Submicroscopic copy-number variations associated with 46,XY disorders of sex development

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Abstract

Background: Mutations in known causative genes and cytogenetically detectable chromosomal rearrangements account for a fraction of cases with 46,XY disorders of sex development (DSD). Recent advances in molecular cytogenetic technologies, including array-based comparative genomic hybridization (aCGH) and multiplex ligation-dependent probe amplification (MLPA), have enabled the identification of copy-number variations (CNVs) in individuals with apparently normal karyotypes.

Findings: This review paper summarizes the results of 15 recent studies, in which aCGH or MLPA were used to identify CNVs. Several submicroscopic CNVs have been detected in patients with 46,XY DSD. These CNVs included deletions involving known causative genes such as DMRT1 or NR5A1, duplications involving NR0B1, deletions involving putative cis-regulatory elements of SOX9, and various deletions and duplications of unknown pathogenicity.

Conclusions: The results of recent studies highlight the significance of submicroscopic CNVs as the genetic basis of 46,XY DSD. Molecular cytogenetic analyses should be included in the diagnostic workup of patients with 46,XY DSD of unknown origin. Further studies using aCGH will serve to clarify novel causes of this condition.

Keywords: Disorders of sex development; Copy-number variations; Multiplex ligation-dependent probe amplification; Comparative genomic hybridization

Introduction

46,XY disorders of sex development (46,XY DSD) are clinically and genetically heterogeneous conditions that lead to genital abnormalities at birth, defective sexual development during puberty, and infertility in adulthood [1,2]. To date, mutations in several genes have been identified in patients with 46,XY DSD. Known causative genes for 46,XY DSD include ATRX, CBX2, DHH, DMRT1, GATA4, MAP3K1, NR0B1 (alias DAX1), NR5A1 (alias SF1), RSP01, SOX9, SRY, WNT4, and WT1 involved in testicular development; AKR1C2/4, AR, CYP11A1, CYP17A1, LHCGR, HSD3B2, HSD17B3, POR, SRD5A2, and STAR involved in androgen production or function; and AMH, AMHR2, INSL3, RXFP2, and the HOXD cluster involved in genital organ formation [1,3,4]. In addition, various chromosomal rearrangements have also been associated with 46,XY DSD [1,3]. However, mutations in known causative genes and cytogenetically detectable chromosomal rearrangements have been identified in only 20% to 30% of cases [1], indicating that other genetic or environmental factors play an important role in the development of 46,XY DSD.

Recent advances in molecular technology, including array-based comparative genomic hybridization (aCGH), multiplex ligation-dependent probe amplification (MLPA), and next-generation sequencing (NGS), have enabled high-throughput analysis of clinical samples. Of these, aCGH is highly useful to detect copy-number variations (CNVs) in the genome of individuals with apparently normal karyotypes [5,6], and MLPA can identify various copy-number alterations in specific disease-associated loci [7]. NGS primarily focuses on identification of nucleotide substitutions. Molecular cytogenetic analyses using aCGH or MLPA revealed the importance of CNVs as the cause of several genetic disorders, although accumulating evidence shows that submicroscopic CNVs can also occur as functionally neutral polymorphisms [6]. Here, we review recent reports on molecular cytogenetic analyses of patients with 46,XY DSD.
Findings
46,XY DSD-associated CNVs identified by molecular cytogenetic analyses

In this review, we summarize the results of 15 recent studies [8-22]. We found these papers through a PubMed search using the key words ‘disorders of sex development’, together with ‘comparative genomic hybridization’, ‘multiplex ligation-dependent probe amplification’, or ‘copy-number variations’. We focused on original articles, in which aCGH or MLPA was used to identify submicroscopic CNVs in patients with 46,XY DSD. The 15 studies showed 28 deletions and 4 duplications as genetic causes of 46,XY DSD, as well as several other CNVs whose association with the disease phenotype remains uncertain (Table 1, parts a and b, and Table 2) [8-22]. Notably, White et al. identified pathogenic CNVs in 3 of 23 patients with 46,XY gonadal dysgenesis [18], and Igarashi et al. detected CNVs in 3 of 24 patients with various types of 46,XY DSD [9]. These data suggest the significance of submicroscopic CNVs in the etiology of 46,XY DSD. All CNVs, except for those on the sex chromosomes, were detected in the heterozygous state. Parental samples of the CNV-positive patients were analyzed in some cases, confirming the de novo occurrence or maternal inheritance of all CNVs examined (Table 1, parts a and b).

