RESEARCH ARTICLE

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A Genome-Wide Approach to Children's Aggressive Behavior: *The EAGLE consortium*

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Individual differences in aggressive behavior emerge in early childhood and predict persisting behavioral problems and disorders. Studies of antisocial and severe aggression in adulthood indicate substantial underlying biology. However, little attention has been given to genome-wide approaches of aggressive behavior in children. We analyzed data from nine population-based studies and assessed aggressive behavior using well-validated parent-reported questionnaires. This is the largest sample exploring children's aggressive behavior to date (N = 18,988), with measures in two developmental stages (N = 15,668 early childhood and N = 16,311 middle childhood/early adolescence). First, we estimated the additive genetic variance of children's aggressive behavior based on genome-wide SNP information, using genome-wide complex trait analysis (GCTA). Second, genetic associations within each study were assessed using a quasi-Poisson regression approach, capturing the highly right-skewed distribution of aggressive behavior. Third, we performed meta-analyses of genome-wide associations for both the total age-mixed sample and the two developmental stages. Finally, we performed a gene-based test using the summary statistics of the total sample. GCTA quantified variance tagged by common SNPs (10–54%). The meta-analysis of the total sample identified one region in chromosome 2 (2p12) at near genome-wide significance (top SNP rs11126630, $P = 5.30 \times 10^{-8}$). The separate meta-analyses of the two developmental stages revealed suggestive evidence of association at the same locus. The gene-based

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analysis indicated association of variation within *AVPR1A* with aggressive behavior. We conclude that common variants at 2p12 show suggestive evidence for association with childhood aggression. Replication of these initial findings is needed, and further studies should clarify its biological meaning.

Key words: genome-wide complex trait analysis (GCTA); meta-analysis; aggression; childhood; population-based

INTRODUCTION

Aggressive behavior in childhood is often an antecedent to violent behavior in adolescence [Raine, 2002]. According to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5), childhood aggressive behavior is a criterion for disruptive behavior disorders such as oppositional defiant disorder (ODD) and conduct disorder (CD) [American Psychiatric Association, 2013]. Aggressive behavior is also implicated in neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) [Hamshere et al., 2013], autism spectrum disorder (ASD) [Mandy et al., 2013], dysregulated behavior [Pope and Bierman, 1999], substance use disorders [White et al., 2013], antisocial personality disorder [Schaeffer et al., 2003], and schizophrenia [Volavka, 2013] later in life. The above supports a cross-disorder prevalence, and to our knowledge this study is the first large-scale, genome-wide association study aiming to identify the specific factors that underlie the neurobiology of aggressive behavior in children.

Aggressive behavior emerges as early as infancy [Lewis and Sullivan, 1990]. It typically increases during the first years of life and decreases before school entrance [Tremblay et al., 2005; Alink et al., 2006]. However, in population-based samples children exhibit heterogeneity in levels of aggressive behavior [NICHD Early Child Care Research Network, 2004]. Developmental and psychological research has emphasized individual differences in trajectories of aggressive behavior over time [Tremblay, 2000; Dishion, 2014], yet very little is known about the genetic underpinnings of these behaviors. Longitudinal twin studies show moderate to high sex- and informer-independent estimates of heritability (51–72%) of aggressive behavior between ages 3 and 12 [Hudziak et al., 2003]. Genetic factors account for approximately 65% of the total phenotypic stability in twins [van Beijsterveldt et al., 2003].

Prior studies of the genetic correlates of aggressive behavior have examined polymorphic variations in multiple candidate genes [Volavka et al., 2004; Bakermans-Kranenburg and van IJzendoorn, 2006; Oades et al., 2008; Takahashi et al., 2012], across different ages (from childhood to adulthood) and types of samples (population-based, clinical), and using differing definitions of aggressive behavior. However, a recent meta-analysis of these studies found no substantive evidence for association between any of these polymorphisms and aggressive behavior [Vassos et al., 2013].

In contrast, there are only a few genome-wide association studies (GWAS) of aggression-related behavior in humans. In adults, there are GWAS studies of aggression-related phenotypes of adult antisocial behavior [Tielbeek et al., 2012] and behavioral disinhibition [McGue et al., 2013], but similar studies in children are scarce. A relatively small study of callous-unemotional behavior in children (N = 2,930) revealed no genome-wide significant hits [Viding et al., 2013], whereas a genome-wide linkage study of conduct problems in childhood (N = 1,295) identified suggestive regions at chromosomes 2 and 19 [Dick et al., 2004]. Similarly, a GWA study on hostility and anger traits in adolescents (N = 1,780) identified only suggestive hits [Merjonen et al., 2011].

