GENETIC ALTERATIONS IN JUVENILE NASOPHARYNGEAL ANGIOFIBROMAS

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Abstract: Juvenile nasopharyngeal angiofibroma (JNA) is a rare benign neoplasm of the nasopharynx that accounts for 0.5% of all head and neck tumors. Although histologically benign in appearance, JNAs are locally aggressive and destructive, spreading from the nasal cavity to the nasopharynx, para-nasal sinuses, and orbit skull base with intracranial extension. The gender selectivity of JNA and the relatively young age at diagnosis suggest hormone-dependent development. Hormonal disorders have been reported in patients with JNA, and androgen and estrogen receptors have been identified in tumor tissue; however, a hormonal influence on JNA is controversial. Recent studies have attempted to further delineate the pathogenesis of JNA through analysis of genetic and molecular changes. Understanding of the molecular mechanisms involved in JNA might improve prevention, prognosis, and treatment of this tumor. In this review, we discuss published studies addressing the possible molecular pathways that might be involved in the development of JNA.

Keywords: juvenile nasopharyngeal angiofibromas; genetic alterations; cytogenetic alterations

Juvenile nasopharyngeal angiofibroma (JNA) is a rare benign neoplasm of the nasopharynx that accounts for 0.5% of all head and neck tumors.\(^1,^2\) Its general incidence is approximately 1:150,000, but it predominantly affects adolescent boys and men between the ages of 14 and 25 years.\(^2,^4\) Rare cases have been reported in men over 25 years old and in adolescent girls.\(^5,^9\)

JNA emerges in the posterolateral wall of the nasal cavity posterior to the pterygopalatine ganglion and may extend through adjacent structures by pressure erosion through bone.\(^10\) These tumors are nonencapsulated entities composed of a proliferating and irregular vascular component within a fibrous stroma consisting predominantly of fibroblasts.\(^1,^11\) Although histologically benign in appearance, JNAs are locally aggressive and destructive spreading from the nasal cavity to the nasopharynx, paranasal sinuses, and orbit skull base with intracranial extension.\(^4,^12\) Evidence of intracranial spread occurs in 10% to 20% of cases\(^4,^13\) and the reported rate of recurrence following treatment varies between 0% and 57%.\(^12,^14,^15\)

Imaging techniques have led to great advances in the diagnosis and treatment of JNA. The prognosis is very good with early diagnosis. However, due to the rather innocuous presenting symptoms (nasal obstruction and epistaxis), diagnosis most often occurs during later stages of the disease.\(^2,^16\) The most common primary treatment is surgical
resection combined with preoperative embolization, and chemotherapy and radiotherapy are recommended as additional treatment options.2,4,17

There are 2 main theories of origin of JNA, proposing fibrous or vascular origin; however, its etiology and pathogenesis remain unknown. Few studies have investigated the genetic and molecular changes underlying JNA. In this review, we discuss published studies addressing the molecular pathways that might be involved in the development of JNA.

STEROID HORMONES AND NUCLEAR RECEPTORS

Steroid hormones have been implicated in the development of many human tumors, including breast and prostate carcinomas, and the presence of steroid hormone receptors in neoplastic tissues has important clinical implications.18

Classical steroid hormones, such as estrogen, progesterone, androgens, glucocorticoids, and mineralocorticoids, are synthesized and secreted by endocrine cells. They travel via the bloodstream to their target cells, enter by diffusion, and bind to members of the superfamily of nuclear hormone receptors which act as ligand-inducible transcription factors. Upon hormone binding, the receptor dissociates from heat-shock proteins, dimerizes, and binds to hormone responsive elements, in DNA, leading to the regulation of hormone responsive genes.19

JNA’s sex selectivity and the relatively young age at diagnosis suggest that its development is hormone dependent.20 Initial studies focused on sex hormone imbalances as a potential mediator of JNA development.20–23 Other studies have focused on the presence of sex hormone receptors in JNA tissue.1,23–28

