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WWP1 germline variants are associated with normocephalic autism spectrum disorder

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Dear Editor,

Autism spectrum disorder (ASD, MIM: 209850) is a group of common but heterogeneous neurodevelopmental disorders with a prevalence of 4–10 per 10,000 individuals[1,2]. About 5% of ASD cases are caused by single-gene variants in *FMR1* (MIM: 309550), *MECP2* (MIM: 300005), or *SHANK3* (MIM: 606230); 10% by copy number variants (CNVs)[2], while the majority is attributed to polygenic inheritance of common variants[3]. In addition, germline *PTEN* mutations have been identified in 2–5% of all ASD patients and ~10% of macrocephalic ASD[4]. Recently, Lee et al.[5] identified germline variants within the E3 ubiquitin ligase *WWP1* (MIM: 602307) gene in *PTEN* mutation negative individuals with neoplastic phenotypes found in PHTS (MIM: 158350).

To establish whether WWP1 could play a role in ASD and neurodevelopment disorders, we analyzed 198 unrelated individuals mainly referred for syndromic or non-syndromic developmental delay and/or ASD of unknown genetic etiology. All individuals were clinically diagnosed with ASD on the basis of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Whole-exome sequencing, validated by Sanger sequencing, identified eight different heterozygous germline mutations (one recurrent in three unrelated patients) of the *WWP1* gene in 10 of 198 unrelated probands via WES (Table 1). None of the variant positive probands had macrocephaly. In two cases, parental origin could not be investigated, therefore, a de novo origin of the mutation, cannot be ruled out. For each patient (6 males and 4 females; ages 3–26), the clinical data have been reassessed. None of the probands had germline *PTEN* mutations or other mutations in genes (*FMR1, SHANK3, MECP2, CDK19*) associated with ASD/intellectual disability (ID). We independently confirmed that *WWP1* variation does not act as a modifier for ASD phenotypes in PHTS with none of ~600 mainly American *PTEN* mutation positive research associated with the *WWP1* locus. Similarly, routine chromosome studies and *FRAXA* locus were normal. GnomAD database analysis revealed that the identified WWP1 variants with the exception of R389S, R893H, and M728L (never detected), existed with a cumulative frequency of 0.00085 in ethnically matched populations (EUR), indicating that they are very rare variants. Specifically, *WWP1* germline variants occurred in 10/396 alleles (allelic freq. = 0.0252) from the 198 unrelated individuals with ASD/ID (Table 1) which is a highly significant difference from European population frequencies from GnomAD ($p < 0.00001$; OR = 30.6 with 95% CI 16.27 and 57.59). We therefore extended the study to a cohort of 1158 individuals from the Italian general population to establish the frequency of *WWP1* variants in this Italian cohort. We detected three *WWP1* rare variants (c.1118G>A, p-Arg373Gln; c.1486G>C, p. Glu496Gln; c.2234A>G, p.Asn745S) (3/2316 alleles: allelic freq. = 0.00129). Notably, *WWP1* variants were again shown to be over-represented in the ASD/ID series, even when compared with the Italian cohort examined ($p < 0.00001$; OR = 19.93 with 95% CI 5.47 and 72.90). The variants are found in all functional domains of the protein (the catalytic C-terminal HECT domain; the N-terminal C2 domain and WW domains) with an over-representation in the HECT domain (4/8). To predict the potential impact of the identified variants on the

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Table 1 Summary of variations in ASD patients carrying *WWP1* mutations.

Patient ID	Sex	Exon	Position (Hg19)	Nucleotide	Amino acid	Domain	GnomAd[a]	dbSNP	Transmission
GM4277	F	Int 7	87414243	c.540-5T>C		NA	0.0027	rs187132881	Mother
GM3474	M	11	87439881	c.1167A>C	p.Arg389Ser	WW1	0	NA	NA
A020	M	14	87443954	c.1583G>A	p.Arg528His	WW4	0.000008	rs554041348	Father
GM6802	F	20	87460703	c.2234A>G	p.Asn745Ser	HECT	0.00003	rs148651938	Mother
GM8105	M	20	87460703	c.2234A>G	p.Asn745Ser	HECT	0.00003	rs148651938	Father
GM-1HSL	M	20	87460703	c.2234A>G	p.Asn745Ser	HECT	0.00003	rs148651938	NA
GM4098	F	20	87460645	c.2176G>A	p.Val726Ile	HECT	0.000023	rs144129917	Mother
GM8302	F	25	87479031	c.2678G>A	p.Arg893His	HECT	0	rs755897749	Father
A036	M	20	87460651	c.2182A>T	p.Met728Leu	HECT	0	NA	Father
A069	M	5	87393781	c.257G>A	p.Arg86His	C2	0.000023	rs371650373	Mother

[a]EUR.

protein we used different tools (PolyPhen2, Mutation Taster, SIFT, MetaLR_pred, and MetaSVM_pred). The recurrent N745S variant has been previously reported by Lee et al.[5]: it is in the HECT domain and is expected to decrease its binding to the N-terminal domain. Analogously, R86H (C2 domain) was also described by Lee et al.[5]. This variant is functionally relevant since it induces a gain-of-function effect in triggering PTEN poly-ubiquitination[5]. With regards to the other five coding variants observed in our ASD cases, one is predicted by in silico analysis to be deleterious (R528H), while the others gave conflicting results.

Our results suggest that germline *WWP1* variants identified in ASD/ID/NDDs may contribute to the pathogenesis of ASD/ID/NDDs. In addition, since the enzymatic activity of WWP1 can be inhibited by the natural compound, indole-3-carbinol[6], our study identifies a possible therapeutic target for individuals with ASD/ID/NDDS.

Web resources

GnomAD, https://gnomad.broadinstitute.org/
PolyPhen2, http://genetics.bwh.harvard.edu/pph2/
Mutation Taster, http://www.mutationtaster.org/
SIFT, https://sift.bii.a-star.edu.sg/
OMIM, https://OMIM.org/.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical declarations

The study was conducted in agreement with the principles of the Declaration of Helsinki. Informed written consent was obtained from each patients. As regards the participation of children in the research, consent and authorization were signed by the parents in accordance with the rules laid down by the Ethics Committee of the Bambino Gesù Hospital in Rome (HYPERLINK "https://urldefense.proofpoint.com/v2/url?u=http-3A__www.ospedalebambinogesu.it_en_home&d=DwMFaQ&c=vh6FgFnduejNhPPD0fl_yRaSfZy8CWbW-nlf4XJhSqx8&r=H8EiHZYdOfzgj3SnkNr1OWfOZuk7IdFteVpx6F9BizvoZAKx_zII-bLuDzKXrCwF8&m=0dvSb4bLNoeGzXhLeNXyRGhxjEoUL6Qd_oj7-reRTsMg&s=FMPyM3gTbUpOHGox37ytL4D0gGUI3gocQlCNX9p-1IE&e=" MailScanner ha rilevato un possibile tentativo di frode proveniente da "urldefense.proofpoint.com" http://www.ospedalebambinogesu.it/en/home).

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