Deletions encompassing known 46,XY DSD-causative genes

Submicroscopic deletions encompassing known causative genes were identified in 18 patients (Table 1, part a). These deletions ranged from 74 kb to 18.0 Mb and caused haploinsufficiency of LHCGR, DMRT1, NR5A1, WT1, or HOXD cluster [8-17]. Of these deletions, those encompassing DMRT1 or NR5A1 were relatively common. Notably, CNVs in cases 3, 16, and 17 were 10 to 18 Mb in length. These results imply that in some cases, deletions ≥10 Mb can be missed by standard karyotyping, although this method is expected to detect CNVs ≥5 to 10 Mb [11]. Actually, the results of karyotyping are affected by the quality of samples and the genomic position of CNVs [11]. Submicroscopic deletions in the 18 patients were associated with both isolated and syndromic 46,XY DSD. Syndromic DSD resulting from these deletions were primarily ascribed to contiguous gene deletions. For example, a deletion in case 16 encompassing WT1 and PAX6 caused 46,XY DSD, Wilms tumor, aniridia, and mental retardation, which is collectively referred to as WAGR syndrome. This WAGR syndrome has been described in several patients with cytogenetically detectable deletions at 11p. The size of submicroscopic deletions in 18 patients roughly corresponded to patients’ phenotypes (isolated or syndromic); 7 of the 9 patients with deletions ≥1.0 Mb manifested additional clinical features such as mental retardation, short stature, and facial dysmorphism, whereas such features were not reported in 9 patients with smaller deletions (Table 1, part a). On the other hand, syndromic 46,XY DSD was also caused by deletions of a single gene with complex functions. Indeed, finger anomalies observed in DSD patients with HOXD cluster-containing deletions are consistent with the fact that HOXD genes, particularly HOXD13 [23], control the development of limb and external genitalia [4]. Although submicroscopic deletions involving known causative genes were frequently identified in patients with syndromic 46,XY DSD (Table 1, part a), previous molecular cytogenetic analyses may be biased toward patients with complex phenotypes. Thus, further studies are necessary to clarify the precise frequency of pathogenic CNVs in patients with isolated and syndromic 46,XY DSD.

Deletions in the upstream regions of known 46,XY DSD-causative genes

Submicroscopic deletions that reside adjacent to known causative genes also underlie 46,XY DSD. To date, deletions involving the upstream regions of SOX9, GATA4, or NR0B1 have been reported to cause 46,XY gonadal dysgenesis (Table 1, part b) [10,12,18-21]. These deletions are predicted to disrupt the cis-regulatory machinery of genes involved in testis formation. For example, SOX9 is regulated by SRY and plays a critical role in the development of testis and long bones [1]. SOX9 abnormalities lead to 46,XY gonadal dysgenesis with or without campomelic dysplasia [1]. It is known that SOX9 expression is tightly regulated by multiple cis-acting enhancers in the upstream and downstream regions and that elimination of the enhancer(s) leads to tissue-specific dysregulation of SOX9 [10,18-20]. Previous studies have mapped SOX9 enhancers for craniofacial tissues to a genomic interval >1.0 Mb apart from the coding region and a testis enhancer to a 32.5-kb region at a position 607 to 640 kb upstream from the start codon [10,18-20]. These enhancer regions seem to contain transcription factor binding sites which are essential to maintain SOX9 expression during development. Since microdeletions in the SOX9 upstream region can result in complete gonadal dysgenesis similar to that observed in patients with SOX9 amorphic mutations, elimination of the distal enhancer seems to completely abolish SOX9 expression on the affected allele [10,18-20]. Deletions in the upstream regions of GATA4 and NR0B1 are also likely to encompass cis-acting enhancers of these genes [12,18,21]. Identification of deletions in the upstream or downstream regions of genes provides clues regarding cis-regulatory mechanisms of each gene.