The first evidence for an association between steroid hormone imbalance and JNA development was the observation that patients with JNA are sexually underdeveloped, and that tumor regresses only after full development of secondary sex characteristics.21 Later studies provided additional evidence of an influence of sex hormones on the development of JNA.24,26,29,30 Schiff,20 studying 4 cases, proposed that JNA may develop due to excess of androgen and reported tumor reduction following treatment with estrogen. Johnsen et al.22 studying 1 case, and Johns et al.23 studying 6 cases, also found a decrease in tumor size with estrogen treatment. Using electron microscopy, Kuttner et al.31 observed histological and cytological alterations of the vascular component and stromal fibroblasts in 4 patients treated with estrogen, suggesting a direct action of the hormone. In fact, during the 1960s and 1970s, presurgical estrogen administration was recommended to shrink the tumor and decrease intraoperative hemorrhage, although the efficacy of these studies was poorly controlled.32

Preoperative estrogen therapy is not currently used because of the variable effect of estrogens on JNA, as well as the secondary feminizing effects and the risk of cardiovascular complications.33 Johnsen et al.22 demonstrated tumor growth after administration of testosterone, which suggests that an androgen blocker might be used to suppress the growth of JNA. Consistent with this idea, Hagen et al.34 showed that the proliferative rate of tumor fibroblasts in culture increased when testosterone was added to the medium, whereas the addition of 2 anti-androgens, cyproterone acetate and flutamide, reduced the proliferative rate. Two studies investigated flutamide in vivo, 1 finding an average tumor reduction of 44% in 4 of 5 cases with JNA35 and the other finding a maximum tumor reduction of 11.1%, suggesting that flutamide treatment is not advantageous at the time of surgery.33

Although the mechanism of action of exogenous estrogen in JNA was unknown, some investigators sought to account for the clinical observation of tumor reduction after hormone therapy by examining the presence of estrogen receptor (ER). Clinical measurement of ER has been performed routinely since the 1970s, although the method has changed over the years. Early assays were based on ligand-binding techniques, radioligand binding, or fluorescent hormone binding; by the 1980s, these methods were supplanted by monoclonal antibody-based techniques, such as immunohistochemistry and immunoenzyme assay.33 The use of different methodologies and monoclonal or polyclonal antibodies may explain the controversial results of studies analyzing steroid receptors in JNA, which are described below (Table 1).

Using binding assays, Johns et al.23 found no evidence of ERs in 6 cases of JNA compared with breast cancer controls and suggested that exogenous administration of estrogen reduces tumor size, but does not have a direct effect on JNA. Alternatively, they proposed that estrogens may reduce tumor size by decreasing the secretion of hypothalamic gonadotropin releasing hormone and therefore testosterone production. In another study of 8 JNA cases, Lee et al.24 used binding assays and found that none of the tumors were
positive for estrogen or progesterone receptors, whereas 3 were positive for androgen receptors (ARs), suggesting that JNA is not dependent on estrogen or progesterone, but no significantly alteration of serum estradiol or testosterone levels was found.

Farag et al\textsuperscript{25} evaluated 7 JNA cases and found that serum levels of dihydrotestosterone, testosterone, and 17β-estradiol were within the normal ranges. Binding assays detected ARs, but not ERs in all 7 JNA cases analyzed. In another study using binding assays, 5 JNA samples were negative for estrogen and progesterone receptors, but highly positive for ARs, suggesting that JNA might be androgen dependent.\textsuperscript{1} Brentani et al\textsuperscript{26} investigated the presence of androgen, estrogen, progesterone, and glucocorticoid receptors in cytosol from 12 JNA cases and found a predominance of progesterone (58\%) and glucocorticoid (84\%) receptors, with smaller number of estrogen (25\%) and androgen (25\%) receptors. In spite of the low levels of steroid receptors, there was a significant association between the presence of progesterone and ARs and high endothelial and fibroblastic cellularity.

Hwang et al\textsuperscript{27} obtained direct evidence for the presence of ARs in 18 of 24 JNA cases. Immunostaining for the receptors was found in stromal and endothelial cells. On the contrary, Gatalica,\textsuperscript{28} studying 8 cases of JNA and 8 samples of nasal turbinate as a control group, found no estrogen or progesterone receptors in any of the 16 samples. Weak nuclear AR immunoreactivity was found in a minority of endothelial and stromal cells although in both tumor and normal samples.

