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The behavioral phenotype in *MECP2* duplication syndrome: A comparison to idiopathic autism

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Abstract

Alterations in the X-linked gene *MECP2* encoding the methyl-CpG-binding protein 2 (MeCP2) have been linked to autism spectrum disorders (ASD). Most recently, data suggest that overexpression of *MECP2* may be related to ASD. To better characterize the relevance of *MECP2* overexpression to ASD-related behaviors, we compared the core symptoms of ASD in *MECP2* duplication syndrome to nonverbal-mental-age-matched boys with idiopathic ASD. Within the *MECP2* duplication group we further delineated aspects of the behavioral phenotype, and also examined how duplication size and gene content corresponded to clinical severity. We compared 10 males with *MECP2* duplication syndrome (ages 3–10) to a chronological and mental age-matched sample of 9 nonverbal males with idiopathic ASD. Our results indicate that boys with *MECP2* duplication syndrome share the core behavioral features of ASD (e.g. social affect, restricted/repetitive behaviors). Direct comparisons of ASD profiles revealed that a majority of boys with *MECP2* duplication syndrome are similar to idiopathic ASD; they have impairments in social affect (albeit to a lesser degree than idiopathic ASD) and similar severity in restricted/repetitive behaviors. Nonverbal mental age did not correlate with severity of social impairment or repetitive behaviors. Within the *MECP2* duplication group, breakpoint size does not predict differences in clinical severity. In addition to social withdrawal and stereotyped behaviors, we also found that hyposensitivity to pain/temperature are part of the behavioral phenotype of *MECP2* duplication syndrome. Our results illustrate that overexpression/increased dosage of *MECP2* is related to core features of ASD.

Introduction

Autism spectrum disorder (ASD) is a complex, heterogeneous disorder that likely results from a variety of complex combinations of genetic and environmental factors. ASD is

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characterized by deficits in social affect, which includes deficits in verbal and nonverbal communication and reciprocal social interaction, accompanied by restricted and repetitive/ stereotyped behaviors (RRB). Candidate gene, genome-wide sequencing strategies, and copy number analysis led to the identification of dosage differences in genes linked to ASD (Geschwind, 2011; Sanders et al., 2011; State & Levitt, 2011). Clinical studies of single gene disorders with a high rate of ASD have allowed for better assessment of etiology and gene function because of the significant downstream effects of these mutations. For example, our clinical studies of the X-linked *MECP2* duplication syndrome, in which the *MECP2* gene encoding the methyl-CpG-binding protein 2 (MeCP2) is overexpressed, informed recent work in mouse models which demonstrated that alterations in *MECP2* levels differentially and specifically affect molecular pathways underlying ASD-related social behavior (Samaco et al., 2012). *MECP2* mutations that dysregulate MeCP2 protein function are also linked to non-syndromic ASD (Carney et al., 2003; Coutinho et al., 2007; Loat et al., 2008; Shibayama et al., 2004). This is supported by a recent study that further demonstrates the relevance of examining the overlap between idiopathic ASD and *MECP2* duplication syndrome. Specifically, individuals with idiopathic ASD and mothers of individuals with ASD were found to overexpress *MECP2* in peripheral leukocytes in the absence of *MECP2* mutations (Kuwano et al., 2011). In addition, postmortem brain studies show higher MeCP2 protein levels in idiopathic ASD in the anterior cingulate, thalamus, and motor cortex. Taken together, these findings suggest that overexpression of *MECP2* may be fundamentally related to some forms of ASD.

