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(LETTER TO THE EDITOR)

A novel *PTCH1* mutation in a patient of nevoid basal cell carcinoma syndrome

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Nevoid basal cell carcinoma syndrome (NBCCS) is a genetically determined disorder in which multiple skin tumors indistinguishable from basal cell carcinoma, palmoplantar pits, malformation of other tissues such as cysts of the jaws, abnormalities of the ribs and vertebrae, and a variety of less common changes, including ectopic calcification, cerebral tumors and so on may be observed. The penetrance is almost 100% with variable expressivity. Because the symptoms may not be discernible in early life stage, the diagnosis of NBCCS is sometimes difficult. We describe a sporadic case of NBCCS with palmoplantar pits, skeletal abnormalies, and intracranial calcification. The patient showed a novel insertion mutation (1408_1409insGGCT) of *PTCH1* gene.

A 4-year-old Japanese boy showed multiple tiny pits and blackish papules which occasionally showed inflammatory changes on the palms and soles (Figure A). He had epilepsy since 4 months old, followed by mild mental and developmental retardation. Frontal boss and mild hypertelorism (interocular-biorbital index=244.4; about 400 in normal) were noted. Chest X-ray examination detected fusions of ribs on the upper portion of thorax (Figure D). Computed tomography revealed dotted calcification of the falx and tentorium (Figure C). Neither jaw cysts nor basal cell carcinomas were detected. A blackish papule on his palm was biopsied, and the histopathological examination revealed basaloid cell nests surrounded by loose stromal

tissue. The lesion was covered by thinned epidermis with slightly defective horny layer (Figure B).

Although NBCCS was suspected, no family history was obtained. We performed PCR-SSCP analysis for the screening of *PTCH1* gene-mutation, which was followed by direct DNA sequencing. Peripheral blood was obtained from the patient, his father and two normal adults. Informed consent was obtained from each subject. Genomic DNA was extracted from peripheral blood leukocytes, and used as template DNA for PCR. PCR primers were designed as previously reported [1], and PCR amplification was performed at 35-40 cycles with Taq DNA polymerase (TAKARA, Ohtsu, Japan). These samples were mixed with the same volume of loading buffer, denatured at 95^{o}C for 5 min, and electrophoresed on 0.5xMDE gel (BMA, Rockland, USA) at 20^{o}C. Then the gel was stained with Gelstar (BMA, Rockland, USA), and observed by transiluminator. These samples were directly sequenced with primers for SSCP analysis by using ABI DNA sequencing system (Applied Biosystems, NJ). Extra band and ordinary band, separated by SSCP analysis, were re-amplified and cloned into TA-cloning plasmid. Then they were sequenced by using M13 and RM13 universal primers. A 4-bases insertion mutation between 1408 and 1409 nucleotides, which induces flameshift and early termination of *PTCH1* protein, was detected on the

patient's exon 9 of *PTCH1* gene (Figure E, F). This was not detected in his father or control adults' gene (Figure E).

NBCCS is a rare autosomal dominant disorder, which is characterized by multiple basal cell carcinomas, various skeletal anomalies and complicated tumors represented by odontogenic jaw cysts. Recently, *PTCH1* gene was shown to be responsible not only for *NBCCS* but also for some cases of sporadic basal cell carcinoma [2]. The mutations have been reported over almost all exons with no hot spots. In addition, it has been reported that loss of function of *PTCH1* could induce basal cell carcinomas in vivo [3], reinforcing the role of *PTCH1* in the pathogenesis of NBCCS. Evans et al [4] and Kimonis et al [5] independently proposed criteria for diagnosis of NBCCS, which are divided into major and minor criteria. These two criteria are essentially the same with the major criteria including multiple BCCs, jaw cyst, palmoplantar pits, ectopic calcification, rib anomaly and family history. However, the frequency of some items, such as multiple BCC and jaw cysts is very rare under 10 years-old, and the ectopic calcification usually arise after 30 years-old. Consequently, the diagnosis of NBCCS is sometimes difficult in childhood by these criteria.