Duplications encompassing known 46,XY DSD-causative genes

Submicroscopic duplications involving NR0B1 were identified in several patients with 46,XY DSD (Table 2) [10,18,22].
| Case/family | Patient | Size of deletion | Locus | Affected DSD-causative genes | Gonadal phenotype | External genitalia | Additional clinical features | Method | Inheritance | Reference |
|-------------|---------|------------------|-------|-----------------------------|-------------------|-------------------|--------------------------|--------|-------------|----------|
| Case 1      | 74 kb   | 2p16.3           | LHCGR | Testes with rete testis, epididymal structures, residual adrenal structures | Ambiguous genitalia | N.D.              | aCGH | De novo    | Richard et al. [8] |
| Case 2      | 8.5 Mb  | 9p24.1-p24.3     | DMRT1 | Streak gonads with ovarian ducts | Female            | MR, schizophrenia | aCGH | N.D.       | Igarashi et al. [9] |
| Case 3      | 10.6 Mb | 9p23-p24.3       | DMRT1 | PGD with dysgerminoma        | Clitoromegany     | Mild MR           | aCGH | N.E.       | Ledig et al. [10]  |
| Case 4      | 9.7 Mb  | 9p23-p24.3       | DMRT1 | GD                           | N.D.              | Hydrops, facial dysmorphism, limb and kidney abnormalities | aCGH | De novo    | Ledig et al. [10]  |
| Case 5      | 821.6 kb| 9p24.3           | DMRT1 | CGD                         | Female            | None              | aCGH | N.E.       | Ledig et al. [10]  |
| Case 6      | 103.2 kb| 9p24.3           | DMRT1 | CGD                         | Female            | None              | aCGH | N.E.       | Ledig et al. [10]  |
| Case 7      | 9.7 Mb/ duplication (26 Mb) | 9p23-p24.3/9p13.1-p23 | DMRT1 | GD                         | N.D.              | None              | Targeted aCGH | De novo   | Tannour-Louet et al. [11] |
| Case 8      | 6.7 Mb  | 9p24.1-pter      | DMRT1 | GD                           | N.D.              | None              | Targeted aCGH | De novo   | Tannour-Louet et al. [11] |
| Case 9      | 0.26 Mb | 9p24.3           | DMRT1 | CGD                         | Female            | None              | Targeted aCGH | N.D.     | Tannour-Louet et al. [11] |
| Case 10     | 0.20 Mb | 9p24.3           | DMRT1 | GD                           | N.D.              | None              | Targeted aCGH | N.E.     | Tannour-Louet et al. [11] |
| Case 11     | 0.24 Mb | 9q33.3           | NR5A1 (SF1) | Abnormal germ cells, no Leydig cells | Ambiguous genitalia | N.D.              | aCGH | Maternal   | Harrison et al. [12] |
| Case 12     | 3.1 to 48 kb | 9q33.3 | NR5A1 (SF1) | Leydig cell hyperplasia, scarce germ cells, carcinoma in situ | Female without uterus, clitoromegaly | N.D.          | Custom MLPA | N.D.     | Barbaro et al. [13] |
| Case 13     | 0.96 Mb | 9q33.3           | NR5A1 (SF1) | N.D.                        | Clitoromegaly, shallow vaginal entrance | Ptosis     | aCGH | De novo    | van Silfhout et al. 2009 [14] |
| Case 14     | 1.54 Mb | 9q33.3           | NR5A1 (SF1) | N.E.                        | Female            | Mild MR, minor dysmorphisms | aCGH | De novo    | Brandt et al. [15]  |
| Case 15     | 3.07 Mb | 9q33.3-q34.11   | NR5A1 (SF1) | Ovotestis                   | Clitoromegaly     | Genitopatellar syndrome | aCGH | N.D.       | Schlaubitz et al. [16] |
| Case 16     | 10 Mb   | 11p12-p14.1     | WT1   | N.E.                        | Female            | WAGR syndrome*    | aCGH | De novo    | Le Caignec et al. [17] |
| Case 17     | 18.0 Mb | 2q31.1-q32.1    | HOXD cluster | N.D.                     | Severe micropenis, hypospadias | Short stature, MR, multiple anomalies | aCGH | N.D.       | Igarashi et al. [9]  |
| Case 18 | 503.2 kb | Yq11.223 | AZFb-c region | CGD | Female | None | aCGH | N.D. | Ledig et al. [10] |
|---|---|---|---|---|---|---|---|---|---|
| Case 19 | 1.193 Mb | 17q24.2-q24.3 | Upstream of SOX9 | CGD | Female | Cleft palate, short stature | SNP array | N.D. | White et al. [18] |
| Case 20 | 3.3 Mb | 17q24.2-q24.3 | Upstream of SOX9 | CGD | Female | Acampomelic campomelic dysplasia, kyphoscoliosis | aCGH | N.D. | Ledig et al. [10] |
| Family 1 | | | | | | | | | |
| Proband | 240 kb | 17q24.3 | Upstream of SOX9 | Small testis (right), streak gonad (left) | Asymmetric external genitalia, urogenital sinus with a phallus | None | MLPA/aCGH | Maternal | Benko et al. [19] |
| Cousin | 240 kb | 17q24.3 | Upstream of SOX9 | Streak gonad with gonadoblastoma (right), ovary (left) | Female | None | MLPA/aCGH | Maternal | Benko et al. [19] |
| Case 21 | 236.3 kb | 17q24.3 | Upstream of SOX9 | Streak gonad (right), gonadal tumor (left) | Female | None | MLPA/aCGH | Maternal | Kim et al. [20] |
| Family 2 | | | | | | | | | |
| Proband | 65.4 kb | 17q24.3 | Upstream of SOX9 | Dysgenic testis\(^b\) | Ambiguous genitalia | None | MLPA/aCGH | Maternal | Kim et al. [20] |
| Sibling | 65.4 kb | 17q24.3 | Upstream of SOX9 | Dysgerminoma (right), streak gonad with a gonadoblastoma (left) | Female | None | MLPA/aCGH | Maternal | Kim et al. [20] |
| Family 3 | | | | | | | | | |
| Proband | 136 kb | 17q24.3 | Upstream of SOX9 | Dysgenic gonad with a gonadoblastoma | Female | N.D. | MLPA/aCGH | Maternal | Kim et al. [20] |
| Maternal relative | 136 kb | 17q24.3 | Upstream of SOX9 | Ovarian dysgerminoma | Female | N.D. | MLPA/aCGH | Maternal | Kim et al. [20] |
| Family 4 | | | | | | | | | |
| Proband | 576.9 kb | 17q24.3 | Upstream of SOX9 | CGD | Female | None | MLPA/aCGH | Maternal | Kim et al. [20] |
| Sibling | 576.9 kb | 17q24.3 | Upstream of SOX9 | CGD | Female | None | MLPA/aCGH | Maternal | Kim et al. [20] |
| Case 22 | 35 kb | 8p23.1 | Upstream of GATA4 | CGD | Female | Adrenal hypoplasia congenita | SNP array | N.D. | White et al. [18] |
| Case 23 | 0.22 Mb | 8p23.1 | Upstream of GATA4 | PGD | Ambiguous genitalia | N.D. | aCGH | Maternal | Harrison et al. [12] |
| Case 24 | 257 kb | Xp21.2 | Upstream of NR0B1 (DAX1) | Testis-like (right), streak gonad (left) | Female | None | aCGH | Maternal | Smyk et al. [21] |