Sub-microscopic *MECP2* duplications were first identified in a girl with atypical Rett Syndrome (RTT) and preserved speech, a boy with severe intellectual disability (ID) and features of RTT, and in members of three families with X-linked ID (Van Esch et al., 2005) (Ariani, et al., 2004; Meins, et al., 2005). Common core features of *MECP2* duplication include: infantile hypotonia, recurrent respiratory infections, severe to profound intellectual disability, limited to absent speech, spasticity, and gastrointestinal motility problems (Friez et al., 2006; Ramocki et al., 2009; Van Esch, 2012; Van Esch, et al., 2005). Dysmorphism and distinct facial features may also be present, as may developmental regression (Van Esch, 2012). Aspects of the behavioral phenotype are still emerging. In our prior studies, we detailed that features of ASD may also be common in the phenotype of *MECP2* duplications and include reduced eye gaze and low social interest (Ramocki, et al., 2009). Other associated features include poor to absent speech, severe ID, and epilepsy. Some participants exhibited regression and lost their prior mobility and hand use. Nearly 50% of our cohort was initially diagnosed with ASD prior to receiving their genetic diagnosis (Ramocki, et al., 2009). Within *MECP2* duplication syndrome, we have not previously assessed other features that are common in ASD such as irritability, anxiety/social withdrawal, hyperactivity, and sensitivity to pain/temperature. We also have not explicitly made comparisons to a matched group of individuals with non-syndromic ASD. These extensions of our previous research are timely since a recent study in a mouse model of *MECP2* duplication syndrome found that doubling *MECP2* levels causes heightened anxiety and ASD features and alters the expression of genes that influence anxiety, social behavior, and pain sensitivity/nociception (Samaco, et al., 2012).

MECP2 duplication syndrome is likely underdiagnosed; and because genomic rearrangements including *MECP2* copy number gain are typically inherited from a maternal carrier, there is a 50% recurrence risk in future sons in most cases. Due to advantageous skewing of X chromosome inactivation patterns in female carriers, *MECP2* duplication syndrome primarily affects males (Ramocki, et al., 2010). More recent data suggest that *MECP2* duplication syndrome accounts for approximately 1% of all cases of X-linked intellectual disability (ID), but when males with ID who have other features of *MECP2*

duplication syndrome are also screened, the likelihood of identifying duplications involving the *MECP2* locus approaches 15% (Friez, et al., 2006; Lugtenberg et al., 2009).

Prior studies have demonstrated considerable phenotypic variability among patients with *MECP2* duplications for reasons that are not completely understood (Ramocki, et al., 2010). It is possible that increases in *MECP2* copy-number from 2 (duplications) to 3 (triplications) may account for some patients with a very severe phenotype (Carvalho et al., 2011; del Gaudio et al., 2006). Despite the fact that Xq28 duplications spanning *MECP2* are non-recurrent rearrangements, the observed phenotypic variability is not explained either by rearrangement size alone or gene content since the smallest region of genomic overlap spans 149 kb, contains the *IRAK1* and *MECP2* genes, and is sufficient to reproduce the core syndrome phenotypes. Specific genotype-phenotype correlation studies of duplication size and scores on standardized instruments used to assess clinical severity have never been reported.

In this study, we extend our previous work to further characterize the behavioral phenotype in *MECP2* duplication syndrome with careful attention to how the triad of core features of autism differs between boys with *MECP2* duplication syndrome and nonverbal-mental-age-matched boys with idiopathic ASD. We have focused on behavioral comparisons related to social affect (inclusive of communication and reciprocal social interaction) and repetitive behaviors. Within the *MECP2* duplication group, we examined associated features including pain tolerance, and aberrant behavior, and also examined how duplication size and gene content corresponded to severity of the behavioral phenotype. By probing certain ASD phenotypes (e.g. social affect, RRB) and associated behaviors (e.g. social withdrawal, pain sensitivity), and the degree of behavioral overlap with non-syndromic ASD, this approach may lead to further insights into MeCP2 protein function, novel therapeutic strategies, testing in pre-clinical mouse models, and potential human clinical trials (Cobb, Guy, & Bird, 2010; Dolen, Carpenter, Ocain, & Bear, 2010; Samaco, et al., 2012; Veenstra-VanderWeele & Blakely, 2012). Finally, clearly defining how *MECP2* duplication syndrome is distinct from idiopathic ASD will enhance early identification and treatment, as well as genetic counseling and testing of family members.