We employed *PCR-SSCP* analysis for the detection of *PTCH1* gene mutation. A 4 bases-pair insertion was detected on the exon 9 of patient's *PTCH1* gene. To the

best of our knowledge, this is a novel mutation, which has not been described before and in the database of PTCH1 (http://www.cybergene.se/cgi-bin/w3-msql/ptchbase/index.html). This mutation could not be detected in his father's gene or in normal adults. Because his parents had divorced, his mother's gene analysis was not available. The familial record, however, indicates that his mother did not show any signs of NBCCS, suggesting that the patient's gene mutation is sporadic. In addition, this mutation is located in sterol-sensing domain associated with trafficking of many proteins including Hedgehog [6], suggesting this domain might play another important role in NBCCS as suggested in a previous report [7].

As seen in our case, basaloid cell nests are observed below thinned epidermis with slightly defective horny layer especially in relatively early life, and notably they show little malignant potency. *Shh* signal is a well-known developmental factor highly expressed in fetus, and associated with vertebrate finger formation [8]. The basal cell proliferation on palmoplantar lesion might be induced by high expression of fetal *shh* in the presence of insufficient *PTCH1* protein. As the expression of *shh* signal declines after birth, basal cell proliferation might be down-regulated with regression of the nests. Consequently, only the defective horny layer might be left after birth in the most of

NBCCS cases. In our case these papules and pits on the palms and soles showed inflammatory changes which might be related to the regression process of the basaloid cell nests.

Treatment of NBCCS includes surgical excision of the tumors and the topical application of anti-cancer agents, such as 5-fluorourasil. Recently, imiquimod, an immune modifier affecting Toll-like receptor 7, was reported to be effective for the treatment of multiple BCCs [9].

Despite of all these treatment modalities in our hands, early detection of CNS tumors or BCCs is essential, because they critically determine the prognosis of NBCCS. Specifically, medulloblastoma, which usually occurs by 3 years old, is most important and the premature termination-codon of *PTCH1,* which was also detected in our case, is reportedly associated with medulloblastoma [10]. Although this case showed several symptoms which fulfill the criteria for the diagnosis of NBCCS, determination of pathogenic mutation is quite useful for the genetic counseling of other family members. In this context, the proper diagnosis is essential especially in early life and for this purpose. Screening by SSCP method was quite useful. These mutation analyses would also contribute for the understanding of this rare multisystem disorder.

