 20% or less?

14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

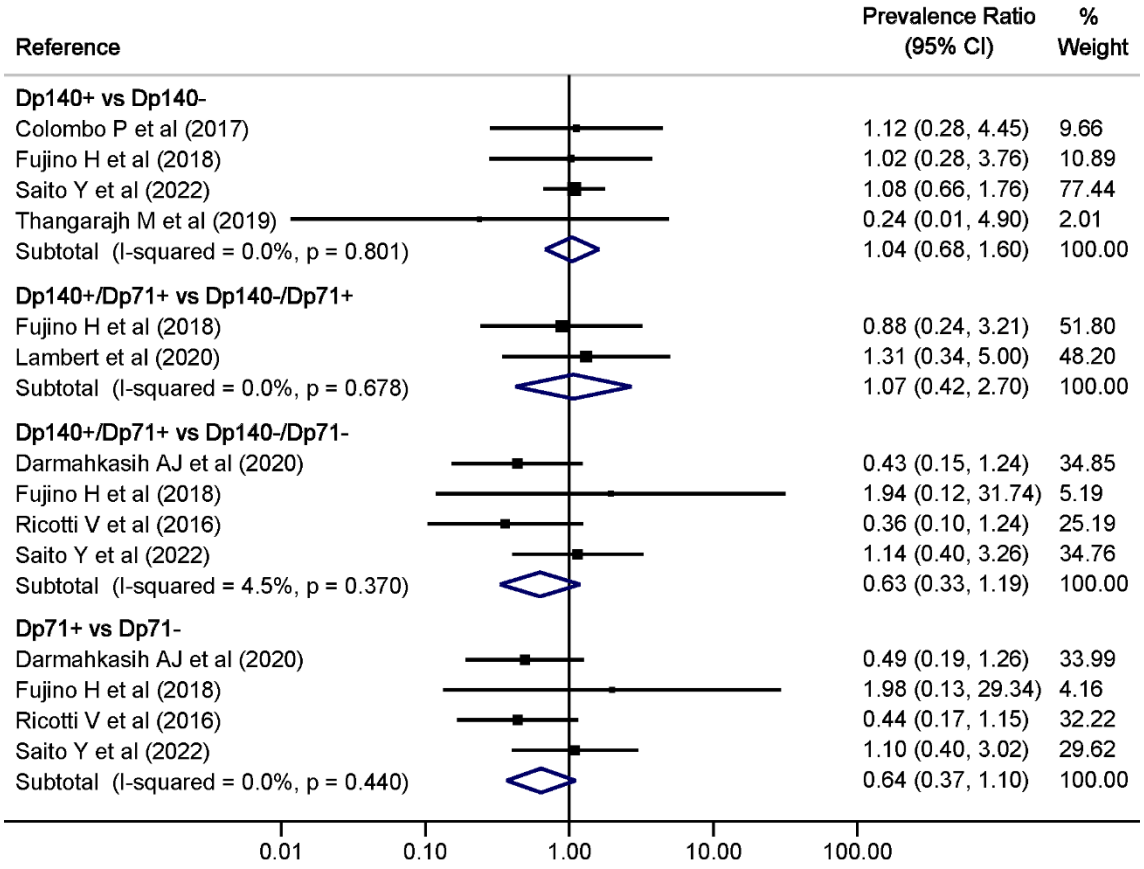
15. Overall bias. If poor, it need comments.

Supplementary table 6. Grades of Recommendation, Assessment, Development, and Evaluation.

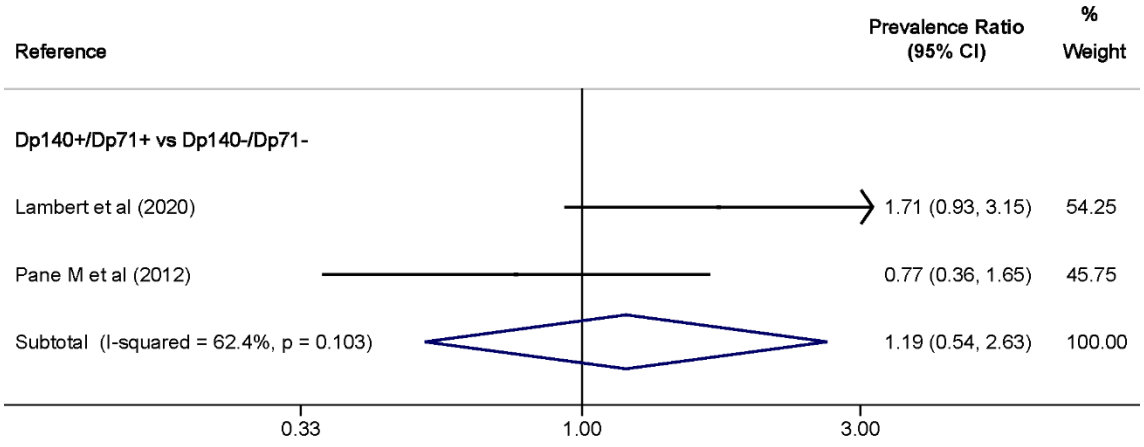
Certainty assessment							Impact	Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**		
A. Developmental disorders								
Dp140+ vs Dp140-								
2	Observational studies	Not serious	Not serious	Not serious	Not serious	Strong association	PR = 0.11 (0.04, 0.34)	MODERATE
Dp71+ vs Dp71-								
2	Observational studies	Not serious	Not serious	Not serious	Not serious	Strong association	PR = 0.22 (0.07, 0.67)	MODERATE
B. Autism spectrum disorder								
Dp140+ vs Dp140-								
3	Observational studies	Not serious	Not serious	Not serious	Not serious	None	PR = 1.05 (0.66, 1.65)	LOW
Dp140+/Dp71+ vs Dp140-/Dp71-								
3	Observational studies	Not serious	Not serious	Not serious	Not serious	None	PR = 0.59 (0.29, 1.19)	LOW
Dp71+ vs Dp71-								
3	Observational studies	Not serious	Not serious	Not serious	Not serious	None	PR = 0.61 (0.35, 1.06)	LOW
C. Attention deficit hyperactivity disorder								
Dp140+ vs Dp140-								
3	Observational studies	Not serious	Not serious	Not serious	Not serious	None	PR = 0.44 (0.10, 1.93)	LOW
Dp140+/Dp71+ vs Dp140-/Dp71-								
3	Observational studies	Not serious	Not serious	Not serious	Not serious	Strong association	PR = 0.40 (0.28, 0.56)	MODERATE
Dp71+ vs Dp71-								
3	Observational studies	Not serious	Not serious	Not serious	Not serious	Strong association	PR = 0.47 (0.36, 0.63)	MODERATE

Supplementary figure 1. Meta-analysis of the prevalence ratio and 95% confidence interval by genotype comparisons of autism spectrum disorders and attention deficit hyperactivity disorder in dystrophinopathies.

A- Autism spectrum disorders.



B- Attention deficit hyperactivity disorder in dystrophinopathies.



Supplementary appendix 1. Search strategy.

- Medline, Scopus, Web of Science, and Cochrane Library

(epidemiology OR prevalence OR survey OR frequency OR question* OR ratio OR rate) AND (“becker muscular dystrophy” OR "duchenne muscular dystrophy" OR dystroph*) AND (“developmental disorders” OR “neurodevelopmental disorders” OR "attention deficit hyperactivity disorder" OR adhd OR “autism spectrum disorder” OR autism OR asd OR depression OR anxiety OR "obsessive-compulsive disorder" OR "obsessive compulsive disorder" OR ocd OR "mental disorders" OR "mental health")

- Grey literature

Not specified