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Research Article

Assessment of Genetic Mutations in ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, RBPJ Genes to Induced Adams-Oliver's Syndrome

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Abstract

In this study we have analyzed 31 people. 11 patients Adams-Oliver's syndrome and 20 persons control group. The genes ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, RBPJ, analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with Adams-Oliver's syndrome. In fact, of all people with Adams-Oliver's syndrome. 10 patients Adams-Oliver's syndrome had a genetic mutation in the genes ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, RBPJ Adams-Oliver's syndrome. Any genetic mutations in the target genes control group did not show.

Keywords: Genetic study; Adams-Oliver's syndrome; Mutations the genes ARHGAP31; DLL4; DOCK6; EOGT; NOTCH1; RBPJ; Real Time-PCR

Introduction

Adams-Oliver's syndrome is a rare genetic disorder that is identified at birth. The early signs of the syndrome are disorders of skin development and organ anomalies. Physical disturbances associated with this syndrome are different among affected people. Most people with Adams-Oliver syndrome have a disorder of skin development. In some cases, the bone under the skin has not evolved. People with Adams-Oliver syndrome usually have allergies and lack of hair growth in the affected area. Hand and foot disorders are common in people with Adams-Oliver syndrome. These disorders often include abnormalities in the fingers and toes, abnormal nails in the fingers and toes, fingers and legs (cynadectal) and abnormal shortness or loss of one of the fingers or legs (ductile or oligodactylus) [1-3] (Figure 1).

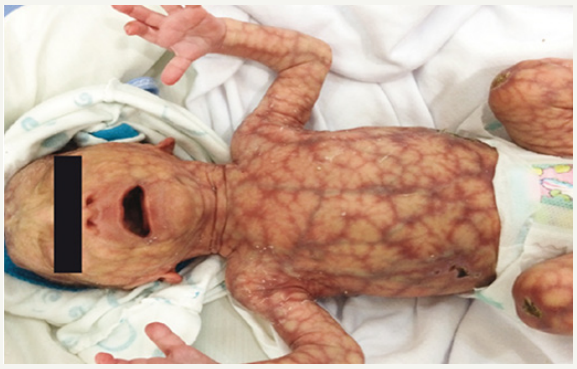

Figure 1: Adams-Oliver's syndrome image with severe skin disorder.

Additionally, people with Adams-Oliver syndrome may also experience high blood pressure in the blood vessels between the heart and the lungs (pulmonary hypertension), which can be life-threatening. Other blood vessel problems and heart defects can also occur in people with Adams-Oliver syndrome. In some cases, people with Adams-Oliver syndrome have nerve problems, delayed growth, learning disabilities, or anomalies in the brain's structure [4,5] (Figure 2).

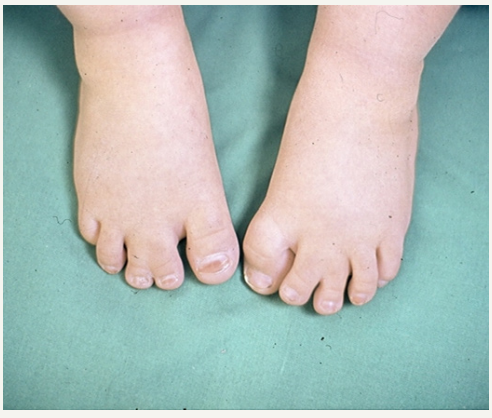

Figure 2: Child's legs with Adams-Oliver syndrome with cinnabar disorder at the toes.

