Androgens and autistic traits: A study of individuals with congenital adrenal hyperplasia

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Abstract

Testosterone promotes male-typical neural and behavioral development in non-human mammals. There is growing evidence that testosterone exerts similar influences on human development, although the range of behaviors affected is not completely known. This study examined the hypothesis that autistic traits are increased following prenatal exposure to abnormally high levels of testosterone caused by congenital adrenal hyperplasia (CAH). Sixty individuals with CAH (34 female, 26 male) and 49 unaffected relatives (24 female, 25 male) completed the Autism Spectrum Quotient (AQ). Females with CAH scored significantly higher than unaffected females on total AQ score, largely due to enhanced scores on subscales measuring social skills and imagination. These results suggest that prenatal exposure to high levels of testosterone influences some autistic traits and that hormonal factors may be involved in vulnerability to autism.

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Androgen levels during early (prenatal or neonatal) development have powerful programming influences on the mammalian brain, and as a consequence, have enduring effects on behavior (Goy and McEwen, 1980; Hines, 2004). Early treatment of female rodents with androgens, including testosterone (T), influences neural characteristics and behaviors that show sex differences. Similarly, in rhesus monkeys, treating pregnant females with T increases male-typical behavior and reduces female-typical behavior in female offspring. In both rodents and non-human primates, the early hormone environment influences social behavior, as well as reproductive behavior (Goy and McEwen, 1980; Hines, 2004).

Autism spectrum conditions (ASCs) are characterized by impairments in reciprocal social interaction, verbal and nonverbal communication, and imaginative play and by strong repetitive behavior and narrow interests, and include classic autism as well as the less severe diagnosis, Asperger’s Syndrome (AS) (American Psychiatric Association, 2000; ICD-10, 1994). As many as 1 in 200 people may have been diagnosed with an ASC, as defined by ICD-10 criteria (Scott et al., 2002). ASCs are more common in males than in females (4:1 for classic autism, 9:1 for AS; Rutter, 1978; Wing, 1981). This sex difference suggests that autistic traits might be influenced by androgen levels prenatally.

Studies relating T measured in amniotic fluid to autistic traits also suggest that T may play a role in ASCs (Baron-Cohen et al., 2004; Knickmeyer et al. 2005). Amniotic fluid T has been found to relate negatively to eye contact in boys at 12 months of age, vocabulary development in 18- and 24-month-olds, and quality of social relationships and range of interests in 4-year-olds, and these characteristics (reduced eye contact, poor social relations, limited interests and impaired vocabulary development) are associated with ASCs. Research examining the ratio of the second to the fourth digit of the human hand (2D:4D ratio), a possible index of prenatal T exposure (Brown et al., 2002), also suggests links between T and ASCs. The 2D:4D ratio is lower in males than in females (Brown et al., 2002; Manning et al., 1998) and is even lower in children with autism (Manning et al., 2001).

A classic approach to studying prenatal influences of androgen on human behavior is to examine situations involving dramatic hormone alteration prenatally (Hines, 2004),

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particularly the autosomal recessive disorder, classical congenital adrenal hyperplasia (CAH). CAH is characterized by an enzyme deficiency (usually of 21 hydroxylase (21-OH)) leading to overproduction of adrenal androgens, including T, beginning prenatally. Worldwide newborn screening suggests that classical CAH caused by 21-OH deficiency occurs in 1 in 14,500 live births (Grumbach et al., 2003). Elevated androgens in female fetuses with CAH typically produce ambiguous (male/female) external genitalia. Girls with CAH are usually diagnosed soon after birth, and treated postnatally with corticosteroids to regulate the hormonal milieu (White and Speiser, 2002). Thus, their primary hormonal abnormality is prenatal. Although CAH affects both sexes, females are of particular interest because they are exposed to elevated T and other androgens for much of gestation; in most male fetuses with CAH, T appears to be in the high normal range and genital appearance is normal (Pang et al., 1980; Wudy et al., 1999).