In the present study, we first estimated the proportion of variance in childhood aggressive behavior accounted for by common genetic variants, using the approaches implemented in the Genome-wide Complex Trait Analysis [GCTA, (Yang et al., 2011)]. It is expected that many genes, each with small effects, affect complex aggressive behavior in childhood. Thus, we used a genome-wide approach to test for genetic associations with parental reports of child's aggressive behavior. We specified a quasi-Poisson regression to model the highly right-skewed distribution of aggressive behavior during childhood. The association analyses were performed in the total sample and in two samples with partially overlapping subjects, characterized by different developmental stages (early childhood and middle childhood/early adolescence). Next, we performed gene-enrichment analysis, hypothesizing that candidate genes previously reported in the literature would be enriched in our genome-wide association study. In summary, data from nine population-based studies were analyzed, and results were combined in a meta-analysis, generating the largest sample used in childhood aggression research to date (N = 18,988).

MATERIALS AND METHODS

Subjects

This study was performed in the framework of the Early Genetics and Lifecourse Epidemiology (EAGLE) consortium (http://research.lunenfeld.ca/eagle/). Information from the participating cohorts is summarized in Table I and a more detailed description of the individual cohorts is presented in the Supplementary Material. Cohorts could participate with data on two developmental stages (early childhood, range: 3–7 years, and middle childhood/early adolescence, range: 8–15 years), whenever data were available. For the cohorts with data on both developmental stages, the largest sample was used for the further analyses (total sample). All children were of North European ancestry. Informed consent was obtained from all participants (parental consent, as appropriate) and study protocols were approved by the local ethics committees.

Genotyping, Quality Control, and Imputation

DNA was extracted from whole blood or buccal cells. Comparisons of genotypes derived from whole blood and from buccal swabs in the same individuals showed excellent concordance indicating that DNA from buccal swabs is of similar quality [Scheet et al., 2012]. For ALSPAC, BC58-T1DGC, GENR, RAINE, TRAILS, and YFS

TABLE I. Descriptives of the Cohorts Participating in the GWAS Meta-Analysis of Childhood Aggression

Study	Questionnaire	N_{total}	$N_{early\ childhood}$	$N_{middle\ childhood/early\ adolescene}$
ALSPAC	SDQ	5,997	5,997	5,752
BC58-T1DGC	Other[a]	2,225	2,225	2,177
BC58-WTCCC2	Other	2,444	2,444	2,366
GENR	CBCL	2,210	2,210	NA
GINI + LISA	SDQ	950	NA	950
NTR	CBCL	1,081	1,081	987
RAINE	CBCL	1,366	1,363	1,366
TRAILS	CBCL	1,280	NA	1,280
YFS	Other	1,435	348	1,435
Total		18,988	15,668	16,311

SDQ, strengths and difficulties questionnaire; CBCL, childhood behavioral checklist; NA, not available.
[a]Comparable items measuring aggressive behavior in general parent-rated questionnaires.

genotyping was performed on Illumina platforms, while for BC58-WTCCC2, GINI + LISA and NTR the Affymetrix platform was used. In all studies included in the meta-analyses, basic quality checks were performed (Supplementary Table S1). Samples were also checked for excess heterozygosity, sex accuracy, relatedness, and missing data. Following these quality control steps, phased genotype data were imputed to build 36 (release 22) of the HapMap reference panel, resulting in more than 2.5 million SNPs for GWAS analysis.

Measurement of Aggressive Behavior

In all cohorts, well-validated questionnaires assessing aggressive behavior in children were mailed to parents of children. In eight out of the nine cohorts, maternal ratings of children's aggressive behavior were obtained. In GINI + LISA, the majority (>80%) of the questionnaires were filled in by the mother. For GENR, NTR, RAINE, and the TRAILS study, aggressive behavior was assessed with the Aggression scale of the Childhood Behavioral Checklist (CBCL). The CBCL 1½–5 [Achenbach and Rescorla, 2000] and CBCL 6–18 version [Achenbach and Rescorla, 2001] were administered for preschool and school-aged children, respectively. Mothers were asked to rate each problem item on a 3-point scale (0 = not true, 1 = somewhat true, and 2 = very true) and the weighted total sum was scored, allowing <25% missing data. Example items are: "My child gets in many fights" and "My child destroys others' things." ALSPAC and the GINI + LISA study used the conduct problem scale of the Strengths and Difficulties Questionnaire (SDQ), such as "My child often fights with other children or bullies them" and "My child often lies or cheats." As previously reported, scores derived from SDQ and CBCL questionnaires are highly correlated and are interchangeable for the assessment of children's behavior problems [Goodman and Scott, 1999]. Finally, BC58-T1DGC, BC58-WTCCC2, and YFS studies used comparable items in general questionnaires rated by the mother, such as "My child's aggressive behavior frequently makes disciplinary action necessary." For YFS study, a 5-point Likert scale was used to rate children's aggressive behavior (1 = "totally disagree" to 5 = "totally agree"). A detailed list of all items used to assess children's aggressive behavior can be found in Supplementary Table S2.