Etiology of the Adams-Oliver Syndrome

The Adams-Oliver syndrome is caused by mutations in the genes ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, and RBPJ. The

ARHGAP31 gene is based on the long arm of chromosome number 3 as 3q13.32-q13.33. The DLL4 gene is based on the long arm of chromosome 15 as 15q15.1. The DOCK6 gene is based on the short arm of chromosome 19 as 19p13.2. The EOGT gene is based on the short arm of chromosome number 3 as 3p14.1. The NOTCH1 gene is based on the long arm of chromosome 9 as 9q34.3. The RBPJ gene is based on the short arm of chromosome number 4 as 4p15.2. Each of these genes plays an important role in fetal growth, and changes in each of them can affect the highly controlled process of embryo development and lead to symptoms of the Adams-Oliver syndrome. The proteins produced by the ARHGAP31 and DOCK6 genes are both involved in the regulation of proteins called GTPases, and they transmit signals that are necessary for the development and development of various aspects of the fetus. The ARHGAP31 and DOCK6 proteins appear to be very important for the development of GTPases during the development of the organs, the skull, and the heart. The process of GTPases is often referred to as molecular switches, because they can be turned on and off. The DOCK6 protein turns on the GTPasese process and the ARHGAP31 protein turns off the GTPases process. The mutation in the DOCK6 gene results in the production of an abnormal or short-lived DOCK6 protein, which is probably not able to illuminate the process of GTPases, which reduces the activity of the embryo's signalling signalling [6-10] (Figure 3). The mutation in the ARHGAP31 gene also reduces the activity of GTPases, resulting in the production of abnormal and short-term ARHGAP31 protein, which is probably not able to extinguish the GTPases process, and this reduction in GTPases activity results in skin problems, bone anomalies, and other characteristics Adams-Oliver syndrome [11].

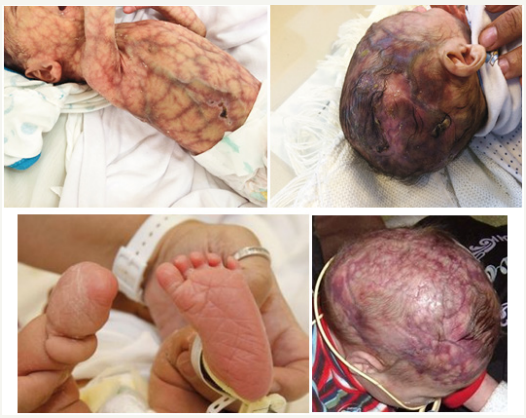

Figure 3: Another view of the newborn with Adams-Oliver syndrome associated with the related disorders.

The proteins produced from the DLL4, NOTCH1, and RBPJ genes form part of the known signaling path called the Notch pathway. The Notch signaling pathway controls the growth of embryonic cells, including: bones, heart, muscles, nerves and blood vessels. The NOTCH1 and DLL4 proteins act as a lock and key, and play a role in stimulating part of the Notch pathway that is critical to the development of blood vessels [12].

The mutations of the NOTCH1 gene and the DLL4 involved in the Adams-Oliver syndrome, probably affect the Notch1 signaling, which may result in some people with this blood vessel syndrome that may develop and develop heart disorders. Researchers believe that Adams-Oliver's syndrome may be due to abnormal growths of blood vessels before birth. Notch1 signalling and other Notch proteins stimulate the RBP-J protein produced from the RBPJ gene to attach specific regions of DNA to control the activity of genes that contribute to cellular evolution of various tissues throughout the body. Slowly The RBPJ gene mutations involved in the Adams-Oliver syndrome alter the RBP-J protein that normally binds DNA. These changes in the gene activity cause a defect in the correct growth of the skin, bones and other tissues, which ultimately lead to the characteristics of the Adams-Oliver syndrome [13]. Little information is available on how the mutation in the EOGT gene causes Adams-Oliver syndrome. The protein produced from the EOGT gene modifies some proteins by transferring molecules called N-acetyl glucosamine to them. The EOGT protein appears to modify the Notch protein, which stimulates the Notch signaling pathway. However, the effect of the change in Notch signaling is unknown. At least three mutations in the EOGT gene have been identified in people with Adams-Oliver syndrome, but how the signs and symptoms of the syndrome are caused by genetic changes in the EOGT gene is still unknown [14] (Figure 4).