The most consistent finding regarding females with CAH is increased male-typical play behavior in childhood, including increased preferences for boys’ toys and for boys as playmates, and decreased preferences for girls’ toys (Hines, 2004). There also is evidence of reduced heterosexual behavior and fantasy (Dittmann et al., 1992; Hines et al., 2004; Money et al., 1984; Zucker et al., 1996), and some support for reduced interest in marriage, motherhood and physical appearance (Dittmann et al., 1990; Money and Ehrhardt, 1972) and increased accuracy in throwing balls and darts at targets (Hines et al., 2003). However, not all sexually differentiated behaviors are affected similarly. For instance, despite changes in gender role behavior in childhood, the great majority of women with CAH have a female gender identity (Dessens et al., 2005). Similarly, although females with CAH show improved targeting ability, their performance on other, more purely cognitive, spatial tasks appears to be unaffected (Hines et al., 2003).

Both CAH and ASCs are rare, making it difficult to determine if ASCs are increased in girls with CAH. However, because autistic traits are normally distributed in the population, it is possible to evaluate whether girls with CAH show increased autistic traits. In the present study, we compared autistic traits in individuals with CAH and their unaffected relatives.

Methods

Participants

Sixty individuals with classical CAH (34 females, 26 males), and 49 unaffected relatives (24 females, 25 males), ages 12 to 45 years, participated in the study. Unaffected relatives were recruited for the control group because they provide a partial control for genetic constitution (other than the genes for CAH) and socioeconomic background (Hines, 1982). All unaffected relative controls were siblings of CAH participants, except one, who was a first cousin. All participants were White-Caucasian, with the exception of two, a male with CAH and his unaffected sister, who were Asian (Indian, Pakistani or Bangladeshi).

Participants with CAH were recruited through endocrinologists at Middlesex and Great Ormond Street Hospitals, London (n = 30, 19 female, 11 male), or via a CAH support group in the U.K. (n = 30, 15 female, 15 male). Unaffected relatives were recruited through the families of individuals with CAH. Almost all participants with CAH had the salt-losing form of the disorder involving 21-OH deficiency (n = 53), but four (two males, two females) had 21-OH deficiency without salt-loss. For the remaining three (one male, two females), medical records confirming 21-OH deficiency and salt-losing status were not available. Written consent was obtained from all participants, and for those younger than 18 years, parents also provided written consent. Physicians who informed their patients of the study did not keep records of who agreed to participate and who declined. Similarly, the support group sent letters to members informing them of the study, but did not record participation rates.

Dependent measures

The Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001) is a self-administered questionnaire measuring the degree to which an individual has traits associated with the autistic spectrum. It includes 50 questions, 10 assessing each of five different areas: social skills, communication, imagination, attention to detail, and attention switching. There are four possible responses to each item (“definitely agree”, “slightly agree”, “slightly disagree”, “definitely disagree”). Some items are worded so that an “agree” response is consistent with an autistic trait and some so that a “disagree” response is. A point is scored if the response is consistent with an autistic trait either slightly or definitely. Thus, higher scores indicate increased autistic traits (i.e., poor social skills, poor communication, poor imagination, exceptional attention to detail, and poor attention-switching/strong focus of attention). (For AQ items, grouped by subscale, see Appendix A).

Data on the AQ (Baron-Cohen et al., 2001) suggest that autistic traits are distributed normally in the general population, with males scoring higher than females. The mean AQ score in the general population for females is 15.4 (SD = 5.7), and for males is 17.8 (SD = 6.8). People with high functioning autism (HFA) or AS have a mean score of 35.8 (SD = 6.5) (maximum possible score = 50), a highly significant difference from the general population. People with HFA or AS also score significantly higher than controls on all subscales, and males score higher than females on all subscales except attention to detail. Test–retest reliability is good. Scores taken two weeks apart do not differ significantly and correlate strongly (r = 0.7, P = 0.002). Internal consistency of items in each subscale, as indicated by Cronbach’s alpha coefficients, is moderate to high (social skills = 0.77; communication = 0.65; imagination = 0.65; attention to detail = 0.63; attention switching = 0.67; Baron-Cohen et al., 2001). The AQ is also a strong predictor of clinical diagnosis among a population referred for clinical evaluation. Among one hundred consecutive referrals to a diagnostic clinic for adults suspected of having AS or HFA, the instrument had good discriminative validity and good screening properties at a threshold score of 26 (Woodbury-Smith et al., 2005).

