Child Behavior Checklist Emotional Dysregulation Profiles in RASopathies

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ABSTRACT

Background: The behavioral phenotype of RASophaties is characterised by wide variability in most domains with risk for psychopathology and high prevalence of attention and social problems. The aim of the study is to investigate affective and behavioral dysregulation in RASopathies compared with a control group.

Method: Affective and behavioral dysregulation was investigated in 72 children with RASopathies and 223 children with typical development. Two Child Behaviour Checklist (CBCL) profiles, Deficient Emotional Self-Regulation (DESR) (elevation between 1 and 2 Standard Deviations (SD) in Anxiety/ Depression, Aggression, Attention (AAA) subscales) and Dysregulation Profile (DP) (elevation of 2 Standard Deviations or more) have been investigated. In a subgroup of the cohort, comparison in CBCL subscales was also performed.

Results: Children with Noonan syndrome (NS), Mazzanti syndrome (MS) and cardiofaciocutaneous syndrome (CFCS) had higher and more often clinically significant CBCL AAA profile than children with Noonan syndrome with multiple lentigines (NSML) and Costello syndrome (CS).

Conclusion: The severity of the scores of the AAA profile marks a possible increased psychopathological risk in RASopathies, highlighting the occurrence of behavioral dysregulation in these patients.

Keywords
Emotional dysregulation profiles, Noonan Syndrome, RASopathies
Introduction

Present study

The aim of the present study is to investigate affective and behavioral regulation in a cohort of 72 individuals affected by RASopathies by using the Child Behavior Checklist (CBCL) questionnaire. Specifically, we used two specific behavioral profiles: CBCL-Deficient Emotional Self-Regulation (DESR) and CBCL-Dysregulation Profile (DP). Our study investigates risk of a complex self-regulation disorder, including both internalizing and externalizing features, in RASopathies. Based on the extensive clinical overlap, on the similar behavioral profile, a subgroup of the cohort (Noonan syndrome/Mazzanti syndrome) is compared with a control group in CBCL subscales.

The RASopathies are a family of disorders resulting from dysregulation of the RAS/MAPK signaling pathway [1-3]. They include Noonan syndrome (NS), Costello syndrome (CS), cardiofaciocutaneous syndrome (CFCS), Noonan syndrome with multiple lentigines (NSML, formerly known as LEOPARD syndrome), Mazzanti syndrome (MS), neurofibromatosis type 1, Legius syndrome and other clinically related disorders. As a group, the RASopathies have an estimated collective prevalence of 1:1500 [4]. Among RASopathies, Noonan syndrome (NS, OMIM 163950) is the most clinically variable due its marked genetic heterogeneity, with disease-causing mutations in PTEN11, SOS1, RAF1, RIT1, LZTR1, and NRAS accounting for more than 90% of molecularly confirmed cases [5,6].

NS and the other RASopathies are characterized by variable cognitive impairment, ranging from moderate/severe intellectual disability (i.e., CS, CFCS) to normal/low average cognitive functioning (i.e., NS and LS) [7,8]. Similarly, the behavioral phenotype of RASopathies is characterized by a wide variability in most domains. Children with CFCS have been shown to be at risk for developing different kind psychopathologies, including high prevalence of attention problems, social problems, and altered thoughts and behaviors (e.g., obsessive thoughts, repetitive acts) [9].

Previous studies highlighted the presence of anxious and somatic symptoms in children diagnosed with NS and CS and occurrence of social and attention problems in children with NS, CS, and CFCS [10,11]. Other studies reported difficulties in “social cognition” in patients affected by NS, especially in emotion recognition and expression (such as inability to express emotions verbally), mood regulation, social behavior, and executive functions. A considerably high proportion of subjects with NS (40-50%) was documented to show attention deficit and hyperactivity (ADHD) symptoms and syndromes [7,12-20]. We recently reported that 70% of our NS patients showed ADHD features and 37% anxiety symptoms or syndromes [21]. Similarly, although no structured study has been conducted in MS, it has been reported that children with this condition exhibit a behavioral profile characterized by ADHD disorder [22,23]. Overall these findings are in line with the role of the RAS-MAPK pathway in the modulation of prefrontal cortex and striatum neurotransmitters involving attention regulation and executive functions [24,25]. Other studies underline that alterations in the striatum might be related to affective problems, such as depression and mood dysregulation [26].