Conflict of Interest Statement

None declared

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Figure legend

Figure 1

A. Multiple tiny blackish papules on the sole (arrow). These papules are observed on the palms and soles.

B. Histopathological examination of palmoplantar papules.

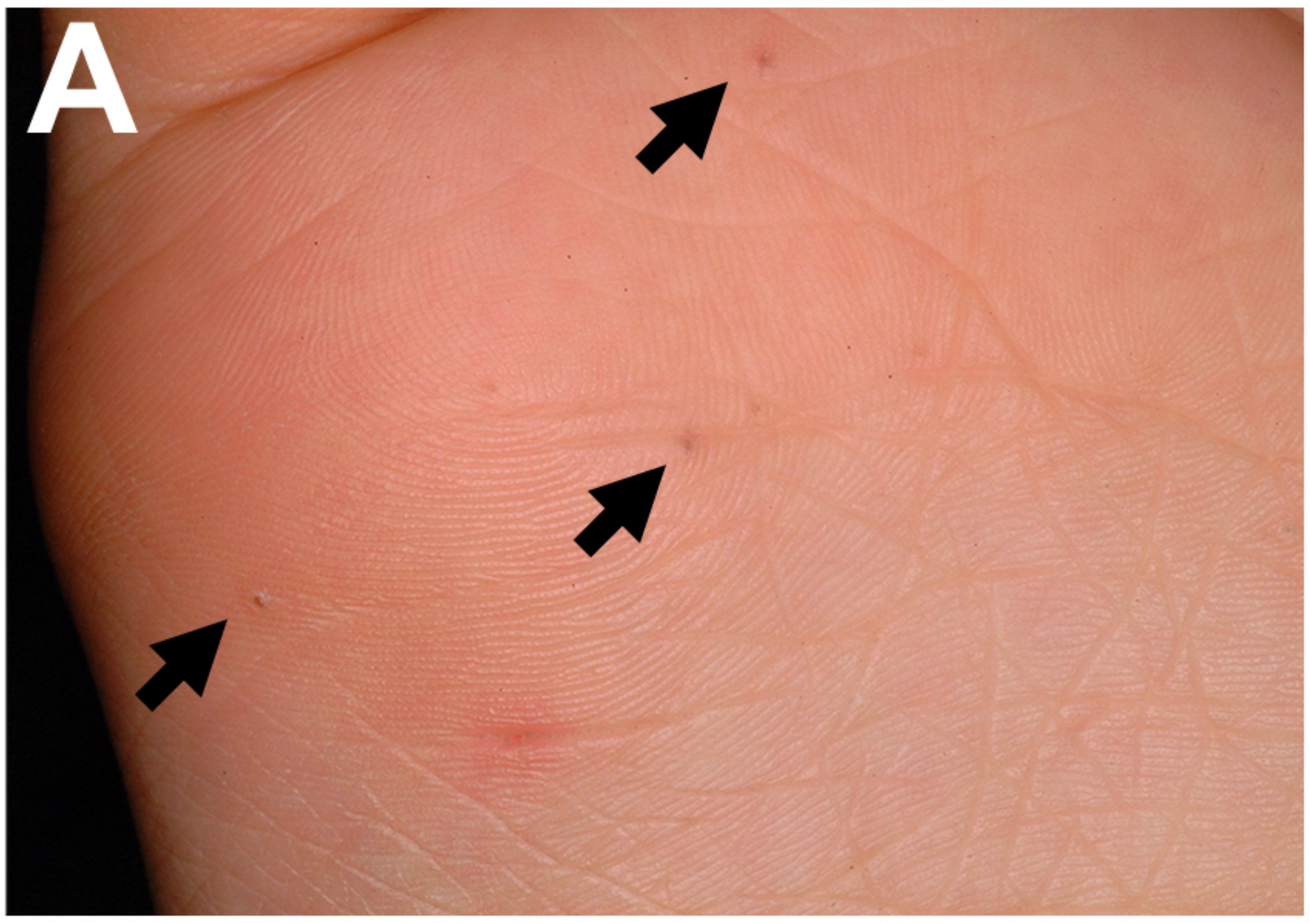
C. Dotted calcifications of the falx (arrow).

