Rare De Novo Germline Copy-Number Variation in Testicular Cancer

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Although heritable factors are an important determinant of risk of early-onset cancer, the majority of these malignancies appear to occur sporadically without identifiable risk factors. Germline de novo copy-number variations (CNVs) have been observed in sporadic neurocognitive and cardiovascular disorders. We explored this mechanism in 382 genomes of 116 early-onset cancer case-parent trios and unaffected siblings. Unique de novo germline CNVs were not observed in 107 breast or colon cancer trios or controls but were indeed found in 7% of 43 testicular germ cell tumor trios; this percentage exceeds background CNV rates and suggests a rare de novo genetic paradigm for susceptibility to some human malignancies.

Heritability is an important determinant of cancer risk; however, even in early-onset human cancers, the vast majority of cases occur without identifiable hereditary or other known risk factors. The role of de novo germline copy-number variations (CNVs) in cancer susceptibility is largely unknown, although de novo mutations in cancer-susceptibility genes (e.g., *APC* [MIM 611731], *RET* [MIM 164761], *RB1* [MIM 614041], and *MEN1* [MIM 613733]) are common. Genomic assessment, especially of de novo germline CNVs in disease-affected individuals, has defined mechanisms of susceptibility for neurodevelopmental and psychiatric disorders, as well as for congenital heart disease. Studies of autism (MIM 209850), schizophrenia (MIM 181500), intellectual disability, and tetralogy of Fallot (TOF [MIM 187500]) have identified a higher incidence of de novo CNVs in disease-affected children than in controls.[1–5] In this study, we explore the role of de novo germline CNVs in early-onset sporadic (nonhereditary) cancers and focus on individuals with early-onset testicular germ cell tumors (TGCTs [MIM 273300]).

TGCTs are the most common solid malignancy in young adult males, and the majority of TGCTs occur between the ages of 15 and 35 years. Most TGCTs are sporadic, but 2% of cases are familial.[6] Family history of disease is among the strongest risk factors for TGCTs: there is an 8- to 10-fold increase in risk for brothers of a TGCT index case and a 4- to 6-fold increase in risk for fathers and sons of a TGCT case.[6] A 1.6 Mb deletion on the long arm of the Y chromosome (i.e., the "gr/gr" deletion in the three azoospermia factor [AZF] regions) has been associated with a 2- to 3-fold increase in TGCT risk.[7] Two TGCT genome-wide association studies have identified common SNPs at the 12q22 locus, encompassing *KITLG* (MIM 184745) and associated with a 2.55- to 3-fold increase in TGCT risk.[8,9] Although these low- to moderate-penetrance susceptibility loci most likely contribute to disease risk, the majority of early-onset TGCTs remain unexplained.

In this study, we ascertained DNA samples from case-parent "trios" composed of a proband with a TGCT (diagnosed at or before 35 years of age), *BRCA1/2* (MIM 113705 and MIM 600185)-mutation-negative breast cancer (MIM 114480) (diagnosed at or before 45 years of age), or early-onset colorectal cancer (CRC [MIM 114500]) (diagnosed at or before the age of 50) and the two unaffected biologic parents of the cases. For additional controls, DNA from unaffected offspring (siblings of cases) was ascertained. Cases with multiple known cancer-affected family members or a known predisposition to cancer were excluded. The research protocol was approved by the institutional review board at the Memorial Sloan-Kettering Cancer Center, and written informed consent was obtained from all subjects. DNA was obtained from saliva and peripheral-blood lymphocytes for purposes of confirmation. Analysis of CNVs was performed by comparative genomic hybridization with the use of the NimbleGen HD2 2.1 million probe microarray platform (oligonucleotides were optimized for hybridization performance, and uniform genome coverage provided a resolution to approximately 25 kb).[4] DNA samples were analyzed in NimbleGen's Icelandic facility, where two-color hybridizations with a single male reference genome were performed. Complete experimental procedures are detailed elsewhere.[4,10,11] For system noise correction, we used local and Lowess normalization and also performed self-self hybridizations by using multiple reference genomes. On the basis of singular value decomposition of the self-self

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Table 1. Identified De Novo Germline CNVs in Testicular Germ Cell Tumor Case-Parent Trios