DSD, disorders of sex development; PGD, partial gonadal dysgenesis; CGD, complete gonadal dysgenesis; GD, gonadal dysgenesis of unknown severity; N.E., not examined; N.D., not described; MR, mental retardation; aCGH, array-based comparative genomic hybridization; MLPA, multiplex ligation-dependent probe amplification. \(^a\)Wilms tumor, aniridia, genitourinary anomalies, and mental retardation syndrome. \(^b\)Dysgenic testis with remnants of ducti deferentes and rete testis and with primitive seminiferous tubules.
| Case | Size of duplication | Locus | Affected DSD-causative gene | Gonadal phenotype | External genitalia | Other clinical features | Method | Reference |
|------|---------------------|-------|-----------------------------|-------------------|-------------------|------------------------|--------|-----------|
| Case 1 | 16.23 Mb | Xp21.1-p22.2 | NR0B1 (DAX1) | CGD | Female | IUGR, facial dysmorphism, disturbance of pulmonary adaption, muscular hypertonia, hearing defect, mental retardation, short stature, macrocephaly | aCGH | Ledig et al. [10] |
| Case 2 | 729 kb | Xp21.2 | NR0B1 (DAX1) | PGD with testicular residues | Clitoromegaly | None | aCGH | Ledig et al. [10] |
| Case 3 | 771 kb | Xp21.2 | NR0B1 (DAX1) | CGD | Female | None | SNP array | White et al. [18] |
| Case 4 | 800 kb | Xp21.2 | NR0B1 (DAX1) | Streak gonad, testicular tissue with atrophic tubules (right) | Ambiguous genitalia | N.D. | Custom MLPA | Barbaro et al. [22] |