D. Fusion of upper ribs (arrow)

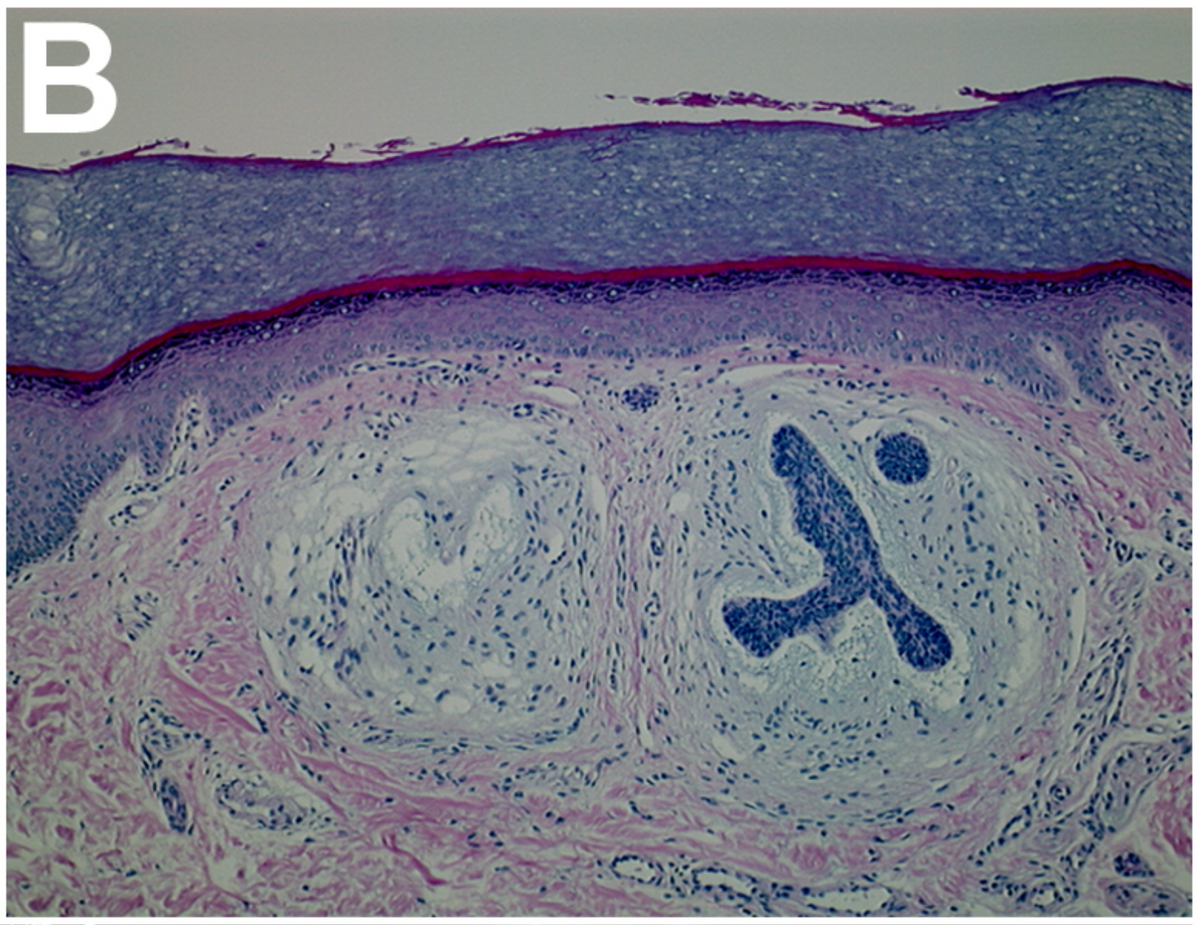
E. SSCP analysis of exon 9. Patient gene shows extraband.

F. Sequencing of the patient's extraband detects 4 base-insertion mutation in the portion of 1409th nucleotide. This mutation induces early termination of *PTCH1* protein.