Genome-Wide Complex Trait Analysis (GCTA)

In ALSPAC (N = 5,997), GENR (N = 2,210), and NTR (N = 1,081) data, SNP-based heritability (SNP h^2) was estimated from observed genotypes using GCTA [Yang et al., 2011]. First, a genetic relatedness matrix between unrelated individuals was estimated. Participants whose relatedness equaled or exceeded 3rd–4th degree relatives (pairwise genetic relatedness >0.025) were excluded. Second, a restricted maximum likelihood method (REML) was used to partition the phenotypic similarity between unrelated individuals into a genetic and residual component. SNP h^2 reflects the proportion of phenotypic variance that can be additively accounted for by common genetic variation [Yang et al., 2013] and indicates the upper limit of variance that can be explained by GWAS efforts. GCTA estimates were adjusted for covariates, that is, sex, age, and the principal components of the genotype data to control for local ancestry and population stratification.

To test possible bias of the GCTA estimates due to the highly right-skewed distribution of aggressive behavior in children, we rank-transformed aggressive scores in the ALSPAC and GENR sample and performed GCTA as described above.

GWAS analyses, Quality Control (QC), and Meta-Analysis (GWAMAs)

GWAS analyses in unrelated participants were conducted within each cohort, excluding family members from each cohort prior to GWA analyses. Aggressive behavior was measured on a continuous scale (with higher scores indicating more aggressive behavior). In all cohorts, the non-standardized aggressive behavior scores showed a right-skewed distribution, with the majority of children scoring low on aggression. Association analyses were performed using quasi-Poisson regression, which can accommodate overdispersion [Faraway, 2005], using the R Stats Package [R Development Core Team, 2013]. The quasi-Poisson regression model was preferred over a simple rank-transformation to obtain directly interpretable effect estimates from each cohort. Specifically, in every cohort the untransformed counts of aggressive behavior

scores were regressed on age, sex and principal components of the genetic data to account for population stratification and allele dosage. SNP allele dosages were obtained from imputed data (for more details see Supplementary Table S1) and used for GWAS analyses. Non-autosomal SNPs were excluded from GWAS analyses. All analyses were performed in R (Project for Statistical Computing [R Development Core Team, 2013], R script available upon request).

Prior to the GWAMAs, we conducted rigorous quality control (QC) to the summary data of each participating cohort, using the EasyQC software package [Winkler et al., 2014]. Because of the different assessment instruments used to measure aggressive behavior (i.e., not all studies had the same scales to measure aggressive behavior in children) it is recommended to use the sample size-weighted z-score method for GWAMA [Whitlock, 2005]. This method is implemented in meta-analysis helper (METAL) [Willer et al., 2010] and it was used to derive overall P values of the association of each SNP to children's aggressive behavior. Firstly, we meta-analyzed the data in the total sample (N = 18,988). Secondly, for studies with aggressive behavior measured at different developmental stages, we followed up with secondary analyses by meta-analyzing separately early childhood samples (range 3–7 years) and middle childhood/early adolescence (range 8–15 years), a distinction that was based on developmental trajectories of aggression in children [Tremblay, 2000]. At the meta-analysis level, SNPs were filtered according to their frequency (minor allele frequency, MAF > 0.05) and imputation quality (MACH $r^2 > 0.30$ or IMPUTE INFO > 0.4). Results were corrected by genomic inflation factor (λ) at study level, for population stratification. We adopted a threshold of $P < 10^{-6}$ for genome-wide suggestive hits and a threshold of $P < 5 \times 10^{-8}$ for genome-wide significant hits. Annotation and in silico functional analysis of our GWAS meta-analyses top hits was performed using SNPnexus [Ullah et al., 2012].

Gene Enrichment Analysis and Candidate Gene Prioritization

We used two recent synopses [Craig and Halton, 2009; Vassos et al., 2013] of candidate gene association studies and identified the candidate genes previously tested for association with aggressive behavior (in adults and/or children), such as catechol-O-methyltransferase (*COMT*), serotonin receptor (*5-HTTLPR*), dopamine receptors and brain-derived neurotrophic factor (*BDNF*). To evaluate their potential association with child aggressive behavior in a gene-based test, we performed the versatile gene-based association analysis (VEGAS) on the summary statistics of our total sample (N = 18,988). In summary, VEGAS accounts for the number of SNPs in a gene (±50 kb form the start/end of the gene) and the linkage disequilibrium between them to estimate an empirical P value for the association of the gene and trait of interest [Liu et al., 2010]. No SNP information was available for candidate genes located on chromosome X, thus these genes were not included in the gene-enrichment analyses. For the 21 candidate genes tested, the Bonferroni-corrected threshold for multiple testing is $0.05/21 = 2.38 \times 10^{-3}$.