Materials and Methods

Participants

The participants were all male and ranged between the ages of 3–10 years. Inclusion criteria for boys with *MECP2* duplication syndrome were: a confirmed genetic diagnosis (see below), independent mobility, and hand use. All participants with an established diagnosis who met our inclusion criteria were offered enrollment and were ascertained as part of their attendance at the first ever worldwide conference on *MECP2* duplication syndrome. We excluded boys who did not have sufficient hand use or mobility to complete the Autism Diagnostic Observation Schedule (ADOS). Ten boys with *MECP2* duplication syndrome were enrolled in this new study, and were not part of our original cohort group (Ramocki, et al., 2009). Nine boys with ASD were selected through the Vanderbilt local clinical research database and formally participated in either the Simons Simplex collection (SSC) (n=4) or the Autism Treatment Network (ATN) (n=5) research registry. All participants were evaluated by a physician to exclude any underlying genetic conditions (also see Genetic Testing section below). Additional inclusion criteria for boys with idiopathic ASD included matching for chronological age, nonverbal mental age, and language level (i.e. nonverbal) to our sample of boys with *MECP2* duplication syndrome. As detailed in other publications, the enrollment criteria for these respective studies is stringent (Lord et al., 2011) (Coury, Jones, Klatka, Winklosky, & Perrin, 2009) and included detailed phenotypic testing to establish the diagnosis of ASD as well as genetic testing (detailed below).

Genetic Testing

All boys with *MECP2* duplication syndrome had an established clinical diagnosis. To independently confirm the diagnosis and to determine the size, genomic extent and gene content for each rearrangement, we designed a tiling-path oligonucleotide microarray spanning 4.6 Mb across the *MECP2* region on Xq28. The custom 4x44k Agilent Technologies (Santa Clara, CA) microarray was designed using the Agilent e-array website (http://earray.chem.agilent.com/earray/). We selected 22,000 probes covering Chromosome X: 150,000,000–154,600,000 (NCBI build 36), including the *MECP2* gene, which represents an average distribution of 1 probe per 250 bp. Probe labeling and hybridization were performed as described (Carvalho, et al., 2011). To estimate duplication size, we calculated the distance between breakpoints/coordinates (i.e. where probe signals traversed from no gain to gain signaling increased dosage of the genomic interval complementary to the interrogating oligonucleotide probe). In boys who had partial triplications, we used weighted correlations to account for the extra copies.

Blood samples were collected from all participants with ASD as per the respective research protocols of the ATN and the SSC. Chromosome microarray analysis (CMA) was performed, as well as DNA testing for Fragile X, and individuals with co-morbid genetic syndromes (e.g. Fragile X, *MECP2* duplication syndrome, RTT, Angelman, etc.) were excluded (also by evaluation from a clinician through the SSC and/or ATN).

Instruments

To assess Social Affect (inclusive of communication and reciprocal social interaction) and Restricted/Repetitive behaviours, the Autism Diagnostic Observation Schedule (ADOS), Module 1 was completed on all participants. ADOS scores are reported according to the research algorithm (No Words) for the domains of Social Affect and Restricted/Repetitive Behaviors (RRB). The Communication and Reciprocal Social Interaction domains are combined as part of the Social Affect domain using this research algorithm (Gotham, Risi, Pickles, & Lord, 2007), which results in improved predictive validity (Gotham et al., 2008). All participants included in this study had sufficient mobility and hand use to complete the ADOS. All psychologists who administered the ADOS attained research reliability.

Visual reception/nonverbal cognitive abilities and fine motor skills (nonverbal cognitive abilities), and expressive/receptive language abilities which comprised "verbal" cognitive abilities were assessed either using the Mullen Scales of Early Learning (MSEL) or the Bayley Scales of Infant and Toddler Development-III (BSID-III). These instruments have been utilized extensively within these populations and in large scale ASD research studies beyond the normative age ranges. In fact, a recent study demonstrated support for the practice of using MSEL age-equivalents to generate nonverbal IQ and verbal IQ scores in a group of individuals with idiopathic ASD beyond the typical age range of the test (Bishop, Guthrie, Coffing, & Lord, 2011). As per standard convention, age equivalents scores from the visual reception/cognitive and fine motor skills subtests were averaged to create the "nonverbal mental age" (NVMA) score, and scores from expressive and receptive language subtests were combined to create the "verbal mental age" (VMA) score (Bishop, et al., 2011; Lord, et al., 2011).The Vineland Adaptive Behavior Scales – II (VABS-II) was completed as a semi-structured interview with parents to assess overall adaptive behavior.