A



B



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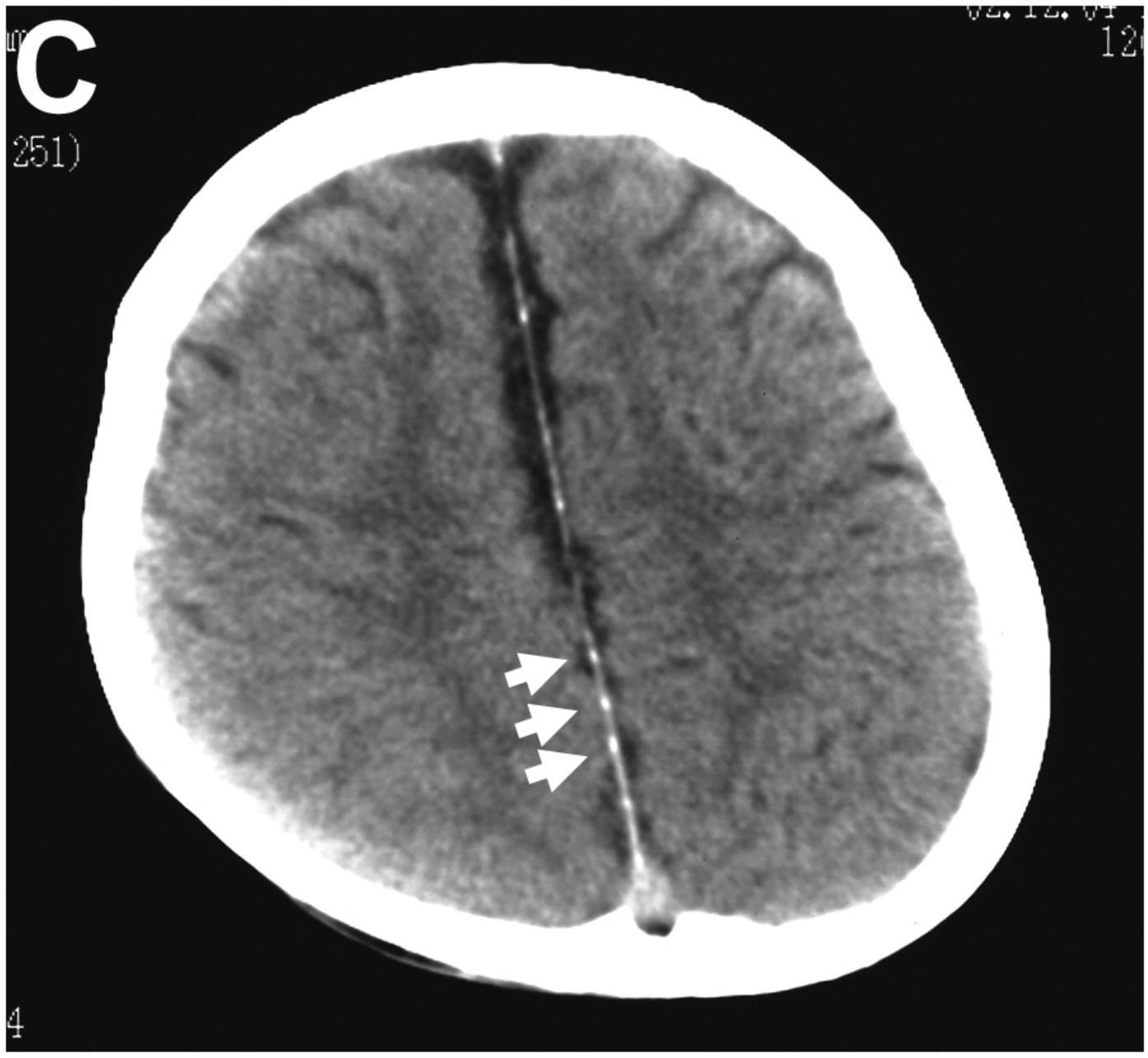

