Warsaw Breakage Syndrome, a Cohesinopathy Associated with Mutations in the XPD Helicase Family Member DDX11/ChlR1

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The iron-sulfur-containing DNA helicases XPD, FANCJ, DDX11, and RTEL represent a small subclass of superfamily 2 helicases. XPD and FANCJ have been connected to the genetic instability syndromes xeroderma pigmentosum and Fanconi anemia. Here, we report a human individual with biallelic mutations in *DDX11*. Defective DDX11 is associated with a unique cellular phenotype in which features of Fanconi anemia (drug-induced chromosomal breakage) and Roberts syndrome (sister chromatid cohesion defects) coexist. The DDX11-deficient patient represents another cohesinopathy, besides Cornelia de Lange syndrome and Roberts syndrome, and shows that DDX11 functions at the interface between DNA repair and sister chromatid cohesion.

A male individual with severe microcephaly, pre- and postnatal growth retardation, and abnormal skin pigmentation (Figure 1 and Figure S1, available online) was suspected to suffer from a chromosomal-instability syndrome. An initial cytogenetic investigation on peripheral blood was performed when the patient was one year old, and a normal male karyotype was observed. The research on patient material was carried out after approval by the institutional review board of the VU University Medical Center, adhered to local ethical standards, and was initiated only after the relevant informed consent had been obtained. Spontaneous and radiation-induced chromosomal instability as well as the frequency of spontaneous sister chromatid exchanges (SCEs) were within the normal range, which effectively excluded ataxia telangiectasia (MIM 208900), Nijmegen breakage syndrome (MIM 251260), and Bloom syndrome (MIM 210900) (data not shown). To test for Fanconi anemia (FA [MIM 227650]), we examined fresh T lymphocyte cultures as well as EBV-immortalized B lymphoblasts for mitomycin C (MMC)-induced chromosomal breakage (Figure 2A and Figure S2). As observed in cells from FA patients, strongly increased breakage was detected in both cell types, supporting an FA diagnosis. However, additional abnormalities in the cohesion of sister chromatids were observed (Figures 2B and 2C): chromosomes with centromeric cohesion defects ("railroads") and total "premature chromatid separation" (PCS). Strikingly, the percentage of metaphases showing total PCS increased to 50%–60% upon exposure to the DNA cross-linking agent MMC or the topoisomerase I inhibitor camptothecin. Cohesion defects have not been reported to occur in FA but are typical for the cohesinopathy Roberts syndrome (RBS [MIM 268300]), in which predominantly centromeric cohesion defects are observed (Figure 2C). The affected individual thus appeared unique in showing a combination of FA- and RBS-like cytogenetic features; e.g., excessive drug-induced chromosomal breakage and cohesion defects. The excessive susceptibilities at the cytogenetic level were also observed in growth-inhibition assays on lymphoblasts (Figure S3). Clonogenic survival of skin fibroblasts confirmed MMC hypersensitivity, whereas sensitivity to irradiation with X-rays or ultraviolet C light was in the normal range (Figure S4). This patient thus seemed to represent a novel cohesinopathy, with cellular features of both FA and RBS in association with a distinct clinical phenotype.

Cohesion defects are not observed in any of the known FA-complementation groups. Therefore, candidate genes were sought for the affected individual, amongst those for which a role in sister chromatid cohesion has been described in the literature. Protein products from candidate genes were investigated by immunoblot analysis of lymphoblast and fibroblast lysates. This revealed normal levels of the acetyltransferase ESCO2 (MIM 609353), which is mutated in RBS.[1] The related acetyltransferase ESCO1 (MIM 609674) also appeared normal (Figure 3A). Additional proteins, including SMC1 (MIM 300040), SMC3 (MIM 606062), SMC5 (MIM 609386), SMC6 (MIM 609387), PDS5A (MIM 613200), PDS5B (MIM 605333), CTF18 (MIM 613201), CTF4/AND-1 (MIM 608126), and DDX11 (MIM 601150), were investigated, but no aberrations in protein levels or mobility were observed (data not shown), with the exception of the helicase DDX11, which was barely detectable in fibroblasts and lymphoblasts from the affected individual (Figure 3A). Sequence analysis of genomic DNA uncovered two mutations in