All parents of boys with *MECP2* duplication syndrome completed the Aberrant Behavior Checklist (ABC). The ABC is a questionnaire that evaluates the presence of specific maladaptive behaviors in 5 categories (irritability, lethargy/withdrawal, inappropriate speech, hyperactivity, and stereotypic behavior). The ABC has been previously utilized in individuals with a variety of genetic syndromes, and has been used extensively in studies of

non-syndromic ASD. ABC raw scores for each of the categories were analyzed, except for the "Inappropriate Speech" category because children in our study had limited speech. Parents of boys with *MECP2* duplication syndrome also completed the Sensory Profile Questionnaire. The results of this entire questionnaire are not reported here, but results of whether or not participants had decreased awareness of pain/temperature are reported.

Statistics

Because of the broad age range of our participants, we initially examined correlations between chronological age and the ADOS Social Affect and RRB scales to determine whether or not performance on the ADOS was related to chronological age. Since these correlations were not significant (p=.52, p=.936), chronological age is not used as a covariate. Because of the small sample size, non-parametric statistics (Mann-Whitney U test) were used to compare differences between boys with *MECP2* duplication syndrome, and boys with idiopathic ASD on our clinical outcome measures. To determine the relationship between duplication size and clinical severity in boys with *MECP2* duplication syndrome, we used bivariate correlations. Since chronological age was not correlated with any outcome variables, partial correlations (controlling for age) were not utilized.

Results

ASD vs. *MECP2* duplication syndrome

As expected, our groups were evenly matched for chronological age (See Table 1). All participants were given the MSEL or the BSID-III, and the VABS-II. Our matching procedures were again confirmed since there were no significant differences in NVMA (p=.55) or VMA (p=.27) between individuals with *MECP2* duplication syndrome, and those with idiopathic ASD (See Table 1). In addition, individuals who were given the Bayley scales scored comparably to those who were given the Mullen Scales of Early Learning. Similarly, there were no significant differences between boys with *MECP2* duplication syndrome and boys with idiopathic ASD on the composite scores of the VABS-II (p=1.00), or any VABS-II subscales (p=.17 to p=.62) (See Table 1).

Our data indicate that four of 10 boys with *MECP2* duplication syndrome had a diagnosis of ASD prior to having a genetic etiological diagnosis. Upon direct evaluation, seven of 10 boys (70%) exceeded total cutoff scores for ASD on the ADOS. Figure 2 shows the ADOS social affect scores of individuals with idiopathic ASD and boys with *MECP2* duplication syndrome. Specifically, although the majority of boys with *MECP2* duplications exceed threshold cutoff scores, and thus are quite similar to boys with ASD, they do not have as many impairments in social affect as compared to boys with ASD [z=2.34; p=.01]. No differences in RRB were noted between the groups (z=.50, p=ns). It is important to note that there were no significant correlations between NVMA and ADOS social affect or RRB profiles (p=.52; p=.94).

Within group descriptions for *MECP2* duplication syndrome

Table 3 shows the general clinical characteristics of boys with *MECP2* duplication syndrome. As is consistent with previous studies, a majority of boys in this cohort have a history of hypotonia and recurrent respiratory infections. Most boys in our sample also have constipation and histories of gastroesophageal reflux. The age of genetic diagnosis of *MECP2* duplication syndrome was very variable, ranging between 16 months to 7 years of age. Children who were older in terms of chronological age received later genetic diagnoses (r=.97; p<.001). We also inquired about sensitivity to pain and temperature. Data from the Sensory Profile Questionnaire and clinical interviews revealed that parents of nine of ten participants in our study reported that their boys either "frequently" or "always" (as opposed

to occasionally, seldom, or never) had a decreased awareness of pain and temperature. Data from the ABC revealed that eight of ten boys with *MECP2* duplication syndrome had clinically elevated scores for the Lethargy/Social Withdrawal subscale, and seven of ten boys had elevated scores for the Stereotyped behaviors subscale. No boys with *MECP2* duplication syndrome had clinical elevations for the Irritability subscale, and only two of ten had elevations for the Hyperactivity subscale of the ABC.