RESULTS

GCTA

After filtering for common variants (MAF > 0.01), the ALSPAC sample ($N_{total} = 5{,}997$), GENR sample ($N_{total} = 2{,}210$), and NTR sample ($N_{total} = 1{,}081$) yielded more than 450,000 directly genotyped SNPs for analysis. After exclusion of close relatives, REML analyses were performed. Common genetic variation explained a quantifiable proportion of variance in the traits analyzed. Specifically, in the ALSPAC sample of 4-year-old children SNP h^2 was 0.10 (SE = 0.06, $P = 0.04$, $N_{unrelated} = 5{,}505$). In the GENR sample of 6-year-old children, we estimated SNP $h^2 = 0.54$ (SE = 0.19, $P = 0.002$, $N_{unrelated} = 2{,}101$) and in the smaller NTR sample of 3-year-old children SNP h^2 was 0.46 (SE = 0.35, $P = 0.09$, $N_{unrelated} = 908$). The 95% confidence intervals (CI) of the GCTA heritabilities in the ALSPAC, GENR, and NTR samples overlap, and this indicates that differences should be interpreted carefully.

Similarly, we estimated GCTA heritability in children in middle childhood/early adolescence participating in ALSPAC study. The estimations were similar to the ones estimated for the preschoolers, although not significant (SNP $h^2 = 0.08$, SE = 0.06, $P = 0.10$, $N_{unrelated} = 5{,}299$).

Sensitivity analysis was performed to assess the effect of the right-skewed distribution of aggressive symptoms in the ALSPAC and GENR samples. GCTA estimations of the rank-transformed aggressive behavior in both samples indicated no substantial bias in our estimations, although the SNP h^2 was notable in the middle childhood/early adolescence developmental stage of the ALSPAC study (SNP $h^2 = 0.12$, SE = 0.06, $P = 0.02$, $N_{unrelated} = 5{,}299$).

GWAS Meta-Analysis

Nine cohorts contributed data for the total meta-analysis (N = 18,988 children, mean age = 8.44 years, SD = 4.16). EasyQC was implemented in all studies and after filtering and quality control, approximately 2.5 million genotyped and imputed SNPs were available per sample. Study-level and meta-level QC results are presented as Supplementary Material (Supplementary Figures S1–3). The meta-analysis revealed a near genome-wide significant locus on chromosome 2p12 ($P = 5.30 \times 10^{-8}$) and several suggestive loci across other chromosomes (i.e., chromosome 3q26, 6p22, 10p12, 17q24). The top SNPs are summarized in Table II (more details can be found in Supplementary Table S3). The Manhattan and quantile–quantile (QQ) plot of GWAS meta-analysis in the total sample are presented in Figures 1 and 2, respectively. The strongest signal was observed for rs11126630 on chromosome 2 residing between *LRRTM4* and *SNAR-H*. Consistently, across all participating studies, the T allele of the rs11126630 was related to lower levels of aggressive behavior in children (e.g., within the GENR study, the presence of the T allele was related to a decrease of 0.04 log counts in aggressive behavior). The effect estimates and standard errors at rs11126630 are presented for each study in Supplementary Table S4. The regional association plot of our GWAS top hit is presented in Figure 3.

For the two follow-up meta-analyses using two age-strata, we first analyzed data from children in early childhood (N = 15,668; mean age = 5.36 years, SD = 1.5). The meta-analysis did not show

TABLE II. Top Signals That Reached Suggestive Genome Wide Significance ($P < 10^{-6}$), Sorted by Ascending *P*, in Total Sample (N = 18,988)

SNP	Chromosome	Position	Allele 1/2	Frequency 1	Direction[a]	# Hits	meta *P*	heterogeneity P^b	Nearest gene
rs11126630	2	77796352	T/C	0.49	−−−−−−−−−	14	5.30e-8	0.78	*LRRTM4; SNAR-H*
rs9372149	6	107152014	A/T	0.32	+++++++++	2	1.18e-6	0.38	*PDSS2*
rs2015436	6	28929137	T/C	0.73	−−+−−−−−−	12	1.57e-6	0.58	*TRIM27*
rs10508552	10	17966561	C/G	0.65	−−−−−+−−−	1	1.72e-6	0.18	*MRC1*
rs7625357	3	169630044	T/C	0.33	+++++−+++	4	4.08e-6	0.10	*MECOM*
rs2079515	17	70917365	C/G	0.47	+++++++++	2	4.29e-6	0.21	*CASC17*

[a]Order of participating studies: NTR, BC58-T1DGC, BC58-WTCCC2, GENR, ALSPAC, GINI + LISA, RAINE, TRAILS, YFS.
[b]*P* value showing heterogeneity between the participating studies.

genome-wide significant results, although there were suggestive SNPs, as summarized in Table III (a more detailed table of the total suggestive hits can be found in Supplementary Table S5). The Manhattan and QQ plot of the GWAS meta-analysis for children in early childhood are presented in Figures S4 and S5, respectively.