Control measures

Age and general intelligence were assessed to determine if participants in the four groups were similar in regard to these background factors. Participants provided written information on their date of birth. The age-scaled vocabulary score, obtained using the age appropriate Wechsler scales, provided the measure of general intelligence.

Procedures

All participants received the AQ by mail, completed it at home, and returned it in a stamped, pre-addressed envelope, along with other questionnaires. They received £20 for completing and returning the questionnaires. Information on age and vocabulary was obtained during a 6-h assessment at the University for which participants were paid £50. Participants were part of a study of CAH and psychological development that also assessed cognitive and perceptual abilities (general intelligence, mathematical ability, mental rotations ability, targeting ability, spatial perception, verbal fluency, perceptual speed, object memory and location memory, fine motor co-ordination, grip strength, hand preferences and language lateralization), personality (physical aggression, verbal aggression, dominance, gender identity, sexual orientation) and recalled childhood gender role behavior (toy, playmate and activity preferences) in 129 participants. (See Hines et al., 2003; Hines et al., 2004; Mathews et al., 2004, for additional details of the study, and published results on spatial abilities, language lateralization, hand preferences,
Table 1
Means and standard deviations for total AQ scores and subscale scores by sex and diagnosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>Subscale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAH</td>
<td>18.44</td>
<td>2.76 2.85</td>
</tr>
<tr>
<td></td>
<td>3.91 4.71 4.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.51 1.94 1.84 2.02 2.01 1.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.0 1.71 2.04 2.00 6.00 4.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.25 1.37 1.43 1.56 2.06 2.11</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAH</td>
<td>17.35</td>
<td>2.08 2.69</td>
</tr>
<tr>
<td></td>
<td>3.81 4.27 4.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.05 1.98 1.76 2.67 1.76 2.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.88 2.36 2.60 3.76 5.16 5.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.21 2.04 2.08 1.94 1.77 1.61</td>
<td></td>
</tr>
</tbody>
</table>

* Differences significantly from mean for female control group, \( P < 0.05 \).
** \( P < 0.001 \).

Statistical analyses

T tests were used to examine specific hypotheses regarding sex differences and CAH-related differences on the AQ. These a priori hypotheses were that: 1. Total scores on the AQ and on subscales measuring social skills, communication, imagination, attention to detail, and attention switching would be higher in unaffected males than in unaffected females; 2. Females with CAH would score higher than unaffected females on the total scale, and on those subscales that showed sex differences favoring males in the current study. When testing these a priori hypotheses, directional (i.e., one-tailed) statistical tests were used. In other cases, two-tailed tests were used. In presenting data, we provide effect sizes (\( d \) values), and \( t \) test results for comparisons of unaffected males versus unaffected females, females with CAH versus those without CAH and males with versus without CAH. As expected, unaffected males had significantly higher total AQ scores than unaffected females. In addition, females with CAH scored higher on total AQ than unaffected females. Total AQ scores of males with and without CAH did not differ.

The planned \( t \) tests comparing subscale scores in unaffected relatives indicated a significant sex difference on the imagination subscale. As expected, males scored higher than females. Sex differences on other subscales were not statistically significant. Planned \( t \) tests comparing females with and without CAH indicated significant differences on the social skills and imagination subscales (females with CAH > unaffected females) and on the attention to detail subscale (unaffected females > females with CAH), but not on the communication or attention switching subscales. The difference on the attention to detail subscale was in the opposite direction to the differences on the total scale, and the social skills and imagination subscales. However, its direction was consistent with the direction of the sex difference, in that unaffected males scored somewhat, though not statistically significantly, lower than unaffected females on attention to detail. For males, there were no significant differences on any of the individual subscales for participants with versus without CAH.