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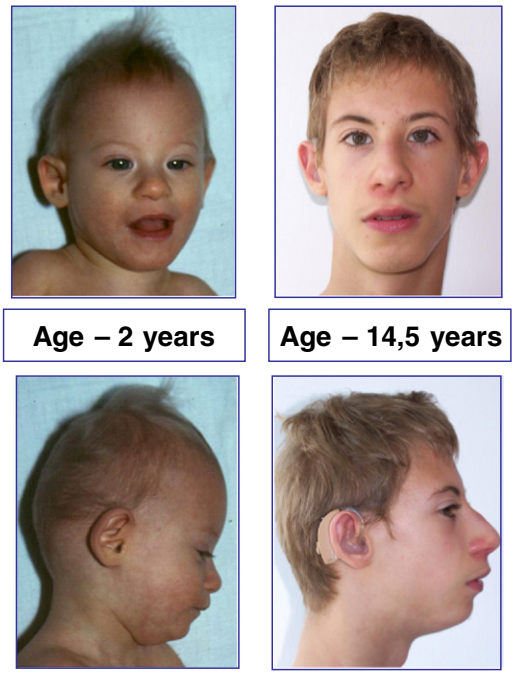

Figure 1. Characteristics of the Affected Individual
The patient is a male from Warsaw, Poland, with severe intrauterine growth retardation (weight 1300 g [< 3rd centile], length 38 cm [< 3rd centile], and head circumference 25 cm [< 3rd centile] at 37 wks of gestation). Several congenital abnormalities were found, including microcephaly, facial dysmorphy (small and elongated face, narrow bifrontal diameter, jugular hypoplasia, bilateral epicanthal folds, relatively large mouth, and cup-shaped ears), high arched palate, coloboma of the right optic disc, deafness due to structural abnormalities of the inner ear (bilateral hypoplastic cochlea), small ventricular septal defect, bilateral clinodactyly of the fifth fingers, syndactyly of the second and third toes, cutis marmorata, and one hypo- and three hyperpigmented patches on the skin. Psychomotor and mental development were mildly retarded. Growth was severely retarded (Figure S1). Genitalia and pubertal development were normal. No malabsorption or pituitary or thyroid insufficiency was found. No hematological abnormalities, immunodeficiency, or malignancy were noted either.

DDX11 (MIM 601150): a splice-site mutation in intron 22 of the maternal allele (IVS22+2T>C) and a 3 bp deletion in exon 26 of the paternal allele (c.2689_2691del) (Figure 3B). The splice-site mutation leads to a deletion of the last 10 bp of exon 22 from the DDX11 cDNA (Figure 3C), and the 3 bp deletion results in the deletion of a highly conserved lysine residue in the DDX11 protein (K897; Figure S5). Given the hardly detectable band on immunoblot (Figure 3A), the mutant protein is probably unstable. Introduction of DDX11 cDNA into lymphoblasts from the affected individual (Figure 4A) rescued the abnormal phenotype, in terms of both chromosomal morphology (cohesion defect; Figure 4B) and sensitivity to growth inhibition by MMC or camptothecin (Figures 4C and 4D). We conclude that the abnormal cellular phenotype of the affected individual is causally related to the mutations that we identified in *DDX11*. Sequence analysis of six additional patients with a clinical phenotype similar to that of the affected individual did not reveal any additional examples of mutations in *DDX11*. To our knowledge, this leaves the presented case as the first and only human example of a patient with a genetic defect in *DDX11*.