Figure 2 shows the mapping of duplications/triplications and the breakpoints of our participants with *MECP2* duplication syndrome. As noted in the figure, duplications within the smallest region of overlap (SRO) are sufficient to cause *MECP2* duplication syndrome. We examined the correlations between breakpoint size and our clinical outcome measures and did not find any statistically significant correlations for ADOS social affect scores (p=.55), ADOS RRB scores (p=.17), NVMA (p=.45), VMA (p=.48), or the VABS-II adaptive behavior composite (p=.35). There were also no significant correlations with any of the subscales of the ABC (p range=.59 to p=.88).

Discussion

In this study, we extended our previous work by comparing boys with *MECP2* duplication syndrome to a chronological and nonverbal mental age matched group of boys with idiopathic ASD with an emphasis on comparing core ASD features and adaptive behavior. Within the *MECP2* duplication group, we further delineated aspects of the behavioral phenotype, and examined whether duplication size corresponded to clinical severity. Our results provide further support that core behavioral aspects/features of ASD (e.g. social affect, RRB) are related to *MECP2* duplication syndrome/overexpression of *MECP2*. In addition, our results suggest that within the *MECP2* duplication group, breakpoint size alone does not predict differences in clinical severity.

Our direct comparisons of ASD profiles revealed many similarities between boys with *MECP2* duplication syndrome, and the matched group of boys with ASD. A majority of boys with *MECP2* duplication syndrome have impairments in social affect, albeit to a lesser degree than a matched sample of kids with idiopathic ASD. There were no differences in RRB between boys with idiopathic ASD and boys with *MECP2* duplication syndrome. Of importance is that the ASD-related behaviors across groups cannot be attributable to low cognition alone since nonverbal mental age did not correlate with severity of ADOS social affect or RRB scores in our study. Further support for the spectrum of ASD-related behaviors being associated with overexpression of *MECP2/MECP2* duplications is found in recent reports of girls with *MECP2* duplications who have a milder cognitive phenotype but still exhibit ASD features (Grasshoff et al., 2011). Beyond ASD, alterations in *MECP2* are connected to a broad range of social impairments, even within the context of "normal" cognitive functioning (Piton et al., 2011) (Ramocki, et al., 2009), further suggesting that low cognition alone cannot account for ASD-related phenotypes, and that common pathophysiological mechanisms may be involved across a broader range of disorders. When taken together with the findings that individuals with idiopathic ASD and mothers of individuals with idiopathic ASD were found to overexpress *MECP2* in peripheral leukocytes in the absence of *MECP2* mutations (Kuwano, et al., 2011), the aggregate data suggest that overexpression of *MECP2* may be fundamentally related to the core social features of ASD. Our data also suggest that the MeCP2 protein may have an important role in "normal" social development.

In further delineating the core behavioral phenotype in *MECP2* duplication syndrome, our data from the ABC suggested that stereotyped behaviors and lethargy/social withdrawal were common. In contrast, levels of irritability were not in a clinically significant range for

any boys with *MECP2* duplication syndrome and aggression does not appear to be a core aspect of the behavioral phenotype. Also, while two of ten boys with *MECP2* duplication syndrome exceeded clinically significant levels for hyperactivity, hyperactivity also does not appear to be a core aspect of the behavioral phenotype in *MECP2* duplication syndrome.

There is increasing evidence that altered levels of MeCP2 may modulate pain sensitivity (Geranton, Fratto, Tochiki, & Hunt, 2008; Samaco et al., 2008). For this reason, we had parents of boys with *MECP2* duplication syndrome complete the Sensory Profile Questionnaire. Parents of nine out of ten boys in our study reported decreased sensitivity to pain and temperature, indicating that sensory hypo-responsiveness may be a core aspect of the phenotype in *MECP2* duplication syndrome. These findings mirror those from a recent study of girls with RTT, where parents reported decreased sensitivity to pain, and decreased/delayed responses in situations likely to cause pain (e.g. falls, burns, injections, etc.) (Downs et al., 2010). Hypo-responsiveness to sensory stimuli has also been reported in a subset of individuals with idiopathic ASD; with a recent study demonstrating that hypo-responsiveness correlates strongly with ASD-related Social Affect profiles, but less so with repetitive behaviors (Foss-Feig, Heacock, & Cascio, 2012). Since the majority of our boys with *MECP2* duplication syndrome "frequently" or "always" had decreased responses to pain and temperature, we did not calculate how severity of impairments in Social Affect corresponded to pain/temperature sensitivity within this study (because of decreased variability in scores), but this would be of importance to examine in future work.