Recent research in clinical non-syndromic samples, has begun to recognize that affective problems like emotional instability, impulsivity, agitation, restlessness, and mood dysregulation are frequently associated with ADHD, and that patients diagnosed with ADHD are frequently reported to show DESR [27-29]. DESR has been defined by Gottman and Katz [30] as the ability to “(a) inhibit inappropriate behavior related to strong negative or positive emotion, (b) self-
soothe any physiological arousal that the strong affect has induced, (c) refocus attention, and (d) organize for coordinated action in the service of an external goal” (p. 373). DESR is characterized by poor self-regulation including symptoms of low frustration tolerance, impatience, quickness to anger, and being easily excited to emotional reactions. Several studies have reported that DESR is associated to a specific dysregulation profile at CBCL, given by the sum of the score at Anxiety-Depression, Aggression, and Attention (AAA) subscales. Specifically, the CBCL DESR profile is defined as a CBCL-AAA score increased by 1–2 standard deviation (180 ≥ CBCL-AAA score < 210) [27-29,31-37]–, while a more severe form of DESR, called CBCL- DP is characterized by a CBCL-AAA score > 210 (CBCL-AAA score increased more than 2 SD).

These affective and behavioral dysregulation profiles are not specific, and they are not associated to the presence of a single disorder, but they are considered as a risk marker of a complex self-regulation disorder, including both internalizing and externalizing features [38]. Moreover, CBCL DP has been associated with severe psychopathology, principally disruptive behavior disorders (DBDs) [34], suicidal behavior [35], substance use disorders [36], with relevant affective storms, reactive aggression and often reduced need of sleep, and significant lower level of school adjustment and occupational stability [34,37].

Given the high rate of ADHD features in NS and related disorders [12–21], the frequent association between ADHD and DESR in samples without genetic syndromes [27], and the urgent need of screening tools to identified early psychiatric features in RASopathies, we propose a potentially useful screening tool to identify risk of deficient emotional regulation in RASopathies.

Methods

Participants

The cohort recruited in the study was followed up at the Department of Pediatrics of the Catholic University (Rome, Italy), Bambino Gesù Children’s Hospital (Rome, Italy), and Clinica Pediatrica, University of Milano-Bicocca, San Gerardo Hospital/MBBM Foundation (Monza, Italy). In all cases, diagnosis of RASopathy was made by experienced medical geneticists and pediatricians. Following clinical assessment, all patients had been screened for mutations within the entire coding sequence of PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF, SHOC2, MEK1, CBL, RIT1, SOS2, and LZTR1 genes. Only individuals with molecularly confirmed diagnosis of NS, NSML, MS, CS, and CFCS were included in the study.

The study cohort comprised 72 children (37 males, 35 females). Chronological age (CA) of children and adolescents ranged from 6 to 19.2 years (median age 10.10, mean 10.71, SD 3.61) and intelligence quotient (IQ) score was between 33 and 131 (median 86.5; mean 80, SD 21.8). Control group (CG) was composed by 207 children with typical development (TD) recruited in the local school (111 males, 96 females). Age range of CG was between 6 and 16 years (median age: 10 years; mean 10, SD 2.17). TD was reported by parents, and the study cohort did not include children born prematurely, or having any suspected or diagnosed neurological condition or learning disability (Table 1).

Assessment

General cognitive abilities were assessed with age-scaled tests based on age, language and cognitive skills including Raven Coloured Progressive Matrices Test [39], Wechsler Preschool and Primary Scale of Intelligence [40], Wechsler Intelligence Scale for Children–Fourth Edition [41] and Leiter International Performance Scale – Revised, brief version [42]. We classified Intellectual abilities according to the Diagnostic and Statistical Manual of Mental Disorders, 2000 (DSM-IV-TR) [43].