DDX11 is the ortholog of yeast Chl1,[2] and siRNA experiments in human cells pointed to a role for DDX11 in sister chromatid cohesion.[3] Nevertheless, it was surprising to find DDX11 deficiency in humans, because *Ddx11* knockout mice are lethal at embryonic day 10.5.[4] The mother of the affected individual had two miscarriages (Figure 5), but no material was available for examination of their possible association with biallelic mutations in *DDX11*. Excessive aneuploidy, as observed in cells from the $Ddx11^{-/-}$ mice,[4] was not observed in cultured lymphocytes from the affected individual (Figure S6), which could indicate that the affected cells may have adapted to DDX11 deficiency or that some residual DDX11 activity may still be present. The affected individual, now 14.5 years old, has thus far not presented with any malignancies, but his mother and grandmother, both carriers of the splice-site mutation, have developed Hodgkin lymphoma and adenocarcinoma of the endometrium, respectively (Figure 5). In addition, his great-grandmother developed cervical cancer and her sister developed uterine cancer. These data suggest that DDX11 may act as a tumor suppressor, which could be related to the proposed role of DDX11 in viral genome maintenance.[5]

DDX11 shares sequence similarity with FANCJ and the related DEAH-box helicases XPD and RTEL,[6] all of which contain a unique iron-sulfur cluster between helicase domain IA and II.[7,8] FANCJ and XPD are connected to the genetic-instability syndromes FA and xeroderma pigmentosum (MIM 278730), respectively, and their inactivation leads to an increased cancer risk. Here, we have shown that inactivation of DDX11 causes a form of genetic instability that combines features observed in FA with those seen in RBS. The DDX11-deficient individual thus represents another example of a human cohesinopathy, besides Cornelia de Lange syndrome (MIM 122470) and RBS. We propose to name the syndrome associated with defective DDX11 "Warsaw breakage syndrome" (WABS), in acknowledgment of the origin of the identified individual with this disorder, and to adopt WABS1 as an alias for DDX11. A detailed insight into the clinical phenotype of WABS awaits the identification of additional patients. Given the hypersensitivity of DDX11/WABS1-deficient cells for MMC and camptothecin, agents that both interfere with DNA replication, DDX11/WABS1 may function at the interface of replication-coupled DNA repair and sister chromatid cohesion.

Supplemental Data

Supplemental Data include six figures and can be found with this article online at http://www.ajhg.org.