Trio	Tumor Type	Pathology	Stage	Age at Diagnosis	Chromosome Location	Amplification or Deletion	Size	Implicated Gene(s)
T68	NSGCT	mixed germ cell tumor containing yolk sac tumor and teratoma	IIIc	27 years	7q11.22	amplification	119 kb	*AUTS2*
T74	NSGCT	mixed germ cell tumor predominantly containing embryonal cell carcinoma and focus of immature teratoma and seminoma	I	27 years	6p21.2	deletion	400 kb	*KIF6*, *KNCK16*, and *KNCK17*
T82	NSGCT	mixed germ cell tumor containing seminoma, embryonal carcinoma, and yolk sac tumor	IIa	23 years	12q24.11	amplification	178 kb	*SSH1*

The following abbreviation is used: NSGCT, nonseminomatous germ cell tumor.

data, the principal components of system noise were determined and the distortion of genetic signal was minimized.[11] We used Kolmogorov-Smirnov (KS) segmentation, minimizing variance to segment the data, and KS statistics to judge the significance of the segments.[4] For a given KS segmentation, states were only computed for probes that passed a series of filters, including exclusion of probes with more than two mappings and those that occurred above a population threshold on the basis of the frequency of polymorphisms over a set of 1,500 high-quality control hybridizations. Once the performance parameters for each hybridization were characterized, the probability that a probe was a "Mendel violator" was calculated with a strict threshold for the Mendel violation p value of 10^{-9}; this was the same stringent protocol used in our recent study of nearly 1,000 autism case-parent trios and controls.[4] For comparison of the de novo events between cases and controls, a two-tailed Fisher's exact test was applied. We used the two-sided binomial test to determine significant deviations of the observed number of CNVs from a theoretically expected distribution of observations.

The median age of the 43 testicular cancer cases was 29 years (range = 19–35 years) at diagnosis; ten individuals had a seminoma, and 33 had a nonseminomatous TGCT. The frequency of de novo CNVs was compared in 60 breast cancer case-parent trios (median age = 35 years; range = 20–45 years), 34 unaffected siblings (median age = 37 years; range = 21–57 years), and 13 trios with early-onset colorectal cancer (median age = 38 years; range = 25–50 years). We identified three de novo deletion or amplification events in three different TGCT probands (Table 1) but did not identify any de novo germline events in the unaffected siblings or in any of the early-onset breast or colorectal cancer trios. Overall, we analyzed 382 genomes for CNVs and found a de novo germline CNV in 7% (3 out of 43) of TGCT index cases and in none of the 107 unaffected siblings or early-onset breast or colorectal cancer trios ($p = 0.02$). Compared to the 7% rate of de novo CNVs observed for testicular cancer, as well as for congenital heart disease and autism,[4] the lack of de novo CNVs in the breast cancer trios ($n = 60$; $p = 0.02$) and the combined breast and colon cancer trios ($n = 73$; $p = 0.01$) is significantly different than what was expected.

In the TGCT trios, the de novo CNVs identified (one deletion and two amplification events) were 119 kb, 178 kb, and 400 kb in size (Figure 1). In one developmentally normal young man who was diagnosed with metastatic nonseminomatous testicular cancer at age 27, a 119 kb amplification was present in region 7q11.22 (Figure 1A). The 7q11 locus has been implicated by cytogenetic and genomic analyses as an area of frequent somatic amplification in testicular germ cell tumors.[12,13] This region also lies near autism susceptibility candidate 2 (*AUTS2*), which codes for a highly conserved neuronal nuclear protein that is developmentally regulated and implicated in autism and intellectual disabilities but that has not been studied in tumors.[14,15] A large 400 kb de novo CNV in 6p21.2 (Figure 1B) encompasses a kinesin family member 6 (*KIF6* [MIM 613919]), which has not yet been implicated in carcinogenesis. Finally, a 178 kb gain-of-copy-number event was observed in 12q24.11 (Figure 1C). This region encompasses the phosphatase slingshot-1 (*SSH1*), which was previously implicated in the cofilin pathway activation and which might play a role in cancer cell motility and invasion.[16]

To validate the specific de novo amplifications and deletions discovered from the HD2 array, we used a Taqman CNV approach, which confirmed the presence of the events in the probands of interest in both saliva- and blood-derived DNA (Figure 2). Next, we used Taqman assays to determine the frequency of each of these genomic events in a larger ascertainment of 113 sporadic TGCT cases; none of the deletions or amplifications was detected, suggesting the rarity of these specific genomic events.