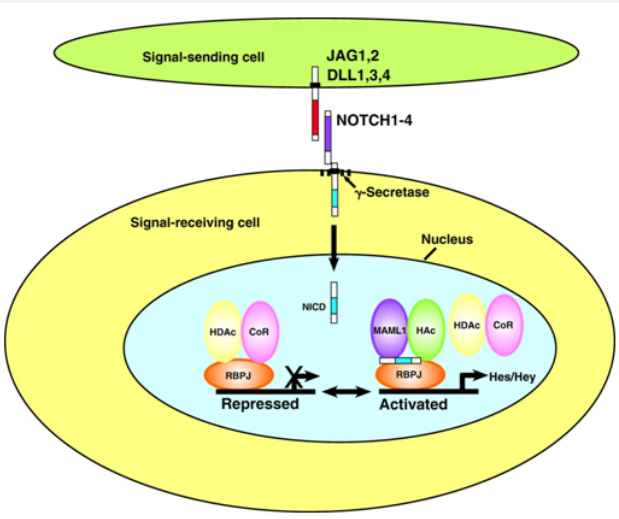

Figure 4: Schematic representation of the Notch1 signaling pathway in the cell.

Adams-Oliver syndrome can have different hereditary patterns. When this syndrome is generated by mutations in the genes ARHGAP31, DLL4, NOTCH1, RBPJ, then they will follow the dominant autosomal inheritance pattern. Therefore, in order to produce this syndrome, ARHGAP31, DLL4, NOTCH1, RBPJ (parent or parent) gene is required, and the chance of having a child with the syndrome in the dominant autosomal state is 50 for any pregnancy % is [15]. If the Adams-Oliver syndrome is caused by the mutation of the DOCK6 and EOGT genes, then the syndrome follows an autosomal recessive pattern of heredity. Therefore, in order to create this syndrome, two copies of the mutated genes DOCK6 and EOGT (one parent and the other mother) are needed and the chance of having a child with Adams-Oliver's syndrome in

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an autosomal recessive state, for each probable pregnancy of 25 % is [15] (Figure 5,6).

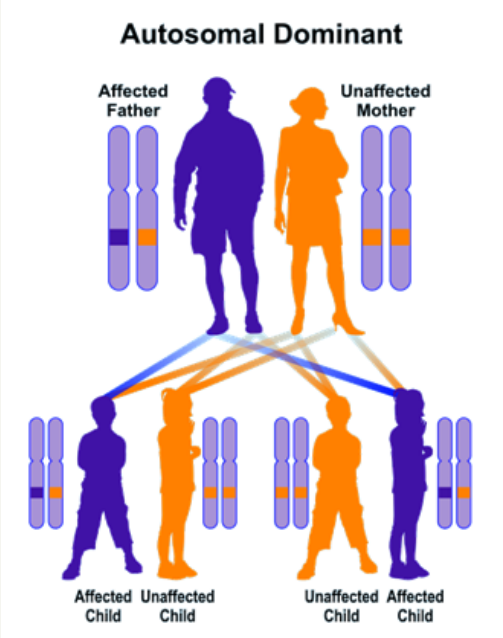

Figure 5: Schematic view of the dominant autosomal inheritance pattern that Adams-Oliver syndrome complies with this pattern by mutation in the genes ARHGAP31, DLL4, NOTCH1, RBPJ.

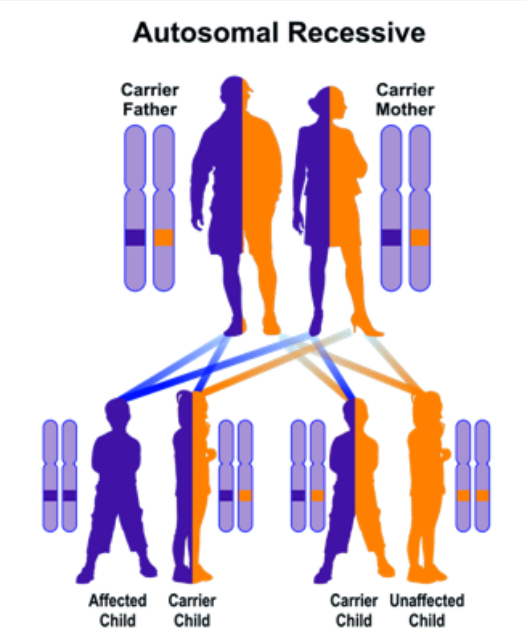

Figure 6: Schematic view of autosomal recessive hereditary pattern that Adams-Oliver syndrome follows with this mutation in DOCK6 and EOGT genes.