The Child Behaviour Checklist (CBCL) [44,45] is a component of the Achenbach System of Empirically Based Assessment and is a widely used method to identify behavior problems in children. In the present study, it was used to assess behavioral alterations and psychopathological features. The school-age version (CBCL/6–18), with 120 questions, is dedicated to children from 6 to 18 years and completed by parents. The checklist consists of a number of statements about the child’s behavior in the past six months. Responses are recorded on a Likert scale, with values equal to 0 (not true), 1 (somewhat or sometimes true), and 2 (very true or often true). Raw scores are converted to gender and age standardized scores (T scores having a mean of 50 and SD 10). Similar questions are grouped into a number of syndromes (e.g., aggressive behavior), and their scores are added up to obtain a global score for that specific syndrome. Syndromes
scores are further obtained to provide scores for internalizing and externalizing problem scales. A score of all questions is also totaled. For each syndrome, problem scale, and total score, tables determine whether the score represents normal, borderline, or clinical behavior. The CBCL AAA profile is defined as DESR by a score of ≥ 180 and <210 (1<SD<2) on the sum of the attention, aggression, and anxious/depressed CBCL scales.

The CBCL-DP profile is defined as positive by a score of >210 (>2 SD) on the sum of the same syndrome scales [32].

The spectrum of cognitive skills and other CBCL indexes had previously been reported for a subset patients of the study cohort [7,11].

### Analyses

To examine differences between NS, NSML, MS, CS, and CFCS in the distribution of CBCL AAA profile score, the following categories were considered: CBCL DESR profile ≥ 180 and <210, CBCL-DP profile >210. Participants with genetically confirmed diagnosis of NS and MS (NS/MS group) were compared with CG in chronological age and CBCL AAA profile score by using T-test (Student’s t-test). The NS/MS group was also compared with CG in CBCL subscales, using repeated measures ANOVA, with CBCL subscales as within-factor and groups as between-factor. In presence of significant differences, post hoc comparisons were analyzed by means of Tukey’s HSD (Honestly Significant Difference) tests. The alpha level was set at p<0.05.

Factors identified preliminarily as possibly associated with NS/MS were further entered stepwise into multivariate logistic regression modeling to compute odds ratios (OR) with their Confidence Intervals, with NS/MS as the outcome measure.

SPSS 21.0 (International Business Machines Corporation, Armonk, NY, USA for Windows) was used for statistical analyses.

### Results

#### Study cohort

The study cohort included 49 patients with a molecularly confirmed diagnosis of NS (PTPN11, n=36; SOS1, n=9; RAF1, n=4). Six patients had clinical diagnosis of CFCS, which was associated with a mutated BRAF (n=5) or MEK1 (n=1) allele, and 7 subjects had a diagnosis of CS confirmed by the presence of a HRAS mutation. Finally, 6 individuals had NSML associated with mutations in PTPN11, and 4 had a diagnosis of MS (mutated SHOC2 allele) (Table 1).

Children with NS, MS and CFCS had higher prevalence of clinically significant CBCL AAA scores than children with NSML and CS. In children with NS, the mean of CBCL AAA score was 180 ± 21 with 43% of cases obtaining a score between 1 and 2 SD (CBCL DESR