DSD, disorders of sex development; PGD, partial gonadal dysgenesis; CGD, complete gonadal dysgenesis; N.D., not described; MLPA, multiplex ligation-dependent probe amplification; aCGH, array-based comparative genomic hybridization; IUGR, intrauterine growth restriction.

Table 2 46,XY DSD-associated duplications identified by molecular cytogenetic analyses
NR0B1 is a transcription factor gene isolated from the dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on Xp21 [24]. Overdosage of NR0B1 is a well-known cause of syndromic 46,XY DSD in individuals with large X chromosomal rearrangements [25]. Recently, molecular cytogenetic analysis by Smyk et al. has shown that submicroscopic duplications encompassing NR0B1 can lead to 46,XY DSD as a sole clinical manifestation [21]. Copy-number gains of NR0B1 were predicted to perturb testicular development by downregulating the protein expression of SF1, WT1, and SOX9 [18].

CNVs of unknown pathogenicity

Genome-wide copy-number analyses identified several submicroscopic CNVs that could be associated with the development of 46,XY DSD. Ledig et al. performed aCGH analysis on 87 patients with 46,XY DSD and identified 31 CNVs which have not been described as polymorphisms [10]. While 7 of the 31 CNVs encompassed known DSD-causative genes, the other CNVs have not been implicated in DSD. Ledig et al. proposed that several genes, including GKAPI, NCOA4, and CTNNA3, are novel candidate genes for 46,XY DSD. Furthermore, Tannour-Louet et al. [11] analyzed 116 patients with 46,XY and 46,XX DSD and 8,951 control individuals, and identified 25 CNVs that may underlie DSD. Of these, 13 CNVs were detected in individuals with 46,XY karyotype. Further studies on large cohorts will clarify the pathogenicity of these CNVs.

Clinical applications of molecular cytogenetic technologies

Molecular cytogenetic analyses using aCGH or MLPA would be beneficial for patients with 46,XY DSD, because identification of pathogenic CNVs could help to predict the disease outcome and possible complications of patients. For example, patients with deletions encompassing WT1 have a high risk of Wilms tumor and renal failure. Furthermore, detection of disease-associated CNVs significantly improves the accuracy of genetic counseling for patients’ families. Molecular cytogenetic analyses, together with mutation screening using NGS, should be included in the diagnostic workup of patients with 46,XY DSD of unknown origin.