Materials and Methods

In this study, 11 patients with Adams-Oliver's syndrome and 20 persons control group were studied. Peripheral blood samples from patients and parents with written permission control were prepared. After separation of serum, using Real Time-PCR technique of tRNA molecules was collected. To isolate dermatitis cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solution in ratio of 1to5with the peripheral blood of patients and controls were mixed. After 70 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 14 min at 480 Gera. The cell sediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrate ratio of 1to2 on ficole (Ficol) was poured in the 480G was centrifuged for 39 minutes. Mono nuclear dermatitis cells also are included, has alower density than ficole and soon which they are based. The remaining erythrocyte has a molecular weight greater than ficole and deposited in test tubes. The supernatant, which contained the mono nuclear cells, was removed, and the 480 Gera was centrifuged for 19 minutes. Finally, the sediment cell, the antibody and dermatitis cells was added after 39 minutes incubation at 5 °C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACSS pam Stem cell culture medium containing the transcription genes ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, RBPJ, and were kept.

To determine the purity of dermatitis cells are extracted, flow cytometry was used. For this purpose, approximately 3×10^2 dermatitis cells were transfer red to 1.5ml Eppendorf tube and then were centrifuged at 2300 rpm for 11 minutes at time. Remove the supernatant culture medium and there maining sediment, 100µl of PBS buffer was added. After adding 5-10µl PE monoclonal anti body to the cell suspension for 70 min at 4 °C, incubated and read immediately by flow cytometry. For example, rather than control anti body dermatitis cells PE, IgG1 negative control solution was used.

Total mRNA extraction procedure includes:

i. 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 7 minutes. Then 200µl chloroform solution to target mix, and then transfer the micro tubes was added, and the shaker well was mixed for 35 seconds. The present mix for 4 minutes at room temperature and then incubated for 40 min at 4 °C on was centrifuged at 15200 rpm era. Remove the upper phase product was transfer reductase new microtube and to the one times the volume of cold ethanol was added. The resulting mixture for 72 hours at -20 °C was incubated.

ii. Then for 35 min at 4 °C on was centrifuged at 15000 rpm era. Remove the super natant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4 °C on by the time we were centrifuged 15000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 20µl sterile water and at a later stage, the concentration of extracted mRNA was determined.

To assessment the quality of mi-RNAs, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and 1µl oligoprimers 18 (dT) was performed.

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Following the PCR reaction 2μM dNTP, 1μg cDNA, Fermentas PCR buffer 1X, 0/75μM MgCl2, 1.25 U/μL Tag DNA at 95 °C for 4 min, 95 °C for 30s, annealing temperature 58 °C for 30s, and72 °C for30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophores is with ethidium bromide staining and color was evaluated.

Results (Figure 7-10)