Secondly, we analyzed data from children in middle childhood/early adolescence (N = 16,311), with a mean age of 11.39 years (SD = 1.86). We did not observe genome-wide significant results, although there were suggestive SNPs as summarized in Table IV (more details in Supplementary Table S6). The Manhattan and QQ plot for children in middle childhood/early adolescence are presented in Figures S6 and S7, respectively.

Comparison of the results of the total mixed-age sample and the two developmental stages indicated that the locus on chromosome 2 (top SNP, rs11126630) showed also suggestive significance ($P = 6.32 \times 10^{-7}$) in the middle childhood/early adolescence stage. The same locus reached suggestive evidence for association in the early childhood stage ($P = 1.27 \times 10^{-5}$). At the suggestive level, we also found overlapping signals between the two developmental stages on chromosome 10q22.

Gene Enrichment Analysis & Candidate Gene Prioritization

The association of aggression levels with candidate genes previously linked to individual differences in aggression as found in the VEGAS gene-based analysis is reported in Table V. Only the *AVPR1A* (arginine vasopressin receptor 1A) gene was significantly

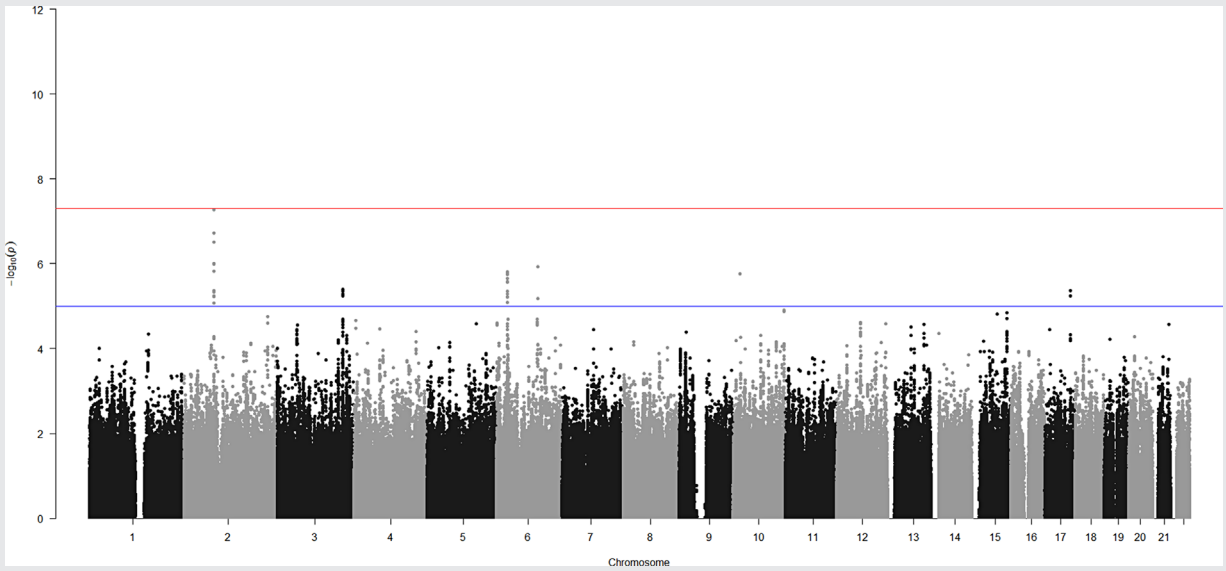

FIG. 1. Manhattan plot of the GWAS meta-analysis of childhood aggressive behavior in the total sample (N = 18,988). The *x*-axis represents the autosomal chromosomes and the *y*-axis shows the −log10(p). The red line indicates the genome-wide significance level ($P = 5 \times 10e\text{-}8$) and the blue line indicates the suggestive significance level ($P = 1 \times 10e\text{-}5$).