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**Table 1: Neurobehavioral characteristics according gene involved in the cohort included in the study.**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>GENE</th>
<th>NUMBER OF CASES</th>
<th>GENDER DISTRIBUTION</th>
<th>MEDIAN AGE AND RANGE (YEARS)</th>
<th>MEDIAN IQ AND RANGE</th>
<th>CBCL INTERNALIZING Mean ± SD</th>
<th>CBCL EXTERNALIZING Mean ± SD</th>
<th>CBCL TOTAL Mean ± SD</th>
<th>AAA profile Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>PTPN11</td>
<td>36</td>
<td>25M/11F</td>
<td>9.75 (6-19.2)</td>
<td>89 (62-115)</td>
<td>57.7 ± 11.7</td>
<td>56.4 ± 10.3</td>
<td>59 ± 12.3</td>
<td>179.3 ± 22</td>
</tr>
<tr>
<td>NS</td>
<td>SOS1</td>
<td>9</td>
<td>4M/5F</td>
<td>11.3 (7.3-16)</td>
<td>92.5 (42-110)</td>
<td>64.8 ± 9.8</td>
<td>55.4 ± 10.9</td>
<td>62.4 ± 11.3</td>
<td>183.3 ± 16.8</td>
</tr>
<tr>
<td>NS</td>
<td>RAF1</td>
<td>4</td>
<td>1M/3F</td>
<td>12.6 (9.8-15)</td>
<td>70 (46-104)</td>
<td>65.7 ± 5.6</td>
<td>58 ± 4.6</td>
<td>64 ± 7.1</td>
<td>188.7 ± 18.1</td>
</tr>
<tr>
<td>NS</td>
<td>all genes</td>
<td>49</td>
<td>30F/19M</td>
<td>10.4 (6-19.2)</td>
<td>88 (42-115)</td>
<td>59.7 ± 11.3</td>
<td>56.3 ± 10</td>
<td>60 ± 11</td>
<td>180 ± 21</td>
</tr>
<tr>
<td>MS</td>
<td>SCHOC2</td>
<td>4</td>
<td>3F/1M</td>
<td>10.3 (9.7-12)</td>
<td>68.5 (50-55)</td>
<td>63.6 ± 6.9</td>
<td>60.5 ± 6.6</td>
<td>66.2 ± 8.8</td>
<td>196.2 ± 52.7</td>
</tr>
<tr>
<td>NSML</td>
<td>PTPN11</td>
<td>6</td>
<td>3F/3M</td>
<td>9.7 (6.5-12.1)</td>
<td>100 (73-131)</td>
<td>54.8 ± 3.9</td>
<td>52.1 ± 10.2</td>
<td>55.8 ± 7.6</td>
<td>165.8 ± 13.6</td>
</tr>
<tr>
<td>CFCS</td>
<td>BRAF</td>
<td>5</td>
<td>5F</td>
<td>12 (6-19)</td>
<td>44 (36-80)</td>
<td>58.8 ± 2</td>
<td>60.2 ± 12</td>
<td>63.6 ± 12</td>
<td>182 ± 11</td>
</tr>
<tr>
<td>CFCS</td>
<td>MEK1</td>
<td>1</td>
<td>1F</td>
<td>12.7</td>
<td>42</td>
<td>58</td>
<td>55</td>
<td>66</td>
<td>184</td>
</tr>
<tr>
<td>CFCS</td>
<td>all genes</td>
<td>6</td>
<td>6F</td>
<td>12.3 (6-19)</td>
<td>44 (36-60)</td>
<td>58.6 ± 2</td>
<td>59.3 ± 2.4</td>
<td>64 ± 1.5</td>
<td>182.3 ± 9.4</td>
</tr>
<tr>
<td>CS</td>
<td>HRAS</td>
<td>7</td>
<td>3M/4F</td>
<td>7.9 (6-18)</td>
<td>51 (33-60)</td>
<td>57.2 ± 7.1</td>
<td>52.1 ± 10.2</td>
<td>55.8 ± 7.6</td>
<td>165.8 ± 13.6</td>
</tr>
<tr>
<td>Entire cohort</td>
<td>-</td>
<td>72</td>
<td>37M/35F</td>
<td>10.10 (6-19.2)</td>
<td>86.5 (33-131)</td>
<td>58.2 ± 9.9</td>
<td>55.9 ± 9.6</td>
<td>60.2 ± 10.5</td>
<td>179.7 ± 20.4</td>
</tr>
<tr>
<td>Control group</td>
<td>-</td>
<td>207</td>
<td>111M/96F</td>
<td>10.0 (6-16)</td>
<td>TD</td>
<td>48.0 ± 9.0</td>
<td>46.0 ± 8.0</td>
<td>45.0 ± 9.0</td>
<td>157.0 ± 8.0</td>
</tr>
</tbody>
</table>

Legend: M, male; F, female; IQ, total intelligence quotient; NS, Noonan syndrome; MS, Mazzanti syndrome; NSML, Noonan syndrome with multiple lentigines (i.e., LEOPARD syndrome); CFCS, cardiofaciocutaneous syndrome; CS, Costello syndrome; CBCL, Child Behavior Checklist; TD Typical Development; AAA profile, Anxiety/Depression Aggression Attention profile; SD, standard deviation
profile), and 10% showing a score above 2 SD (CBCL-DP profile). Among children with MS (CBCL AAA score mean 196.2 ± 52.7), only one participant had a CBCL AAA score in average, while two children presented a CBCL DESR profile, and one child had a CBCL-DP profile.