There was variability in the age of genetic diagnosis in our boys with *MECP2* duplications. Of note, however, is that older boys received later diagnoses, suggesting that in recent years, there may be increased awareness of the features of *MECP2* duplication syndrome leading to formal genetic testing and diagnosis. Still, 40% of our cohort had an original diagnosis of an autism spectrum disorder. Our cohort group for this study consisted of boys with mobility and hand use, introducing a selection bias as compared to those boys who exhibited significant regression and were hospitalized. Although most boys in our sample also had hypotonia and recurrent respiratory infections, it may have been more challenging to sort out the distinct aspects of the phenotype of *MECP2* duplication syndrome in these participants. Careful inquiry regarding family history may lead to an increased diagnostic yield in that often more than one family member was affected across generations; sometimes diagnoses of severe ASD were all that had been made.

Another goal of this study was to examine whether or not duplication size in boys with *MECP2* duplication syndrome corresponded to clinical severity (e.g. NVMA, VMA, ADOS scores, adaptive behavior). As is consistent with previous studies (Lugtenberg, et al., 2009; Van Esch, et al., 2005), we did not find any significant correlations with duplication size and any of our clinical outcome measures. While this lack of significance may be attributable, in part, to our small sample size, another plausible explanation is that varying levels of MeCP2 protein or *MECP2* transcript may account for the differences in clinical severity amongst our patients. For example, two brothers BAB3158 and BAB3159, have identical rearrangements, but differing levels of clinical severity, particularly with regard to ASD-related Social Affect (communication and reciprocal social interaction) and lethargy/social withdrawal. We previously reported a series of 30 male patients with *MECP2* duplications (Carvalho, et al., 2009), in which the duplication sizes were between 250 kb and 2.6 MB. The smallest region of overlap (SRO) in our series combined with our unpublished results surmised from 44 patients is approximately 140 kb encompassing *HCFC1, IRAK1, MECP2* as well as all known *MECP2* cis-regulatory regions (Liu & Francke, 2006). By comparison, case BAB2977, despite carrying the largest duplication rearrangement, does not include the entire pre-defined SRO which may help explain why his clinical parameters are not as severe as all other patients who have the entire SRO duplicated (Figure 2).

While this study clearly extends our prior work, it does have limitations. First, we acknowledge that our sample size was small and this may limit the generalizability of our findings. As noted above, we included boys with *MECP2* duplication syndrome who were mobile and had hand use, and thus were less severely affected than the broader population of individuals with *MECP2* duplications. In addition, ASD is a very heterogeneous disorder, and although this study focused on the identification of common behaviors, we did not investigate common biomarkers (e.g. *MECP2* expression levels) across idiopathic ASD and *MECP2* duplication syndrome. Given recent research demonstrating altered *MECP2* expression levels in idiopathic ASD, this approach has relevance in identifying possible sensitive diagnostic markers and treatment targets/outcomes and provides for a better examination of the potential contribution of individual genes such as *MECP2* to specific behaviors such as ASD-related social impairment. Future studies should also examine the physiological correlates of ASD-related social impairment and features such as anxiety in *MECP2* duplication syndrome and idiopathic ASD, given that *CRH*, which encodes corticotrophin releasing hormone, is a target gene of MeCP2 (Samaco, et al., 2012).

In spite of these limitations, the present study illustrates that overexpression/increased dosage of *MECP2* is related to core features of ASD. In fact, both increases and decreases in *MECP2* gene dosage and expression are related to ASD features (e.g. regression, stereotyped behaviors, social withdrawal) across mouse and human studies (Ramocki & Zoghbi, 2008; Samaco et al., 2009) (Coutinho, et al., 2007; Samaco, et al., 2008), demonstrating the importance of tight regulation of *MECP2*. Together, these studies should provide a potential framework for investigating treatments which could have broader impact for individuals with idiopathic ASD and those with *MECP2* duplications.