Saylam et al\textsuperscript{36} performed immunohistochemical analysis on 27 JNA samples and found that 7.4\% of the cases were positive for the ER and 33.3\% of the cases were positive for progesterone receptors. Montag et al\textsuperscript{32} analyzed 13 JNA cases by immunohistochemistry and found that all were positive for ER-β in stromal pericytic and endothelial cells. Five cases were positive for AR in stromal cells and none of the cases demonstrated staining for ER-α or progesterone receptor. Nowadays, ER determinations are performed with an antibody-based method, typically using a monoclonal antibody against the ER-α protein. These methods fail to detect ER-β, which may account for the discrepancies in the literature regarding ER in JNAs and other mesenchymal tumors.\textsuperscript{32}

In spite of the reports of hormonal disorders in patients with JNA and the presence of androgen and/or ERs in tumor tissues, apparently no alterations of hormonal serum levels were observed, and the hormonal influence on JNA is still controversial.\textsuperscript{20–28,31}

**GROWTH FACTORS**

The secretion of proangiogenic growth factors is essential to growth and metastasis of solid tumors.\textsuperscript{37} The highly angiogenic response that accompanies JNA has motivated several investigators to examine the presence of growth factors associated with the angiogenic process in JNA.

Polypeptide growth factors promote growth by both paracrine and autocrine mechanisms. Most of the receptors for these growth factors are trans-membrane tyrosine-specific protein kinases.\textsuperscript{38,39} Vascular endothelial growth factor (VEGF) is the most prominent proangiogenic growth factor in tumor biology. It is frequently expressed by tumor cells and is also expressed by many normal tissues.\textsuperscript{37}

Brieger et al\textsuperscript{40} conducted an immunohistochemical examination of 10 JNAs and found frequent expression of VEGF in stromal cells and vessels (80\%). Furthermore, VEGF expression

<table>
<thead>
<tr>
<th>Method</th>
<th>AR</th>
<th>ER</th>
<th>PR</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Binding assay</td>
<td>3/8</td>
<td>0/8</td>
<td>0/8</td>
<td>Lee et al\textsuperscript{34}</td>
</tr>
<tr>
<td>Binding assay</td>
<td>7/7</td>
<td>0/7</td>
<td>–</td>
<td>Farag et al\textsuperscript{25}</td>
</tr>
<tr>
<td>Binding assay</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>Antonelli et al\textsuperscript{1}</td>
</tr>
<tr>
<td>Binding assay</td>
<td>3/12</td>
<td>3/12</td>
<td>7/12</td>
<td>Brentani et al\textsuperscript{26}</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>18/24</td>
<td>0/24</td>
<td>2/24</td>
<td>Hwang et al\textsuperscript{27}</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>8/8*</td>
<td>0/8</td>
<td>0/8</td>
<td>Gatalica\textsuperscript{28}</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>–</td>
<td>2/27</td>
<td>9/27</td>
<td>Saylam et al\textsuperscript{36}</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>5/13</td>
<td>13/13</td>
<td>0/13</td>
<td>Montag et al\textsuperscript{32}</td>
</tr>
</tbody>
</table>

*Weak expression in less than 5\% of the cells.

Abbreviations: AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor.
was associated with proliferation and increased vessel density, suggesting that it might promote vascularization in JNAs. Recently, Schuon et al. used immunohistochemistry to evaluate the subcellular distribution of several angiogenic factors and showed that increased levels of basic fibroblast growth factor (bFGF), transforming growth factor-β1 (TGFβ1), and VEGF receptor-2 (VEGFR-2) are associated with high vessel densities in JNA.

Using immunohistochemistry, Saylam et al. observed VEGF expression in 24 of 27 (88.9%) JNA samples, including all recurrent cases. In addition, all 27 samples were positive for proliferating cell nuclear antigen, a marker of tumor proliferation that may be useful in predicting tumor behavior and prognosis. 36, 37

Basic fibroblast growth factor is a mitogenic factor for many cell types of mesodermal and neuroectodermal lineage. Its biological functions include angiogenesis, tissue development, differentiation, and modulation of neural function. 38 Schiff et al. found evidence of bFGF expression in JNA tissues from 3 patients. More recently, Schuon et al. observed a correlation between high bFGF levels in stromal cells and the fibrous tissue component in a series of 13 JNAs, suggesting that this angiogenic factor might be involved in the pathogenesis of JNA.