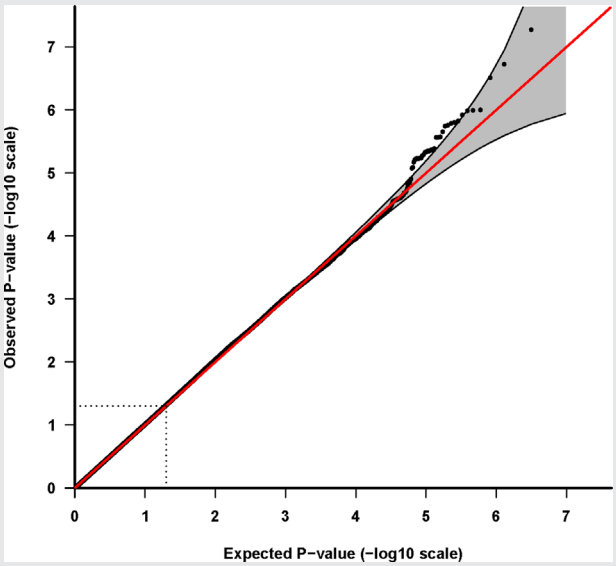

FIG. 2. Quantile–quantile (QQ) plot illustrating probability values from GWAS meta-analysis of aggression in the total sample (N = 18,988). The red line indicated the distribution under the null hypothesis and the shaded area indicates the 95% confidence band.

associated with childhood aggression levels ($P = 1.61 \times 10^{-3}$). The association persisted after correcting for multiple testing (Bonferroni correction; new threshold of significance $P < 2.38 \times 10^{-3}$).

DISCUSSION

Twin and family studies have suggested a significant heritability of aggressive behavior, while genome-wide association studies have indicated potential biological pathways leading to extreme aggression in adolescence and adulthood. This is the first large-scale, genome-wide approach attempting to discover novel genes implicated in children's aggressive behavior in population-based samples.

As part of the EAGLE consortium, we gathered the largest sample to date (total N = 18,988) from which to explore genetic correlates of children's aggressive behavior. We estimated the proportion of phenotypic variance explained by additive effects of common SNPs, using the GCTA approach. Our findings suggest that common genetic variation contributes to the phenotypic variation in aggressive behavior, with GCTA heritability estimates ranging between 10% and 54% in different cohorts during early childhood. The heterogeneity of the point GCTA estimates between cohorts could reflect that genotype data were obtained by different genotyping platforms (Illumina or Affymetrix), containing only partially overlapping SNPs (only for the GWAS analyses the genotype data were imputed). Second, GCTA heritability is an estimation of the ratio of

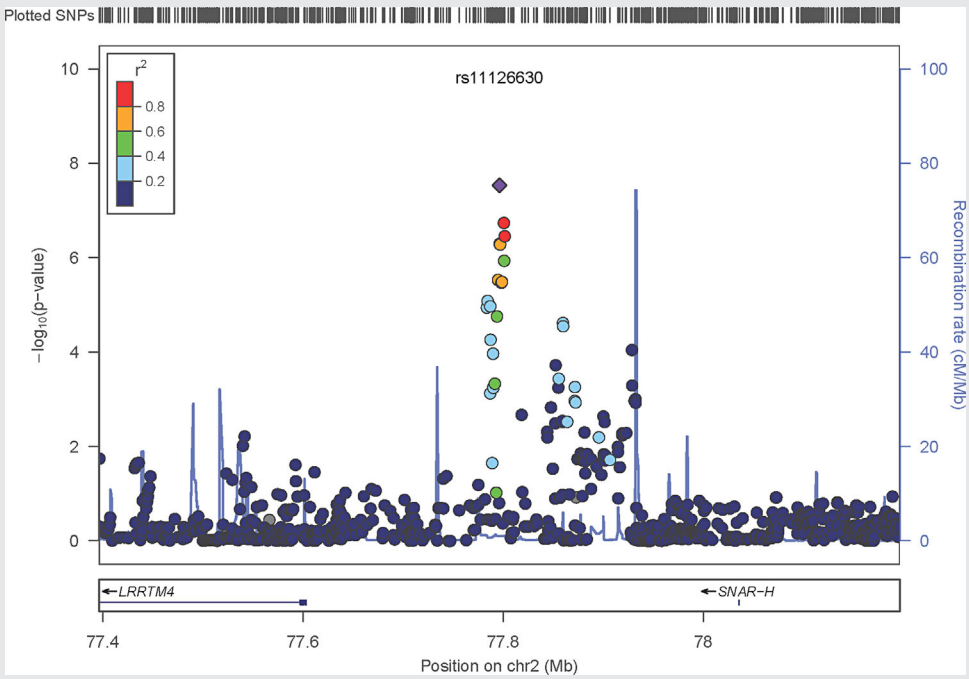

FIG. 3. Regional association plot of the genome-wide significant hit, in total sample. The top SNP is indicated with a diamond and the flanking SNPs, in circles, are colored according to their linkage disequilibrium (LD) with rs11126630. The plot was constructed by HapMap, CEU population (Northern and Western European ancestry).