Acknowledgments

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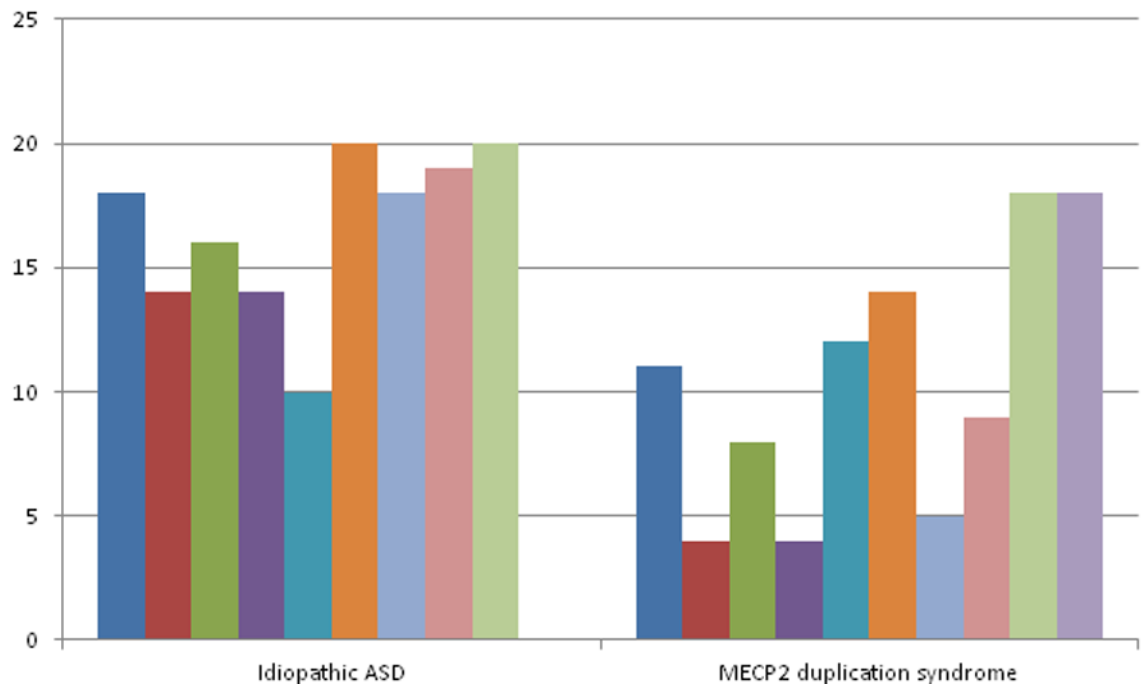
ASD-related Social Affect – ADOS scores