TGFβ1 is a polypeptide growth factor that is produced in fibroblasts, macrophages, and endothelial cells. It helps in the regulation of the cell cycle, production of extracellular matrix, and angiogenesis induction while increasing its own expression. 39 TGFβ1 seems to play a role in the pathogenesis of JNA; Nagai et al., 40 Dillard et al., 41 Saylam et al., 36 and Schuon et al. 42 observed VEGF expression in 24 of 27 (88.9%) JNA cases analyzed. In addition, Schuon et al. also found positive staining for TGFβ1 in stromal cell nuclei and cytoplasm of JNA tissue from 19 patients, and Saylam et al. also found positive staining for TGFβ1 in 14 of 27 JNA cases analyzed. In addition, Schuon et al. observed a good correlation between stromal TGFβ1 expression and increased vessel density in JNA, suggesting that TGFβ1 is important for stromal as well as for vessel growth promotion in these tumors.

Bone morphogenic proteins (BMPs) are members of the TGFβ superfamily and have a complex role in regulating cell growth and differentiation via the BMP-Smad signaling pathway. 43 Overexpression of BMP-4 has been found in patients with fibrodysplasia ossificans progressiva and in various bone and soft tissue sarcomas. 44, 45 Immunohistochemical analysis by Zhang et al. revealed strong expression of BMP-4 in JNAs. However, the level of expression was similar to that observed in nasal polyps, suggesting that this factor might not have an important role in JNA. Nagai et al. also observed strong expression of TGFβ3 in JNA samples. However, there was no statistically significant difference in its expression between JNA and nasal polyps.

Platelet-derived growth factor (PDGF) stimulates tumor growth and progression through effects on tumor and stromal cells. 46 Nagai et al. found that PDGF-B mRNA is overexpressed in 50% of JNAs. PDGF-B is mitogenic for the endothelial cells of small capillaries and stimulates the synthesis of matrix components, suggesting that PDGF-B might contribute to neovascularization and fibrosis in JNAs.

Insulin-like growth factors (IGFs) are polypeptide growth factors with functional homology to insulin, and have a variety of putative functions, including stimulation of cell growth, cell division, and apoptosis. 47 IGFs function via the receptor IGF-1R. IGF-1R has been implicated in tumorigenesis, having been found to promote cell survival in both tissue culture and animals. 48 Immunohistochemical investigation revealed no expression of IGF-1R in JNAs. 49 However, 1 study using Northern blot found overexpression of IGFII in 53% of JNAs, suggesting that IGFII might regulate the growth of these tumors. 50 In an attempt to identify the mechanism underlying the overexpression of IGFII in JNAs, Coutinho-Camillo et al. analyzed the genomic imprinting and methylation status of IGFII and H19 genes in 27 samples of JNA. Loss of imprinting of IGFII gene was found in 50% of the informative cases and hypomethylation of the H19 gene was found in 75% of the samples, suggesting that alterations in the IGFII/H19 imprinted region may play a role in JNA.

In addition to its well-known effect in neural tissue, nerve growth factor (NGF), a member of the neurotrophin family, has been shown to regulate mast cell differentiation and angiogenesis in inflammatory conditions through its receptor...
NGF immunoreactivity has been demonstrated in fibroblast stromal cells and endothelial cells of JNA, suggesting that it may promote vascular growth in JNA. 51

Altogether, these studies evaluating growth factors suggest that specifically VEGF, TGFβ1, and IGFII seem to play a role in the development of JNA. The observed alterations of growth factor pathways in JNAs are summarized in Table 2.

### CHROMOSOMAL ABNORMALITIES

The use of molecular genetic techniques such as loss of heterozygosity analysis, fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH) has improved the detection of chromosome alterations. These techniques are helpful in defining chromosomal regions that may harbor amplified oncogenes or deleted tumor suppressor genes. 58,59

Schick et al 50 obtained the first evidence of genetic imbalances in angiofibromas using CGH. In a pilot study of 3 patients with JNA, they detected an additional chromosome X and a loss of chromosome Y in 2 male patients. They also found losses on chromosomes 17, 19p, and 22q and gains on chromosomes 3q, 4q, 5q, 6q, 7q, 8q, 12p, 12q, 13q, 14q, 18q, and 21q. Concurrent chromosomal gains on 8q12–q22 were observed in all 3 patients. Recently, Heinrich et al 51 used CGH and detected several chromosomal aberrations in JNAs; they also found that DNA gains are remarkably more common than DNA losses in these tumors.