TABLE III. Top Signals That Reached Suggestive Genome Wide Significance ($P < 10^{-5}$), Sorted by Ascending *P*, in Early Childhood (N = 15,668)

SNP	Chromosome	Position	Allele 1/2	Frequency 1	Direction[a]	# Hits	meta *P*	heterogeneity P^b	Nearest gene
rs2763339	10	69929889	T/G	0.36	+++++++	3	2.21e-6	0.88	*COL13A1*
rs11760485	7	4361919	T/C	0.38	+++++++	2	1.18e-6	0.48	*SDK1*
rs17086954	6	156016170	A/G	0.68	+++++++	1	5.91e-6	0.45	*LOC101928923*
rs1577595	6	93694060	A/C	0.29	+++++++	3	9.63e-6	0.87	*TSG1*
rs589804	2	4469035	T/G	0.51	++++++-	1	9.65e-6	0.80	*LOC727982*

[a]Order of participating cohorts: ALSPAC, BC58-T1DGC, BC58-WTCCC2, GENR, NTR, RAINE, YFS.
[b]*P* value showing heterogeneity between the participating studies.

TABLE IV. Top Signals That Reached Suggestive Genome Wide Significance ($P < 10^{-5}$), Sorted by Ascending *P*, in Middle Childhood/Early Adolescence (N = 16,311)

SNP	Chromosome	Position	Allele 1/2	Frequency 1	Direction[a]	# Hits	meta *P*	heterogeneity P^b	Nearest gene
rs11126630	2	77796352	T/C	0.52	−−−−−−−−	10	6.32e-7	0.70	*LRRTM4*; *SNAR-H*
rs11700808	21	19160726	A/C	0.54	−+−+++++	7	1.12e-6	0.45	*LOC101927797*
rs9787796	11	131986383	T/C	0.15	+++++++++	3	1.68e-6	0.41	*OPCML*
rs3843585	10	71374757	T/C	0.62	+−−+−−−−	4	2.91e-6	0.26	*COL13A1*
rs12153160	5	152931393	A/C	0.84	+−−−−−−−	5	5.10e-6	0.54	*GRIA1*
rs11977715	7	94955639	A/G	0.22	++++++++−	1	7.81e-6	0.75	*ASB4*

[a]Order of participating cohorts: GINI + LISA, ALSPAC, RAINE, TRAILS, YFS, BC58-T1DGC, BC58-WTCCC2, NTR.
[b]*P* value showing heterogeneity between the participating studies.

TABLE V. Association of Candidate Genes, as Indicated in the Literature, With Levels of Aggressive Behavior, Using a Gene-Based Test (VEGAS)

Gene name	Chromosome	N SNPs	Start position (bp)	Stop position (bp)	Gene- based *P*
Serotonin pathway					
SLC6A4	17	63	25549031	25586841	0.61
HTR1A	5	43	63292033	63293302	0.40
HTR1B	6	134	78228666	78229839	0.91
TPH1	11	79	17999113	18018885	0.59
TPH2	12	179	70618892	70712488	0.07
Dopaminergic pathway					
DRD1	5	144	174800280	174803769	0.26
DRD2	11	195	112785526	112851211	0.97
DRD3	3	119	115330246	115380589	0.36
DRD4	11	50	627304	630703	0.52
DBH	9	177	135491305	135514287	0.30
COMT	22	147	18309308	18336530	0.78
SLC6A3	5	140	1445909	1498538	0.79
Adrenergic receptors					
ADRB1	10	94	115793795	115796657	0.11
NET1	10	116	5444517	5490426	0.75
SLC6A2	16	162	54248056	54295201	0.78
Stress response					
NR3C1	5	160	142637688	142795270	0.42
FKBP5	6	82	35649344	35764692	0.87
Other					
NOS1	12	243	116135361	116283965	0.09
SLC2A1	1	135	43163632	43197434	0.28
AVPR1A	12	91	61826482	61832857	1.61e-3
BDNF	11	104	27633017	27699872	0.42

phenotypic variation explained by common SNPs, thus is dependent on sample characteristics [Visscher and Goddard, 2015]. Environmental factors differentially influence gene expression in different samples, thereby changing phenotyping variance or the characteristics of the genetic relatedness matrix among cohorts. Finally, it is possible that heritability estimations change with age, as has been previously shown for autistic like traits [St Pourcain et al., 2014] and general cognitive ability [Trzaskowski et al., 2014].