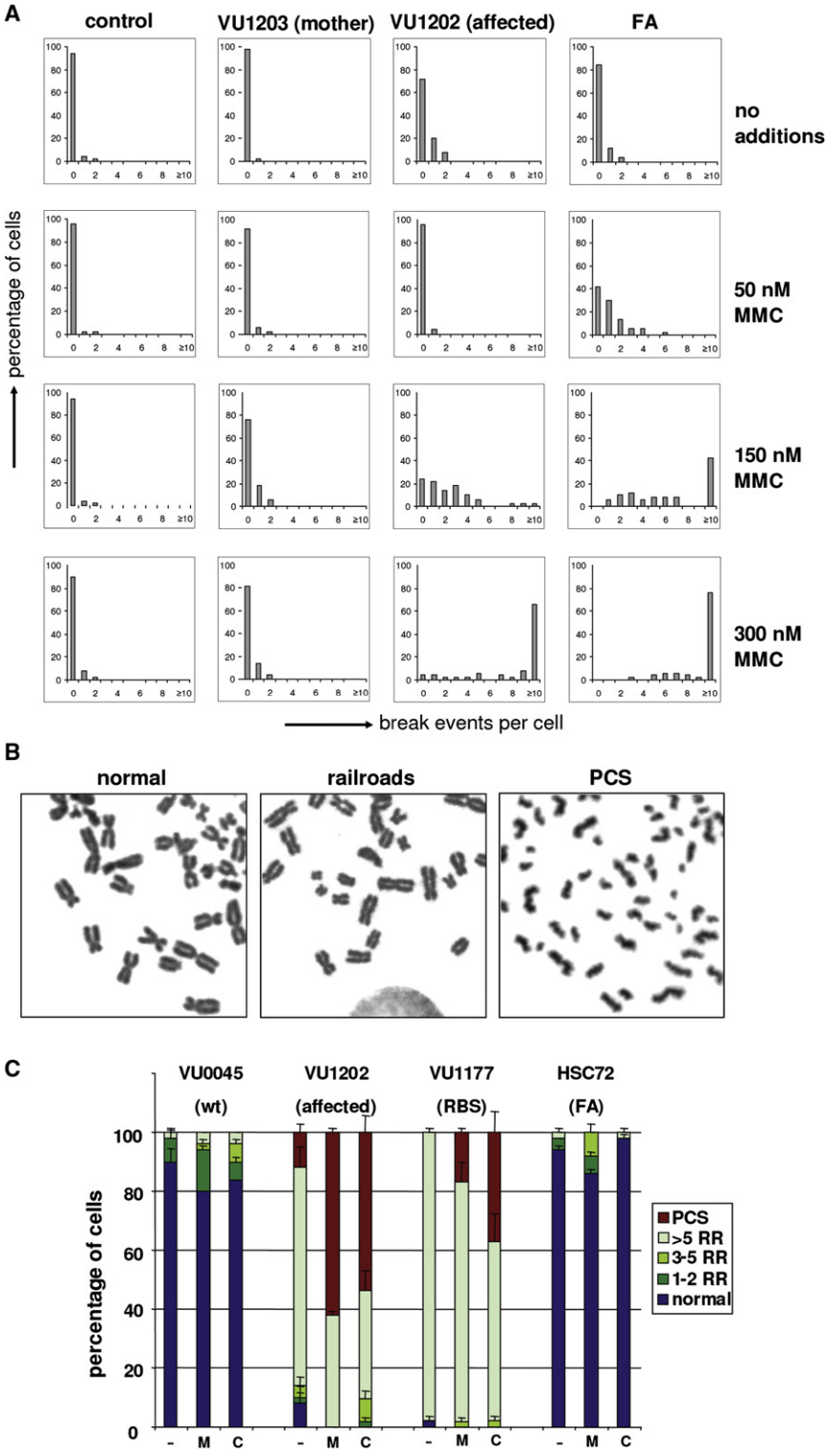

Figure 2. Chromosomal Breakage and Cohesion Defects in Cells Derived from the Affected Individual

(A) Chromosomal breakage after MMC treatment of T lymphocyte cultures from the affected individual (VU1202), his mother, a healthy control, and an individual with Fanconi anemia. Percentages of cells with up to $\geq$ 10 break events per cell are indicated.

(B) Representative pictures of metaphases with railroad chromosomes (RR) and total premature chromatid separation (PCS).

(C) RR and PCS are frequently observed in lymphoblasts from an individual with Roberts syndrome and in those from the affected individual. Percentages of cells with normal chromosomes, RR, and PCS are shown in untreated cells and in cells treated for 48 hr with 150 nM MMC (M) or 2.5 nM camptothecin (C). Patient VU1177 is homozygous for c.1105insA in *ESCO2*, and patient HSC72 is homozygous for a deletion of exon18 to 28 in *FANCA*. The figure shows the average of two experiments with standard deviation.