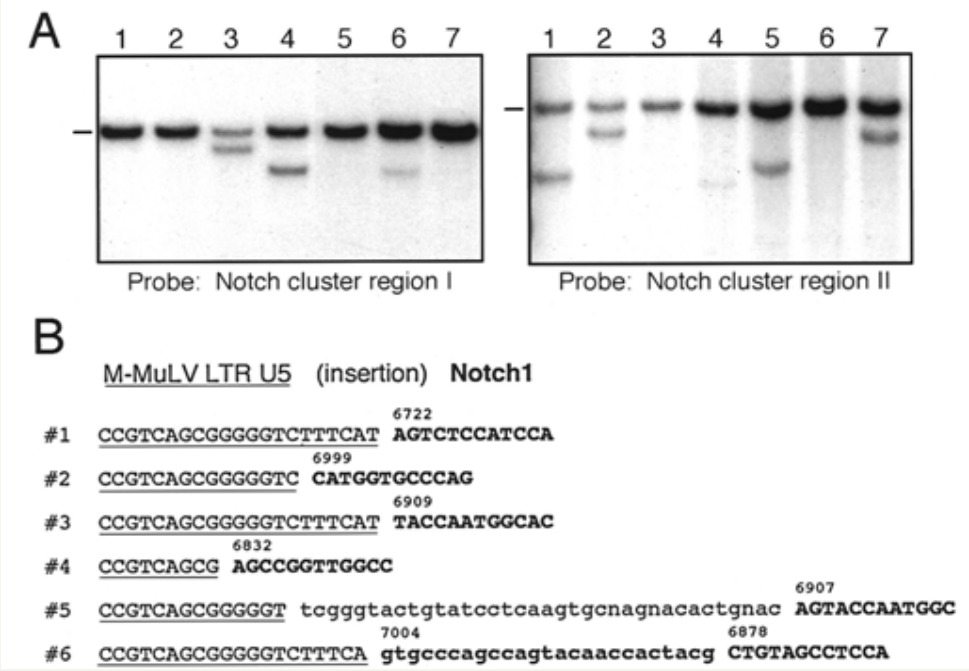

Figure 7: Schematic of band formation pattern in NOTCH1 gene along with nucleotide sequence.