The accepted cut-off on the AQ that strongly correlates with a diagnosis of an ASC is 32 in the general population (Baron-Cohen et al., 2001), and 26 in individuals referred for clinical evaluation because of suspected HFA/AS (Woodbury-Smith et al., 2005). Both values are substantially above the mean score of 18.4 for the group of females with CAH. In addition, only two participants had total AQ scores of 32 or above, and these were two brothers, one of whom had CAH and one of whom did not. Eight participants had scores of 26 or above, including four control males, one male with CAH and three females with CAH.

Table 2
Effect sizes (\( d \)), \( t \) and \( P \) values for comparisons of total AQ scores and subscale scores for male versus female controls, and for females and males with versus without CAH

<table>
<thead>
<tr>
<th>Scale</th>
<th>Comparison</th>
<th>CF vs. CAHF</th>
<th>CM vs. CAHM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( d )  ( t )  ( P )</td>
<td>( d )  ( t )  ( P )</td>
<td>( d )  ( t )  ( P )</td>
</tr>
<tr>
<td>Total AQ</td>
<td>0.55 1.89 0.033*</td>
<td>0.50 1.82 0.037*</td>
<td>−0.25 −0.89 NS</td>
</tr>
<tr>
<td>Social skills</td>
<td>0.38 1.31 NS</td>
<td>0.63 2.43 0.018*</td>
<td>−0.14 −0.50 NS</td>
</tr>
<tr>
<td>Communication</td>
<td>0.32 1.10 NS</td>
<td>0.50 1.81 NS</td>
<td>0.05 0.17 NS</td>
</tr>
<tr>
<td>Imagination</td>
<td>1.01 3.49 0.001</td>
<td>1.07 3.89 0.001</td>
<td>0.02 0.07 NS</td>
</tr>
<tr>
<td>Attention to detail</td>
<td>−0.44 −1.53 NS</td>
<td>−0.63 −2.39 0.020</td>
<td>−0.50 −1.80 NS</td>
</tr>
<tr>
<td>Attention switching</td>
<td>0.40 1.40 NS</td>
<td>−0.02 −0.10 NS</td>
<td>−0.28 −0.98 NS</td>
</tr>
</tbody>
</table>

Note. CF, control females; CAHF, females with CAH; CM, control males; CAHM, males with CAH; NS, not significant. Statistically significant comparisons are indicated by italics.

* One tailed test because of a directional a priori hypothesis.

Results

The AQ

Table 1 shows means and standard deviations for total AQ scores and subscale scores by sex and diagnosis. Table 2 shows effect sizes (\( d \) values), and \( t \) test results for comparisons of unaffected males versus unaffected females, females with CAH versus those without CAH and males with versus without CAH. As expected, unaffected males had significantly higher total AQ scores than unaffected females. In addition, females with CAH scored higher on total AQ than unaffected females. Total AQ scores of males with and without CAH did not differ.

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Because a pair of siblings obtained the two highest scores on the AQ, we examined the correlation between sibling AQ scores in the sample as a whole. This correlation was positive and significant for total AQ ($r = 0.38, P = 0.02$) and for social skills ($r = 0.42, P = 0.01$), and positive, but not significant, for the other subscales ($r = 0.18$ for communication, $r = 0.05$ for imagination, $r = 0.21$ for attention to detail, and $r = 0.09$ for attention switching).

Control variables (age and vocabulary)

Age did not correlate with total AQ scores ($r = 0.17$, ns), but vocabulary did ($r = -0.27, P = 0.005$); higher levels of autistic traits were associated with reduced vocabulary scores. However, none of the four groups of participants differed from any other in either age or vocabulary. For age, mean (SD) values in years were 20.37 (6.61) for females with CAH, 20.97 (8.30) for males with CAH, 19.95 (5.88) for unaffected female controls, and 19.50 (7.66) for unaffected male controls. For age-scaled vocabulary scores, mean (SD) values were 8.68 (2.60) for females with CAH, 9.23 (2.66) for males with CAH, 9.29 (2.58) for unaffected female controls and 9.12 (2.20) for unaffected male controls.