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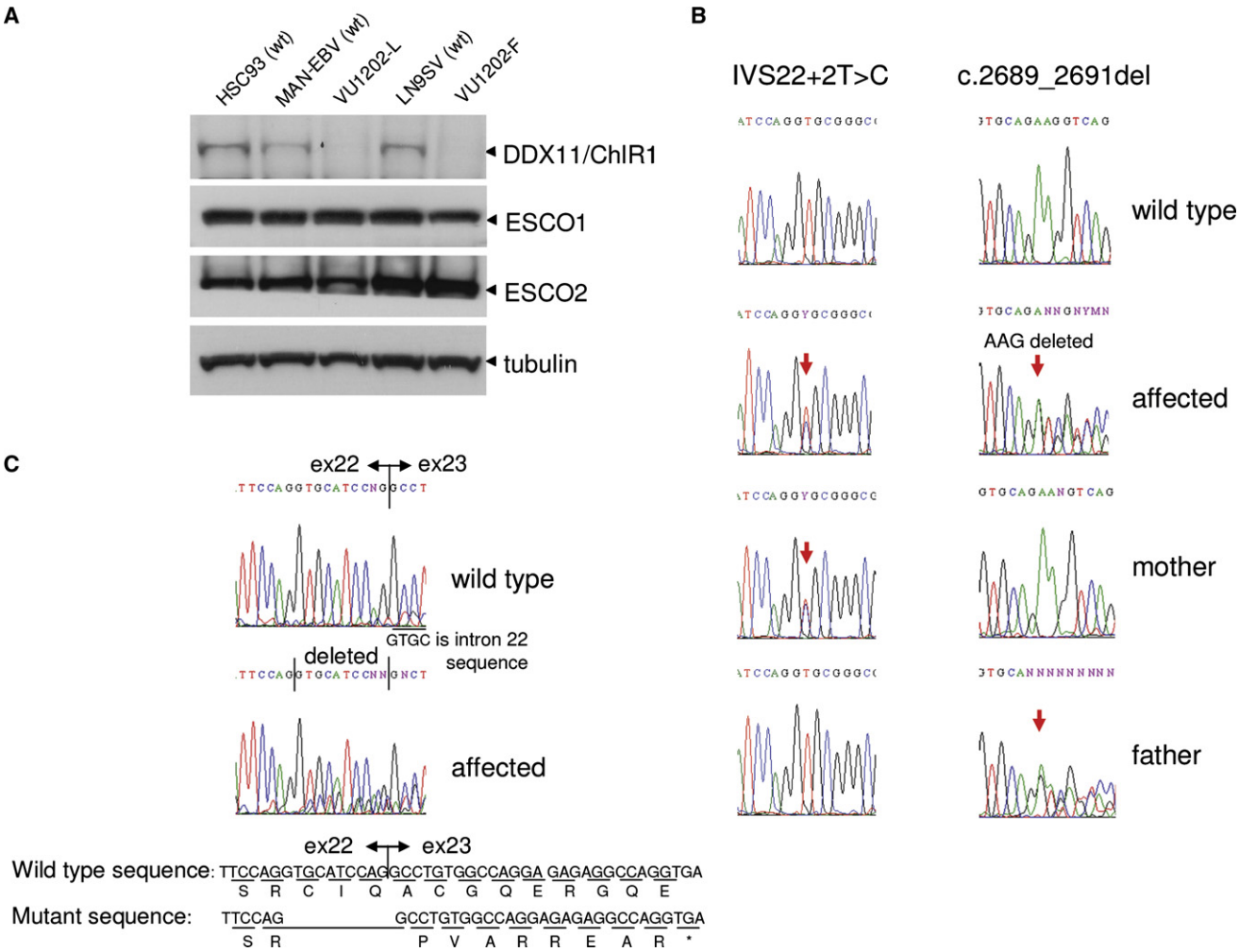

Figure 3. *DDX11* Mutations in the Affected Individual and His Parents
(A) Immunoblotting revealed normal ESCO1 and ESCO2 but hardly detectable DDX11 protein levels in lymphoblasts (VU1202-L) and fibroblasts (VU1202-F) from the affected individual.
(B) Sequence analysis on genomic DNA revealed the splice-site mutation IVS22+2T>C in the *DDX11* gene of the affected individual and his mother (red arrow). In the paternal allele, a 3 bp deletion was found (red arrow), which deleted a conserved lysine residue (K897) from the DDX11 protein.
(C) Sequencing on cDNA of the affected individual showed that the splice-site mutation IVS22+2T>C leads to a 10 bp deletion of exon 22 sequence from the DDX11 cDNA, resulting in a frameshift at codon 754 and a premature stop at codon 763.