Schick et al 52 using FISH analysis also detected a significant loss of the Y chromosome in 6 of 7 JNA cases. Five cases demonstrated a gain of chromosome X and a gain of the AR gene (located on chromosome Xq11–q12). The finding of an additional copy of the AR gene in JNAs is consistent with the immunohistological findings of high AR levels in JNAs. However, amplification of the AR gene was not detected.

Brunner et al 53 used CGH to analyze 7 cases with JNA and detected a variety of abnormalities affecting 18 different chromosomes in 6 of 7 JNA cases. Frequent chromosomal gains were observed on chromosomes 4q, 6q, and 8q and complete loss of the Y chromosome was observed in 4 patients. Loss of Xq21.3q27 was detected in 1 patient.

The presence of abnormalities on the sex chromosomes in patients with JNA, including loss of the Y chromosome, might be of great importance. Loss of the Y chromosome was reported in all studies. It has also been reported in chronic myeloid leukemia and in transitional cell carcinoma cell lines. 64,65 Furthermore, loss of both chromosome Y and chromosome X have been found to be associated with metastases, local invasion, and high proliferation rate in pancreatic endocrine tumors. 66

A high number of chromosomal abnormalities in JNAs, as detected in these studies, may alter cell cycle control. Recently, Schick et al 57 using genome DNA microarray and metaphase-CGH, detected chromosomal aberrations in several chromosomes including frequent gains at chromosomes 4q, 6, 12, and X and losses of chromosomes 8, 16, 17, 22, and Y. They also detected amplifica-

<table>
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<th>Growth factor</th>
<th>Method of investigation</th>
<th>Implication in JNA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>Immunohistochemistry</td>
<td>Expressed in stromal cells and vessels; may be related to vascularization</td>
<td>Brieger et al 40, Saylam et al 36</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Immunohistochemistry</td>
<td>Expression may be related to angiogenesis and ECM production</td>
<td>Nagai et al 47, Dillard et al 48, Saylam et al 36, Schuon et al 41</td>
</tr>
<tr>
<td>bFGF</td>
<td>Western blot and immunohistochemistry</td>
<td>High levels of expression correlate with high vessel density</td>
<td>Schiff et al 55, Schuon et al 41, Nagai et al 47</td>
</tr>
<tr>
<td>PDGF-B</td>
<td>Northern blot</td>
<td>Overexpression might contribute to neovascularization and fibrosis</td>
<td>Nagai et al 47</td>
</tr>
<tr>
<td>IGFII</td>
<td>Northern blot and PCR-RFLP</td>
<td>Expression may be related to cell growth and cell division</td>
<td>Coutinho-Camillo et al 55</td>
</tr>
<tr>
<td>NGF</td>
<td>Immunohistochemistry</td>
<td>Expression in stromal and endothelial cells may be related to vascular growth</td>
<td>Zhang et al 41</td>
</tr>
</tbody>
</table>

Abbreviations: VEGF, vascular endothelial growth factor; TGF, transforming growth factor; bFGF, basic fibroblast growth factor; PDGF-B, platelet-derived growth factor B; IGF, insulin-like growth factor; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; NGF, nerve growth factor.
tion of AUKRA (STK15) and MDM2 genes which may be involved in chromosomal instability.

In summary, several studies have demonstrated numerous chromosomal alterations in JNAs, particularly, gains at chromosomes 4, 6, 8, and X and losses of chromosomes 17, 22, and Y (Table 3). These data provide important information regarding the location of tumor suppressor genes (losses) and oncogenes (gains) that are potentially involved in JNA. However, the target gene or genes for those deletions and gains is still unknown.

### TUMOR SUPPRESSOR GENES

The increased frequency of JNA among patients with familial adenomatous polyposis (FAP) suggests an interesting association between the 2 conditions.68,69 Germline mutations in the adenomatous polyposis coli (APC) tumor suppressor gene located on chromosome 5q21 are responsible for development of FAP.70 APC mutations are also present in the majority of sporadic colorectal tumors.71 The protein encoded by the APC gene can interact with other proteins in different cellular compartments.72 The APC protein regulates the level of β-catenin, which acts both as a submembranous component in cadherin-mediated cell–cell adhesion and as a downstream transcriptional activator in the Wnt signaling pathway.72,73

Ferouz et al69 used the technique of polymerase chain reaction–single strand conformation polymorphism (PCR-SSCP) and investigated the presence of APC mutations in 5 cases of JNA, but found no evidence of APC mutation. Guertl et al74 investigated JNAs for mutations or allelic loss of the APC gene using direct sequencing analysis of PCR products. None of the 11 cases exhibited mutation of the APC gene, and none of the 9 informative cases (heterozygous) carried an allelic loss (Table 4).