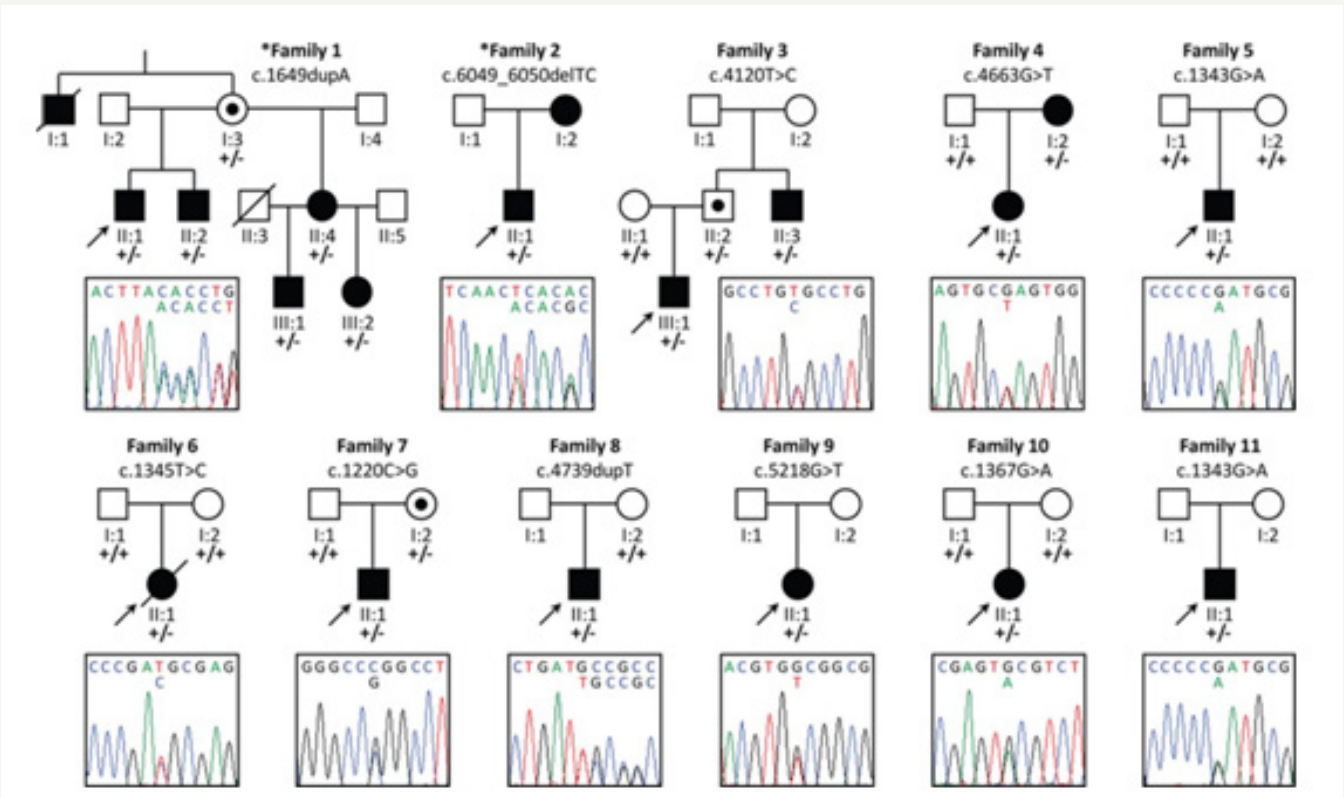

Figure 8: Schematic of the types of mutations created in target genes in patients with Adam-Oliver syndrome.

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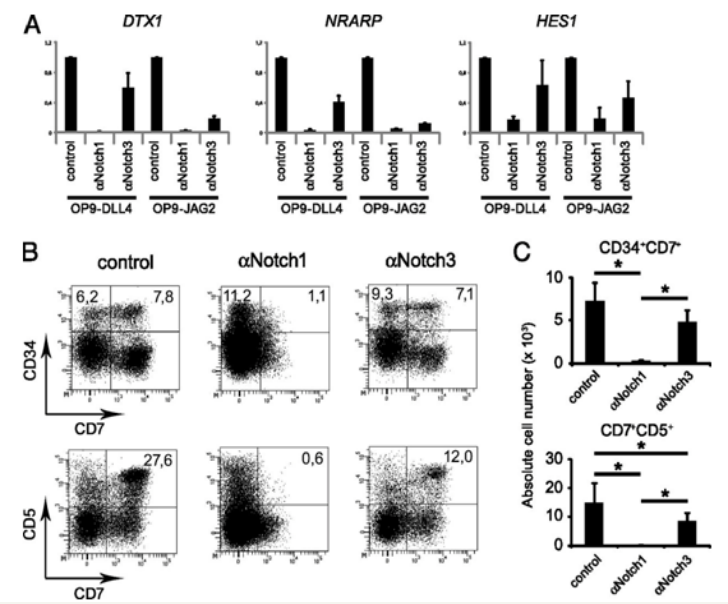

Figure 9: Schematic of distribution pattern of NOTCH1 and DLL4 genes in the control group and Adams-Oliver syndrome patients.

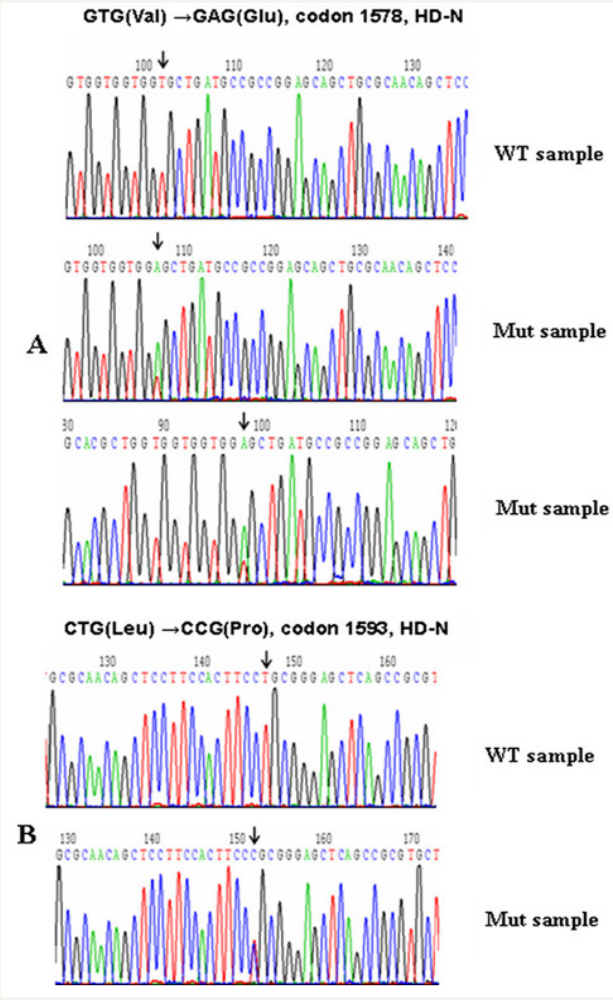

Figure 10: Schematic of NOTCH1 gene mutations with nucleotide sequence in the control group and patients with Adams-Oliver syndrome.