In the CFCS group, the mean of CBCL AAA score was 182 ± 1: more than half (67%) of children had a score above 180 in CBCL AAA, and all these subjects obtained a score between 1 and 2 SD (CBCL DESR profile). Concerning CS and NSML, only one patient (respectively 14% and 16%) obtained a score between 1 and 2 SD (CBCL DESR profile); the rest obtained a score in average. Overall, 48% of children with RASopathies had a clinically significant CBCL AAA profile score (mean 194 ± 14.7): 40% of cases showed CBCL DESR profile while only 8% of children had the CBCL-DP profile.

### Comparisons between groups

Based on the extensive clinical overlap, on the similar behavioral profile fifty-three children with NS and MS were included in a single population cohort (NS/MS) and matched to 207 CG on the basis of their chronological age ($t_{1,258}=1.56, p=0.12$).

The comparisons between groups documented statistically significant higher CBCL AAA scores in the NS/MS group (182±21.9 vs. 157.4± 8.0, $t_{1,256}=13.14, p=0.00001, \eta^2=0.4$). Concerning syndrome scale of CBCL, comparisons showed significant main effect of Group ($F_{1,256}=155.94, p<0.0001$). The Group × syndrome scale interaction was also significant ($F_{7,1280}=8.76, p<0.00001$). As shown in Table 2, the NS/MS group obtained always significant higher score than CG in any syndrome subscale considered ($p$ always < 0.001).

Also regarding DSM oriented scale, a significant main effects of Group was found ($F_{1,256}=148.02, p<0.0001$) with higher scores documented in the syndromic group. The Group × DSM oriented scale interaction was significant ($F_{7,1280}=6.76, p<0.00001$) and post-hoc comparisons indicated that in any DSM oriented subscale NS/MS obtained higher score ($p$ always <0.001). Furthermore, main effect of Group was found significant in CBCL internalizing, externalizing, and total problem scale ($F_{1,256}=95.96, p<0.00001$) with higher scores in NS/MS group. Also in these comparisons, the Group × CBCL internalizing, externalizing and total problems interaction were significantly different ($F_{2,516}=9.53, p<0.00001$) since the NS/MS obtained in any subscale considered always significant higher score than CG ($p$ always < 0.00001) (Table 2).

### Multivariate regression modeling

By multivariate logistical regression modeling we tested for independent and significant association of CBCL subscales-score in NS/MS group versus CG (Table 3). Factors (n=8) preliminarily associated with outcome at bivariate analysis were entered stepwise into regression modeling in order of preliminary significance. After, 9 factors (including CBCL Syndrome Scales + CBCL anxiety-depression score + aggressive score (ADA)) were included in the model and two factors were found to be significantly and independently associated with NS and MS diagnosis: 1) CBCL attention problems score, and 2) CBCL–anxiety-depression score + aggressive score (CBCL-ADA score) (Table 3).

Of note a greater CBCL-AAA score (CBCL-DESR profile) was significantly and independently associated with NS and MS diagnosis even when controlled for CBCL total score (DESR profile in NS/MS vs CG, OR=1.13, p<0.0001; CBCL total score in NS/MS vs CG, OR=1.00, p=0.9, by binary logistic regression modeling).

### Discussion

The present paper aimed to analyze risk of affective and behavioral dysregulation in children affected by RASopathies. To this goal, the emotional aspects were investigated by using the CBCL–AAA profile. Our previous study suggested that children affected by RASopathies might have greater levels of psychopathology, and that the presence of psychiatric features might be independent from the presence/absence of intellectual disabilities, and academic or medical problems [11]. Particularly, we recently observed that children with NS have a higher prevalence of attention deficit/hyperactivity and anxiety symptoms and syndromes when compared to typically developed children [21]. Consistently with our previous observations, a recent paper reported significant problems on both the Attention and Social CBCL subscales in NS compared to healthy children [19].

To our knowledge, this is the first study documenting the use of the CBCL-dysregulation profile to screen children diagnosed with RASopathies for alteration of affective and emotional regulation. Our findings indicate that children with RASopathies show affective and
behavioral dysregulation risk, as measured by the CBCL-AAA profile.