Investigation of 16 JNAs for mutations of APC and the β-catenin gene revealed β-catenin gene transcripts in 75% of the cases.75 Nuclear accumulation of β-catenin was diffusely present in stromal cells but not in endothelial cells, suggesting that APC/β-catenin pathway may be involved in the pathogenesis of JNA, and that stromal cells are the neoplastic cells of JNAs.75 Zhang et al51 also found strong expression of β-catenin in JNAs and confirmed its immunolocalization in tumoral tissue when compared with nasal polyps.

Valanzano et al76 presented genetic evidence that JNA is an integral FAP tumor. Analyzing the sequence of the APC gene and the presence of recurrent β-catenin mutations in matched blood and tumor DNA from a JNA affected FAP carrier, they found 2 frameshift mutations in the β-catenin binding regions of the APC gene. One of these mutations was found in both blood and JNA tissue and the other was detected only in JNA DNA.

β-Catenin can function as a coactivator of the AR.80 Nuclear accumulation of mutated β-catenin and AR protein is thought to increase tumor androgen sensitivity, which might account for the

### Table 3. Chromosomal abnormalities frequently observed in juvenile nasopharyngeal angiofibromas.

<table>
<thead>
<tr>
<th>Chromosomal alteration</th>
<th>Method of investigation</th>
<th>No. of cases analyzed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gains on 6q, 7q, 8q, 12p, 13q, 14q, 18q, 21q, and X</td>
<td>CGH</td>
<td>3</td>
<td>Schick et al60</td>
</tr>
<tr>
<td>Losses on 17, 19p, 22q, and Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent alteration: 8q12–q22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gain of X and loss of Y</td>
<td>FISH</td>
<td>7</td>
<td>Schick et al62</td>
</tr>
<tr>
<td>Alters in 18 different chromosomes</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent alterations:</td>
<td>CGH</td>
<td>7</td>
<td>Brunner et al63</td>
</tr>
<tr>
<td>Gains on 4q, 6q, and 8q</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Losses of Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Several chromosomal aberrations:</td>
<td>CGH</td>
<td>17</td>
<td>Heinrich et al61</td>
</tr>
<tr>
<td>Gains on chromosomal arms 1p, 9q, 10q, 12q, 16q, 17q, 19p, 19q, 20q, and 22q</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Losses on chromosome 4 in one case</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gain on X and gains and losses on Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aberrations in several chromosomes</td>
<td>CGH</td>
<td>29</td>
<td>Schick et al67</td>
</tr>
<tr>
<td>Recurrent alterations:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gains on 4q, 6, 12, and X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Losses of 8, 16, 17, 22, and Y</td>
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</tbody>
</table>

Abbreviations: CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization.
fact that JNA develops almost exclusively in adolescent males.

The tumor suppressor gene TP53 is a transcription factor that has been implicated in the regulation of several biological pathways controlling cell growth, transcription, apoptosis, senescence, and genomic stability. Loss of normal p53 function results in uncontrolled cell growth. Mutations in p53 are frequently observed in human neoplasms (http://www-p53.iarc.fr). Only 2 studies have investigated the occurrence of genetic alterations of TP53 in JNA. Using Northern blot, Nagai et al observed increased mRNA expression of the TP53 gene in 32% of the JNA cases, although mutation analysis has not been performed. Using FISH and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, Schick et al detected losses of the tumor suppressor gene TP53 in 5 of 7 cases with JNA. Using Western blot and immunohistochemistry, which detect only mutated p53, they did not detect p53 in JNAs. Further investigation is necessary to determine whether or not inactivation of TP53 plays a role in JNA.

ONCOGENES

The phosphoprotein encoded by the c-myc gene is involved in numerous cellular processes ranging from cell growth, proliferation, loss of differentiation to apoptosis. Furthermore, c-myc has a potent angiogenic activity, inducing fibroblasts to build up an immature vascular network.