Discussion

This study tested the hypothesis that prenatal levels of androgens influence autistic traits, by measuring these traits in individuals with CAH, a disorder causing increased adrenal androgen production, beginning prenatally. We predicted that AQ scores would be higher in control males than in control females, and in females with CAH compared to unaffected females.

Our findings for sex differences on the AQ and its subscales resemble prior findings. A prior study of 76 males and 98 females from the general population found that males scored higher than females on the total AQ ($d = 0.38$) and on the social skills ($d = 0.21$), communication ($d = 0.37$), imagination ($d = 0.47$), and attention switching ($d = 0.38$) subscales, but not on the attention to detail subscale ($d = -0.09$; Baron-Cohen et al., 2001). We also found a significant sex difference in total AQ scores, and subscale scores showed similar effect sizes for sex differences to those seen in the prior study. However, not all of the sub-scale sex differences were statistically significant in the current study, perhaps because of the smaller sample size.

We also found some, though not universal, support for the hypothesis that prenatal exposure to high levels of androgens, caused by CAH in females, increases scores on the AQ. As noted above, prior research has found that males score higher than females on the total AQ and on all subscales except attention to detail (Baron-Cohen et al., 2001). In the current study, females with CAH scored significantly higher on the total AQ and on subscales measuring social skills and imagination. In addition, effect sizes for these androgen-related differences were moderate to large in size. Nevertheless, although females with CAH generally showed higher levels of autistic traits than their unaffected female relatives, the opposite was true on the subscale measuring attention to detail, on which they scored lower. This result may have occurred because attention to detail, although higher in autistic than in non-autistic individuals, is not higher in males than in females. In addition, although females with CAH generally showed a shift toward more male-typical responses on the AQ and its subscales, data for the attention switching subscale did not conform to this pattern. Despite prior evidence that males score higher on attention switching than females (Baron-Cohen et al., 2001) and observation of a moderate sex difference on attention switching in the current study ($d = 0.40$), females with and without CAH showed essentially no differences on this subscale ($d = -0.02$). Therefore, although our results suggest that prenatal T may increase some male-typical characteristics associated with autism, they also suggest that this effect is limited to a subset of such characteristics. Prior findings suggest that females with CAH show more male-typical behavior in a variety of other areas, including childhood play interests, and sexual orientation, but not in all areas (Hines, 2004). Our results resemble these prior findings in suggesting that some, but not all, sexually dimorphic characteristics related to autism are altered in females with CAH.

Males with CAH did not differ from unaffected males on the total AQ or on any of its subscales. This is consistent with expectations, given that males with CAH typically do not show alterations in sexually differentiated behaviors (Hines, 2004), perhaps because their T levels are within the normal range prenatally (Pang et al., 1980; Wudy et al., 1999).

Although we found an association between CAH-related exposure to elevated androgen prenatally and increases in some autistic spectrum characteristics that are more common in males than in females, none of the females in our study had AQ scores that met the cut-off for suspicion of a clinical diagnosis among the general population. This suggests that high levels of androgen may contribute to autism related traits, but that other factors are more important for the development of a diagnosable condition on the autistic spectrum. Genes are prominent among the other factors that have been suggested to cause autism (Muhle et al., 2004), and, in our sample, total AQ scores showed a significant positive correlation for sibling pairs, suggesting a genetic, or other familial, contribution. It seems likely that high levels of androgen require augmentation by as yet unknown genetic (or other) characteristics to produce a clinically diagnosed ASC.