Summary and conclusions

Recent studies revealed that submicroscopic CNVs constitute a fraction of the genetic causes of both isolated and syndromic 46,XY DSD. ACGH and MLPA appear to be useful for molecular diagnosis of patients with 46,XY DSD. Furthermore, genome-wide copy-number analyses using aCGH will serve to identify novel causes of 46,XY DSD.
12. Harrison SM, Granberg CF, Keays M, Hill M, Grimstby GM, Baker LA (2014) DNA copy number variations in patients with 46,XY disorders of sex development. J Urol 192(6):1801–1806, doi:10.1016/j.juro.2014.06.040
13. Barbaro M, Cools M, Looijenga LHJ, Drop SLS, Wedell A (2011) Partial deletion of the NR5A1 (SF1) gene detected by synthetic probe MLPA in a patient with XY gonadal disorder of sex development. Sex Dev 5(6):181–187, doi:10.1159/000328282
14. van Stiphout A, Boot AM, Dijkstraen T, Hoek A, Nijman R, Sikkema-Raddatz B, van Ravenssawij-Arts CMA (2009) A unique 970 kb microdeletion in 9q33.3, including the NR5A1 gene in a 46,XY female. Eur J Med Genet 52(2–3):157–160, doi:10.1016/j.ejmg.2009.02.009
15. Brandt T, Blanchard L, Desai K, Nimkarn S, Cohen N, Edelmann L, Mehta L (2013) 46,XY disorder of sex development and developmental delay associated with a novel 9q33.3 microdeletion encompassing NR5A1. Eur J Med Genet 56(11):619–623, doi:10.1016/j.ejmg.2013.09.006
16. Schlaubitz S, Yatsenko SA, Smith LD, Keller KL, Vissers LE, Scott DA, Cai WW, Reardon W, Abdul-Rahman OA, Lammer EJ, Lifchez CA, Magenis E, Veltman JA, Stankiewicz P, Zabel BU, Lee B (2007) Ovotestes and XY sex reversal in a female with an interstitial 9q33.3-q34.1 deletion encompassing NR5A1 and LMX1B causing features of genitopatellar syndrome. Am J Med Genet A 143A(10):1071–1081, doi:10.1002/ajmg.a.31685
17. Le Caignec C, Delnatte C, Vermeesch JR, Boceno M, Joubert M, Lavenant F, Thomas E, Labalme A, Rival J-M (2007) Complete sex reversal in a WAGR syndrome patient. Am J Med Genet A 143A(22):2692–2695, doi:10.1002/ajmg.a.31997
18. White S, Ohnesorg T, Notini A, Roeszler K, Hewitt J, Daigag H, Smith C, Turbitt E, Gustin S, van den Berg J, Miles D, Western P, Arboleda V, Schwamcher V, Gordon L, Bell K, Bengtsson H, Speed T, Hutson J, Warne G, Harley V, Koopman P, Vilain E, Sinclair A (2011) Copy number variation in patients with disorders of sex development due to 46,XY gonadal dysgenesis. PLoS One 6(3), e17793, doi:10.1371/journal.pone.0017793
19. Benko S, Gordon CT, Mallet D, Sreenivasan R, Thauvin-Robinet C, Brendel H, Uhl C, Malin V, Huet F, Molven A, Malm V, Huret F, Houge G, Morel Y, Lyonnet S (2011) Disruption of a long distance regulatory region upstream of SOX9 in isolated disorders of sex development. J Med Genet 48(12):825–830, doi:10.1136/jmedgenet-2011-100255
20. Kim GJ, Sock E, Buchberger A, Just W, Denzer F, Hoepffner W, German J, Cole T, Mann J, Seguin JH, Zifof W, Cortesan C, Schmidlde H, Rostasy M, Kramer M, Kaltenbach S, Rosier B, Georg I, Troppmann E, Teichmann AC, Safelfder A, Widholz SA, Weacker P, Hoit O, Camerino G, Radi O, Wegner M, Arnold HH, Scherer G (2015) Copy number variation of two separate regulatory regions upstream of SOX9 causes isolated 46,XY or 46,XX disorder of sex development. J Med Genet. doi:10.1136/jmedgenet-2014-102864
21. Smyk M, Berg JS, Purley A, Curtis FK, Fernandez BA, Bilen-Willner GA, Lupski JR, Cheung SW, Stankiewicz P (2007) Male-to-female sex reversal associated with an ~250 kb deletion upstream of NR0B1 (DAX1). Hum Genet 121(1):63–70, doi:10.1007/s00439-007-0373-8
22. Barbaro M, Cicognani A, Balasso A, Lofgren A, Baldazzi L, Wedell A, Oncan M (2008) Gene dosage imbalances in patients with 46,XY gonadal DSD detected by an in-house-designed synthetic probe set for multiplex ligation-dependent probe amplification analysis. Clin Genet 73(5):453–464, doi:10.1111/j.1399-0004.2008.00980.x
23. Warot X, Fromental-Ramain C, Fraulob V, Chambon P, Dollé P (1997) Gene dosage-dependent effects of the Hoxa-13 and Hoxd-13 mutations on morphogenesis of the terminal parts of the digestive and urogenital tracts. Development 124(23):4781–4791
24. Zanaria E, Muscatelli F, Bardoni B, Strom TM, Gioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER et al (1994) An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 372(6507):635–641, doi:10.1038/372635a0
25. Ogata T, Matsuo N (1996) Sex determining gene on the X chromosome short arm: dosage sensitive sex reversal. Acta Paediatr Jpn 38(4):390–398