Deregulation of c-myc is commonly found in a broad range of malignancies. Detection of intensive crosstalk between β-catenin, AR, and c-myc, and CGH findings of chromosome 8 alterations have stimulated investigation of the occurrence of genetic alterations involving the protooncogene myc (8q24) in JNAs.

A Northern blot study of the expression of c-myc mRNA in 25 JNA cases found no differences between the expression of c-myc in normal and tumor tissue. On the contrary, using RT-PCR, which is a more sensitive technique, Schick et al found c-myc mRNA overexpression in 3 of 7 JNAs. FISH analysis revealed loss of c-myc in 7 cases. However, in 3 advanced JNAs, they found gains of c-myc, which were associated with increased mRNA and protein levels, suggesting involvement of the c-myc oncogene in the aggressive growth behavior of these tumors (Table 4).

Members of the fos family (c-Fos, FosB, and its smaller splice variants, Fra-1 and Fra-2) dimerize with Jun protein to form the AP-1 transcription factor complex, which leads to activation of a number of AP-1-dependent target genes involved in cell proliferation or death, differentiation, and inflammation. c-fos has oncogenic activity and is frequently overexpressed in tumor cells. Nagai et al investigated the mRNA expression of c-fos gene by Northern blot and observed increased expression of the c-fos gene in 14% of JNA cases.

The protooncoprotein c-kit is a tyrosine kinase receptor that belongs to the family of PDGF receptors. Expression of c-kit and activation mutation of the c-kit gene have been detected in some mesenchymal tumors, such as gastrointestinal stromal tumors. High levels of c-kit expression have been used as a target for treatment with

<table>
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<th>Gene</th>
<th>Method of investigation</th>
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<tbody>
<tr>
<td>β-catenin</td>
<td>Immunohistochemistry and sequencing</td>
<td>Mutations in β-catenin would abrogate controlled growth and impair cell differentiation</td>
<td>Abraham et al, Zhang et al, Valanzano et al</td>
</tr>
<tr>
<td>TP53</td>
<td>Northern blot and FISH</td>
<td>Although overexpression of p53 mRNA has been found, p53 pathway may be inactivated via interaction with β-catenin</td>
<td>Nagai et al, Schick et al</td>
</tr>
<tr>
<td>c-myc</td>
<td>FISH, RT-PCR, immunohistochemistry, and Western blot</td>
<td>Upregulation of c-myc may be involved in aggressive tumor growth of JNAs</td>
<td>Schick et al</td>
</tr>
<tr>
<td>c-fos</td>
<td>Northern blot</td>
<td>Overexpression of c-fos mRNA may be related to cell proliferation</td>
<td>Nagai et al</td>
</tr>
<tr>
<td>c-kit</td>
<td>Immunohistochemistry</td>
<td>Strongly expressed in stromal cells; may be used as a target for treatment with STI571</td>
<td>Zhang et al</td>
</tr>
<tr>
<td>GSTM1</td>
<td>PCR</td>
<td>Loss of gene expression; polymorphism might affect the risk of developing JNA</td>
<td>Gautham et al</td>
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</table>

Abbreviations: FISH, fluorescence in situ hybridization; RT-PCR, reverse transcriptase-polymerase chain reaction; PCR, polymerase chain reaction.
STI571. Immunohistochemical analysis showed strong expression of c-kit in the stromal cells of JNA samples, suggesting that c-kit expression may be of clinical importance to patients with JNA.51

Mutations of the ras gene have been associated with a wide range of human solid tumors.90 Members of the ras gene family (Ki-ras, Ha-ras, and N-ras) are structurally related and encode a protein (p21) with an important role in the regulation of normal signal transduction and cell growth.91 Mutations within the Ki-ras and Ha-ras genes were investigated in 28 JNAs using PCR-SSCP and DNA sequencing.92 However, there was no evidence of mutation within codons 12, 13, 59, and 61.