The CBCL AAA profile has been widely analyzed in several studies in cohorts of children without genetic syndromes [32,34,35,37] and found to be associated with ADHD and with a mood disorder diagnosis, especially with affective and behavioral dysregulation. Also, the CBCL DESR profile has been shown to be related to maladaptive behaviors in response to negative emotions or frustration, elevated irritability, impulsivity and anger, high rates of anxiety and disruptive disorders [32,46], and has been associated with an increased risk of developing a major depressive disorder during childhood and adolescence [31,47]. CBCL-DP profile resulted associated with severe psychopathology and poor adjustment, and has been associated with an increased risk of developing a pediatric bipolar disorder [34,35,37]. Also, longitudinal studies suggested that mood and behavioral dysregulation in childhood may be a putative predictor of future overall psychopathology and maladjustment [35,48].

Subjects with NS and MS were found to be significantly impaired in all CBCL syndrome and DSM oriented scales when compared to CG. Moreover, group with NS/MS significantly differed to CG also for the presence of DESR. Indeed, CBCL-DESR profile was found to be significantly and independently associated with NS/MS diagnosis by multivariate logistic regression model: 1] CBCL attention deficit/hyperactivity problems, 2] CBCL-oppositional defiant problems, and 3] CBCL-conduct problems.

Table 2: CBCL scores in NS/MS group versus control group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>NS/MS N=53</th>
<th>control group N=207</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent rated behaviors (CBCL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syndrome Scales</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Anxious/Depressed</td>
<td>59.5 ± 9.05</td>
<td>52.8 ± 4.00</td>
<td>0.000029</td>
</tr>
<tr>
<td>- Withdrawn/Depressed</td>
<td>59.0 ± 8.99</td>
<td>53.4 ± 4.79</td>
<td>0.00032</td>
</tr>
<tr>
<td>- Somatic Complaints</td>
<td>61.1 ± 9.11</td>
<td>54.3 ± 5.15</td>
<td>0.00029</td>
</tr>
<tr>
<td>- Social Problems</td>
<td>61.1 ± 8.43</td>
<td>53.8 ± 3.87</td>
<td>0.00029</td>
</tr>
<tr>
<td>- Thought Problems</td>
<td>57.8 ± 8.55</td>
<td>51.8 ± 3.79</td>
<td>0.00030</td>
</tr>
<tr>
<td>- Attention Problems</td>
<td>63.6 ± 9.14</td>
<td>52.7 ± 3.73</td>
<td>0.00029</td>
</tr>
<tr>
<td>- Rule-Breaking Behavior</td>
<td>56.9 ± 6.25</td>
<td>52.0 ± 3.33</td>
<td>0.000173</td>
</tr>
<tr>
<td>- Aggressive Behavior</td>
<td>58.5 ± 7.47</td>
<td>52.7 ± 3.06</td>
<td>0.00029</td>
</tr>
<tr>
<td>Internalizing Problems</td>
<td>59.9 ± 11.07</td>
<td>48.0 ± 9.00</td>
<td>0.00020</td>
</tr>
<tr>
<td>Externalizing Problems</td>
<td>56.6 ± 9.81</td>
<td>46.0 ± 8.05</td>
<td>0.00020</td>
</tr>
<tr>
<td>Total Problems</td>
<td>60.5 ± 11.60</td>
<td>45.0 ± 9.00</td>
<td>0.00020</td>
</tr>
<tr>
<td>Parent rated behaviors (CBCL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSM-oriented scales</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Affective Problems</td>
<td>61.4 ± 9</td>
<td>53.4 ± 4.5</td>
<td>0.00018</td>
</tr>
<tr>
<td>- Anxiety Problems</td>
<td>61.2 ± 8.5</td>
<td>54.4 ± 5</td>
<td>0.00018</td>
</tr>
<tr>
<td>- Somatic Problems</td>
<td>60.7 ± 9.7</td>
<td>54.5 ± 5.4</td>
<td>0.00024</td>
</tr>
<tr>
<td>- Attention Deficit/Hyperactivity Problems</td>
<td>62 ± 8.1</td>
<td>52.2 ± 3.2</td>
<td>0.00018</td>
</tr>
<tr>
<td>- Oppositional Defiant Problems</td>
<td>57.2 ± 7.2</td>
<td>52.2 ± 3.2</td>
<td>0.000133</td>
</tr>
<tr>
<td>- Conduct Problems</td>
<td>56.9 ± 6.3</td>
<td>51.6 ± 3.2</td>
<td>0.000061</td>
</tr>
</tbody>
</table>

Legend: SD standard deviation; N number; pT point T; NS Noonan syndrome; MS Mazzanti syndrome
* post hoc Unequal NHSD

Table 3: Multivariate logistic regression model of factors associated with NS/MS group vs control group.