Because CAH is a clinical disorder, interpretation of our results could be confounded by difficulty in differentiating between the effects of elevated androgen prenatally and other characteristics of CAH. For instance, fetuses with CAH are also exposed to low levels of glucocorticoids and high levels of adrenocorticotropic hormone (ACTH) and postnatal treatment may produce glucocorticoid excess (Grumbach et al., 2003). However, these hormonal abnormalities occur in males as well as in females with CAH, whereas we found evidence that only females showed an increase in scores on the AQ. Thus, the pattern of results we observed corresponds more closely to the pattern of T abnormality than to the pattern of glucocorticoid or ACTH abnormality. Some researchers...
also have suggested that parents, being aware that their daughters with CAH had virilized genitalia at birth, may treat these daughters differently, in a manner that encourages the development of male-typical characteristics (Quadagno et al., 1977). However, research involving behavioral observation of parents interacting with their children with CAH does not support these suggestions; in fact, parents appear to encourage feminine behavior more in their daughters with CAH than in their unaffected daughters (Pasterski et al., 2005). Also, higher levels of androgen prenatally predict more male-typical behavior in healthy female offspring, who do not have any genital abnormality, suggesting that hormone–behavior relationships are seen in the absence of genital virilization and any consequent changes in parental behavior (Hines et al., 2002; Udry et al., 1995). In addition, in contrast to the large body of prior research leading to the hypothesis that T influences autistic traits, there is no theoretical basis for hypothesizing that either changes in glucocorticoids or ACTH or differential treatment by parents would increase autistic traits. For these reasons, we think the most likely explanation for the association we have observed between prenatal androgen exposure and some autistic spectrum traits in females is an influence of androgen on the developing brain.

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Appendix A. The AQ

All items are answered using the following response scale: Definitely agree, Slightly agree, Slightly disagree, Definitely disagree

SOCIAL SKILLS
1. I prefer to do things with others rather than on my own.
11. I find social situations easy.
13. I would rather go to a library than a party.
15. I find myself drawn more strongly to people than to things.
22. I find it hard to make new friends.
26. I find it easy to work out what someone is thinking or feeling just by looking at their face.
44. I enjoy social occasions.
47. I enjoy meeting new people.
48. I am a good diplomat.

COMMUNICATION
7. Other people frequently tell me that what I’ve said is impolite, even though I think it is polite.
17. I enjoy social chit-chat.
18. When I talk, it isn’t always easy for others to get a word in edgeways.
26. I frequently find that I don’t know how to keep a conversation going.
27. I find it easy to “read between the lines” when someone is talking to me.
31. I know how to tell if someone listening to me is getting bored.
33. When I talk on the phone I’m not sure when it’s my turn to speak.
35. I am often the last to understand the point of a joke.
38. I am good at social chit-chat.
39. People often tell me that I keep going on and on about the same thing.

IMAGINATION
3. If I try to imagine something, I find it very easy to create a picture in my mind.
8. When I am reading a story, I can easily imagine what the characters might look like.
20. When I am reading a story, I find out difficult to work out the characters’ intentions.
21. I don’t particularly enjoy reading fiction.
24. I would rather go to the theatre than a museum.
40. When I was young, I used to enjoy playing games involving pretending with other children.
41. I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.)
42. I find it difficult to imagine what it would be like to be someone else.
50. I find it very easy to play games with children that involve pretending.

ATTENTION TO DETAIL
5. I often notice small sounds when others do not.
6. I usually notice car number plates or similar strings of information.
9. I am fascinated by dates.
12. I tend to notice details that others do not.
23. I notice patterns in things all the time.
28. I usually concentrate more on the whole picture, rather than the small details.
29. I am not very good at remembering phone numbers.
30. I don’t usually notice small changes in a situation, or a person’s appearance.
49. I am not very good at remembering people’s date of birth.

ATTENTION SWITCHING
2. I prefer to do things the same way over and over again.
4. I frequently get so strongly absorbed in one thing that I lose sight of other things.
10. In a social group, I can easily keep track of several different people’s conversations.
16. I tend to have very strong interests which I get upset about if I can’t pursue.
25. It does not upset me if my daily routine is disturbed.
32. I find it easy to do more than one thing at once.
34. I enjoy doing things spontaneously.
37. If there is an interruption, I can switch back to what I was doing very quickly.
43. I like to plan any activities I participate in carefully.
46. New situations make me anxious.

Responses of ‘Definitely agree’ or ‘Slightly agree’ score 1 point for items 1, 3, 8, 10, 11, 14, 15, 17, 18, 24, 25, 27, 28, 29, 30, 31, 32, 34, 36, 37, 38, 40, 44, 47, 48, 49, 50.

References