Her-2/neu, also known as c-erbB-2, encodes a transmembrane tyrosine kinase protein related to the epidermal growth factor receptor family. Amplifications of the Her-2/neu oncogene have been reported for several different tumors.93 However, FISH analysis of 7 JNA samples showed no evidence of Her-2/neu gene amplification.77

**OTHER GENES**

Glutathione S-transferases (GSTs) are a family of enzymes that detoxify hydrophobic electrophiles, including polycyclic aromatic hydrocarbon carcinogens that have been implicated in the pathogenesis of lung cancer. The glutathione S-transferase M1 (GSTM1) gene within the mu class of human GSTs has been shown to be polymorphic, and individuals who are homozygous for a null allele have the GSTM1-null genotype.94 When GSTM1 is not expressed, there is increased risk of malignancy of the upper aerodigestive tract. Gautham et al79 found that 3 of 8 JNA samples did not express the GSTM1 gene. Some studies have shown that the combination of the GSTM1-null genotype and CYP1A1, NAT2, or GSTP1 polymorphism confers a greater risk of lung cancer than does the GSTM1-null genotype alone. Future investigations into JNA development should focus on assessing the risk related to multiple combinations of genetic polymorphisms that may individually identify individuals at high risk.94

The Cas family of multidaptor and scaffold molecules has an essential role in intracellular signaling events. Although these proteins do not have enzymatic or transcriptional activity, they exert spatiotemporal control of signaling events through their ability to undergo changes in phosphorylation and to associate with effector proteins in multimolecular complexes. p130Cas has a well-established role in cell motility as a component of the integrin signaling machinery.95 However, immunohistochemical analysis of p130Cas revealed no expression in JNAs.51

**CONCLUSIONS AND FUTURE PERSPECTIVES**

There are important unanswered questions regarding the histogenesis of JNA. Does JNA originate in vascular endothelial cells or in fibroblasts? The biphasic nature of JNA is poorly understood. Do the stromal and vascular components proliferate and grow together or is 1 component responsible for growth and the other component merely a bystander? There have been speculations on the origin of JNA (fibrous or vascular) since the 19th century (reviewed by Schick et al96). Because of its excessive vascularity some investigators have considered JNA as a vasoproliferative malformation. Sternberg97 and Hubbard98 proposed that JNA is a specific type of hemangioma and Schif in 1959 considered JNA as an ectopic vascular tissue and suggested that pituitary estrogen axis imbalance contributes to its growth. Histological investigations by Beham et al99 suggested that JNA is a vascular malformation; according to Schick et al,96 the vascular component could be explained embryologically as due to vascular atavism.

Several of the angiogenic markers, growth factors, and proliferation markers, discussed in this review, have been found to be associated with the pathogenesis of JNA, and some were also described in vascular anomalies100–102; therefore, these markers do not identify a potential origin of this benign tumor.

Recent immunohistochemical results indicated that VEGF, its transcriptional regulator hypoxia-inducible factor-1 (HIF-1), and other proangiogenic factors are frequently localized to JNA stromal cells, suggesting that deregulated vessel growth is driven by stromally derived growth factor.36,40,41,48 Moreover, the frequent presence of β-catenin mutations in JNA and the confinement of nuclear β-catenin expression to nuclei of stromal cells reinforce the hypothesis that the stromal component is the key neoplastic element of JNAs.

Recent genetic analysis of JNA clarified some details of the tumor’s genetic abnormalities and provided evidence for a role of androgens in JNA biology. The partial or complete losses of the Y chromosome and gains of the AR gene due to gains
of chromosome X are indicative of an androgen-related pathophysiological process in JNA. It was recently proposed that there is an interplay between AR, VEGF, and HIF in prostate cancer; this also appears possible in JNA. Furthermore, β-catenin functions as an AR coactivator protein. The combined effect of β-catenin gene mutation to increased AR expression has been suggested as an explanation of its occurrence in adolescent males.

However, the question of whether the initiating event in the development of these tumors occurs in the endothelium or in the stroma remains, and to date, no single theory can explain all the characteristics of nasofibromas including the predilection for the male sex.

The next step in clarifying the histogenesis of JNA is laser-microdissection of fibroblasts and vessels and separate analysis of their genomic profile and allelic imbalances. A recent study using microdissection and RT-PCR found similar mRNA expression in endothelial cells and fibroblasts of some analyzed transcripts, suggesting that the cell of origin could not be differentiated. New studies using global genomic-based analysis will contribute to better understanding the origin and interactions between vascular and fibroblastic components of this particular neoplasm.

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