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Discussion and Conclusion

According to the results of sequencing the genome of patients with Adams-Oliver's syndrome, and the genetic mutations ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, RBPJ genes found that about 100% of patients with Adams-Oliver's syndrome, they have this genetic mutations. Patients with Adams-Oliver's syndrome, unusual and frightening images in the process of Adams-Oliver's syndrome, experience. Lot epigenetic factors involved in Adams-Oliver's syndrome. But the most prominent factor to induce Adams-Oliver's syndrome, mutations is ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, RBPJ genes. This gene can induce the birth and can also be induced in the adulthood (Figure 11).

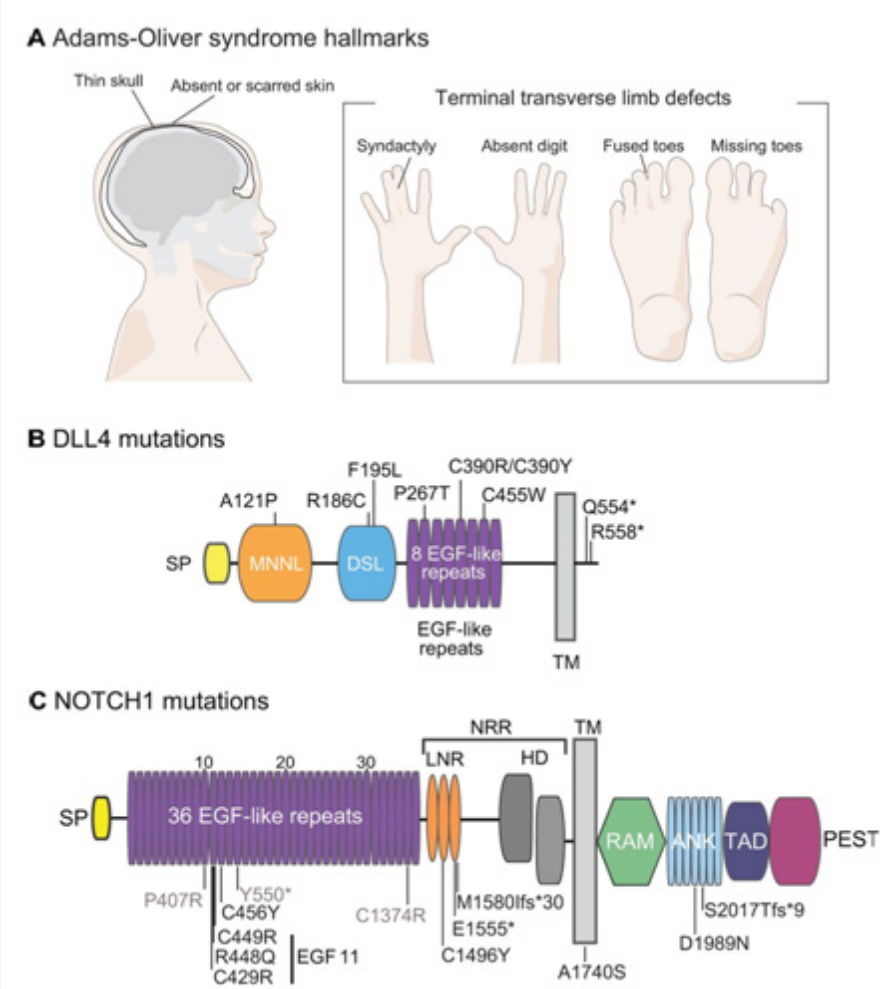

Figure 11: Schematic of the clinical signs of the Adams-Oliver Syndrome with mutations in the NOTCH1 and DLL4 genes.

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