Figure 1.
ADOS social affect scores for idiopathic ASD and *MECP2* duplication syndrome
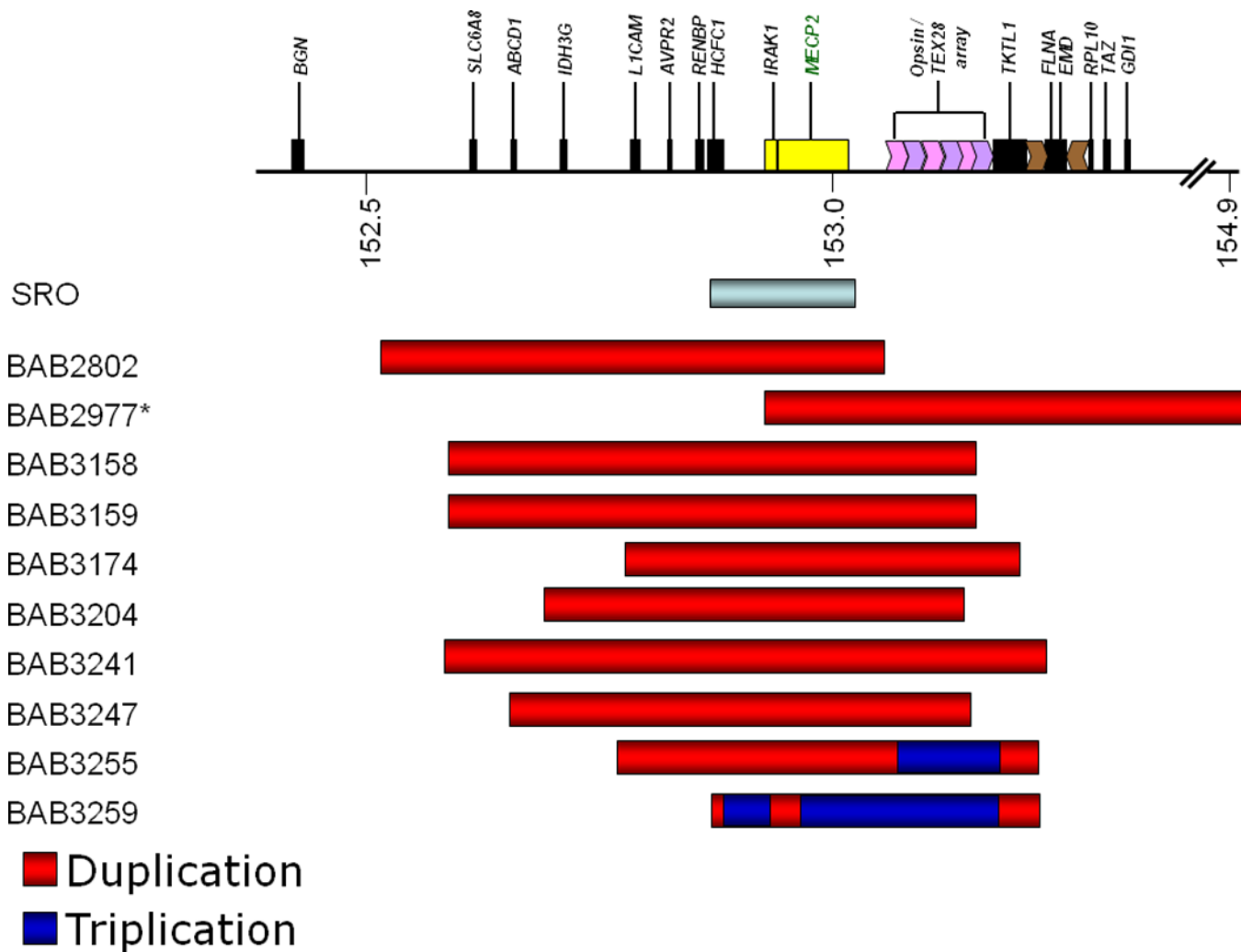
Figure 2.
SRO represents the smallest region of overlap (in yellow) that extends from *IRAK1* past *MECP2*. Duplications in this region alone are sufficient to cause *MECP2* duplication syndrome.

Table 1

Demographics

	Chronological Age (M, SD)	Nonverbal Mental Age (M, SD)	Verbal Mental Age (M, SD)	Vineland Adaptive Behavior Composite Standard Score (M, SD)
Idiopathic ASD	5.30 years, 1.96 years	18.67 months, 6.17 months	10.89 months, 3.90 months	55.00, 9.15
***MECP2* duplication syndrome**	5.64 years, 2.63 years	17.68 months, 6.56 months	13.35 months, 5.28 months	55.00, 4.30

Table 2

Aberrant Behavior Checklist subscale raw scores

	Irritability (M, SD)	Lethargy/Social Withdrawal (M, SD)	Stereotypy (M, SD)	Hyperactivity (M, SD)
Idiopathic ASD (n=4)	18.75, 12.09	11.50, 4.93	11.50, 2.89	31.25, 5.56
***MECP2* duplication syndrome (n=10)**	3.10, 2.77	9.70, 6.98	8.80, 7.98	8.30, 5.16

Table 3

Clinical Findings in boys with *MECP2* duplication syndrome

Patient Number	Age at *MECP2* genetic Diagnosis	Constipation	GER	Infantile Hypotonia	Recurrent Infections	Pain Insensitivity
BAB2802	4 yo	X	X	X	X	X
BAB2977	33 months		X	X	X	
BAB 3158	7 yo	X	X	X	X	X
BAB 3159	3 yo	X	X	X	X	X
BAB 3174	7 yo	X		X	X	X
BAB 3204	7 yo	X	X		X	X
BAB 3241	19 months	X	X	X	X	X
BAB 3247	17 months	X	X	X	X	X
BAB 3255	18 months	X	X	X	X	X
BAB 3259	16 months	X	X	X	X	X