<table>
<thead>
<tr>
<th>Factors</th>
<th>NS/MS (mean ± SD)</th>
<th>control group (mean ± SD)</th>
<th>OR (95% CI)</th>
<th>Statistics</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBCL-Attention Problems</td>
<td>63.6 ± 9.14</td>
<td>52.7 ± 3.73</td>
<td>1.24 [1.15–1.34]</td>
<td>28.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CBCL-Anxiety-depression + CBCL-oppositional score (ADA)</td>
<td>118 ± 14.6</td>
<td>104 ± 5.77</td>
<td>1.07 [1.01–1.13]</td>
<td>6.56</td>
<td>0.01</td>
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</table>
prolonged problems score and 2) CBCL anxiety-depression score + aggressive score (ADA) (Table 3). This finding indicates that attention problems and behavioral dysregulation/affective problems are important psychopathological features to investigate in children diagnosed with NS/MS. Finally, our results indicated that children with NS, MS and CFCS had higher and more often clinically significant CBCL-AAA and CBCL total scores than children with LS, CS and CG. Our findings confirm previous evidences [11,19,21] showing the presence of attention disorders in children with NS compared to the general population during juvenile age, and document a considerably high prevalence of behavioral dysregulation profile in RASopathies (considering CBCL Anxiety scale score + CBCL Aggressive scale score). This affective and behavioral dysregulation profile appears to be present in the RASopathies independently from the presence of intellectual disability.

Deficits in emotional and behavioral regulation, especially irritability and aggressiveness, are usually associated with cognitive impairment in subjects affected by genetic syndromes with intellectual disability [49,50]. It is worth to note that in this case the behavioral dysregulation profile does not seem to be related to the cognitive impairment. Indeed, NS and NSML patients with similar IQ seems to be characterized by very different degrees of affective and behavioral problems, with NS being significantly more impaired on CBCL AAA profiles compared to subjects with NSML. Consistent with this observation, CS and CFCS, which are generally more severely cognitively impaired [7], appear to have an opposite pattern in the emotional regulation profile, with CFCS presenting positivity for DESR profile in several cases and CS, never presenting this profile.

From a neurobiological perspective, the high proportion of attention problems found in children with NS and MS might represent the result of dysfunctional inhibitory brain circuits at the level of prefrontal cortex and striatum, which have been documented to lead to executive function and attention deficits [24,25]. Similarly, the high proportion of positivity on CBCL AAA profiles in both disorders could be explained by functional alterations involving the striatum with direct impact on affective problems, such as depression and mood dysregulation [26]. We suggest to include the evaluation of behavioral dysregulation risk in children with RASopathies since better definition of this profile may be a putative predictor of future overall psychopathology and maladjustment.

**Limitations**

Our findings should be evaluated taking into account some limitations. First, the study did not allow a complete psychopathological evaluation of cases and controls, but permitted to collect a complete rating of symptoms by parents that are commonly used as a screening for mood disorders. Secondly, investigation of emotional dysregulation by using questionnaire generally used to investigate internalizing and externalizing problems could be a limit to define deeply these features. Moreover, the analyzes were based on cross-sectional data, and the present findings do not allow to predict the evolution of the mood and emotional dysregulation profile of children affected by RASopathies at follow-up.

Finally, these analyses should be validated in larger patient cohorts specifically including the diverse RASopathies disorders. These data are expected to drive future, adequately powered prospective studies with the aim of better characterizing the developmental psychopathology of RASopathies.

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**Authors’ Contribution**

L.S., A.P., C.C., S.G., M.D. participated in the research design, the data analysis and writing of the article. V.S., T.M., V.S., P.F. participated in the data analysis and writing of the article. M.M., C.MP., D.MC., S.A., Z.G. participated in the performance of the research.
Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.