Previously published heritability estimates based on twin studies of childhood aggressive behavior indicated moderate to high heritability (51–72%) [Hudziak et al., 2003] while GCTA estimates of adult antisocial behavior were not significantly different than zero (SNP $h^2 = 0.55$, SE = 0.41, $P = 0.07$, $N_{cases} = 160$ and $N_{controls} = 2{,}012$) [Tielbeek et al., 2012]. Our GCTA estimates however, support a polygenic contribution of common variants to children's aggressive behavior. Large sample sizes will be required to identify the responsible genes [Trzaskowski et al., 2013] in addition to in depth analysis of rare variation.

Our study revealed a near genome-wide significant locus on chromosome 2p12 (top SNP: rs11126630, $P = 5.30 \times 10^{-8}$). This SNP is located near *LRRTM4* (leucine rich repeat transmembrane neuronal 4) and *SNAR-H* (small ILF3/NF90-associated RNA H) genes. *LRRTM4* regulates excitatory synapse development [Siddiqui et al., 2013] while *SNAR-H* is implicated in the transcription process and is also expressed in neurons [Parrott and Mathews, 2007]. In silico studies showed that variation in 2p12 can affect regulatory transcription factor binding sites in the *LRRTM4* gene promoter [Lauren et al., 2003]. Another suggestive locus ($P = 1.57 \times 10^{-6}$) was 6p22, near the *TRIM27* gene. The top SNP rs9257616 (chromosome 6p22.1) has been previously associated with social communication traits, in a population sample of 8-year-old children [St Pourcain et al., 2013]. The *TRIM27* gene promotes apoptosis and it has been associated with neurotoxicity of dopaminergic neurons in mouse models [Liu et al., 2014]. How these genes may be implicated in the neurophysiology of children's aggressive levels should be a research priority. Replication efforts of our results in independent samples are needed, yet we note that this study is consistent with a previous smaller genome-wide linkage study on conduct disorder that pointed to similar regions on chromosome 2 [Dick et al., 2004].

The results of this GWA meta-analysis of aggressive behavior in children did not show overlap with the suggestive SNPs previously reported for aggression-related traits in adults [Tielbeek et al., 2012; McGue et al., 2013]. The differences in the phenotypes measured in adults (behavioral inhibition and antisocial behavior, respectively) do not allow for direct comparisons with aggressive behavior in children.

Meta-analyses in the two developmental stages (early childhood and middle childhood/early adolescence) did not reveal genome-wide significant results. These analyses indicate that even small differences in sample size can impact on the power to detect genetic associations at the genome-wide significance level. Furthermore, in the partially overlapping developmental stages, genetic variation at the 2p12 region is also enriched, as in the analyses with the total sample. This could imply that the associated region has a relatively stable and less age-dependent effect. At the suggestive level of significance, we also found an association of the 10q22 region with levels of aggressive behavior in both developmental stages. Microdeletions in this region have been previously associated with cognitive and behavioral problems [Balciuniene et al., 2007].

Finally, the *AVRP1A* gene was enriched in our gene-based analysis. It is possible that functional variation in the arginine vasopressin receptor is responsible for higher levels of aggressive behavior, as it has already been shown in rodents [Ferris et al., 1997]. Variation in *AVPR1A* has also been of high interest for social behavior [Ebstein et al., 2012; Charles et al., 2014]. The results of our analyses on candidate genes previously associated with aggression also indicate that it is unlikely that common variants in these genes play a significant role in explaining individual variation in children's aggressive behavior. Since commercial genotyping arrays do not capture structural variation (i.e., copy number variation, variable number tandem repeats or micro-inversions) or the effect of rare SNPs, further studies on these candidate genes (such as *DRD4* and *5HTTLPR*) can be valuable to investigate their potential influence on aggressive behavior in children [Wilkening et al., 2009].

This study has limitations. First, aggressive behavior was reported predominantly by mothers, which can be a source of reporting bias [Najman et al., 2001]. Future studies could use multiple reporters to estimate children's aggressive behavior more precisely. Second, although the sample size is the largest to date for childhood aggression, compared to adults studies of psychiatric traits, the sample size is low, limiting the power to detect associations of common variants with small effects. In developmental and behavioral research, this is a considerable challenge. Third, this was a discovery study, thus we analyzed all available data in all cohorts, leaving no cohorts for replication. Future steps should include efforts to introduce additional cohorts to our consortium, increase the sample size and test whether our findings are replicated in independent samples.

In summary, we found evidence that common additive genetic variants explain variance in parent-rated, non-clinical aggressive behavior in children. Our discovery GWAS meta-analysis identified genes with potential biological links to aggression. The acquisition of even larger sample sizes through continued collaboration between groups and consortia will be crucial for the replication of these genetic loci and their contribution to child aggressive behavior.

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