Utilizing an agnostic genomic scan of case-parent trios, we demonstrate de novo germline CNVs as a mechanism of human cancer susceptibility. In other human diseases for which de novo germline CNVs have been implicated (e.g. autism and congenital heart disease), similar rates of de novo events were identified (7%–9%) and were considerably higher than the expected background rates of 1%–2% for such events.[1,2,4,5] On the basis of 1,500 controls analyzed with the same array platform, the

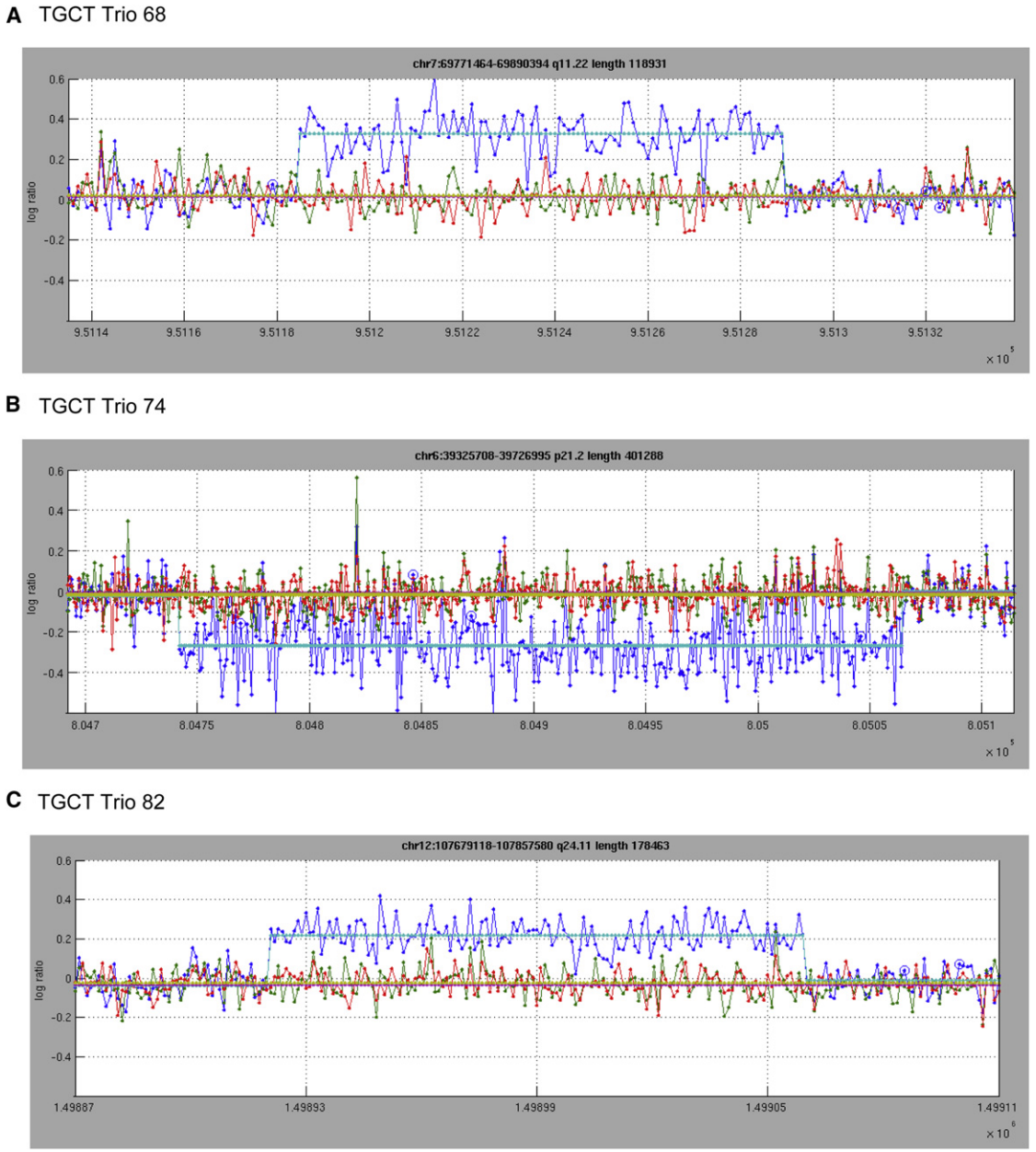

Figure 1. Representation of De Novo Events in Testicular Germ Cell Tumors
The three de novo events include (A) an amplification in 7q11.22, (B) a deletion in 6p21.2, and (C) a duplication in 12q24.11. In each panel, the probe log-ratio values for the mother, father, and proband appear in red, green, and blue, respectively. The probability that a probe "violates" Mendelian inheritance was calculated, and polarity was assigned to this probability depending on whether the probe in the proband was detecting a duplication (positive) or a deletion (negative).

implicated genomic regions were not in areas of frequent copy-number polymorphisms and repeat events were not identified in 113 sporadic TGCT cases, suggesting that these genomic events appear to be rare. Given sample-size limitations, replication of our study in an independent set of TGCT trios would provide further confirmation of our findings.