Web Resources

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

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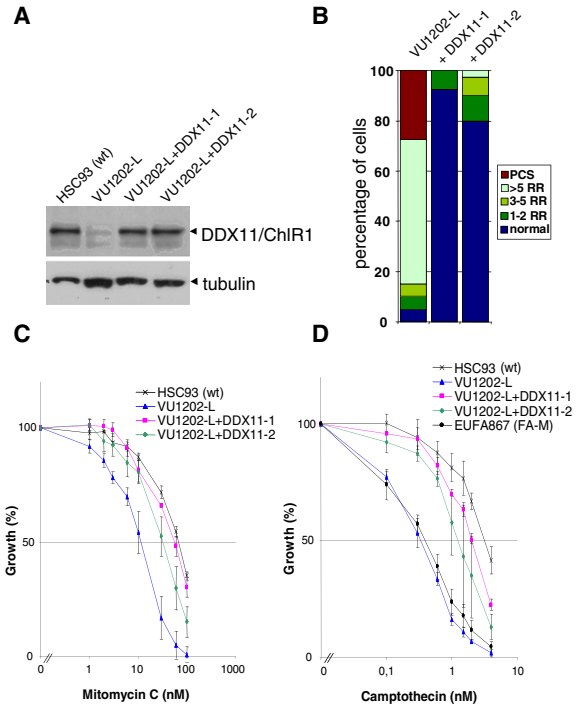

Figure 4. DDX11 Deficiency Underlies the Abnormal Cellular Phenotype in the Affected Individual

(A–D) Two independent transfections of DDX11 cDNA into lymphoblasts of patient VU1202 (A) restored normal DDX11 protein levels and chromosomal cohesion defects (B), as well as hypersensitivity to growth inhibition by MMC (C) and camptothecin (D). (C) and (D) show the average of two independent experiments, with the standard error of the mean shown.

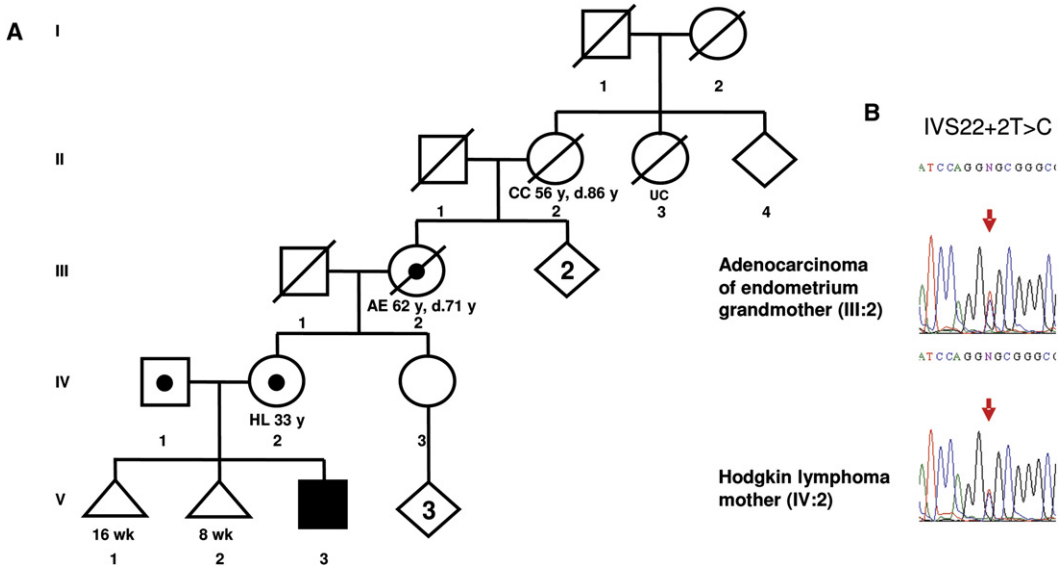

Figure 5. Pedigree of the Affected Individual and Cancer Incidence in the Family

(A) Pedigree of the affected individual (V:3). The mother of the patient (IV:2) had two miscarriages in the first trimester (V:1 and V:2) and developed Hodgkin lymphoma (HL) at age 33. The patient's grandmother (III:2), also a carrier of the splice-site mutation IVS22+2T>C, was diagnosed with an adenocarcinoma of the endometrium (AE) at the age of 62. The patient's great-grandmother (II:2) was diagnosed with cervical cancer (CC) at age 56, and her sister (II:3) was diagnosed with uterine cancer (UC). Of the latter two cases, the *DDX11* mutation status is unknown. There is no evidence for cancer predisposition in the family of the father.

(B) The splice-site mutation IVS22+2T>C was detected in the tumors of the mother and grandmother.