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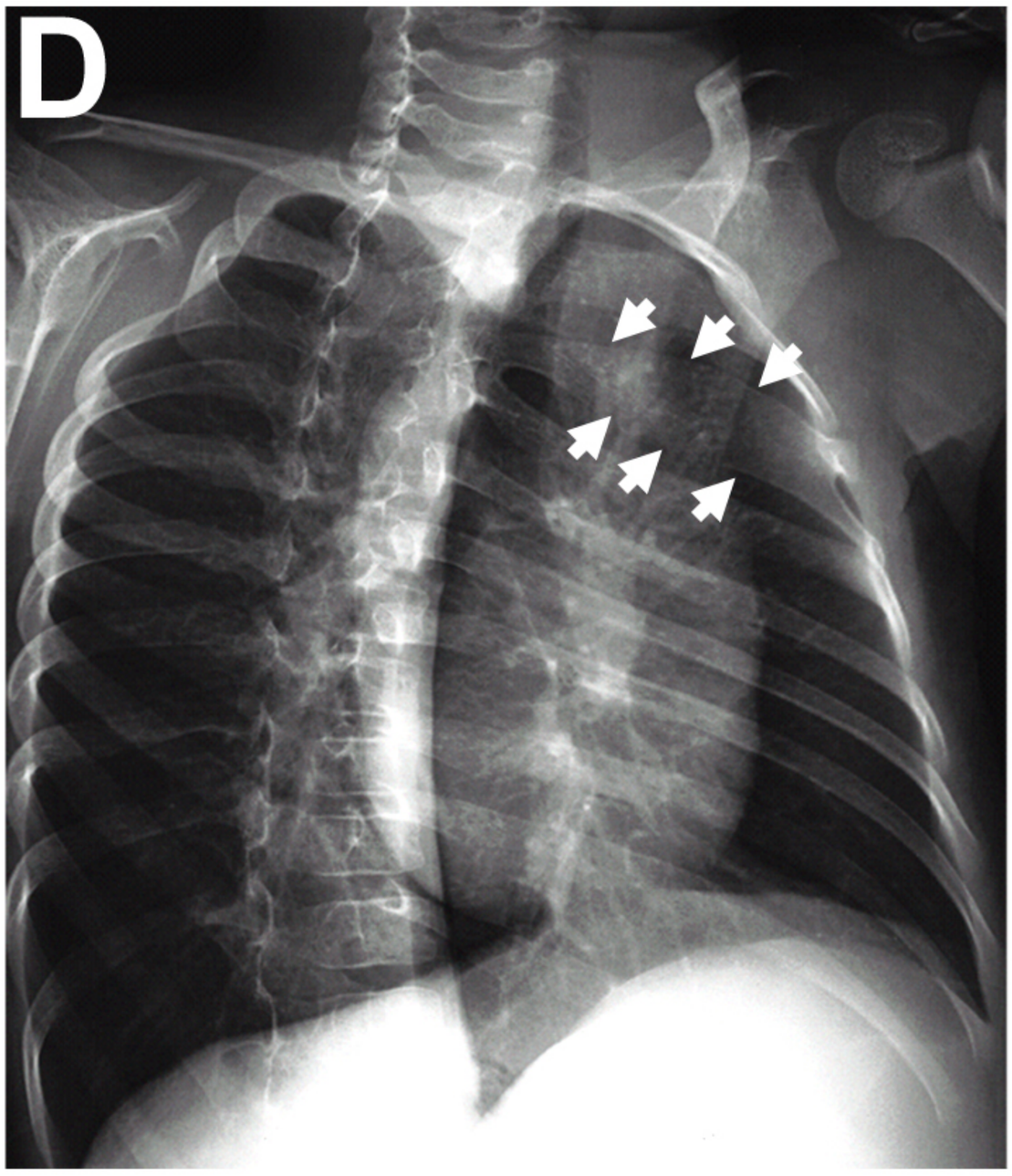

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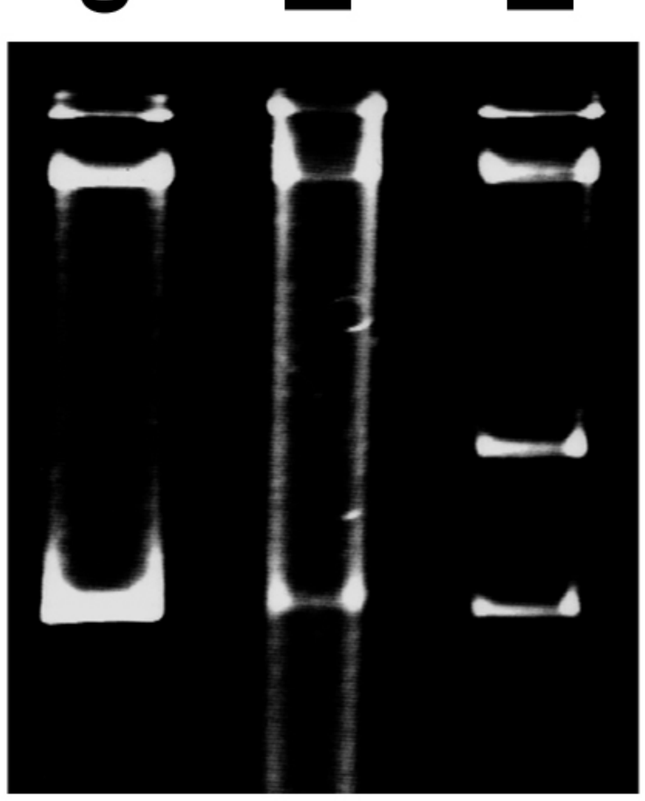

exon 9

F