In contrast, no de novo CNVs were identified in a cohort of early-onset sporadic breast or colorectal cancer case-parent trios. The de novo mutation paradigm might be important but less readily evident in early-onset human diseases in which the natural history of the disease would normally result in decreased fecundity. Currently curable by surgery, radiation, and/or chemotherapy,[17] TGCTs were previously a fatal cancer affecting young men before they were able to reproduce. This observation might explain the absence of detection of germline events in the relatively rare Mendelian TGCT pedigrees ascertained to date. In addition, in sperm samples banked prior to treatment and in samples obtained after treatment, many TGCT individuals retain qualitative and/or quantitative defects in spermatogenesis.[17] We also speculate that the paradigm of a de novo germline etiology of disease might be less applicable to late-onset cancers and more relevant

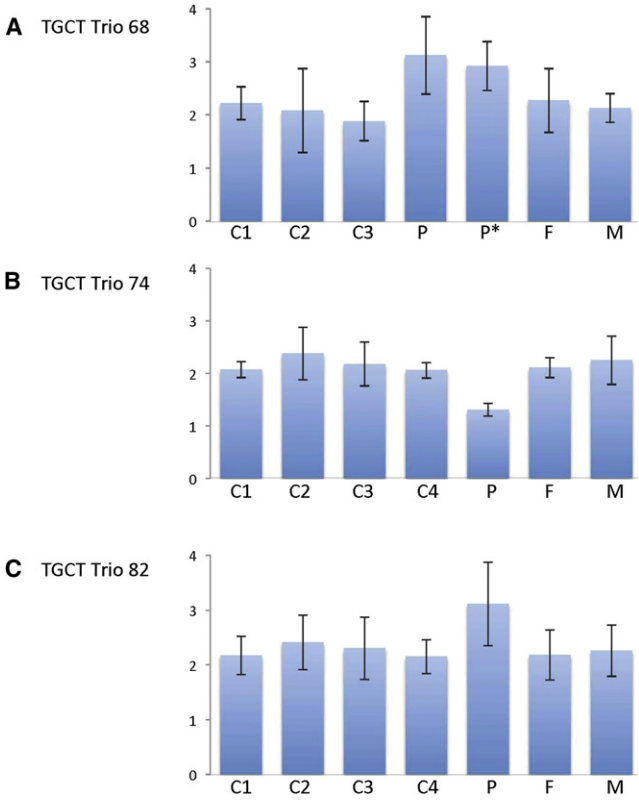

Figure 2. Validation of De Novo Events in Testicular Germ Cell Tumors

(A–C) Taqman CNV assays confirm the presence of the de novo amplifications or deletions in the three TGCT trios. In trio 68, both saliva and blood were obtained from the proband (P and P* for saliva and blood, respectively) for confirmation of the amplification. The amplification or the deletion is present in the proband (P) and absent in the parents (M and F) and controls (C1–C4). Error bars indicate the standard deviation of the copy number for the replicates.

to very early-onset cancers for which a purifying selection is more likely to eliminate the heritable susceptibility factors. These findings might act to limit the vertical transmission and accumulation of de novo germline structural aberrations causing susceptibility to this disease. If correct, this might in part explain the seemingly lower frequency of de novo events in adult-onset breast and colon cancer cases.

The identification of the genetic basis of TGCTs, as well as other cancers, will require additional analysis of de novo mutational and structural genomic events with the use of next-generation-sequencing technologies. However, the findings reported here indicate that a case-parent trio design for the identification of de novo mutations in early-onset sporadic cases might represent a new paradigm for discovering mechanisms of susceptibility to human neoplasia.

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Web Resources

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

References

1. Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., Yamrom, B., Yoon, S., Krasnitz, A., Kendall, J., et al. (2007). Strong association of de novo copy number mutations with autism. Science *316*, 445–449.
2. Xu, B., Roos, J.L., Levy, S., van Rensburg, E.J., Gogos, J.A., and Karayiorgou, M. (2008). Strong association of de novo copy number mutations with sporadic schizophrenia. Nat. Genet. *40*, 880–885.
3. Stefansson, H., Rujescu, D., Cichon, S., Pietiläinen, O.P., Ingason, A., Steinberg, S., Fossdal, R., Sigurdsson, E., Sigmundsson, T., Buizer-Voskamp, J.E., et al.; GROUP. (2008). Large recurrent microdeletions associated with schizophrenia. Nature *455*, 232–236.
4. Levy, D., Ronemus, M., Yamrom, B., Lee, Y.H., Leotta, A., Kendall, J., Marks, S., Lakshmi, B., Pai, D., Ye, K., et al. (2011). Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron *70*, 886–897.
5. Greenway, S.C., Pereira, A.C., Lin, J.C., DePalma, S.R., Israel, S.J., Mesquita, S.M., Ergul, E., Conta, J.H., Korn, J.M., McCarroll, S.A., et al. (2009). De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. Nat. Genet. *41*, 931–935.
6. Krausz, C., and Looijenga, L.H. (2008). Genetic aspects of testicular germ cell tumors. Cell Cycle *7*, 3519–3524.
7. Nathanson, K.L., Kanetsky, P.A., Hawes, R., Vaughn, D.J., Letrero, R., Tucker, K., Friedlander, M., Phillips, K.A., Hogg, D., Jewett, M.A., et al. (2005). The Y deletion gr/gr and susceptibility to testicular germ cell tumor. Am. J. Hum. Genet. *77*, 1034–1043.
8. Kanetsky, P.A., Mitra, N., Vardhanabhuti, S., Li, M., Vaughn, D.J., Letrero, R., Ciosek, S.L., Doody, D.R., Smith, L.M., Weaver, J., et al. (2009). Common variation in KITLG and at 5q31.3 predisposes to testicular germ cell cancer. Nat. Genet. *41*, 811–815.

9. Rapley, E.A., Turnbull, C., Al Olama, A.A., Dermitzakis, E.T., Linger, R., Huddart, R.A., Renwick, A., Hughes, D., Hines, S., Seal, S., et al.; UK Testicular Cancer Collaboration. (2009). A genome-wide association study of testicular germ cell tumor. Nat. Genet. *41*, 807–810.
10. Lee, Y. R.M., Kendall J. (2011). in *arXiv:1105.0900v1*.
11. Lee, Y.H., Ronemus, M., Kendall, J., Lakshmi, B., Leotta, A., Levy, D., Esposito, D., Grubor, V., Ye, K., Wigler, M., and Yamrom, B. (2012). Reducing system noise in copy number data using principal components of self-self hybridizations. Proc. Natl. Acad. Sci. USA *109*, E103–E110.
12. Korkola, J.E., Heck, S., Olshen, A.B., Reuter, V.E., Bosl, G.J., Houldsworth, J., and Chaganti, R.S. (2008). In vivo differentiation and genomic evolution in adult male germ cell tumors. Genes Chromosomes Cancer *47*, 43–55.
13. Rodriguez, E., Mathew, S., Reuter, V., Ilson, D.H., Bosl, G.J., and Chaganti, R.S. (1992). Cytogenetic analysis of 124 prospectively ascertained male germ cell tumors. Cancer Res. *52*, 2285–2291.
14. Sultana, R., Yu, C.E., Yu, J., Munson, J., Chen, D., Hua, W., Estes, A., Cortes, F., de la Barra, F., Yu, D., et al. (2002). Identification of a novel gene on chromosome 7q11.2 interrupted by a translocation breakpoint in a pair of autistic twins. Genomics *80*, 129–134.
15. Kalscheuer, V.M., FitzPatrick, D., Tommerup, N., Bugge, M., Niebuhr, E., Neumann, L.M., Tzschach, A., Shoichet, S.A., Menzel, C., Erdogan, F., et al. (2007). Mutations in autism susceptibility candidate 2 (AUTS2) in patients with mental retardation. Hum. Genet. *121*, 501–509.
16. Wang, L.H., Xiang, J., Yan, M., Zhang, Y., Zhao, Y., Yue, C.F., Xu, J., Zheng, F.M., Chen, J.N., Kang, Z., et al. (2010). The mitotic kinase Aurora-A induces mammary cell migration and breast cancer metastasis by activating the Cofilin-F-actin pathway. Cancer Res. *70*, 9118–9128.
17. Feldman, D.R., Bosl, G.J., Sheinfeld, J., and Motzer, R.J. (2008). Medical treatment of advanced testicular cancer. JAMA *299*, 672–684.